The effect of aerobic training on growth, muscle development and heart condition in Atlantic salmon (*Salmo salar*) post-smolts in large-scale semi-closed containment systems.

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Content

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Abstract

Exposing fish to moderate water currents as a form of exercise is often perceived as positive for fish welfare, growth, muscle development and heart condition. This thesis examines what effect rearing Atlantic salmon (*Salmo salar*) post-smolts in a flow through semi-closed raceway system (Preline) has on growth, mortality, feed conversion, and development of white skeletal and heart muscle. The experiment consists of three phases: 1. Smolt in freshwater 2. Post-smolt in seawater (Preline vs. control facilities) and 3. On-growing in seawater. A total of 321.412 smolts (101 g SEM ± 4.2) were distributed in two groups. Fish in the Preline system was exposed to a 10-20 cm/s water current and a traditional open cage system were used as control. Temperature, salinity and oxygen varied between the two systems, as this was a large-scale experiment and water conditions were difficult to control. After four months in the systems, the control fish had a significantly higher weight, length and condition factor compared to the Preline group. This was linked with higher temperatures at the control site. The Preline group had a 2.44 times higher frequency of small muscle fibres in the white skeletal muscle at the end of the post-smolt phase. This was associated with the Preline fish being exposed to a constant water flow, promoting aerobic exercise. Furthermore, the increased recruitment of white muscle fibres might increase the growth potential for further production. There were significantly higher elevated mRNA levels of MEF2C and GATA4, indicating cardiomyocyte hypertrophy, in the Preline group at the end of the post-smolt phase. This was also associated with aerobic exercise and can possibly help the fish cope with stressful situations they might be exposed to in a farming environment.

1 Introduction

1.1 Background

The production of Atlantic salmon (*Salmo salar*) post-smolts have traditionally been conducted in open net pen systems in the sea. Post-smolt refers to the period after the fish have been transferred from freshwater to seawater, until they reach a weight of approximately 1 kg. The seawater stage is considered to be the most critical phase of production, since about 20 % of the fish will not make it to market size (Hjeltnes et al., 2017). Throughout the seawater phase, many factors contribute to this loss of biomass, including; poor smolt quality, disease and treatments. Some of the measures that have been done to prevent production related losses is to bring forth new and innovative farming technology. These include the option of using semi-closed or closed rearing systems in parts or throughout the seawater production phase, to reduce the time fish spend in open cage systems.

1.2 *Closed aquaculture technology*

A closed, or semi-closed, aquaculture system can be defined as a fish producing system that has an impenetrable or close to impenetrable barrier between the fish and the surrounding environment (Iversen et al. 2013). During the last couple of years, several new variants of semi-closed or closed farming technologies have been launched, which can differ in size and shape, and volumes may vary from 1000 m^3 to 21 000 m^3 (Iversen et al., 2013, Teknologirådet, 2013) Construction material can vary from enclosed bags, to more rigid material like polyethylene (PE), fibreglass, steel and concrete. These constructions can either be land based, or placed in the sea. Most of the current semi-closed rearing systems in the sea do not have an extensive treatment of the inlet water, and creates only minor differences in the environment inside and outside of the system. One of the expected advantages with semi-closed farming technology in the sea is the lowered energy costs compared with land based post-smolt systems (Iversen et al., 2013, Teknologirådet 2012) as a result of minimal lifting height of the water. The degree of control over the water quality and farming environment, by controlling the depth of the water intake, is most likely going to be greater in a closed farm rather than a traditional open net pen.

Salmon lice (*Lepeophtheirus salmonis*) represents one of the biggest challenges in the Norwegian salmon aquaculture industry, which inflicts large economic costs and subsequent losses, along with potential negative effects on wild salmonid populations (Costello, 2009). By pumping water from below the surface layers, closed farming technology should in theory avoid

salmon lice infections (Rosten et al., 2011). Closed farming technology can also potentially be largely helpful in reducing environmental impacts from aquaculture. This includes reducing organic waste emissions, prevent spreading of fish pathogens, and reduce farmed escapees (Rosten et al., 2011). This study will focus on a semi-closed seawater raceway system called Preline, developed by Preline Fish Farming System in close collaboration with Lerøy Seafood Group. The system is constructed as a large oval pipe, with a deep-water intake and outlet at 35 m, which forms a constant deep-water flow through the system.

1.3 Aerobic exercise

In closed or semi-closed aquaculture systems it is possible to control the water current, as opposed to the traditional open net pen cages. This especially applies to flow-through raceway systems. Salmonids are active species of fish and can be made to swim against a constant current. This makes them ideal subjects for studies that test the effects of aerobic training. Producing a robust fish, by making it constantly swim towards a current on low to moderate speeds, can give several advantages. Factors that can be influenced by a training regime are, among others, increased growth (East and Magnan, 1987, Totland et al., 1987, Farrell et al., 1990, Jørgensen and Jobling, 1993, Young and Cech Jr, 1994, Castro et al., 2011, Solstorm et al., 2015) reduced aggressive behaviour (Adams et al., 1995, Brännäs, 2009, Solstorm et al., 2016), better feed conversion (Leon, 1986, East and Magnan, 1987, Christiansen et al., 1992), and, development of skeletal and cardiac muscle (Walker and Emerson, 1978, Totland et al., 1987, Bugeon et al., 2003, Martin and Johnston, 2005, Rasmussen et al., 2011, Castro et al., 2013).

Increase in growth as an effect of exercise has been documented in many studies for salmonid fish species. Swimming at relatively low speeds, 1.5 body lengths per seconds (BL/s) or less, have proven to be the most effective in improving growth rate (Walker and Emerson, 1978, East and Magnan, 1987, Totland et al., 1987, Farrell et al., 1990, Jørgensen and Jobling, 1993, Young and Cech Jr, 1994, Castro et al., 2011, Solstorm et al., 2015). The particular water current that causes the best growth rate seems to vary between life stages. Jørgensen and Jobling (1993) found that exposing fish to water currents of 1.5 BL/s gave the best growth in juvenile Atlantic salmon, when compared to 0, 1.0 and 2.0 BL/s, while Castro et al. (2011) found a better growth in pre-smolts reared at 0.8-1.0 BL/s, either by interval or continuous currents. For post-smolts, Solstorm et al. (2015) found increased growth for groups reared at 0.2 and 0.8 BL/s compared to a water velocity of 1.5 BL/s.

Salmonids tend to form dominant hierarchies and show aggressiveness towards each other (Winberg et al., 1991, Adams et al., 1995). Fish that are swimming freely in still water tend to form dominant hierarchies and show increased aggression, which can lead to less food for subordinate fish (Adams et al., 1995, Brännäs, 2009) and greater differences in size within the fish population (Jobling et al., 1993, Brännäs, 2009). Aggressive behaviour can cause skin damage and lesions to other individuals, and further, studies have shown that high aggression can lead to increased spontaneous activity (Christiansen et al., 1991, Jobling et al., 1993, Solstorm et al., 2016). This can in turn lead to high energetic costs and increase the feed conversion ratio of the population. Solstorm et al. (2016) showed in their study that moderate water velocities (0.8 BL/s) created less movement and interactions between fish, compared to slower velocities (0.2 BL/s). Fish that are swimming towards a constant current tend to form schools, which in turn can lead to reduced aggressive behaviour. This can result in more available food for subordinate fish and, consequently, a higher growth rate in the population (East and Magnan, 1987, Totland et al., 1987, Farrell et al., 1990, Christiansen et al., 1991, Christiansen et al., 1992, Jobling et al., 1993, Jørgensen and Jobling, 1993, Adams et al., 1995, Brännäs, 2009). The feed conversion ratio (FCR) is of uttermost importance to fish farmers and can be described as the amount of mass gained by the fish relative to the amount of feed consumed (Jackson, 2009). Several studies have shown that exercise decreases the feed conversion ratio for different salmonid species (Leon, 1986, East and Magnan, 1987, Christiansen et al., 1992). Although swimming and exercise demands a certain amount of energy, the fish eat more food as appetite is stimulated, and weight gain is achieved faster with less food used (Davison, 1989).

Due to all the studies showing the effect of training on growth, aggression and feed conversion, it is particularly interesting to identify how these factors are affected in post-smolts reared in a large-scale raceway system.

1.4 Skeletal muscle

The growth of muscle in fish differs from that of mammals as the recruitment of muscle fibres last through large parts of the life cycle, rather than just hypertrophy of the existing fibres (Stickland, 1983). The bulk of the myotome in most fish, and thereby the most relevant for the

aquaculture industry, consists of white muscle fibres of the skeletal muscle, which is mainly used for rapid anaerobic swimming (Alexander, 1969). The diameter of white and red muscle fibres in fish rarely exceeds 200 and 50 μm, respectively (Weatherley et al., 1988). Thus, further individual growth must come from the recruitment of new muscle fibres. For some fish species, like the Atlantic salmon, the recruitment of muscle fibres is massive throughout the life cycle, starting at approximately 5000 white skeletal muscle fibres per myotome at hatching, approximately 180,000 during smoltification and exceeding 1 million fibres when reaching a weight of 4 kg (Johnston, 1999). However, it seems that as fish grow, the contribution to growth from muscle fibre recruitment decreases, while the contribution from hypertrophy increases (Weatherley et al., 1980, Stickland, 1983). During myogenesis, the formation of muscular tissue, myoblast nuclei are absorbed by expanding muscle cells during post-embryotic growth to keep a constant ratio between the volume of nuclei and the volume of cytoplasm (Koumans et al., 1994). Through this process, muscle fibres are formed by the fusion of myoblasts on existing muscle cells, which further leads to the formation of myotubes. Muscle fibre growth and recruitment can be achieved and influenced by factors like diet, exercise training and temperature (Walker and Emerson, 1978, Totland et al., 1987, Stickland et al., 1988, Bugeon et al., 2003, Martin and Johnston, 2005, Ibarz et al., 2011, Rasmussen et al., 2011).

Rasmussen et al. (2011) performed an exercise experiment on rainbow trout for 9 weeks with a water velocity of 0.9 BL/s. By using histological image analysis, they found that exercised fish showed signs of hyperplasia of the white skeletal muscle, as there was a higher amount of muscle fibres in the size class of 0-20 μm compared to the control group, even though this could not be statistically confirmed. This may in turn indicate that fish exposed to an exercise training regime, may have a higher recruitment of muscle fibres than fish that are not. Several other studies have confirmed that a training regime of 0.4-1.6 BL/s stimulates muscle fibre hypertrophy and thereby increasing the overall body weight of the fish (Walker and Emerson, 1978, Totland et al., 1987, Bugeon et al., 2003, Martin and Johnston, 2005, Ibarz et al., 2011).

Since there is a lack of research on white skeletal muscle fibre recruitment in Atlantic salmon as an effect of exercise, the current experiment wanted to investigate the effect of training on muscle fibre hyperplasia in a semi-closed raceway system.

1.5 Heart muscle

Poor cardiac function is a factor that may contribute to high mortality rates after transfer to seawater, and lack of sustained exercise may be one of the reasons for this. The heart of teleost fish species is located ventrally towards the head, and is separated from the abdominal cavity by septum transversum. It consists of several valves and chambers that keep the blood flow running in a constant direction. The oxygen depleted blood gathers in the sinus venosus, from the blood vessel venae hepaticae and to venae cardinalis communis (Kryvi and Poppe, 2016). Furthermore, the blood is transported to the atrium, which pumps the blood into the ventricle of the heart. From there, powerful muscle contractions pump the blood through bulbus arteriosus and the ventral aorta, to the gills for oxygen supply, and further out to the rest of the body. The ventricle, which has an irregular form and consists for most fish species of a spongious myocardium, makes up most of the weight of the heart (Santer et al., 1983). However, more active fish species, such as salmonids or scombridae, have a compact layer of myocardium surrounding the spongious layer and a pyramidal shaped heart that can give powerful contractions (Davie and Farrell, 1991). This can in turn generate a higher blood pressure that supports an increase in the cardiovascular demands of active fish.

Growth of cardiac muscle is similar to that of skeletal muscle as it is driven by cardiomyocyte hypertrophy and hyperplasia. This growth may be stimulated from exercise training, and it has been shown that relative ventricular mass can increase as a result of sustained swimming (Castro et al., 2013). A plastic response like this is most likely to satisfy the higher energetic demands of the skeletal muscle in active fish, along with an improved contractile ability. For mammals, cardiac growth is driven by cardiomyocyte hypertrophy (Soonpaa et al., 1996). It has been shown that both hypertrophy and hyperplasia take place as a response to chronic anemia and sexual maturation in rainbow trout (Clark and Rodnick, 1998, Simonot and Farrell, 2009). Exercise training has also shown to increase relative ventricular mass, although not as much as through chronic anaemia and sexual maturation (Castro et al., 2013). It is likely that this increase is due to both cardiomyocyte hyperplasia and hypertrophy.

Vascular endothelial growth factor (VEGF) is probably the most important factor influencing the formation of new blood vessels in vertebrates (Yancopoulos et al., 2000), and is one of the main driving forces in improving capillarity through prolonged exercise in fish (Iemitsu et al., 2006, Castro et al., 2013). It is more than likely that exercise induced cardiac growth is a result

of hypertrophy as well as hyperplasia. In mammals, cardiomyocytes will stop proliferating soon after birth, and further growth to cope with an increasing workload comes from cardiac hypertrophy (Soonpaa et al., 1996). This is mostly driven by the expression levels of cardiac transcription factors such as GATA4, Myocyte-specific enhancer factor 2C (MEF2C) and the homeobox transcription factor Csx/Nkx2-5 (Kolodziejczyk et al., 1999, Akazawa and Komuro, 2003)

Cardiac health is one of the main factors that are influenced by the effects of aerobic exercise in fish. Constant, moderate movement over a longer period of time can affect several mechanisms in cardiac responses including cardiac growth, contractility, vascularization, energy metabolism and myokine production in different salmonid species (Davie et al., 1986, Farrell et al., 1990, Farrell et al., 1991, Castro et al., 2013). Castro et al. (2013) showed that fish kept at a constant current of 1.31 body lengths/s had higher protein levels of PCNA, which can be used as an indicator of cardiac growth being driven by cardiomyocyte hyperplasia. In addition to this, the fish had elevated cardiac mRNA levels of MEF2C, GATA4 and ACTA1. This suggested an increase in cardiomyocyte tissue due to enlargement of these specific cells located here (cardiomyocyte hypertrophy). Furthermore, the fish showed elevated mRNA levels of VEGF and EPO, suggesting a more efficient oxygen supply network. Other effects that was influenced by aerobic exercise during this study was potentially improved contractile function, a higher capacity for lipid oxidation and a significant enlargement of mitochondrial size, which suggest an enhanced energetic support system. It would be interesting to see if a study like this can be recreated in a large-scale system, and how this influences the expressed mRNA levels of these genes. Therefore, expressed mRNA levels of MEF2C, GATA4 and VEGF in a semi-closed system, compared to a traditional cage, were investigated in the current study.

1.6 Objectives

Currently, there is little information on the effects of rearing post-smolts in semi-closed containment systems, when regarding growth potential, survival, development and welfare indicators. However, there is huge potential with this type of raceway system to reduce infections by pathogens, such as salmon lice, and improve other factors like cardiac health, immune response and overall body composition through aerobic training. The aim of this project was to uncover any possible differences between fish reared in a semi-closed raceway and a traditional open cage system. The main factors that will be evaluated in this study include growth pattern, development of white skeletal muscle and heart condition as an effect of aerobic exercise.

The experiment was based on the following hypotheses:

$H0_1$: Rearing Atlantic salmon post-smolts in semi-closed raceway systems and exposing them to a 10-20 cm/ water current has no significant effect on growth (weigth, length and condition factor (CF)).

$H0_2$: Rearing Atlantic salmon post-smolts in semi-closed raceway systems and exposing them to a 10-20 cm/ water current has no significant effect on muscle fibre hyperplasia.

$H0_3$: Rearing Atlantic salmon post-smolts in semi-closed raceway systems and exposing them to a 10-20 cm/ water current has no significant effect on expressed mRNA levels of MEF2C, GATA4 and VEGF.

2 Materials and methods

2.1 Fish material and rearing conditions

The Atlantic salmon smolts used in this experiment were reared at Sjøtroll Havbruk AS facilities located at Kjærelva, Fitjar. A total number of 321.412 fish were used in the experiment, which had the same genetic and biological background, originating from the strain Salmobreed QTL duo, yearling smolts (1+). The eggs were incubated at 5.8 ^{o}C, and hatched at 513 degree days. First feeding began in early May 2015 (387 degree-days post hatching) and took place under conditions of constant light (LL) and heated water (approximately 14 ^{o}C). During the freshwater period, the fish were kept indoors in green 7m rearing tanks (70m^{3}) at ambient water temperature (Table 2.1) and at constant light (until January 11th, 2016, start photoperiod treatment). A commercial dry diet (EWOS, Bergen, Norway) was fed to all fish according to temperature and fish size.

Table 2.1. Monthly temperature from the June 2015 to June 2016 during the freshwater phase of the production.

Month	**Minimum, ^{o}C**	**Average, ^{o}C**	**Maximum, ^{o}C**
06-2015	15,3	17,2	18,6
07-2015	17,0	20,3	25
08-2015	15,5	17,8	21,8
09-2015	13,8	15,3	16,4
10-2015	10,8	13,0	14,1
11-2015	7,1	8,8	10,9
12-2015	3,9	5,5	6,9
01-2016	2,4	3,6	4,5
02-2016	1,8	2,6	3
03-2016	3,0	3,8	4,7
04-2016	4,3	5,8	7,6
05-2016	6,8	8,3	9,5

Smolts were produced according to standard protocols for yearling smolts. A photoperiod regime that is known for stimulating smoltification in salmonids was initiated at 11 January (Handeland and Stefansson, 2001). This treatment included a decrease in day length from LD24:0 to LD12:12 for 8 weeks (January 11th to March 7th), followed by another 8 weeks on LD24:0 (March 8th to April 30th). By the end of April, all fish showed morphological sings indicating a normal smoltification, including: lowered condition, dark fin margins, silvery scales and high NKA-activity (Stefansson et al., 2008). The fish were then ready for transfer to seawater and was transferred by well boat (Mowistar) to their respective facilities.

2.2 Experimental facilities

The Preline semi-closed raceway system is located at Sagen in Samnanger, in the Trengereid fjord (Hordaland, Norway) (Figure 2.1). This location has a depth of 100 m and is well protected from wind, waves and have good water circulation. The Preline platform is 50 x 12 x 8 m and holds approximately 2000 m^3 water volume (max water flow 400 m^3/min, water exchange rate 5-6 min, water current 10-20 cm/s). Water current was measured by Lerøy Vest AS using a Vector 3D Acoustic Velocimeter (Nortek AS, Norway). During the experimental period the water was collected from a depth of 35 m and circulated via the inlet pipe to the outlet pipe that creates a one-way water current through the system. A traditional open 160m conical circular cage was used as control and was located at Skorpo (Hardanger, Norway) (Figure 2.2). This location had a depth of approximately 250 m and the cages consisted of an Akvaline ring with a 60m deep pen (Norwegian name: spissnot). A facility at Buholmen (Hordaland, Norway) was used for the Preline group during the on-growing phase. The system was similar to the one at Skorpo.

Figure 2.1. Preline location at Sagen, Trengereid fjord and a diagram of the Preline semi-closed system (Sveier et al., 2015).

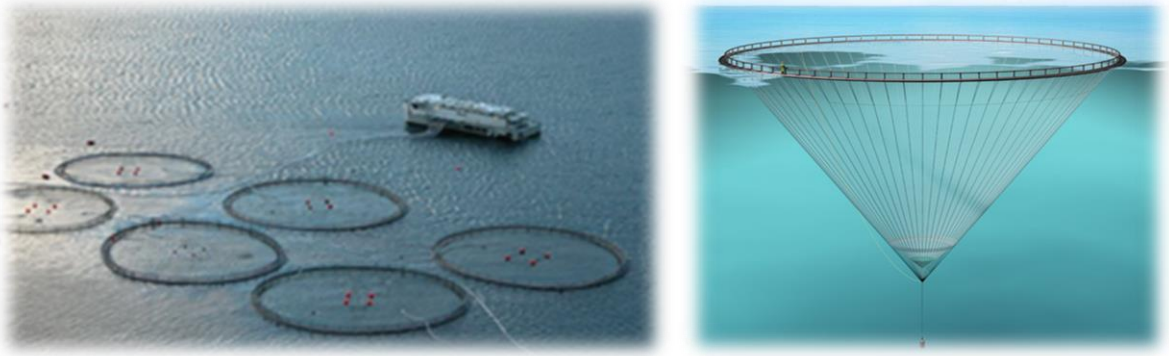

Figure 2.2. Control sea cage facilities at Skorpo, Hardanger fjord and a diagram of the open netpen system used at Skorpo (Sveier et al., 2015).

2.3 Experimental design

The experiment consisted of three different phases;

1. Freshwater: This phase was conducted at the Sjøtroll Havbruk AS facilities located at Kjærelva, Fitjar. Fish from this facility were divided into two separate groups; Preline and Control.

2. Post-smolt in seawater: A total of 157 126 and 164 286 fish were transferred to the Preline and control facilities on April 30th and May 5th, 2016, respectively.

3. On-growing in seawater (adult): The Preline fish were transferred by well boat to a traditional sea cage facility at Buholmen in Hordaland, Norway on August 31st 2016, while the control fish were kept at the Skorpo facility. A schematic representation of the experimental protocol is depicted in Figure 2.3.

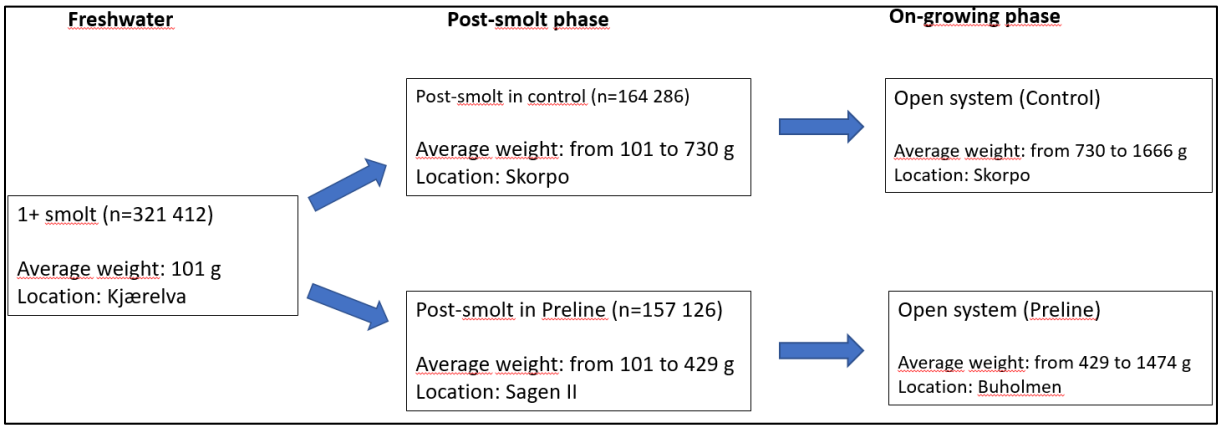

Figure 2.3 Schematic representation of the experimental protocol. One sampling was conducted during the freshwater phase (April 15th 2016) and three during the post-smolt phase at Preline and control facilities, after one (June 1/2nd), two (June29/30th) and four months (August 29/30th) in seawater.

All husbandry practices at the farms were conducted in accordance with standard protocol for Lerøy Vest AS. Oxygen concentrations, feeding, salinity and temperature were controlled by automatic systems (OxyGuard Commander, Sterner) at the facilities and all data was registered daily (Fishtalk, AkvaGroup, Bryne). Oxygen, temperature and salinity were registered at 3m, 8m and 15m in the open cage systems at both Skorpo and Buholmen, and in the inlet and outlet water in the Preline system. All groups were checked twice per day and dead fish were removed. The fish in both treatments were fed commercial freshwater/seawater dry diets (EWOS, Norway) from automatic feeders (AkvaGroup) throughout the study.

2.4 Sampling protocol

There was one sampling conducted during the freshwater phase on April 15th 2016. Three samplings were conducted during the post-smolt phase at each facility, after one month (Control – June 1st and Preline – June 2nd), two months (Preline – June 29th and Control – June 30th) and four months (Preline – August 29th and Control – August 30th) in seawater. The sampling protocol for the post-smolt phase was the same as the freshwater phase. Weight estimations based on feed output (Fishtalk calculations, FCE=1.1) was conducted by Lerøy Vest AS throughout the post-smolt phase. Further, weight estimations (Fishtalk calculations, FCE=1.1) were conducted from the start of the on-growing phase to November 31st.

Each time during sampling in phase 1, Freshwater and 2, Post-smolt in seawater, a total of 30 fish were randomly selected by the use of a large net (Norwegian: Storhov) which was lowered down to 5 m in the Preline and cage systems. Thereafter, feed was thrown over the net to attract fish and then the net was quickly raised to collect fish. Further, the fish was humanely euthanized with $NaCO_3$-buffered tricaine methanesulphonate (MS222, Sigma-Aldrich, St Louis, MO, USA) anesthetic. Size (weight, g and fork length, cm) for all individuals was measured (Mettler Toledo 2000 and length scale). Muscle samples (3-5mm thick) were taken posterior to the dorsal fin and stored on buffered formalin for histological image analysis of muscle fibre size and distribution in a predefined circular area of 1000 μm in diameter. In addition, the ventricle of the heart was sagittally separated into two parts and emptied of blood. The heart samples were stored on buffered formalin and RNA later for histological image analysis and molecular analysis, respectively. Further, heart, gill, muscle and head kidney samples were stored in RNA later in order to screen for selected Salmonid pathogens (SAV/PRV) using real-time RT-PCR analyses.

2.5 TGC, condition factor and feed conversion ratio

Since the Preline and control facilities were located in different places, which varied in seawater temperature, a weight model incorporating growth rate/day dependent on the daily temperature was employed (Thermal Growth Coefficient, TGC). This model takes into account the optimal season temperature for fish growth and was calculated for the post-smolt and on-growing phase using the following equation:

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

The condition factor (CF) of the randomly selected individuals were calculated for each treatment, by using the following equation:

A relative percentage increase in weight was calculated for the two groups during the on-growing phase, from estimated weight values.

CF = (weight/(length)^3) *100

The feed conversion ratio (FCR) was calculated for the post-smolt and on-growing phase using the following equation:

FCR = (Biomass gained / feed consumption) *100

2.6 Histological image analysis

Muscle and heart samples stored on buffered formalin were sent to Fish Vet Group Norge, Oslo, Norway, for embedding, sectioning, staining and mounting. The sections were then scanned at Høyteknologisenteret (Bergen, Norway), using a ZEISS Axio Scan.Z1 slide scanner (Oberkochen, Germany). The sections were analysed using ZEN 2.3 (blue edition, ZEISS Oberkochen, Germany). A circular area of 1000 µm in diameter in the epaxial white skeletal muscle was randomly chosen in approximately the same area for each section (Figure 2.4). In each predefined area, muscle fibre size was measured in µm for the greatest possible distance in each fibre, including those who only had parts inside the circle (Figure 2.5). Muscle fibre diameter was thereafter sorted into 20 µm interval groups (from 0-20 µm up to >220 µm). Frequency tables were made for each of the post-smolt phase sampling points.

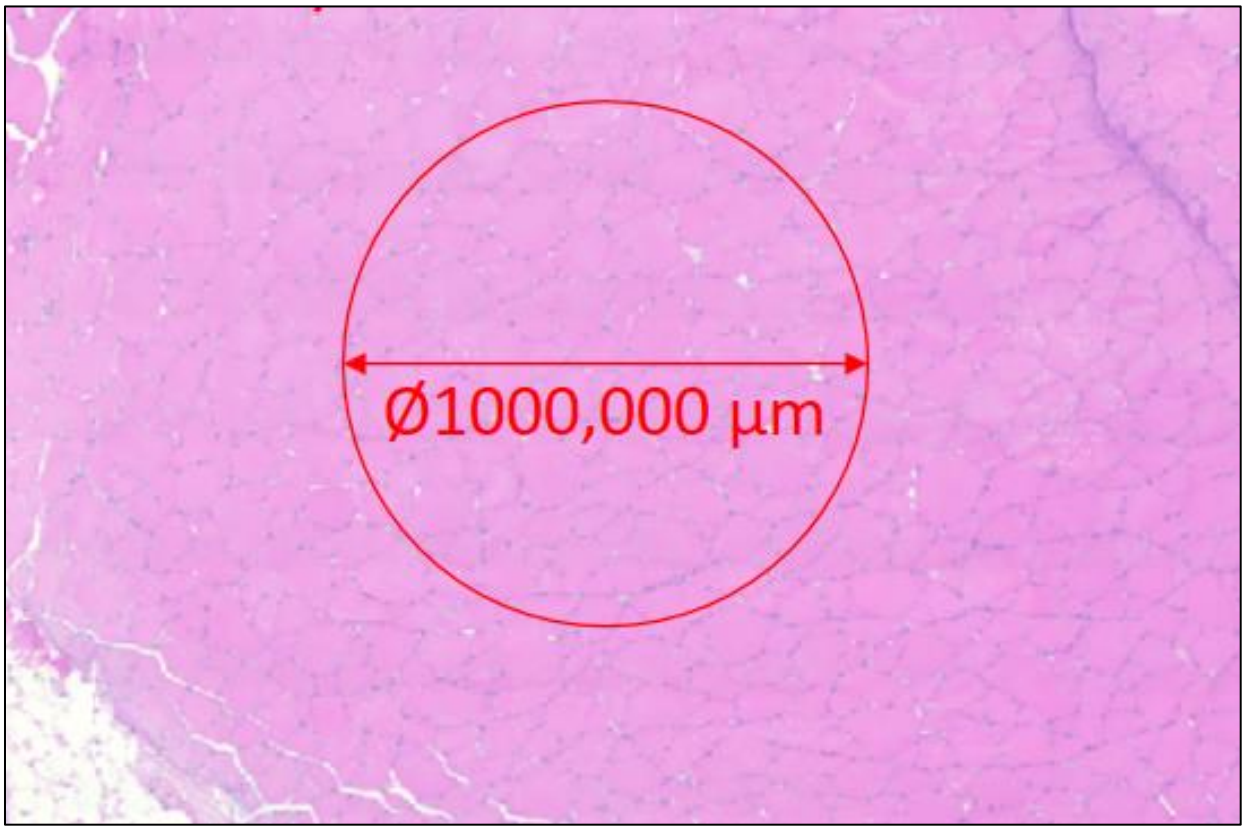

Figure 2.4: In each histological section, a predefined area for the analysis of muscle fibre size and distribution was randomly chosen, in the white skeletal muscle, in approximately the same area for each section.

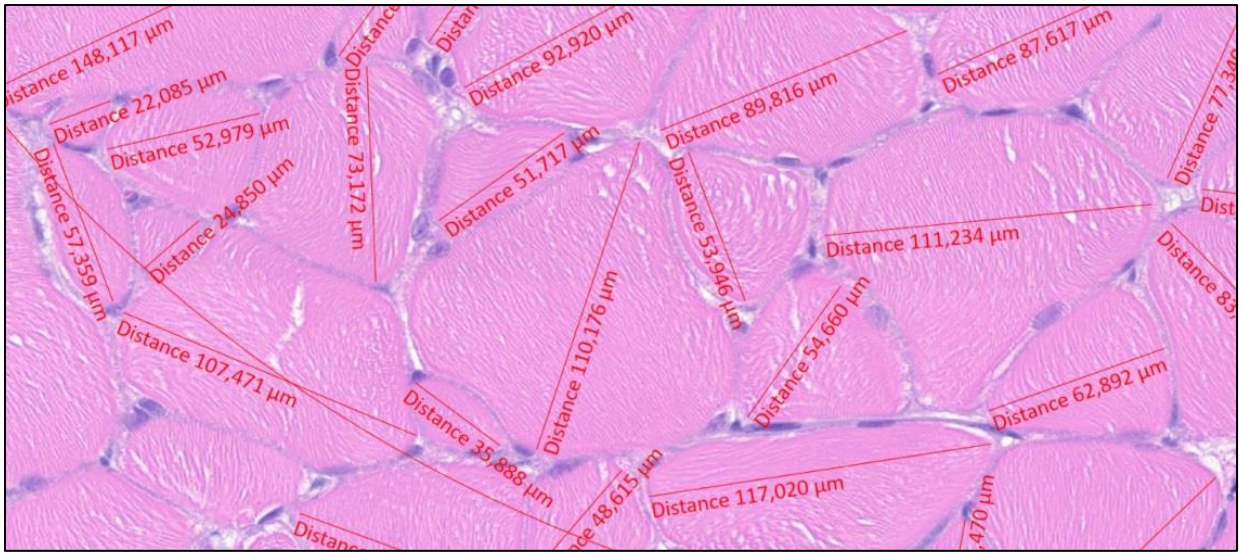

Figure 2.5: Example of measured fibre diameter in all muscle fibres within a predefined area in the white skeletal muscle. All fibres were measured for the greatest possible distance.

2.7 Histopathology and pathological real time RT-PCR screening

As a parallel to the analysis of muscle fibre size, each section was investigated for histopathological signs of degeneration and necrosis in red and white skeletal muscle, and heart muscle. Based on this histopathological investigation, a total of 90 heart samples from both sites, collected in the freshwater (n=30) and last sampling of the post-smolt phase (n=60) (all stored in RNA later), were screened for Piscine orthoreovirus (PRV) and salmonid alphavirus (SAV), using real time RT-PCR analysis (Nylund et al., 2015). RNA from heart was extracted as described below (2.8.1 RNA precipitation). All RNA samples were stored at -20°C until further use.

The AgPt-ID^{TM} one step RT-PCR kit (Applied assistant) was used to test the extracted RNA from heart tissues for presence of SAV and PRV. The following real time RT-PCR assays were used: the PRV-M2 assay targeting the M2 segment capsid protein of PRV (Nylund et al., 2015), and the nsP1assay, targeting the nsP1 gene of SAV (Andersen et al., 2007). The real-time PCR protocol is described in Gunnarsson et al. (2017). The housekeeping gene elongation factor 1 alpha ($EF1A_A$ assay) was used as an internal control (Olsvik et al., 2005). Standard curves were generated using 10-fold serial dilutions of RNA in three parallels. Regression analysis, standard curve slopes s (cycle threshold, Ct, versus log quantity), amplification efficiency E ($E = [10^{1/(-slope)}] - 1$), and the coefficient of determination, R^2, were calculated for all assays. Each run of the real-time RT-PCR consisted of 45 cycles and the samples were considered positive when the fluorescence signal increased above a set threshold of 0.1. Negative controls, RNA extraction controls (lacking target RNA) and no template control, were included in all runs at a rate of 1 control per 10 samples to avoid false positives.

2.8 Molecular analysis of heart

2.8.1 RNA precipitation

For isolation of total RNA, heart samples were purified using a QIAsymphony nucleic acid purification robot (Qiagen, Hilden, Germany). A standard protocol for general purification from the manufacturer was followed. A total of 90 heart samples from the freshwater phase (n=30) and the last sampling (n=60) in the post-smolt phase was purified. Before the QIA symphony procedure, tissue samples had to be disrupted and homogenized. Tubes were prepared with 6-7 mg of zirconium oxide beads (1.4 µm) and 600 µl of RLT plus lysis buffer. A piece of heart

tissue (20-25 mg) was cut off, squeezed slightly to remove access RNA later, weighed and put into tubes containing buffer. Samples were then homogenized for 15 seconds at 5000 rpm in a Precellys 24 (Bertin technologies, Versailles, France). Homogenization samples were then left at least 5 minutes at room temperature for foam subsidence and placed in the refrigerator until sufficient number of samples was prepared for RNA purification. The total RNA concentration and purity was measured using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Massachusetts, United States).

2.8.2 Reverse transcriptase synthesis (cDNA synthesis)

cDNA from the freshwater fish and from the last two samplings in the post-smolt phase was synthesized as follows. Most of the steps were conducted with a Hamilton Microlab Starlet robot (Hamilton, Nevada, United States). The following components were added to a nuclease-free microcentrifuge tube: 1 µl of oligo$(dT)_{20}$ (50µM), 500 ng of total RNA, 1 µl 10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP and dTTP at neutral pH) and sterile, distilled water to make a total volume of 13 µl. Further, this mixture was put in C1000 Touch Thermal Cycler (Bio-Rad Laboratories, CA, USA) and heated to 65°C for 5 min and then incubated on ice for 1 min. Then 4 µl of 5X First-Strand Buffer, along with 1 µl 0.1 M DTT, 1 µl RNaseOUT™ Recombinant RNase Inhibitor and 1 µl SuperScript™ III RT (200 units/µl) was added and samples were incubated at 50°C for 60 minutes. Starting from pooled cDNA, a dilution series with the following concentrations was done with nuclease free water: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128. Based on this dilution series, an optimal dilution of all samples was determined to be 1:7.5. The mastermix (MM) for each gene consisted of SYBR Green (78.84 µl), specific primers (5.4 µl x 2, forward and reverse) and nuclease free water (36.36 µl).

2.8.3 qPCR

Quantitative PCR (q-PCR) was performed to quantify the mRNA abundance of selected genes using the C1000 Touch Thermal Cycler, CFX96 Real-Time System (Bio-Rad Laboratories, CA, USA) in conjunction with the software CFX Manager (version 3.1, Bio-Rad). The qPCR reactions were performed in a total volume of 10µl containing 4.38 µl Gene Expression Master Mix (Bio-Rad Laboratories, CA, USA), 0.3 µl of forward and reverse primer (200 nm final concentration) (Table 2.2), 0.2 µl of nuclease free water and 3 µl of cDNA diluted 1:7.5 The qPCR reactions were performed in 96-well plates (Bio-Rad) and the following thermal cycling protocol was used: 3 min at 95°C, 37 repetitions of 15 sec at 95°C and 1 min at 60°C and in the

end 10 sec at 95°C, 5 sec at 65°C and 5 sec at 95°C. All samples were run in duplicates and to confirm absence of DNA contamination and residues, "non-template control (NTC)" (Bio-Rad Laboratories, CA, USA) was included to the all plates. Furthermore, each plate had duplicate sample of pooled cDNA that was included on each plate and used for correction of differences between plates. Targets genes and reference gene for each individual sample were run in duplicates on separate plates. Prior to analyzing mRNA expression on all samples, all primer set were validated by running two-fold dilution series made from a representative pool of cDNA taken from selected samples covering all time points and different groups.

The amplification efficiency (E) was determined by the slope of a regression line (threshold cycle (ct) values versus log cDNA dilution) from the dilution curve. The efficiency was calculated based on the following formula (Pfaffl, 2004)

$$E = 10^{(-1/slope)}$$

Normalization of the genes expression based on target versus reference gene was calculated as follows (Pfaffl, 2004):

$$Ratio = E\,(CT_{mean})ref \,/\, E\,(CT_{mean})target$$

Ct - threshold cycle for each individual sample

E ref – qPCR efficiency of the reference gene.

E target – qPCR efficiency of the target gene.

Ct mean – average Ct value of duplicate reaction of individual sample

Table 2.2 Primer sequences for each qPCR assay (heart samples)

Gene	Primer sequences (5' → 3')		Accession no.	Reference
Ef1a	F	CCCCTCCAGGACGTTTACAAA	AF321836	(Olsvik et al., 2005)
	R	CACACGGCCCACAGGTACA		
MEF2C	F	CACCGTAACTCGCCTGGTCT	GU252207	(Castro et al., 2013)
	R	GCTTGCGGTTGCTGTTCATA		
GATA4	F	TCTCCATTCGACAGCTCCGT	HM475152	(Castro et al., 2013)
	R	CATCGCTCCACAGTTCACACA		
VEGF	F	AGACAGCCCACATACCCAAG	NM_001124417	(Castro et al., 2013)
	R	GAAGACGTCCACCAGCATCT		

2.9 Statistical analysis

All data sets were tested for normality using the Kolmogornov-Smirnov test. The Hartley F max test was used to test for homogeneity of variances for all groups. All the collected data from the experiment was analysed using the statistical program STATISTICA 13.2. To determine the level of significance for weight, length and condition factor between treatments, a one-way ANOVA was conducted. A one-way ANCOVA was conducted on the white skeletal muscle fibre size, to determine the level of significance between treatments, where length was used as a covariate factor. This was to remove the effect of size as a factor. A one-way ANOVA was conducted to see if there were any differences between the fish that showed symptoms of degeneration and necrosis in red and white skeletal muscle, and heart muscle. The need for homogeneity of variances was fulfilled for all groups, except for the molecular analysis of heart muscle. Therefore, a non-parametric ANOVA (Kruskal-Wallis ANOVA) was conducted for the cardiac muscle mRNA expression of MEF2C, GATA4 and VEGF, to determine the level of significance between treatments. A non-parametric ANOVA (Kruskal-Wallis ANOVA) was also conducted to determine the level of significance between SAV-positive and negative fish, within the last sampling of the control group. All statistical results are given in Appendix I. Differences were considered significant when $p<0.05$. All data in tables and figures are given as mean ± standard error of mean (SEM).

3 Results

Temperature, salinity and oxygen varied within the net pen at the open cage facilities and the mentioned parameters were registered daily at three different depths: 3 m, 8 m and 15 m. Temperature, salinity and oxygen for the open cage systems, both at Skorpo and Buholmen, are given as average of these three depths. For the Preline facility, these parameters were monitored at the water inlet and outlet, and the registrations from the outlet water are given in the results. In order to compare the systems, all registered data for these parameters are given from May 5th 2016 to November 31st 2016.

3.1 Temperature

An increase in temperature from about 7,5°C on May 5th, to 15 and 12,3 °C on August 31st, was seen in the control and Preline facilities, respectively (Figure 3.1). After the Preline fish was transferred to Buholmen for the on-growing period the temperature gradually increased from 15,3 °C on August 31st, peaking at 16,9 °C September 21st, and after that gradually decreasing to 8,7 °C on November 31st. For the control group the same trends were seen, from 15 °C on August 31st, peaking at 16,4 °C September 21st, and further, gradually decreasing to 8,6 °C on November 31st.

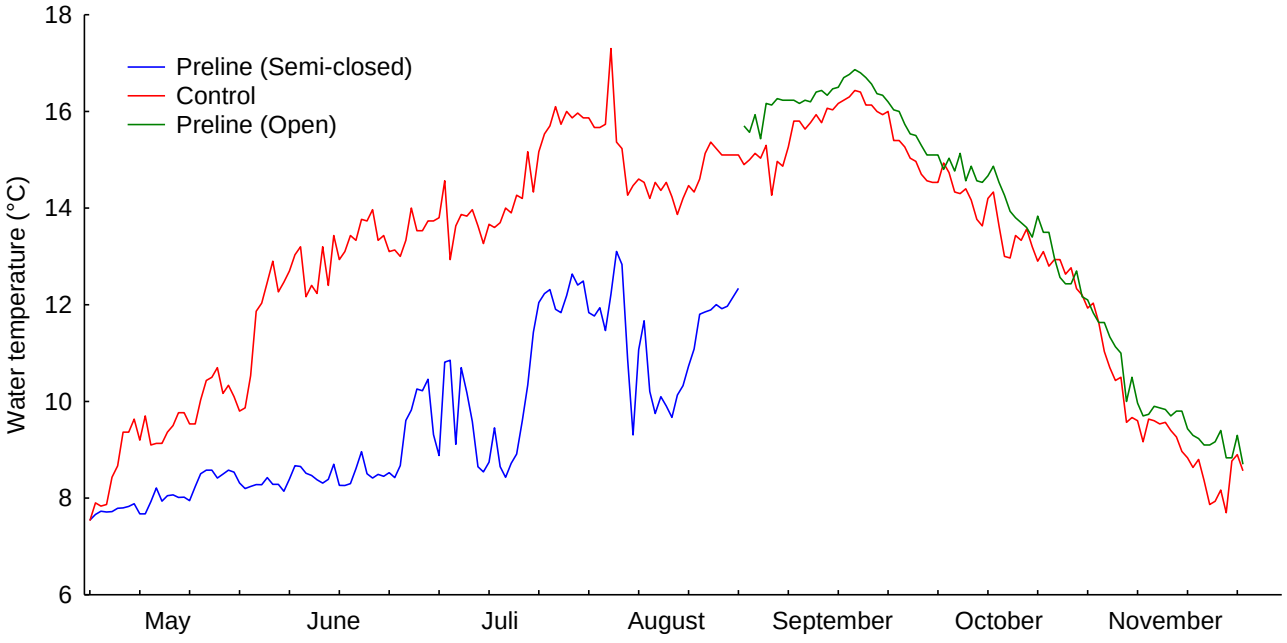

Figure 3.1: Average water temperature (°C) at the Preline and control (red) facilities from May 5th to November 31st. The blue line represents average temperature in the Preline system during the post-smolt phase, from May 5th to August 31st, whereas the green line represents the on-growing period at Buhomen from August 31st to November 31st.

3.2 Salinity

The registered salinity at the two facilities was higher for Preline than the control during both the post-smolt phase and the on growing phase (Figure 3.2). The salinity during the post-smolt phase varied between 26,2 and 34,7 ppt, and averaged at 31,7 ppt, in the Preline system. In the control cage it varied between 21,6 and 27,5 ppt and averaged at 24,2 ppt. During the on-growing period the lowest registered salinity was 26.9 and 20.1 ppt and highest 30.5 and 27.6 ppt in Preline (Buholmen) and control groups, respectively. The average during this period was 28.8 ppt for Preline and 24.6 ppt for control.

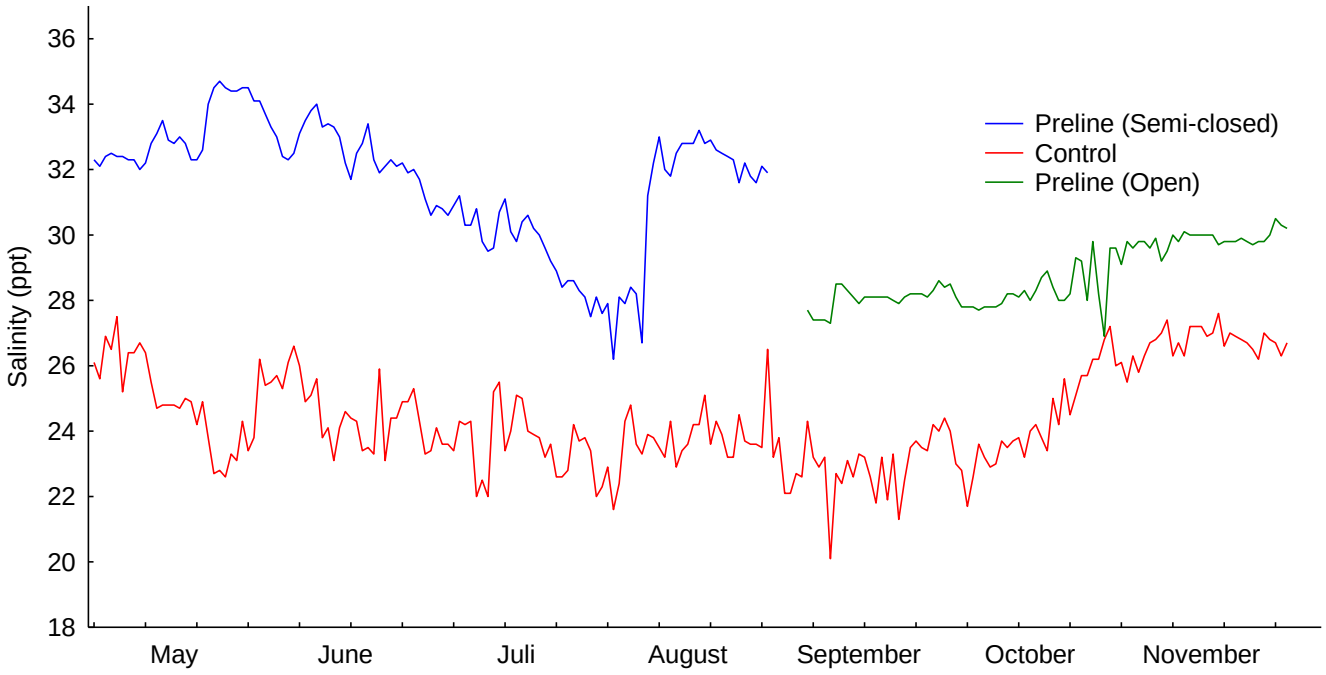

Figure 3.2: Salinity (ppt) at the Preline and control (red) facilities from May 5th to November 31st. The blue line represents the salinity in the Preline system during the post-smolt phase, from May 5th to August 31st, whereas the green line represents the salinity during on-growing period at Buholmen from August 31st to November 31st.

3.3 Oxygen

The lowest oxygen concentration registered in the outlet water at the Preline facility during the post-smolt phase was 72,2%, whereas the highest registered concentration was 131,56 %. For the control group the concentrations were 75,7 % and 111,6 % (Figure 3.3). The average O_2-concentration was 102.74 % and 97.21 % in Preline and control, respectively, during this period. During the on growing phase the lowest registered O_2-concentration was 83,8 % for Preline (Buholmen) and 78 % for the control fish. The highest O_2-concentration was registered at 99,4 % in Preline and 96 % in control. The average O_2-concentration was 92.96 % and 86.63 % in Preline and control groups, respectively, during this period.

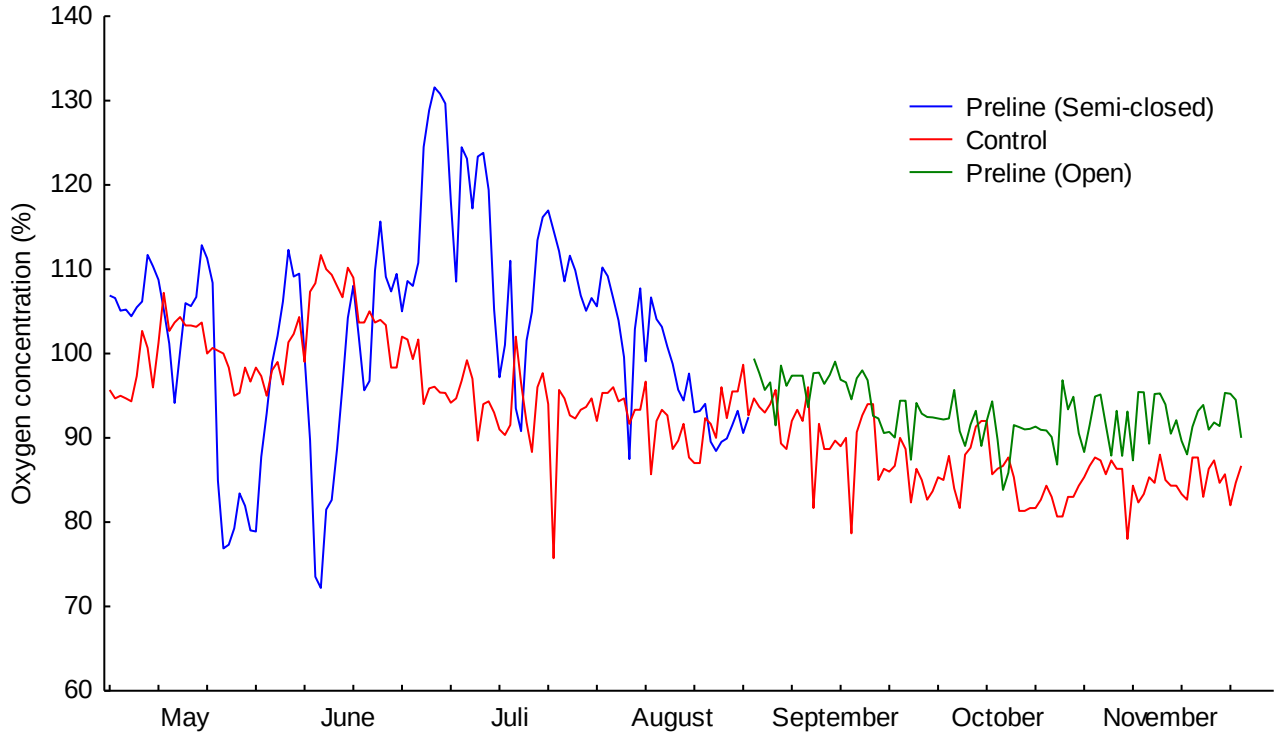

Figure 3.3: Oxygen concentration (%) at the Preline and control (red) facilities from May 5th to November 31st. The blue line represents the O_2-concentration in the Preline system during the post-smolt phase, from May 5th to August 31st, whereas the green line represents the O_2-concentration during on-growing period at Buholmen from August 31st to November 31st.

3.4 Mortality

Mortality was registered from April 30th at the Preline facility and from May 5th at the control facility during the post-smolt phase. From the first registered point at both facilities, the accumulated mortality was 0.54 and 0 % for Preline and control, respectively. The Preline facility had a higher mortality rate throughout the post-smolt phase, and by the end of August the rate had changed to 1.34 and 0.98 % for Preline and control, respectively (Figure 3.4). Notably, three months after the post-smolt phase the mortality in the control group had increased to 3.52%, while it had increased to 2.48% in the Preline (Buholmen) group.

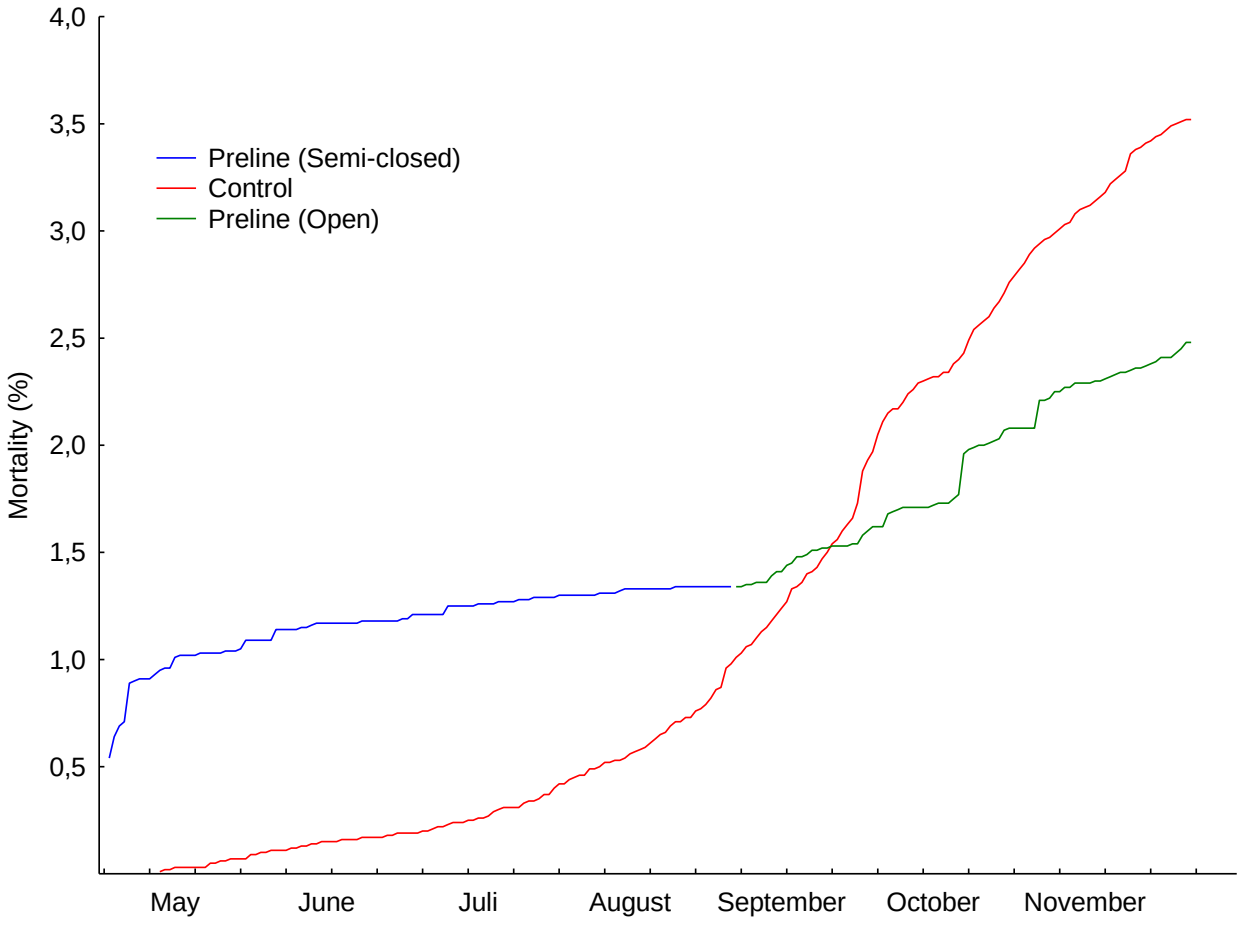

Figure 3.4: Accumulated mortality (%) in the Preline and control (red) group from April 30th (Preline) and May 5th (control) to November 30th. The blue line represents the post-smolt phase of the Preline group, from April 30th to August 31st, whereas the green line represents the on-growing period at Buholmen from August 31st to November 31st.

3.5 Weight

Mean weight (mean ±SEM) from the freshwater sampling (FW) on April 15th was measured to 101 ± 4.2 g. From the weight measurements that was conducted during the post-smolt samplings, there was a significant increase ($p<0.001$) in mean weight from sampling 1 (June 1/2nd) to sampling 3 (August 29/30th) for both groups. For the Preline fish the increase was from 130.51 ± 10.9 g to 429.27 ± 15.4g and 125.01 ± 4.4g to 730 ± 57.2g for the control group (Figure 3.5). There were significant differences in mean weight between the Preline and control groups ($p<0.001$) at sampling 2 and 3 during the post-smolt phase.

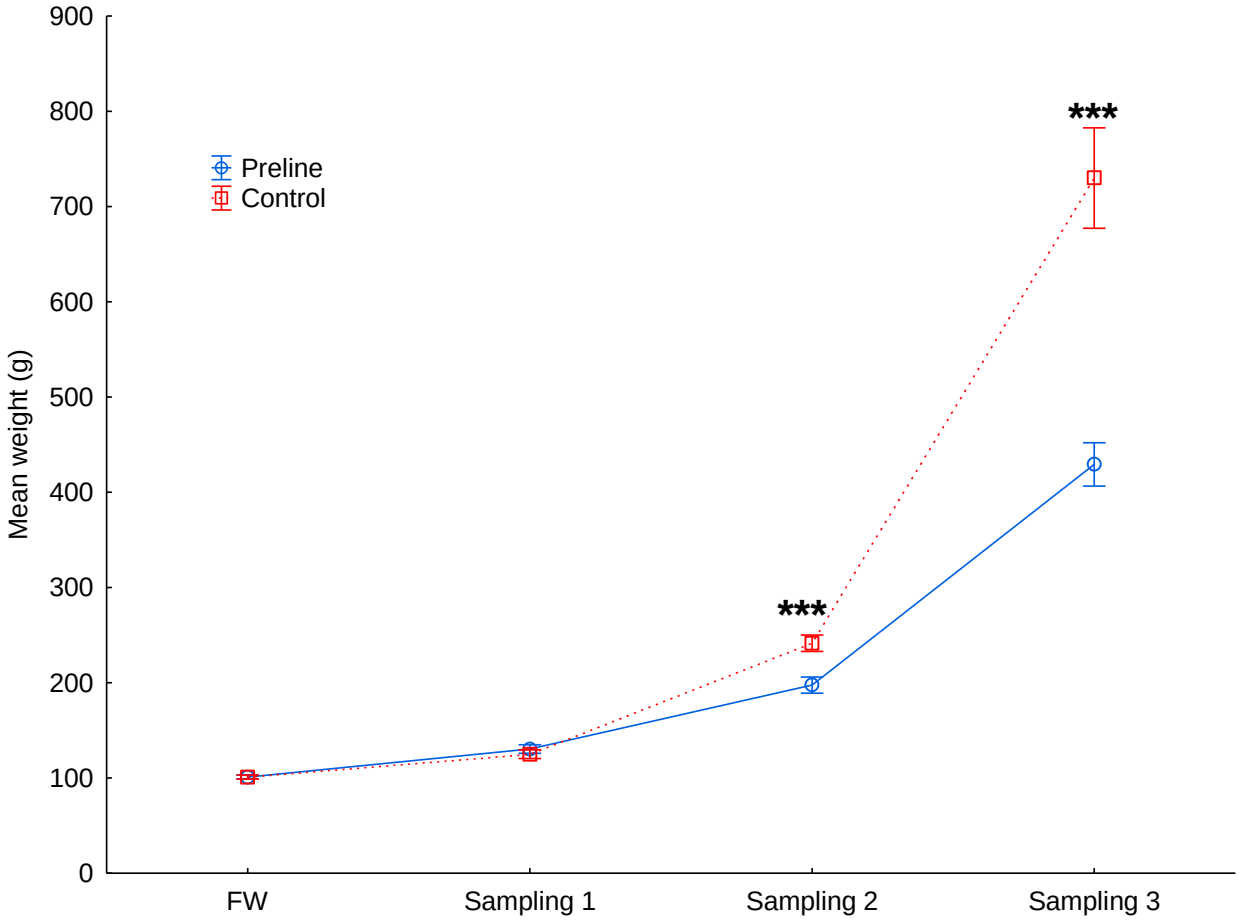

*Figure 3.5: Mean weight in Preline and control groups from the freshwater sampling (FW) on April 15th, and during the post-smolt phase, after sampling 1 (June 1/2nd), sampling 2 (June29/30th) and sampling 3 (August 29/30th). Asterisk indicates the level of significance between treatments; *p<0.05, **p<0.01, ***p<0.001. Each data sampling point is given as mean±SEM, n=30.*

An estimation of average weight based on feed output (Fishtalk calculations, FCE=1.1) was done by the Lerøy Vest AS. From June 1/2nd to August 29/30st there was an increase in mean weight, from 132 to 443.6 g in the Preline group, while the fish at the control facility had an increase in mean weight, from 136.7 to 733.5 g, during the same period (Figure 3.6). On November 31st, three months after the post-smolt phase, the mean weight of the two groups was estimated to 1474.7 g and 1666 g for Preline (Buholmen) and control, respectively (Figure 3.6). During the on-growing period the Preline group had a 232.44 % increase in weight, from August 31st to November 31st, while the Control group had a 122.73 % increase during the same period.

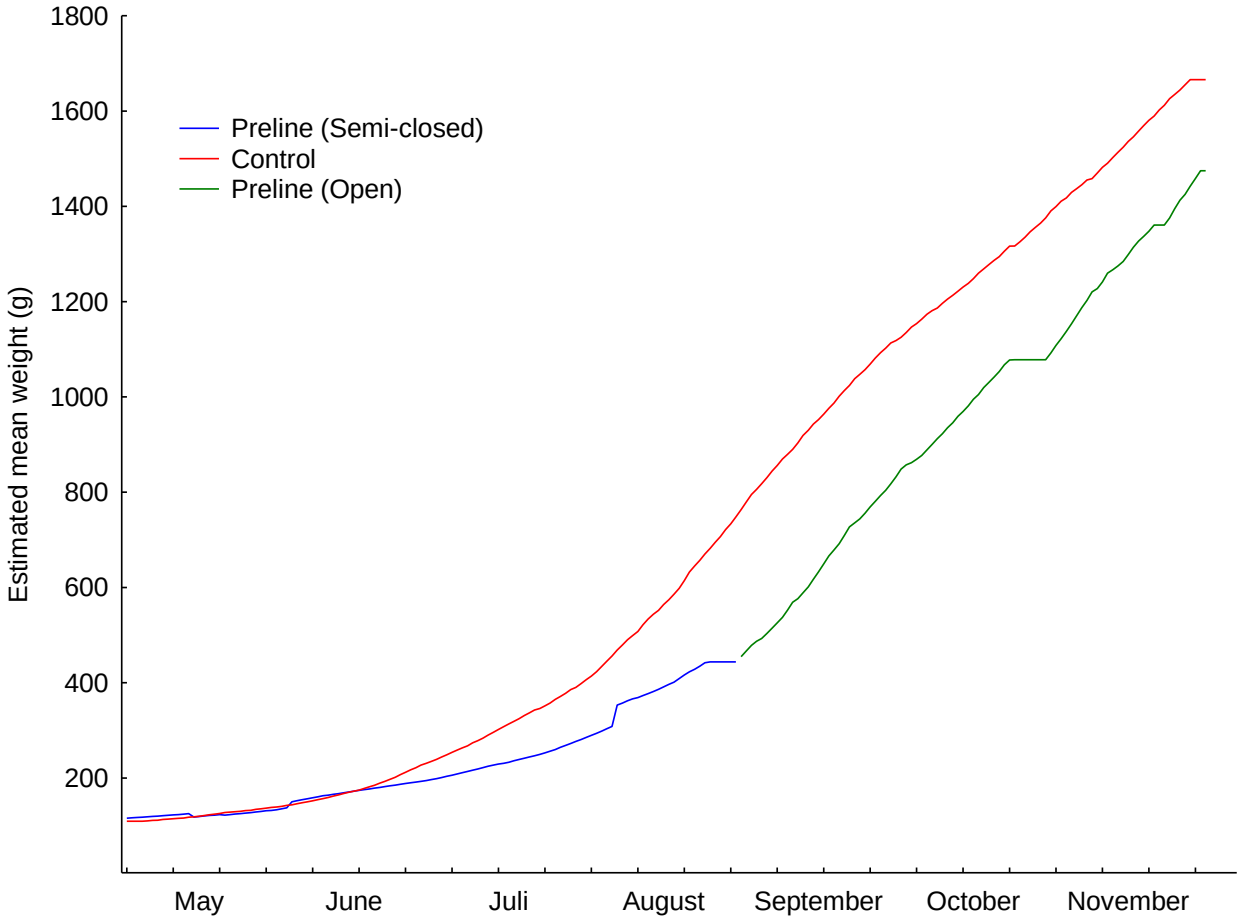

Figure 3.6: Estimated mean weight (Fishtalk calculations, FCE=1.1) for Preline and control (red line) groups from May 5th to November 31st. The blue line represents the weight gain in the Preline system during the post-smolt phase, from May 5th to August 31st, whereas the green line represents the weight gain during on-growing period at Buholmen from August 31st to November 31st.

3.6 Length

Mean length (mean ±SEM) from the freshwater sampling (FW) on April 15th was measured to 20.6 ± 0.2 cm. From the length measurements that was conducted during the post-smolt samplings, there was a significant increase ($p<0.001$) in mean length from sampling 1 (June 1/2nd) to sampling 3 (August 29/30th) for both groups. This increase was from 22.9 ± 0.2 cm to 38.7 ± 0.9 cm in the control group and from 23.2 ± 0,5 cm to 33.4 ± 0.3 cm in the Preline group (Figure 3.7). There were significant differences in mean length between the Preline and control groups ($p<0.001$) at sampling 2 and 3, during the post-smolt phase.

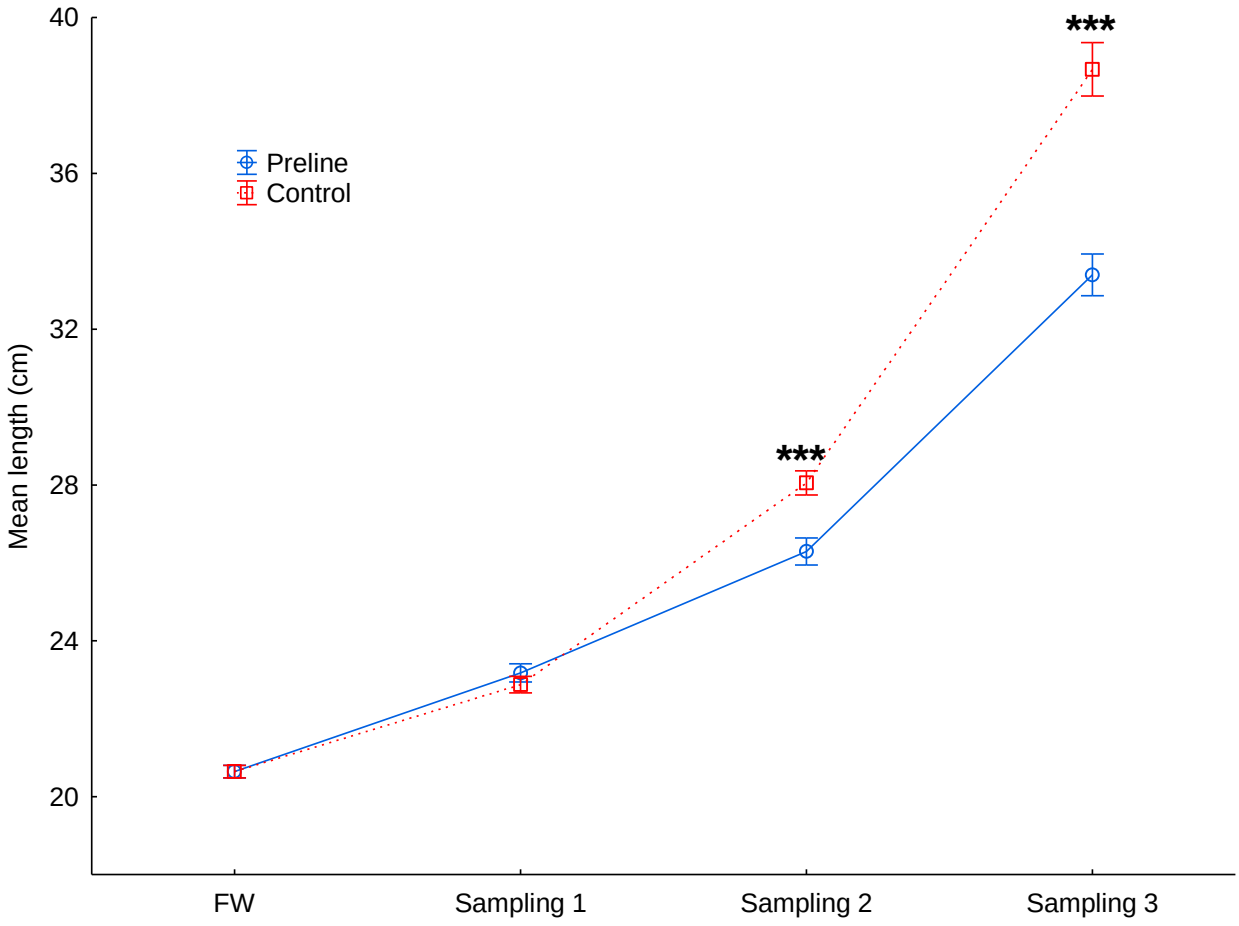

*Figure 3.7: Mean length of sampled fish in Preline and control groups from the freshwater sampling (FW) on April 15th, and during the post-smolt phase, after sampling 1 (June 1/2nd), sampling 2 (June29/30th) and sampling 3 (August 29/30th). Asterisk indicates the level of significance between treatments; *p<0.05, **p<0.01, ***p<0.001. Each data sampling point is given as mean±SEM, n=30.*

3.7 Condition factor

The average (mean ±SEM) condition factor was calculated to 1.15 ± 0.01 from the freshwater sampling (FW) conducted on April 15th. From sampling 1 (June 1/2nd) to sampling 3 (August 29/30th) the condition factor significantly increased ($p<0.001$) from 1.03 ± 0.01 to 1.20 ± 0.02 in the control group and from 1.04 ± 0.01 to 1.12 ± 0.02 in the Preline group (Figure 3.8). There was a significant difference in CF at sampling 3 in the post-smolt phase ($p<0.05$).

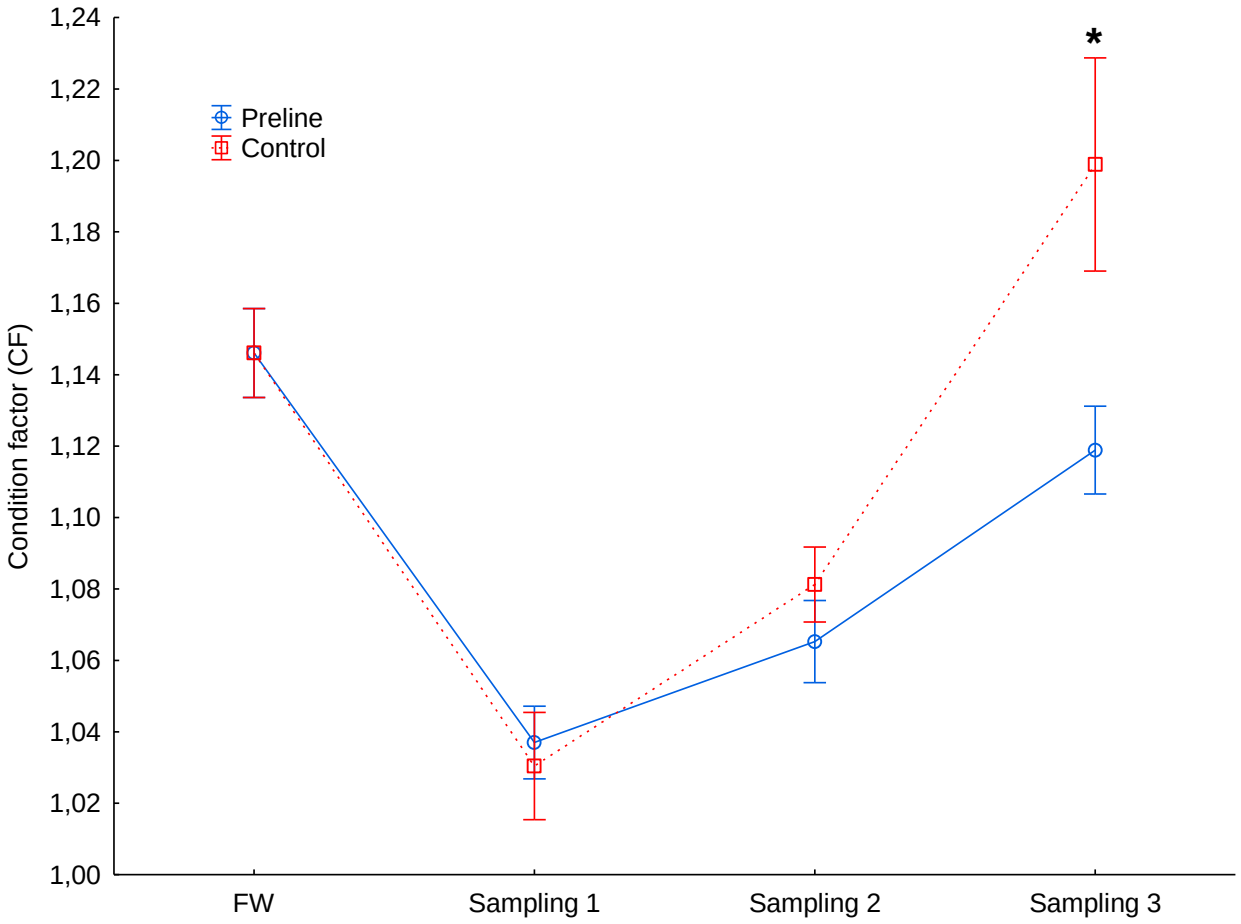

*Figure 3.8: Condition factor (CF) of sampled fish in Preline and control groups from the freshwater sampling (FW) on April 15th, and during the post-smolt phase, after sampling 1 (June 1/2nd), sampling 2 (June29/30th) and sampling 3 (August 29/30th). Asterisk indicates the level of significance between treatments; * p<0.05, ** p<0.01, *** p<0.001. Each data sampling point is given as mean±SEM, n=30.*

3.8 FCR and TGC

The feed conversion ratio (FCR) was found to be 1.04 in the Preline group and 1.08 in the control group, during the post-smolt phase (May 5th – August 31st) (Table 3.1). During the on-growing phase the FCR changed to 1.03 in the Preline (Buholmen) group and stayed the same in the control group, 1.08.

Table 3.1 Feed Conversion Ratio (FCR) in Preline and Conrol groups during the post-smolt (May 5th – August 31st) and on-growing phase (August 31st – November 30th).

Group	FCR
Preline (post-smolt)	1.04
Control (post-smolt)	1.08
Preline (on-growing)	1.03
Control (on-growing)	1.08

TGC for the sampled fish was calculated to 3.149 and 2.778 for control and Preline, respectively, during the post-smolt phase (Table 3.2). TGC was also calculated from estimated values (Fishtalk calculations, FCE=1.1), to 3.041 and 2.850 for control and Preline during the same period (June 1/2nd to August 29/30th). After the Preline fish was transferred to Buholmen, for the on-growing phase, the TGC value decreased in the control group (2.318) and increased in the Preline group (3.001) (Table 3.2).

Table 3.2 Thermal Growth Coefficient (TGC) in Preline and control groups during the post-smolt (May 5th – August 31st) and on-growing phase (August 31st – November 30th). TGC was calculated from weight samplings, and estimated weight values.

Group	TGC (weight from sampling)	TGC (estimated weight)
Preline (post-smolt)	2.778	2.850
Control (post-smolt)	3.149	3.041
Preline (on-growing)	-	3.001
Control (on-growing)	-	2.318

3.9 Histological image analysis

3.9.1 Sampling 1 (June 1/2nd)

At the first sampling point (June 1/2nd) the control fish had a significantly ($p<0.05$) higher frequency of muscle fibres in the range of 0-20 and 140-160 µm, while the Preline fish had had a significant ($p<0.01$) higher frequency in the 40-60 µm interval (Figure 3.9). The average number of fibres per predefined area was 258 ± 7.4 for control and 266.7 ± 9.1 for Preline, at this sampling point.

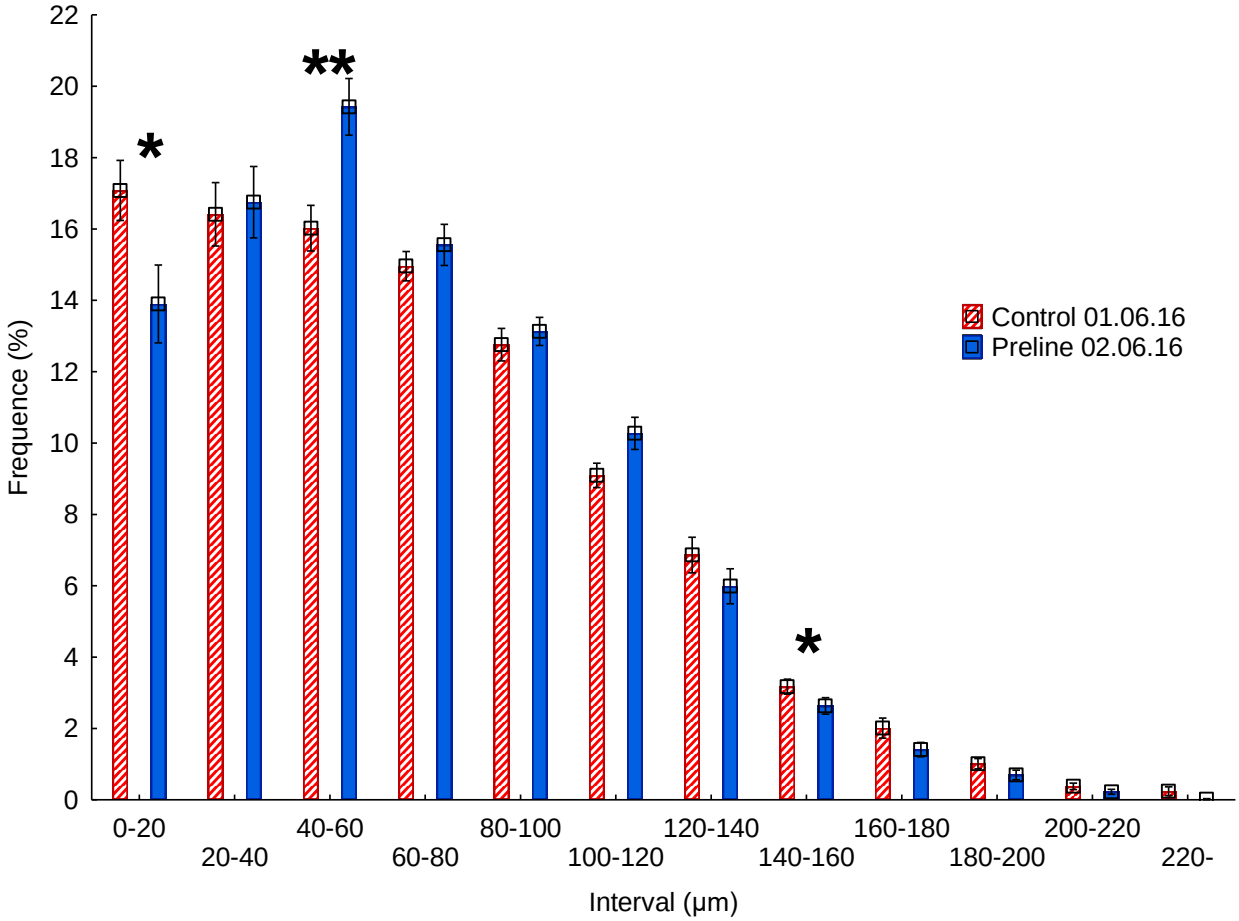

*Figure 3.9: Frequency (%) of muscle fibres in 20 µm interval groups in Preline and control from sampling 1 (June 1/2nd) during the post-smolt phase. Asterisk indicates the level of significance of treatment between groups; * p<0.05, ** p<0.01, *** p<0.001.*

3.9.2 Sampling 2 (June29/30th)

At sampling 2 (June29/30th) the control group had a significantly ($p<0.001$) higher frequency of muscle fibres in the 20-40 µm interval, while the Preline group had a significantly ($p<0.05$) higher frequency in the 100-120, 120-140 and 160-180 µm interval groups (Figure 3.10). The average number of fibres per predefined area was 312.2 ± 14.4 for control and 257.5 ± 7.4 for Preline, at this sampling point.

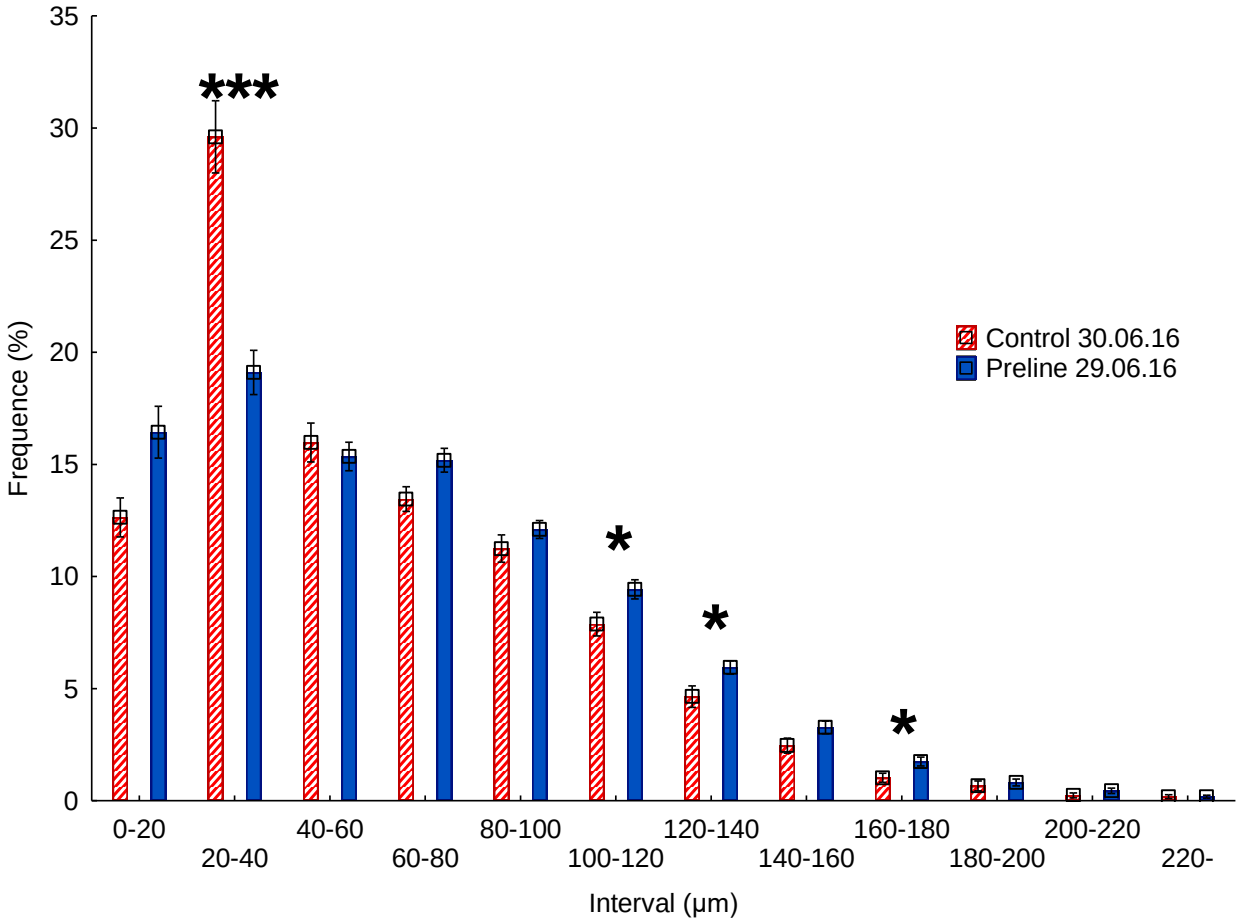

*Figure 3.10: Frequency (%) of muscle fibres in 20 µm interval groups in Preline and control from sampling 2 (June29/30th) during the post-smolt phase. Asterisk indicates the level of significance of treatment between groups; * p<0.05, ** p<0.01, *** p<0.001.*

3.9.3 Sampling 3 (August 29/30th)

During sampling 3 (August 29/30th), at the end of the post-smolt phase, the fish in the Preline group had a significantly ($p<0.05$) higher frequency of the smallest muscle fibres, ranging from 0-20 and 20-40 µm, compared to the control fish. In the 0-20 µm interval, this equals a 2.44 times higher frequency. The control group had a significantly ($p<0.001$) higher frequency in the 60-80 µm interval group (Figure 3.11). The average number of fibres per predefined area was 259 ± 12.9 for control and 265.5 ± 10.9 for Preline, at this sampling point.

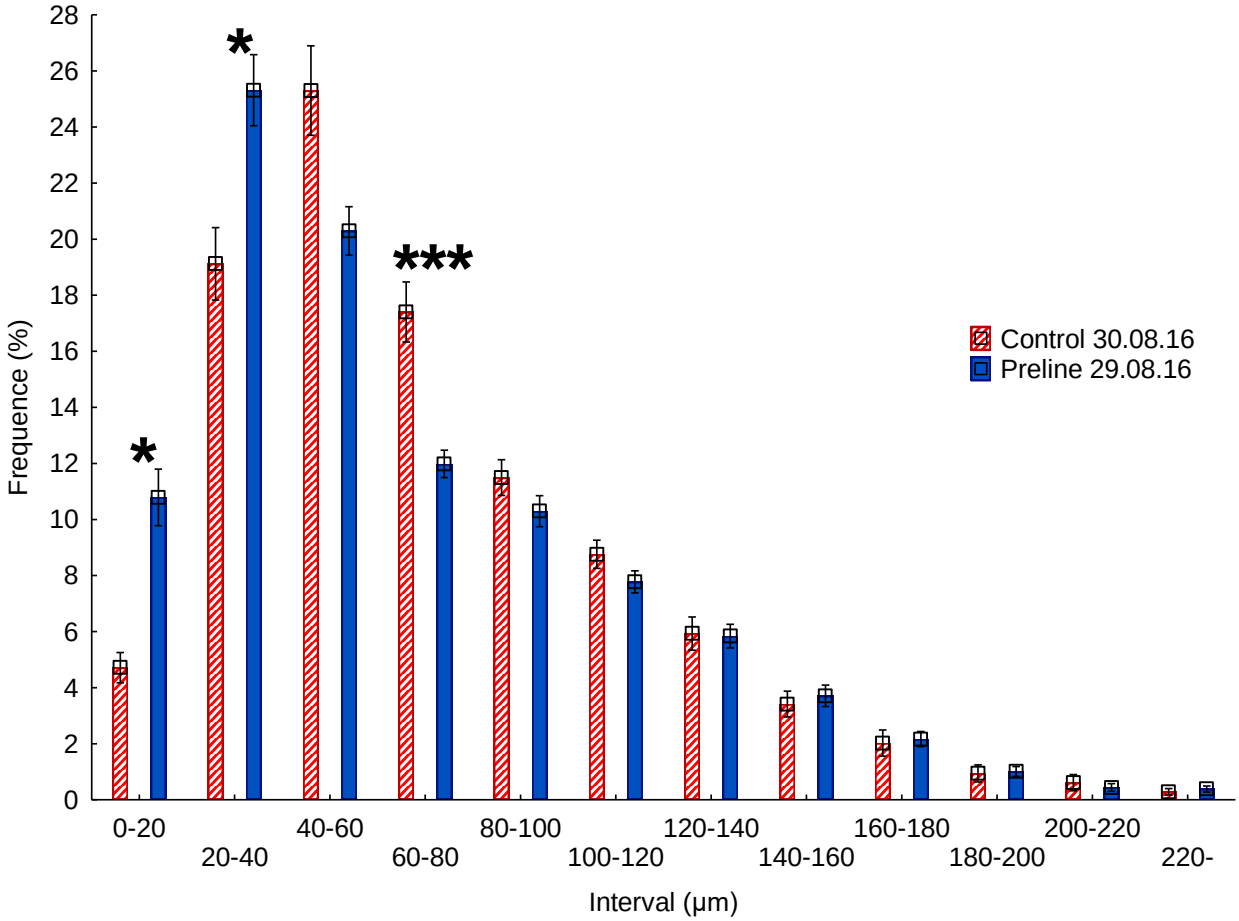

*Figure 3.11: Frequency (%) of muscle fibres in 20 µm interval groups in Preline and control from sampling 3 (August 29/30th) during the post-smolt phase. Asterisk indicates the level of significance of treatment between groups; * p<0.05, ** p<0.01, *** p<0.001.*

3.10 Histopathology and pathological real time RT-PCR screening

8 of the 30 fish from sampling 3 (August 29/30th), during the post-smolt phase, in the control group showed histological signs of degeneration and necrosis in red and white skeletal muscle, and heart muscle (Figure 3.13-3.15). However, through real time RT-PCR, positive results for salmonid alphavirus (SAV) was found in 5 fish in the control group (16,67 %) from sampling 3 during the post-smolt phase (Figure 3.12). These were five of the fish that also showed histopathological symptoms of degeneration and necrosis. Positive results for PRV was found in all groups tested, freshwater (80,0 %), Preline (80,0 %) and control (73,33 %). There were no significant differences in muscle fibre size distribution between fish that showed histological signs of degeneration and necrosis in red and white skeletal muscle, and heart muscle, and those that did not, within the control group.

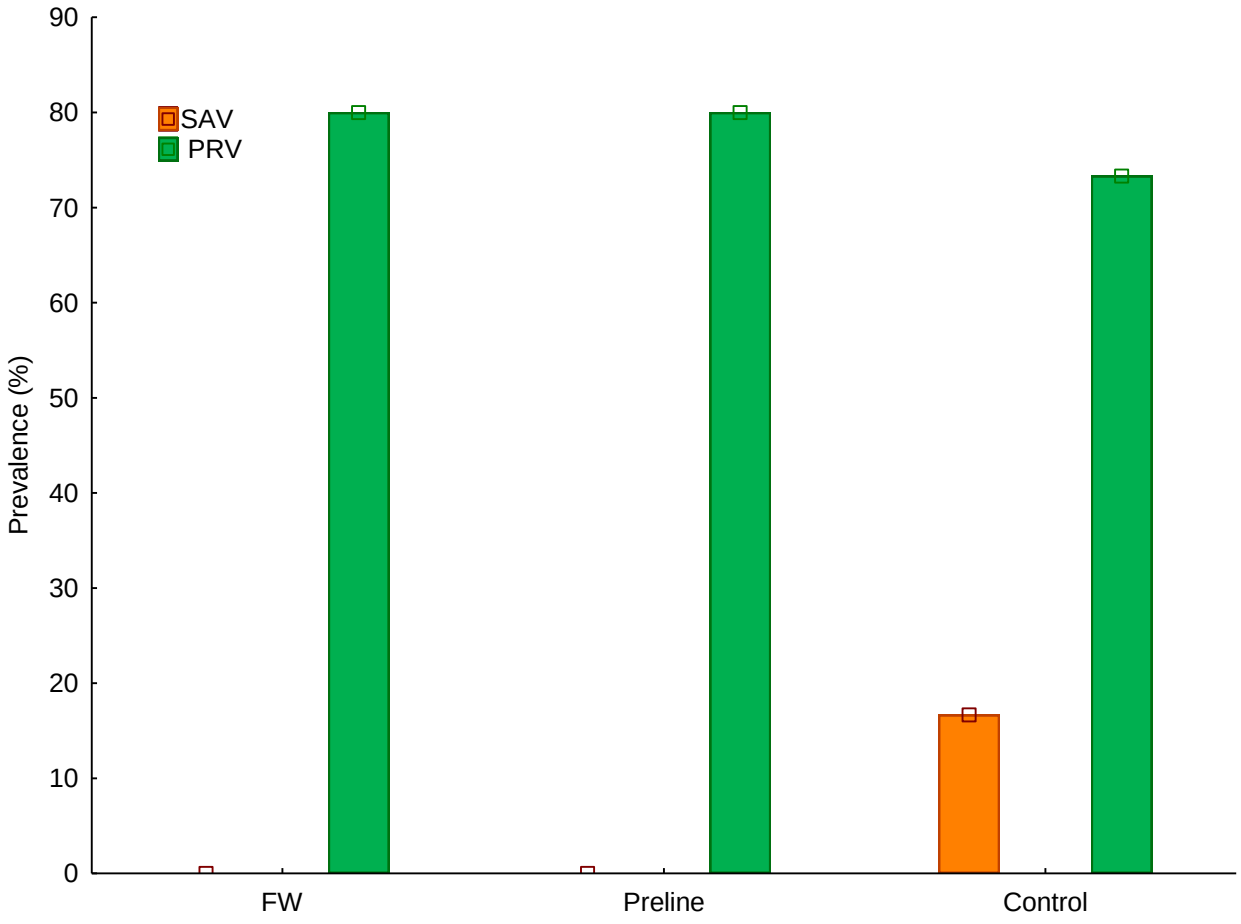

Figure 3.12: Prevalence (%) of Piscine orthoreovirus (PRV) and salmonid alphavirus (SAV) in Freshwater (FW), and in Preline and Control from sampling 3 (August 29/30th) during the post-smolt phase

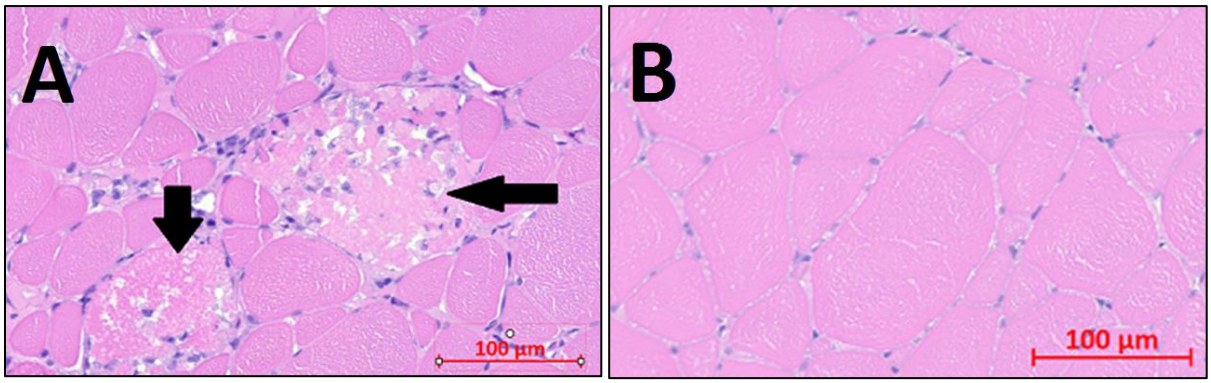

Figure 3.13A: Black arrows are showing degenerated white skeletal muscle fibres. Scale bar at 100 µm. Figure 3.13B Showing normal white skeletal muscle fibres. Scale bar at 100 µm.

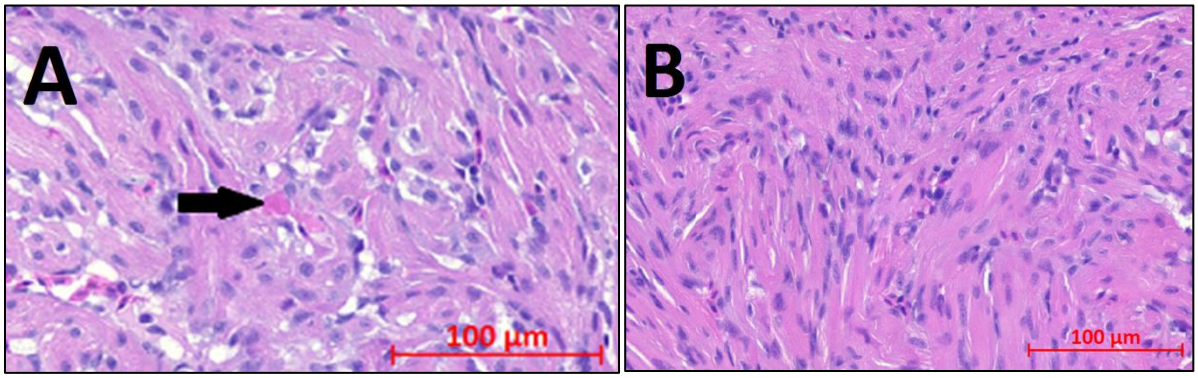

Figure 3.14A: Black arrow is showing a necrotic cardiac muscle fibre. Scale bar at 100 µm. Figure 3.14B Showing normal cardiac muscle fibres. Scale bar at 100 µm.

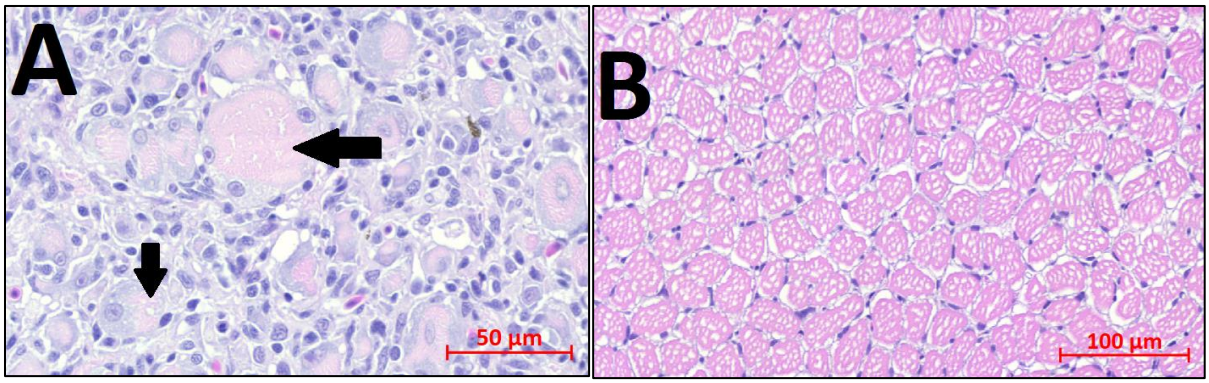

Figure 3.15A: Black arrows are showing degenerated red skeletal muscle fibres. Scale bar at 50 µm. Figure 3.15B Showing normal red skeletal muscle fibres. Scale bar at 100 µm.

3.11 Molecular analysis of heart

There were significant ($p<0.001$) higher mRNA expression levels of MEF2C, GATA4 and VEGF in freshwater group (FW), compared to Preline and control, from sampling 3 (August 29/30th). The Preline fish had significant ($p<0.01$) higher expressed mRNA levels of MEF2C compared to control at sampling 3 (Figure 3.16). Furthermore, there was a significant difference ($p<0.05$) in expressed mRNA levels of GATA4 between Preline and control at sampling 3 (Figure 3.17). No significant differences were found for mRNA levels of VEGF, between Preline and control at sampling 3 (Figure 3.18). No significant differences were found for the expressed mRNA levels of MEF2C within the control group, when SAV-positive fish were compared with SAV-negative. However, significant ($p<0.05$) differences were found for the mRNA expression levels of GATA4 and VEGF within the control group.

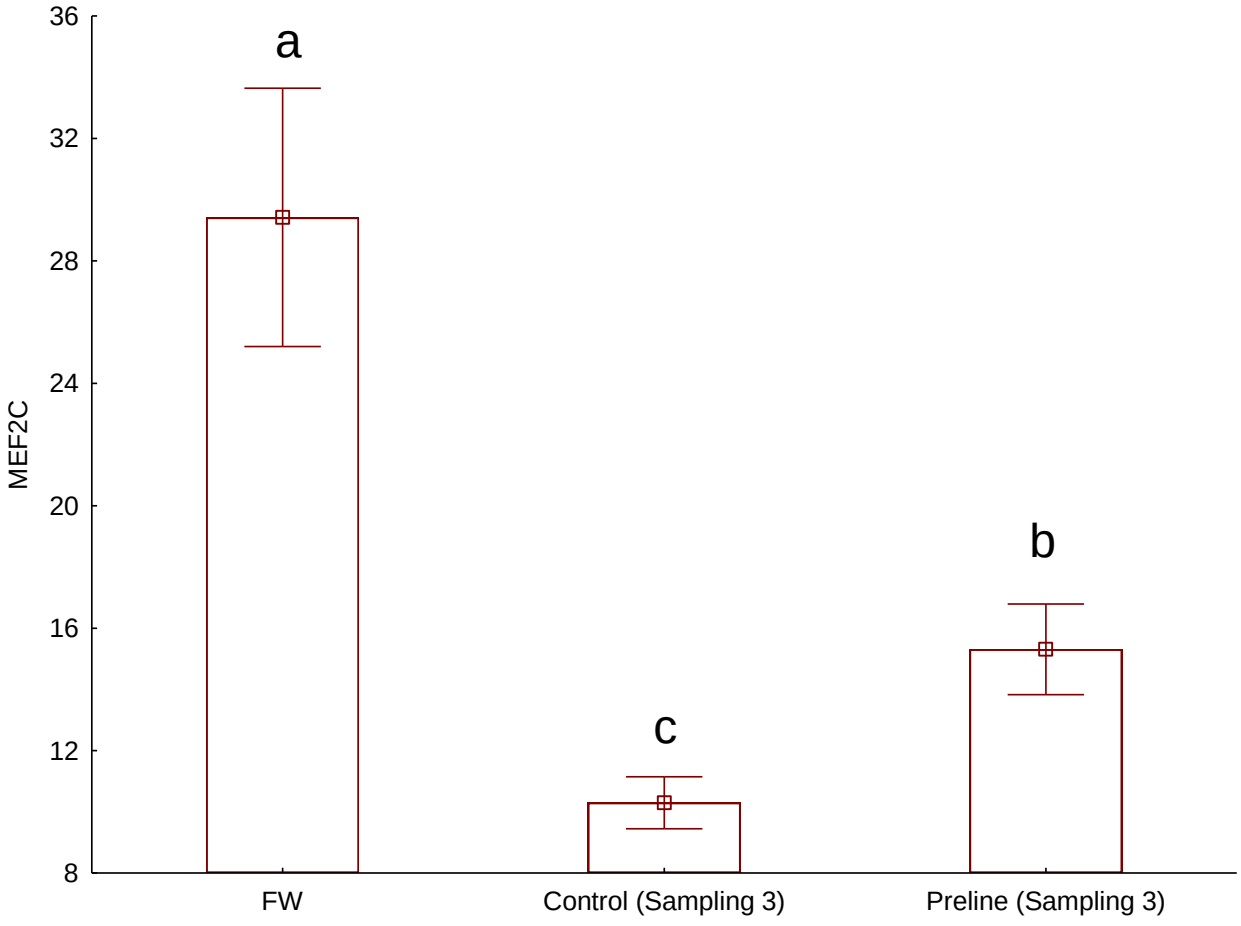

Figure 3.16: Expressed mRNA levels of MEF2C, relative to housekeeping gene (EF1a), in freshwater (FW), and control and Preline fish from sampling 3 (August 29/30th), during the post-smolt phase. Different letters represent significant differences in mRNA expression levels between groups ($p<0.05$).

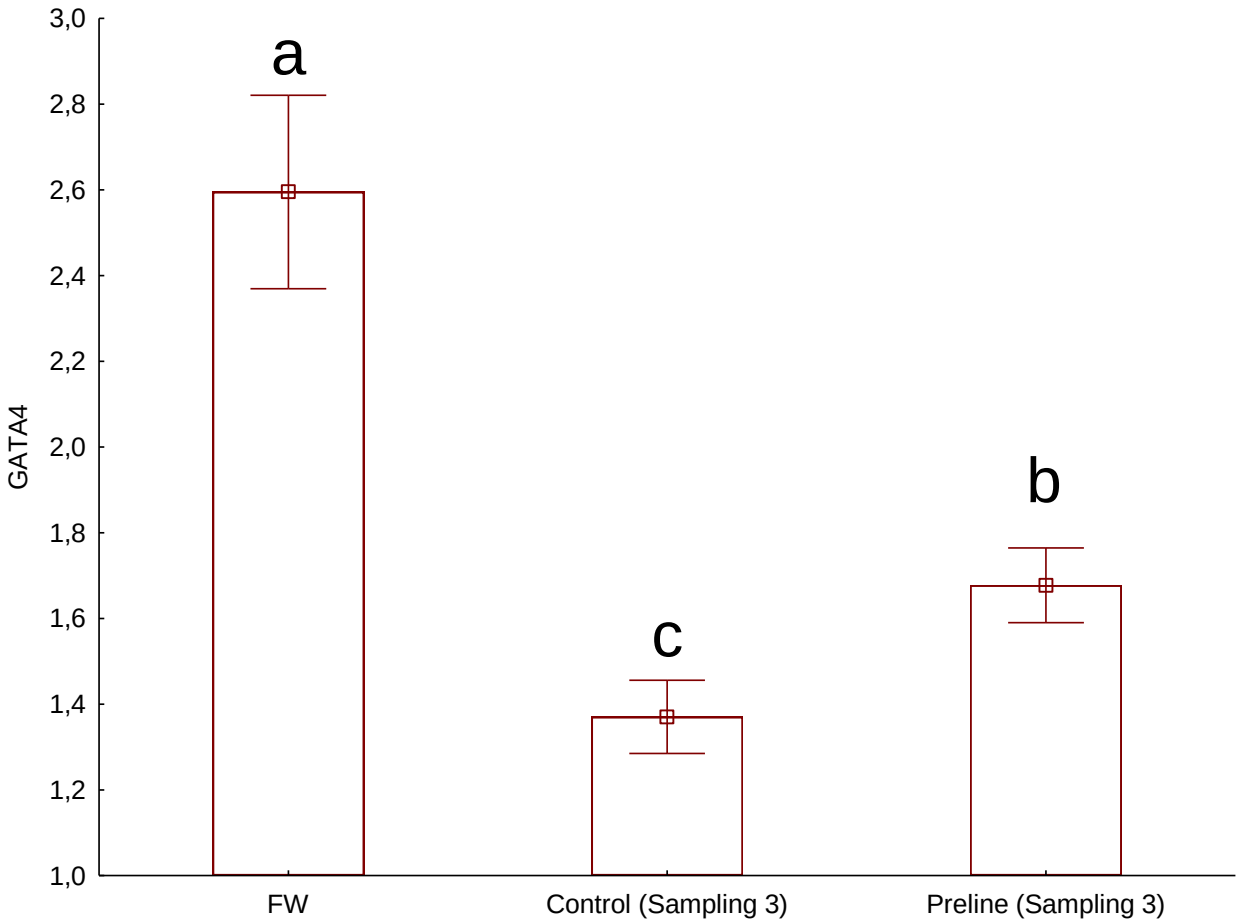

Figure 3.17: Expressed mRNA levels of GATA4, relative to housekeeping gene (EF1a), in freshwater (FW), and control and Preline fish from sampling 3 (August 29/30th), during the post-smolt phase. Different letters represent significant differences in mRNA expression levels between groups ($p<0.05$).

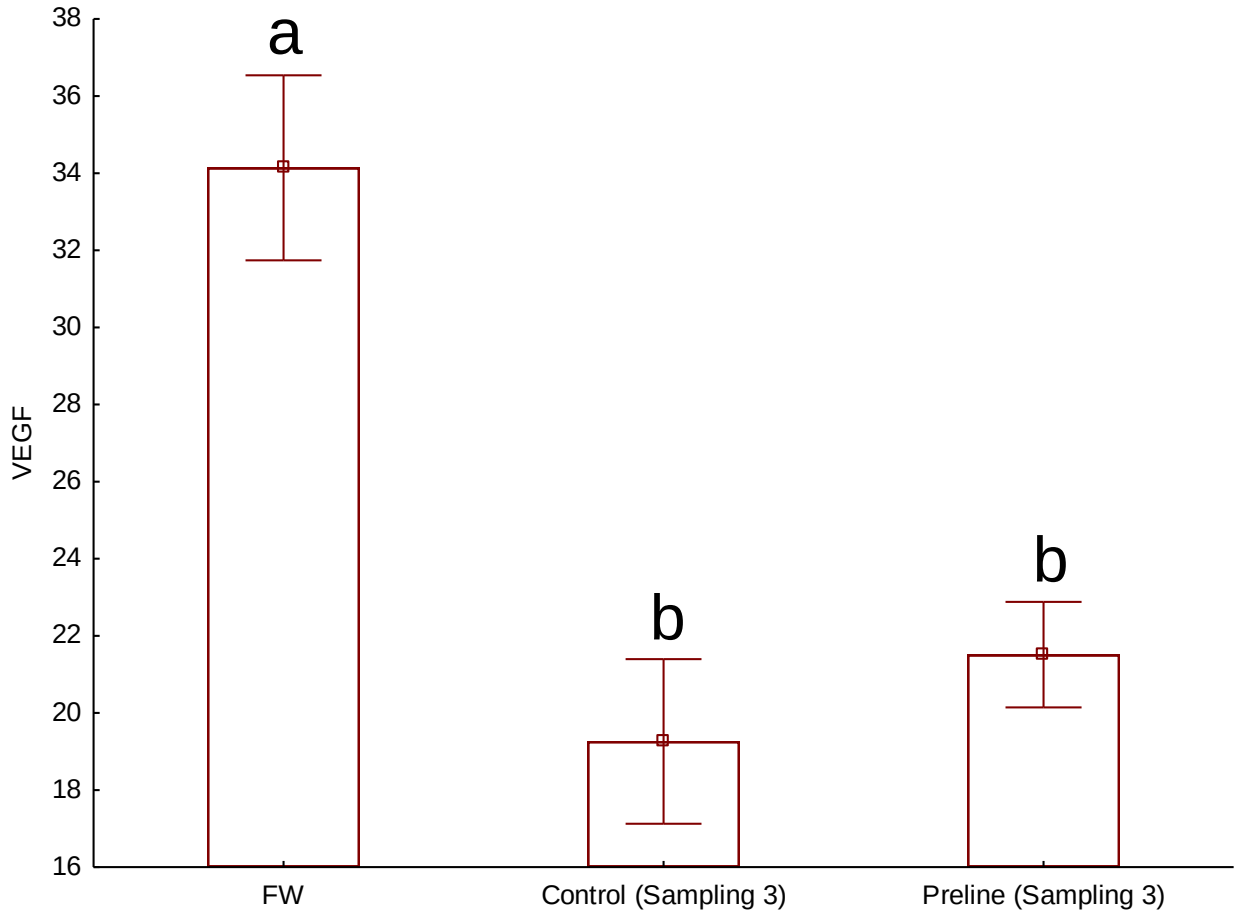

Figure 3.18: Expressed mRNA levels of VEGF, relative to housekeeping gene (EF1a), in freshwater, and control and Preline fish from sampling 3 (August 29/30th), during the post-smolt phase. Different letters represent significant differences in mRNA expression levels between groups ($p<0.05$).

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4 Discussion

4.1 Discussion of methods

In order to compare the two groups, it was essential that the fish originated from the same genetic background and rearing conditions during the freshwater stage, and had a similar experimental period in seawater. Even though the environmental conditions and physical parameters cannot be controlled in this experiment, the results presented in this thesis show field results as they would be in a real production situation. Hence, this study can be regarded as a large-scale follow-up on studies that test the effect of training in small scale lab experiments. Optimally, the sampling would have been conducted on the same day. However, this was not logistically possible due to long distances between locations.

4.1.1 Water parameters

Since this project is conducted at full scale production, similar environmental conditions between experimental groups is difficult to achieve, in contrast to small scale lab experiments. All the three environmental parameters that was measured (temperature, salinity, oxygen) varied between Preline and control, both during the post-smolt and the on-growing phase. This variation was mostly due to the two different systems, but also differences between locations. Since the water intake of the Preline system was at a depth of 35 m, and the fish in the control facility were exposed to surface layers, one would expect differences in temperature, salinity and oxygen. The effect of this causes different temperature controlled growth patterns (see 4.1.2). Further, fish in the open system distribute themselves over various depths, so knowing which exact parameter they were exposed to is difficult. However, the measured data of oxygen and salinity was within normal levels and should not have affected growth and survival in Preline and control (Duston, 1994, Boeuf and Payan, 2001, Thorarensen and Farrell, 2011).

4.1.2 Growth

Temperature is regarded as one of the main factors influencing growth (Fry, 1971). Since the average temperature during the post-smolt phase (9.5 °C in Preline, 12.9 °C in control) varied between facilities, a difference in final weight and length was expected. The estimations based on feed output (Fishtalk calculations, FCE=1.1) correspond with the weight measurements that we conducted during the post-smolt sampling. Therefore, we expect that the weight estimations conducted during the on-growing phase are credible. TGC in this study was not calculated from individually tagged fish, which would be optimal. To compensate for this, 30 random sampled fish were used. It should also be noted that TGC should be used with caution when temperature exceeds the optimum of 15-16 °C (Jobling, 2003), which it did during the on-growing period. Growth rate starts to decrease at temperatures above optimum, so calculating TGC is most accurate between 4-14 °C. This will most likely not affect the TGC, as the period with temperatures above 16 °C was short and at approximately the same time for both groups.

4.1.3 Histology

The fish in this project is the third generation reared in the Preline system and is a continuation of an ongoing project (SFI, CtrlAQUA). For the analysis of muscle fibre size, the first generation was examined in the same way as the current by Sveier et al. (2015). Therefore, the results from this study will be compared to the previous generation, to see if there are any similarities that can be observed. Compared to Sveier et al. (2015), the predefined area in which muscle cells was counted was doubled, to get more data to analyse. To best compare these two studies, the methods used were similar, including measuring fibre diameter for the furthest possible distance. Since the sectioning can affect how the fibres will appear in a histological section, measuring the fibres for the shortest possible distance was considered. However, since there was a large number of individuals and a large predefined area with a great number of fibres, the current method was considered to be optimal.

4.1.4 Histopathology

Other facilities in the same fjord system as the control facility had detected SAV in their fish, so it was suspected that SAV might be present in the control fish as well. Since SAV causes necrosis and degeneration in heart and skeletal muscle (Bruno et al., 2013), each histological section was investigated for such symptoms. Symptoms were only found in the control group at the last sampling point in the post-smolt phase. Therefore, real time RT-PCR analysis was used to detect if SAV was present. It was discussed whether the fish that showed signs of necrosis and degeneration from histology, and those were SAV was present, should be included in the study. Therefore, a statistical test (one-way ANOVA) was conducted, to establish potential differences between fish with and without symptoms. No significant differences were found for muscle fibre size within the control group, when fish that showed symptoms were compared with fish without symptoms. Therefore, these fish were not excluded from the study. Further, no significant differences were found for the expressed mRNA levels of MEF2C within the control group. However, significant ($p<0.05$) differences were found for the mRNA expression levels of GATA4 and VEGF within the control group, so SAV-positive fish were excluded for the analysis of these genes. PRV was detected in both Preline (80%) and control (73.33%), and similar to SAV, it causes necrosis and degeneration in heart and skeletal muscle (Bruno et al., 2013). However, since SAV was detected in 5 of the 8 fish that showed histological signs of necrosis and degeneration, it was presumed that SAV was the cause.

4.1.5 Heart

Heart weight was planned to be measured, in order to calculate relative ventricular mass (RVM), but problems with waves at the open cage facility made it impossible to get accurate weighings. 30 random sampled fish were brought back to Høyteknologisenteret (Bergen, Norway) to do the measuring there, but by that time, the blood inside the heart had coagulated and was impossible to get out. Therefore, data from heart weight are not presented in this study. Along with this, it should have been done a molecular analysis of all heart samplings. It would have been interesting to see how the expressed mRNA levels develop over time, by also analysing the first and second sampling in the post-smolt phase, but because of limited resources and time, this was not possible. However, the samples are stored on -80 °C and will be analysed on a later occasion.

4.1.6 Statistical analysis

Since this is a large-scale experiment, it was not possible to run several experimental group replicates. The total number of fish transferred to the Preline and control facilities were 157 126 and 164 286, respectively. Sampling in large-scale projects can be challenging with regards to ensureing homogenous sample material. Therefore, 30 fish were chosen for each sampling point which is based on what is common when pathogen screenings are conducted in large scale systems (Handeland, 2017, pers. comm.). TGC and FCR are observational values calculated from data given by Lerøy Vest AS, for each phase. Since there is only one replicate for each treatment, no statistical analysis could be conducted for these values. To get more data to analyse statistically, the predefined area in the histological sections were doubled from what Sveier et al. (2015) analysed in their study. Since the size of the fish effects the frequency of small and large muscle fibres (Stickland, 1983), removing the effect of this covariate factor was necessary. Length was chosen as the measurement for size, since this factor is more stable than weight. Comparisons between generation one (Sveier et al., 2015) and the current generation gives the histological analysis of muscle fibres a timely parallel, which contribute to strengthening the interpretation of the results from the current study.

4.2 Discussion of results

4.2.1 Growth and feed conversion

Temperature is regarded as one of the main factors influencing growth (Fry, 1971). Temperature is controlling the rate of metabolic functions, and thereby, influencing the efficiency of increased biomass from feed energy transformation (Handeland et al., 2008). This was evident in the current experiment, as fish from the control group had a significant higher final weight, length and condition factor, compared to the Preline group, at the end of the post-smolt phase. It can be assumed that this difference in growth is mostly due to exposure to different temperatures between experimental groups. The control group was exposed to higher temperatures (12,9 °C on average), whereas the Preline fish were reared in colder water (9,5 °C on average) pumped up from approximately 35 m. Since the temperature profiles differed between locations, a weight model incorporating growth rate/day dependent on the daily temperature was employed (Thermal Growth Coefficient, TGC), which takes into account the different rearing temperatures. It should be noted that during some parts of the on-growing phase, the water temperature exceeded 16 °C, which can affect the TGC value (Jobling, 2003). However, the temperature profiles between the groups during the on-growing phase did not differ as much as during the post-smolt phase and both exceeded 16 °C around the same time, thus, making them comparable. This model showed that the Preline fish still had a lower growth rate during the post-smolt phase. This was in contrast to what was found in Sveier et al. (2015), where TGC was similar in Preline and control (2.141). However, the TGC in Preline (2.778) is still around what is considered average (2.7), calculated and reviewed from several papers by Thorarensen and Farrell (2011). What is noteworthy is that after the Preline fish were transferred to Buholmen, for the on-growing phase, the TGC increased and exceeded that of the control group, and therefore, grew faster. The Preline fish had a higher relative increase in growth (232.44 %), compared to the control group (122.73 %), during this period. Studies have shown that fish kept at low temperatures (2-6 °C) can compensate for a set-back in growth, by rapidly increasing growth when exposed to warmer ambient temperatures (11-14 °C), and catch up with fish constantly kept at ambient temperatures. (Mortensen and Damsgård, 1993, Nicieza and Metcalfe, 1997, Maclean and Metcalfe, 2001). This might explain why the Preline fish showed a higher relative increase in growth, even though the fish in these studies were exposed to abnormally low temperatures. Furthermore, it cannot be ruled out that the decrease we see in TGC for the control group is due to SAV or PRV.

Studies have shown that moderate exercise creates more efficient feed conversion in fish groups (Leon, 1986, East and Magnan, 1987, Christiansen et al., 1992). This was evident in the current study, as the observed feed conversion ratio was lower in the Preline fish group, compared to the control group. It is likely that the FCR was influenced by the training regime that the Preline fish were exposed to. The observed values in the current study are supported by other studies showing the direct effect of training, where appetite is stimulated and weight gain is achieved faster with less feed used (Davison, 1989), and studies showing decreased FCR as a result of exercise (Leon, 1986, East and Magnan, 1987, Christiansen et al., 1992). Along with this, studies have shown fewer interactions between individuals and reduced aggressive behaviour when groups are exposed to constant currents (Christiansen et al., 1991, Jobling et al., 1993, Solstorm et al., 2016). This is also, most likely, affecting the FCR in the Preline and control groups.

4.2.2 Mortality

Daily measurements from Lerøy Vest AS displayed a higher mortality rate directly after transfer to seawater in the Preline group, compared to control. Studies have described that fish used in exercise experiments tend to have an increased mortality rate during the first few days of exposure to an exercise regime (Davison and Goldspink, 1977, Totland et al., 1987). This might explain why the Preline fish showed a higher accumulated mortality than the control group directly after transfer to seawater. However, it is also likely that this accumulated mortality was due to complications during transportation, as the fish were transferred to seawater at different times. Furthermore, we can see from Figure 3.4 that the accumulated mortality in the control cage is gradually increasing over time, while the rate is quite stable in the Preline group. This was also shown in Totland et al. (1987), where raceway reared fish had a 1.2 % mortality rate after two weeks of the experiment. After 8 months, the mortality rates between raceway and control groups had changed to 4.4 % and 8.8 %, respectively. After the Preline fish were transferred to an open system, the control group exceeded Preline in accumulated mortality, and by the end of November the mortality rate was 3.52 % in control and 2.48% in Preline. One can speculate whether this difference is due to the training effect the Preline group were exposed to, and thereby producing a more robust fish, or if other factors were contributing. The pathological screening in this experiment showed that 16,67 % of the control fish showed positive results for a SAV. This virus is the cause of pancreas disease and can reduce appetite, growth and increase morality (Bruno et al., 2013). This might have contributed to an increase in accumulated mortality in the control group, especially during the on-growing phase.

4.2.3 Skeletal muscle

From the histological analysis, we can see how the distribution of muscle fibre size changes as the fish grow. After one month in seawater, both groups have many fibres distributed in the interval 0-20 µm. After four months in seawater, the frequency of fibres in this interval had decreased in both groups, indicating less recruitment of new muscle fibres. As the fish grow, the rate of hyperplasia in skeletal muscle decreases (Weatherley et al., 1980, Stickland, 1983), which explains what is observed in the current study. However, the Preline fish showed a significant higher frequency of small muscle fibres (0-20 µm) at sampling 3, after four months in seawater. The same result was also found by Sveier et al. (2015) in the first generation reared in the Preline system (Figure 4.1). This can potentially be an indication of higher muscle fibre recruitment, compared to the control fish, as a result of aerobic training. Studies showing the effect of training on muscle fibre recruitment in salmonids are marginal. However, it has been shown, although not significant, tendencies for higher frequencies of small muscle fibres as a result of exercise (Rasmussen et al., 2011).

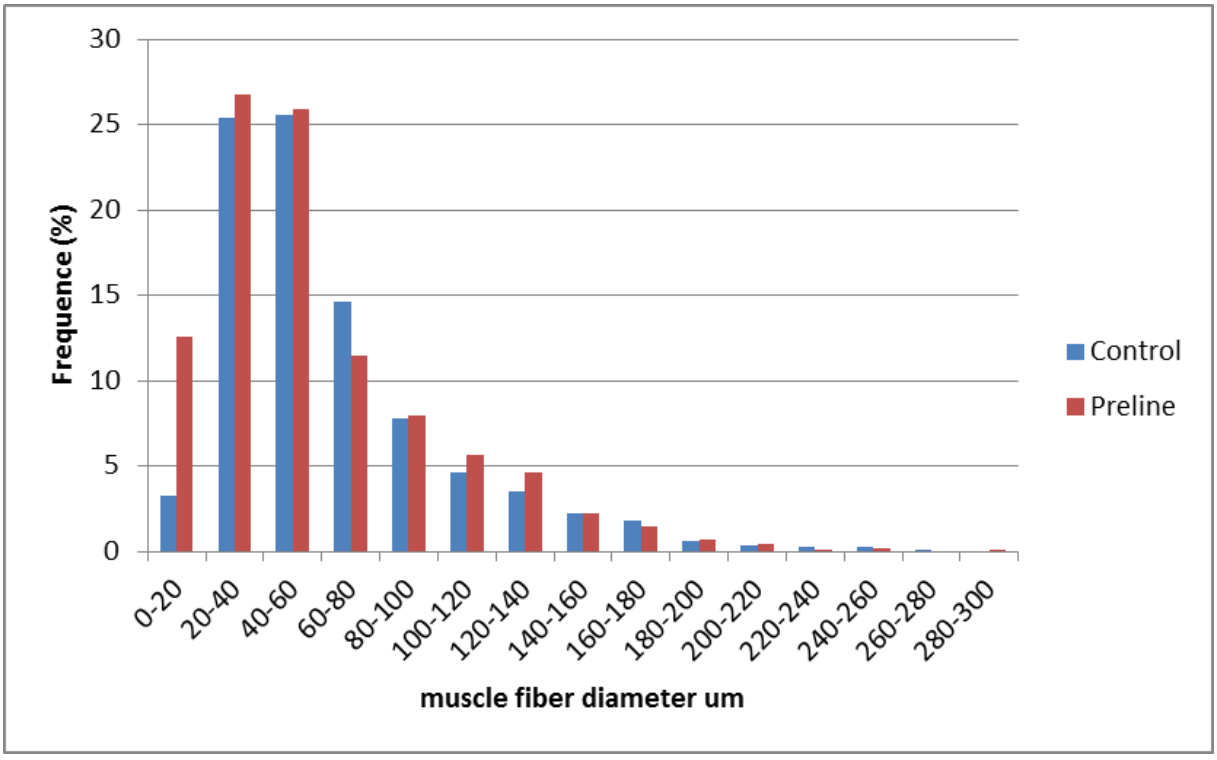

Figure 4.1: Frequence (%) of muscle fibres in 20 µm interval groups from 0-300 µm in Preline and control generation 1. The figure is taken from Sveier et al., 2015.

The larger part of the fish muscle consists of white skeletal muscle. When the fibres in this muscle group contract, the fish gain high speed, but becomes exhausted after a short while. The ATP produced in the white skeletal muscle is mainly by hydrolysis of stored phosphocreatine, as the muscle cells contain few mitochondria (Videler, 1993). Even though white muscle fibres are mainly used for anaerobic swimming, studies have shown that they provide power for speeds that is less than what is known as the critical swimming speed (U_{crit}), for some salmonid species (Johnston and Moon, 1980b, Burgetz et al., 1998). As the current experiment unfolded, the water velocity in the Preline system relative to fish size decreased from approximately 0.45 – 0.85 BL/s in early May, to about 0.30-0.60 BL/s at the end of the post-smolt phase. Several studies have found that muscle fibre recruitment increase with higher water velocities for different fish species (Johnston and Moon, 1980a, Johnston and Moon, 1980b, Rome and Alexander, 1990), but at the same time U_{crit} decreases as the fish grow (Remen et al., 2016). Furthermore, as fish get larger, the contribution to growth by muscle fibre recruitment decreases, and muscle fibre hypertrophy increases (Weatherley et al., 1980, Stickland, 1983). Since this study counted and measured muscle fibres within a predefined area, it would in principle imply that the number of muscle fibres inside this area should decrease from the start to the end of the experiment. This trend was not observed for either of the groups. Therefore, it is reasonable to assume that the difference in frequency in the muscle fibre interval group 0-20 μm is due to muscle fibre recruitment, as a result of aerobic training.

Several studies have indicated that water velocities above 0.40 BL/s can give positive effects on growth through muscle fibre hypertrophy (Walker and Emerson, 1978, Totland et al., 1987, Bugeon et al., 2003, Martin and Johnston, 2005, Ibarz et al., 2011). Totland et al. (1987) performed a more or less similar study as the current, where they tested what effect rearing Atlantic salmon in a raceway system had on muscle growth and composition, compared to a traditional net pen system. The experimental fish used in this study showed improved appetite and consequently 40 % higher weight than the reference fish at the end of the experiment. The white skeletal muscle of the raceway fish was the muscle type that was mostly effected and showed an increased muscle fibre size through hypertrophy, compared to the control group. An interesting note, when regarding muscle fibre hypertrophy, is that the Preline fish did not have a significant lower frequency of muscle fibres in the size class 120- 220 or larger μm at the end of the post-smolt phase, compared to the control group. This is noteworthy, since the number of larger muscle fibres tend to increase with the size of the fish, while the number of small

fibres decrease (Weatherley et al., 1980, Stickland, 1983). The Preline fish had a significantly lower weight and length, and were exposed to lower temperatures than the control group during the post-smolt phase, which could give reason to believe that they would also have fewer of the larger muscle fibres. Since this is not the case, it can be assumed that there has been an effect of training, causing increased muscle cell hypertrophy. After the Preline fish were transferred to Buholmen for the on-growing period, the TGC exceeded that of the control group. This increase could possibly be due to hypertrophy of newly recruited muscle fibres which were more present in the Preline fish, compared to control. If this is the case, there is a huge potential in rearing post-smolts in raceway systems, to increase the individual growth potential as a result of aerobic training.

4.2.4 Heart condition

No significant differences were found between groups when regarding expressed mRNA levels of VEGF. However, significant elevated mRNA levels of MEF2C and GATA4 were found in the Preline group compared to the control fish. MEF2C, GATA4 and VEGF are transcription factors known to show elevated mRNA levels when exposed to a training regime (Akazawa and Komuro, 2003, Iemitsu et al., 2006, Castro et al., 2013). Elevated mRNA levels of MEF2C and GATA4 can be an indication of increase in cardiomyocyte hypertrophy as an effect of training (Kolodziejczyk et al., 1999, Akazawa and Komuro, 2003, Castro et al., 2013). There was a significant decrease in expressed mRNA levels of all the genes evaluated in this study, from the freshwater stage to the end of the post-smolt phase. This could possibly be due to differences between freshwater and seawater stages, or that freshwater fish were exposed to a higher water velocity (Handeland, 2017, pers. comm.), giving a training effect. In Castro et al. (2013), it was found significant elevated expressed mRNA levels of MEF2C, GATA4 and VEGF in a group exposed to a high water velocity (1.3 BL/s), compared to a control group. The current study did not show significant differences in expression levels of VEGF, only in MEF2C and GATA4. It seems that higher water velocities provide a greater impact on the expressed mRNA levels of these genes, which might explain why no differences were found in VEGF mRNA levels. The water current in the Preline system had an effect on the expressed mRNA levels of MEF2C and GATA4, which is supported by the results from Castro et al. (2013). However, in Castro et al. (2013) the expression levels in the moderate velocity group (0.65 BL/s) showed no significant differences, compared to control. Although this water velocity might be more comparable to the results found in the current study, Castro et al. (2013)

did their study on juvenile Atlantic salmon, i.e. smaller fish. Since U_{crit} has been found to decrease with size (Remen et al., 2016), it might be possible that the current study is comparable to a water velocity in between 0.65 and 1.3 BL/s.

Improved contractile function is most likely regulated by the expressed levels of genes that are involved in the excitation-contraction process and in handling fluxes of Ca^{2+}, in mammals (Morán et al., 2003, Rolim et al., 2007). Castro et al. (2013) found an up-regulation of genes related to the excitation-contraction process, which suggests improved contractility. If exercise training can lead to increased cardiac output as a result of increased ventricular mass and improved contractile function (Farrell et al., 1991, Castro et al., 2013), the Preline fish might be able to handle stressful situations better than untrained control fish. Cardiac failure is a factor contributing to accumulated mortality in the seawater stage of production (Hjeltnes et al., 2017). Therefore, it can be largely helpful with an improved heart condition, in a farming environment, when the fish undergoes treatments, is transported or handled in any way.

5 Conclusions

The fish in the Preline system had a lower weight, length and condition factor, compared to the control group during the post-smolt phase, due to differences in temperature. TGC was lower during this phase in the Preline group, suggesting a lower growth rate regardless of temperature. $H0_1$ can thereby be rejected, but as an effect of temperature, rather than aerobic exercise. When regarding muscle fibre size and distribution, we see the same trends as Sveier et al. (2015) did in their study. The Preline fish had, at the end of the post-smolt phase, a 2.44 times higher frequency of muscle fibres in the smallest interval group (0-20 μm), compared to the control fish. This, accompanied by what we know from literature on effects of training on muscle and muscle development, indicates that there is increased rate of muscle fibre recruitment. Therefore, we can reject $H0_2$ as it is shown clear indications of increased hyperplasia in the Preline fish. Furthermore, the Preline fish did not have a lower amount of large muscle fibres (>100 μm) at the end of the post-smolt phase, indicating hypertrophy of white skeletal muscle as an effect of aerobic exercise. The increase in TGC for the Preline fish during the on-growing phase, is possibly a result of hypertrophy of the newly recruited muscle fibres. This suggests that the trained fish have a greater growth potential, after the post-smolt period, than untrained fish, which is beneficial for further production. Results from this study suggest an increased

rate of cardiomyocyte hypertrophy in response to aerobic exercise in raceway systems, compared to traditional sea cages, as the Preline fish had higher expressed mRNA levels of MEF2C and GATA4. Hence, $H0_3$ can be rejected for MEF2C and GATA4, but not for VEGF, since there were no significant differences in expressed mRNA for this gene between experimental groups. Increased growth of the heart ventricle can in turn potentially increase the robustness of the fish. This can be helpful in a farming environment, as the fish are exposed to a lot of stressors which demand a good heart condition.

Future perspectives

It is important with this type of large scale experiment to repeat over several years, compare results and look for trends between generations. One possible issue that should be investigated further is whether fish produced in Preline fish farming system have a higher metabolic use of lipids as a result aerobic exercise. Different studies have shown that fish species that normally have a high swimming activity, such as Scombridae and Salmonides favour the use of lipids for metabolic functions (Magnoni and Weber, 2007). It would also be interesting to see if the same results for muscle fibre size distribution and heart condition can be observed for under yearling (0+) smolts, as water temperatures would be different during the autumn and winter. If similar trends could be shown, it would strengthen the results found in the current study. Further, following up the fish throughout the on-growing period with the same analyses, to see how aerobic training during the post-smolt phase can affect the fish in later life stages. If increased recruitment of white muscle fibres increases the individual growth potential, and if this growth potential lasts until slaughter, it can be of great value for the salmon farming industry.

References

Adams, C. E., Huntingford, F. A., Krpal, J., Jobling, M. & Burnett, S. J. 1995. Exercise, agonistic behaviour and food acquisition in Arctic charr, *Salvelinus alpinus*. *Environmental Biology of Fishes,* 43**,** 213-218.

Akazawa, H. & Komuro, I. 2003. Roles of cardiac transcription factors in cardiac hypertrophy. *Circulation research,* 92**,** 1079-1088.

Alexander, R. M. 1969. The orientation of muscle fibres in the myomeres of fishes. *Journal of the Marine Biological Association of the United Kingdom,* 49**,** 263-290.

Andersen, L., Bratland, A., Hodneland, K. & Nylund, A. 2007. Tissue tropism of salmonid alphaviruses (subtypes SAV1 and SAV3) in experimentally challenged Atlantic salmon (*Salmo salar* L.). *Archives of Virology,* 152**,** 1871-1883.

Boeuf, G. & Payan, P. 2001. How should salinity influence fish growth? *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 130**,** 411-423.

Bruno, D., Noguera, P. & Poppe, T. 2013. *A Colour Atlas of Salmonid Diseases,* Netherlands, Springer Netherlands.

Brännäs, E. 2009. The effect of moderate exercise on growth and aggression depending on social rank in groups of Arctic charr (*Salvelinus alpinus* L.). *Applied Animal Behaviour Science,* 119**,** 115-119.

Bugeon, J., Lefevre, F. & Fauconneau, B. 2003. Fillet texture and muscle structure in brown trout (*Salmo trutta*) subjected to long-term exercise. *Aquaculture Research,* 34**,** 1287-1295.

Burgetz, I. J., Rojas-Vargas, A., Hinch, S. G. & Randall, D. J. 1998. Initial recruitment of anaerobic metabolism during sub-maximal swimming in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology,* 201**,** 2711-2721.

Castro, V., Grisdale-Helland, B., Helland, S. J., Kristensen, T., Jørgensen, S. M., Helgerud, J., Claireaux, G., Farrell, A. P., Krasnov, A. & Takle, H. 2011. Aerobic training stimulates growth and promotes disease resistance in Atlantic salmon (*Salmo salar*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 160**,** 278-290.

Castro, V., Grisdale-Helland, B., Helland, S. J., Torgersen, J., Kristensen, T., Claireaux, G., Farrell, A. P. & Takle, H. 2013. Cardiac molecular-acclimation mechanisms in response to swimming-induced exercise in Atlantic salmon. *PloS one,* 8**.**

Christiansen, J. S., Jørgensen, E. H. & Jobling, M. 1991. Oxygen consumption in relation to sustained exercise and social stress in Arctic charr (*Salvelinus alpinus* L.). *Journal of Experimental Zoology,* 260**,** 149-156.

Christiansen, J. S., Svendsen, Y. S. & Jobling, M. 1992. The combined effects of stocking density and sustained exercise on the behaviour, food intake, and growth of juvenile Arctic charr (*Salvelinus alpinus* L.). *Canadian Journal of Zoology,* 70**,** 115-122.

Clark, R. J. & Rodnick, K. J. 1998. Morphometric and biochemical characteristics of ventricular hypertrophy in male rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology,* 201**,** 1541-1552.

Costello, M. J. 2009. The global economic cost of sea lice to the salmonid farming industry. *Journal of fish diseases,* 32**,** 115-118.

Davie, P. S. & Farrell, A. P. 1991. The coronary and luminal circulations of the myocardium of fishes. *Canadian journal of zoology,* 69**,** 1993-2001.

Davie, P. S., Wells, R. M. & Tetens, V. 1986. Effects of sustained swimming on rainbow trout muscle structure, blood oxygen transport, and lactate dehydrogenase isozymes: evidence for increased aerobic capacity of white muscle. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology,* 237**,** 159-171.

Davison, W. 1989. Training and its effects on teleost fish. *Comparative Biochemistry and Physiology Part A: Physiology,* 94**,** 1-10.

Davison, W. & Goldspink, G. 1977. The effect of prolonged exercise on the lateral musculature of the brown trout (*Salmo trutta*). *Journal of Experimental Biology,* 70**,** 1-12.

Duston, J. 1994. Effect of salinity on survival and growth of Atlantic salmon (*Salmo salar*) parr and smolts. *Aquaculture,* 121**,** 115-124.

East, P. & Magnan, P. 1987. The effect of locomotor activity on the growth of brook charr, *Salvelinus fontinalis* Mitchill. *Canadian Journal of Zoology,* 65**,** 843-846.

Farrell, A., Johansen, J., Steffensen, J., Moyes, C., West, T. & Suarez, R. 1990. Effects of exercise training and coronary ablation on swimming performance, heart size, and cardiac enzymes in rainbow trout, *Oncorhynchus mykiss*. *Canadian journal of zoology,* 68**,** 1174-1179.

Farrell, A., Johansen, J. & Suarez, R. 1991. Effects of exercise-training on cardiac performance and muscle enzymes in rainbow trout, *Oncorhynchus mykiss*. *Fish Physiology and Biochemistry,* 9**,** 303-312.

Fry, F. 1971. 1 The effect of environmental factors on the physiology of fish. *Fish physiology,* 6**,** 1-98.

Gunnarsson, G., Karlsbakk, E., Blindheim, S., Plarre, H., Imsland, A., Handeland, S., Sveier, H. & Nylund, A. 2017. Temporal changes in infections with some pathogens associated with gill disease in farmed Atlantic salmon (*Salmo salar* L). *Aquaculture,* 468**,** 126-134.

Handeland, S. & Stefansson, S. 2001. Photoperiod control and influence of body size on off-season parr–smolt transformation and post-smolt growth. *Aquaculture,* 192**,** 291-307.

Handeland, S. O., Imsland, A. K. & Stefansson, S. O. 2008. The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. *Aquaculture,* 283**,** 36-42.

Hjeltnes, B., Bornø, G., Jansen, M. D., Haukaas, A. & Walde, C. 2017. Fiskehelserapporten 2016. Veterinærinstituttet 2017 (In Norwegian)

Ibarz, A., Felip, O., Fernández-Borràs, J., Martín-Pérez, M., Blasco, J. & Torrella, J. R. 2011. Sustained swimming improves muscle growth and cellularity in gilthead sea bream. *Journal of Comparative Physiology B,* 181**,** 209-217.

Iemitsu, M., Maeda, S., Jesmin, S., Otsuki, T. & Miyauchi, T. 2006. Exercise training improves aging-induced downregulation of VEGF angiogenic signaling cascade in hearts. *American Journal of Physiology-Heart and Circulatory Physiology,* 291**,** H1290-H1298.

Iversen, A., Andreassen, O., Hermansen, Ø., Larsen, T. A. & Terjesen, B. F. 2013. Oppdrettsteknologi og konkurranseposisjon. Fiskeri- og kystdepartementet. (In Norwegian)

Jackson, A. 2009. Fish in-fish out (FIFO) ratios explained. *Aquaculture Europe,* 34**,** 5-10.

Jobling, M. 2003. The thermal growth coefficient (TGC) model of fish growth: a cautionary note. *Aquaculture Research,* 34**,** 581-584.

Jobling, M., Jørgensen, E. H., Arnesen, A. M. & Ringø, E. 1993. Feeding, growth and environmental requirements of Arctic charr: a review of aquaculture potential. *Aquaculture international,* 1**,** 20-46.

Johnston, I. A. 1999. Muscle development and growth: potential implications for flesh quality in fish. *Aquaculture,* 177**,** 99-115.

Johnston, I. A. & Moon, T. W. 1980a. Endurance exercise training in the fast and slow muscles of a teleost fish (*Pollachius virens*). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology,* 135**,** 147-156.

Johnston, I. A. & Moon, T. W. 1980b. Exercise training in skeletal muscle of brook trout (*Salvelinus fontinalis*). *Journal of Experimental Biology,* 87**,** 177-194.

Jørgensen, E. H. & Jobling, M. 1993. The effects of exercise on growth, food utilisation and osmoregulatory capacity of juvenile Atlantic salmon, *Salmo salar*. *Aquaculture,* 116**,** 233-246.

Kolodziejczyk, S. M., Wang, L., Balazsi, K., Derepentigny, Y., Kothary, R. & Megeney, L. A. 1999. MEF2 is upregulated during cardiac hypertrophy and is required for normal post-natal growth of the myocardium. *Current biology,* 9**,** 1203-1206.

Koumans, J., Akster, H., Witkam, A. & Osse, J. 1994. Numbers of muscle nuclei and myosatellite cell nuclei in red and white axial muscle during growth of the carp (*Cyprinus carpio*). *Journal of fish biology,* 44**,** 391-408.

Kryvi, H. & Poppe, T. 2016. *Fiskeanatomi,* Bergen, Fagbokforlaget. (In Norwegian)

Leon, K. A. 1986. Effect of exercise on feed consumption, growth, food conversion, and stamina of brook trout. *The Progressive Fish-Culturist,* 48**,** 43-46.

Maclean, A. & Metcalfe, N. 2001. Social status, access to food, and compensatory growth in juvenile Atlantic salmon. *Journal of Fish Biology,* 58**,** 1331-1346.

Magnoni, L. & Weber, J.-M. 2007. Endurance swimming activates trout lipoprotein lipase: plasma lipids as a fuel for muscle. *Journal of Experimental Biology,* 210**,** 4016-4023.

Martin, C. & Johnston, I. 2005. The role of myostatin and the calcineurin-signalling pathway in regulating muscle mass in response to exercise training in the rainbow trout *Oncorhynchus mykiss* Walbaum. *Journal of experimental biology,* 208**,** 2083-2090.

Morán, M., Saborido, A. & Megías, A. 2003. Ca2+ regulatory systems in rat myocardium are altered by 24 weeks treadmill training. *Pflügers Archiv,* 446**,** 161-168.

Mortensen, A. & Damsgård, B. 1993. Compensatory growth and weight segregation following light and temperature manipulation of juvenile Atlantic salmon (*Salmo salar* L.) and Arctic charr (*Salvelinus alpinus* L.). *Aquaculture,* 114**,** 261-272.

Nicieza, A. G. & Metcalfe, N. B. 1997. Growth compensation in juvenile Atlantic salmon: responses to depressed temperature and food availability. *Ecology,* 78**,** 2385-2400.

Nylund, A., Karlsen, C. R., Good, C., Jørgensen, S. M., Plarre, H., Isaksen, T. E., Handeland, S. O., Wollseth, K. & Ottem, K. F. 2015. Review of microparasites that could represent a future problem for production of salmonids in closed or semi-closed containment systems. Bergen.

Olsvik, P. A., Lie, K. K., Jordal, A.-E. O., Nilsen, T. O. & Hordvik, I. 2005. Evaluation of potential reference genes in real-time RT-PCR studies of Atlantic salmon. *BMC Molecular Biology,* 6**,** 21.

Pfaffl, M. W. 2004. *Quantification strategies in real-time PCR,* Fivephoton Biochemicals.

Rasmussen, R. S., Heinrich, M. T., Hyldig, G., Jacobsen, C. & Jokumsen, A. 2011. Moderate exercise of rainbow trout induces only minor differences in fatty acid profile, texture, white muscle fibres and proximate chemical composition of fillets. *Aquaculture,* 314**,** 159-164.

Remen, M., Solstorm, F., Bui, S., Klebert, P., Vågseth, T., Solstorm, D., Hvas, M. & Oppedal, F. 2016. Critical swimming speed in groups of Atlantic salmon *Salmo salar*. *Aquaculture Environment Interactions,* 8**,** 659-664.

Rolim, N. P., Medeiros, A., Rosa, K. T., Mattos, K. C., Irigoyen, M. C., Krieger, E. M., Krieger, J. E., Negrão, C. E. & Brum, P. C. 2007. Exercise training improves the net balance of cardiac Ca2+ handling protein expression in heart failure. *Physiological genomics,* 29**,** 246-252.

Rome, L. C. & Alexander, R. M. 1990. The influence of temperature on muscle velocity and sustained performance in swimming carp. *Journal of Experimental Biology,* 154**,** 163-178.

Rosten, T. W., Ulgenes, Y., Henriksen, K., Terjesen, B. F., Biering, E. & Winther, U. 2011. Oppdrett av laks og ørret i lukkede anlegg - forprosjekt. Utredning for Fiskeri og havbruksnæringens forskningsfond (FHF). (In Norwegian)

Santer, R., Walker, M. G., Emerson, L. & Witthames, P. 1983. On the morphology of the heart ventricle in marine teleost fish (Teleostei). *Comparative Biochemistry and Physiology Part A: Physiology,* 76**,** 453-457.

Simonot, D. & Farrell, A. 2009. Coronary vascular volume remodelling in rainbow trout *Oncorhynchus mykiss*. *Journal of fish biology,* 75**,** 1762-1772.

Solstorm, F., Solstorm, D., Oppedal, F., Fernö, A., Fraser, T. & Olsen, R. E. 2015. Fast currents reduce production performance of post-smolt Atlantic salmon. *Aquaculture environment interactions,* 7, 125-134.

Solstorm, F., Solstorm, D., Oppedal, F., Olsen, R. E., Stien, L. H. & Fernö, A. 2016. Not too slow, not too fast: water currents affect group structure, aggression and welfare in post-smolt Atlantic salmon *Salmo salar*. *Aquaculture Environment Interactions,* 8**,** 339-347.

Soonpaa, M. H., Kim, K. K., Pajak, L., Franklin, M. & Field, L. J. 1996. Cardiomyocyte DNA synthesis and binucleation during murine development. *American Journal of Physiology-Heart and Circulatory Physiology,* 271**,** H2183-H2189.

Stefansson, S. O., Mccormick, S., Ebbesson, L. O. & Björnsson, B. T. 2008. *Smoltification*. In "Fish Larval Physiology" (Finn A and Kapoor B, eds)

Stickland, N. 1983. Growth and development of muscle fibres in the rainbow trout (*Salmo gairdneri*). *Journal of Anatomy,* 137**,** 323.

Stickland, N., White, R., Mescall, P., Crook, A. & Thorpe, J. 1988. The effect of temperature on myogenesis in embryonic development of the Atlantic salmon (*Salmo salar* L.). *Anatomy and embryology,* 178**,** 253-257.

Sveier, H., Tangen, S. & Handeland, S. O. 2015. Postsmolt of Atlantic salmon (*Salmo salar*) production in floating raceway system. Expectations and preliminary results. Aquaculture Europe, EAS, Rotterdam, The Netherlands, 20-23. October 2015.

Teknologirådet 2013. Fremtidens lakseoppdrett. Oslo. ILAS Grafisk (In Norwegian)

Thorarensen, H. & Farrell, A. P. 2011. The biological requirements for post-smolt Atlantic salmon in closed-containment systems. *Aquaculture,* 312**,** 1-14.

Totland, G., Kryvi, H., Jødestøl, K., Christiansen, E., Tangerås, A. & Slinde, E. 1987. Growth and composition of the swimming muscle of adult Atlantic salmon (*Salmo salar* L.) during long-term sustained swimming. *Aquaculture,* 66**,** 299-313.

Videler, J. J. 1993. *Fish swimming,* Springer Science & Business Media.

Walker, M. G. & Emerson, L. 1978. Sustained swimming speeds and myotomal muscle function in the trout, *Salmo gairdneri*. *Journal of Fish Biology,* 13**,** 475-481.

Weatherley, A., Gill, H. & Lobo, A. 1988. Recruitment and maximal diameter of axial muscle fibres in teleosts and their relationship to somatic growth and ultimate size. *Journal of Fish Biology,* 33**,** 851-859.

Weatherley, A., Gill, H. & Rogers, S. 1980. The relationship between mosaic muscle fibres and size in rainbow trout (*Salmo gairdneri*). *Journal of Fish Biology,* 17**,** 603-610.

Winberg, S., Nilsson, G. E. & Olsén, K. H. 1991. Social rank and brain levels of monoamines and monoamine metabolites in Arctic charr, *Salvelinus alpinus* (L.). *Journal of Comparative Physiology A,* 168**,** 241-246.

Yancopoulos, G. D., Davis, S., Gale, N. W., Rudge, J. S., Wiegand, S. J. & Holash, J. 2000. Vascular-specific growth factors and blood vessel formation. *Nature,* 407**,** 242-248.

Young, P. S. & Cech Jr, J. J. 1994. Effects of different exercise conditioning velocities on the energy reserves and swimming stress responses in young-of-the-year striped bass (*Morone saxatilis*). *Canadian Journal of Fisheries and Aquatic Sciences,* 51**,** 1528-1534.

Appendix I

Weight, length and condition factor

TABLE I. Test results from a one-way ANOVA on weight for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	979341,7	1	979341,7	1595,255	0,000000
Behandling-2	455,0	1	455,0	0,741	0,392857
Error	35606,7	58	613,9		

TABLE II. Test results from a one-way ANOVA on weight for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	2891526	1	2891526	1321,029	0,000000
Behandling-3	29004	1	29004	13,251	0,000582
Error	126953	58	2189		

TABLE III. Test results from a one-way ANOVA on weight for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	20158604	1	20158604	407,5520	0,000000
Behandling-4	1356578	1	1356578	27,4263	0,000002
Error	2868834	58	49463		

TABLE IV. Test results from a one-way ANOVA on length after for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	31813,64	1	31813,64	21202,43	0,000000
Behandling-2	1,35	1	1,35	0,90	0,346793
Error	87,03	58	1,50		

TABLE V. Test results from a one-way ANOVA on length after for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	44303,40	1	44303,40	13688,94	0,000000
Behandling-3	46,46	1	46,46	14,36	0,000362
Error	187,71	58	3,24		

TABLE VI. Test results from a one-way ANOVA on length for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	77911,27	1	77911,27	6849,775	0,000000
Behandling-4	417,65	1	417,65	36,719	0,000000
Error	659,71	58	11,37		

TABLE VII. Test results from a one-way ANOVA on condition factor after for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	64,11417	1	64,11417	13002,42	0,000000
Behandling-2	0,00065	1	0,00065	0,13	0,717474
Error	0,28599	58	0,00493		

TABLE VIII. Test results from a one-way ANOVA on condition factor for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	69,11486	1	69,11486	19001,80	0,000000
Behandling-3	0,00382	1	0,00382	1,05	0,309430
Error	0,21096	58	0,00364		

TABLE IX. Test results from a one-way ANOVA on condition factor for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	80,58043	1	80,58043	5154,859	0,000000
Behandling-4	0,09595	1	0,09595	6,138	0,016167
Error	0,90665	58	0,01563		

TABLE X. Test results from a one-way ANOVA on length increase from sampling 1 (June 1/2nd) to 3 (August 29/30th), during the post-smolt phase, for the control groups.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	56826,04	1	56826,04	7341,010	0,00
Behandling	3743,02	1	3743,02	483,538	0,00
Error	448,97	58	7,74		

TABLE XI. Test results from a one-way ANOVA on weight increase from sampling 1 (June 1/2nd) to 3 (August 29/30th), during the post-smolt phase, for the control groups.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	10965512	1	10965512	260,9824	0,000000
Behandling	5490278	1	5490278	130,6702	0,000000
Error	2436944	58	42016		

TABLE XII. Test results from a one-way ANOVA on CF increase from sampling 1 (June 1/2nd) to 3 (August 29/30th), during the post-smolt phase, for the control groups.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	74,54606	1	74,54606	4453,088	0,000000
Behandling	0,42563	1	0,42563	25,426	0,000005
Error	0,97094	58	0,01674		

TABLE XIII. Test results from a one-way ANOVA on length increase from sampling 1 (June 1/2nd) to 3 (August 29/30th), during the post-smolt phase, for the Preline group.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	48008,13	1	48008,13	9351,291	0,00
Behandling	1566,73	1	1566,73	305,176	0,00
Error	297,76	58	5,13		

TABLE XIV. Test results from a one-way ANOVA on weight increase from sampling 1 (June 1/2nd) to 3 (August 29/30th), during the post-smolt phase, for the Preline group.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	4700350	1	4700350	583,1495	0,00
Behandling	1338839	1	1338839	166,1033	0,00
Error	467496	58	8060		

TABLE XV. Test results from a one-way ANOVA on CF increase from sampling 1 (June 1/2nd) to 3 (August 29/30th), during the post-smolt phase, for the Preline group.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	69,71894	1	69,71894	18238,75	0,000000
Behandling	0,10057	1	0,10057	26,31	0,000004
Error	0,22171	58	0,00382		

Skeletal muscle

TABLE XVI. Test results from a one-way ANCOVA for the muscle fibre interval group 0-20 µm for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	4,618	1	4,6183	0,161075	0,689669
Length	17,000	1	16,9999	0,592917	0,444473
Behandling-1	162,057	1	162,0566	5,652145	0,020812
Error	1634,287	57	28,6717		

TABLE XVII. Test results from a one-way ANCOVA for the muscle fibre interval group 20-40 µm for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	324,980	1	324,9804	12,98821	0,000659
Length	128,456	1	128,4564	5,13391	0,027279
Behandling-1	7,341	1	7,3407	0,29338	0,590175
Error	1426,208	57	25,0212		

TABLE XVIII. Test results from a one-way ANCOVA for the muscle fibre interval group 40-60 µm for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	70,8182	1	70,8182	4,48852	0,038494
Length	1,5539	1	1,5539	0,09849	0,754796
Behandling-1	174,7049	1	174,7049	11,07295	0,001538
Error	899,3249	57	15,7776		

TABLE XIX. Test results from a one-way ANCOVA for the muscle fibre interval group 60-80 µm for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	109,9223	1	109,9223	14,98322	0,000282
Length	18,6419	1	18,6419	2,54103	0,116453
Behandling-1	8,0299	1	8,0299	1,09453	0,299885
Error	418,1727	57	7,3364		

TABLE XX. Test results from a one-way ANCOVA for the muscle fibre interval group 80-100 µm for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	65,2480	1	65,24799	12,07159	0,000985
Length	8,0944	1	8,09435	1,49754	0,226084
Behandling-1	3,1641	1	3,16415	0,58540	0,447359
Error	308,0899	57	5,40509		

TABLE XXI. Test results from a one-way ANCOVA for the muscle fibre interval group 100-120 µm for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	5,6196	1	5,61957	1,160578	0,285884
Length	2,3995	1	2,39955	0,495564	0,484322
Behandling-1	18,8676	1	18,86763	3,896624	0,053237
Error	275,9967	57	4,84205		

TABLE XXII. Test results from a one-way ANCOVA for the muscle fibre interval group 120-140 µm for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	27,2642	1	27,26417	4,249186	0,043841
Length	61,3262	1	61,32616	9,557828	0,003079
Behandling-1	18,7803	1	18,78027	2,926950	0,092550
Error	365,7307	57	6,41633		

TABLE XXIII. Test results from a one-way ANCOVA for the muscle fibre interval group 140-160 µm for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1,82960	1	1,829597	1,322829	0,254888
Length	6,40235	1	6,402350	4,629004	0,035687
Behandling-1	5,66352	1	5,663515	4,094815	0,047713
Error	78,83638	57	1,383094		

TABLE XXIV. Test results from a one-way ANCOVA for the muscle fibre interval group 160-180 µm for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	0,7217	1	0,721676	0,400939	0,529137
Length	2,3819	1	2,381942	1,323327	0,254799
Behandling-1	6,2974	1	6,297416	3,498633	0,066557
Error	102,5980	57	1,799965		

TABLE XXV. Test results from a one-way ANCOVA for the muscle fibre interval group 180-200 µm for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	0,15641	1	0,156405	0,267934	0,606724
Length	0,55095	1	0,550945	0,943812	0,335404
Behandling-1	1,70603	1	1,706027	2,922557	0,092789
Error	33,27344	57	0,583745		

TABLE XXVI. Test results from a one-way ANCOVA for the muscle fibre interval group 200-220 µm for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	0,67387	1	0,673866	3,733334	0,058312
Length	0,89249	1	0,892491	4,944553	0,030152
Behandling-1	0,48761	1	0,487607	2,701429	0,105764
Error	10,28849	57	0,180500		

TABLE XXVII. Test results from a one-way ANCOVA for the muscle fibre interval group 220- µm for sampling 1 (June 1/2nd), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	0,18166	1	0,181658	0,785443	0,379206
Length	0,22968	1	0,229680	0,993079	0,323203
Behandling-1	0,85688	1	0,856883	3,704947	0,059247
Error	13,18300	57	0,231281		

TABLE XXVIII. Test results from a one-way ANCOVA for the muscle fibre interval group 0-20 µm for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	397,011	1	397,0114	13,63110	0,000500
Length	159,710	1	159,7099	5,48353	0,022715
Behandling-1	57,017	1	57,0166	1,95762	0,167186
Error	1660,148	57	29,1254		

TABLE XXIX. Test results from a one-way ANCOVA for the muscle fibre interval group 20-40 µm for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	0,022	1	0,0221	0,00043	0,983600
Length	154,474	1	154,4736	2,98613	0,089395
Behandling-1	954,855	1	954,8553	18,45831	0,000068
Error	2948,632	57	51,7304		

TABLE XXX. Test results from a one-way ANCOVA for the muscle fibre interval group 40-60 µm for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	43,672	1	43,67203	2,478808	0,120924
Length	1,625	1	1,62526	0,092249	0,762443
Behandling-1	2,542	1	2,54166	0,144263	0,705490
Error	1004,235	57	17,61816		

TABLE XXXI. Test results from a one-way ANCOVA for the muscle fibre interval group 60-80 µm for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	86,8207	1	86,82070	9,759163	0,002804
Length	4,4843	1	4,48431	0,504063	0,480615
Behandling-1	25,6696	1	25,66958	2,885414	0,094838
Error	507,0906	57	8,89633		

TABLE XXXII. Test results from a one-way ANCOVA for the muscle fibre interval group 80-100 µm for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	30,7945	1	30,79454	3,802772	0,056092
Length	0,1049	1	0,10489	0,012952	0,909790
Behandling-1	9,6354	1	9,63538	1,189859	0,279948
Error	461,5814	57	8,09792		

TABLE XXXIII. Test results from a one-way ANCOVA for the muscle fibre interval group 100-120 µm for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	16,7431	1	16,74306	2,383230	0,128178
Length	0,0682	1	0,06822	0,009710	0,921849
Behandling-1	30,2079	1	30,20785	4,299827	0,042646
Error	400,4458	57	7,02537		

TABLE XXXIV. Test results from a one-way ANCOVA for the muscle fibre interval group 120-140 µm for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1,5082	1	1,50820	0,310164	0,579760
Length	2,0693	1	2,06934	0,425563	0,516797
Behandling-1	26,4535	1	26,45346	5,440202	0,023233
Error	277,1675	57	4,86259		

TABLE XXXV. Test results from a one-way ANCOVA for the muscle fibre interval group 140-160 µm for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1,7383	1	1,738261	0,595768	0,443386
Length	0,0152	1	0,015192	0,005207	0,942729
Behandling-1	8,2204	1	8,220449	2,817457	0,098719
Error	166,3080	57	2,917683		

TABLE XXXVI. Test results from a one-way ANCOVA for the muscle fibre interval group 160-180 µm for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1,02047	1	1,020475	0,835761	0,364463
Length	0,09832	1	0,098316	0,080520	0,777622
Behandling-1	5,67424	1	5,674242	4,647164	0,035340
Error	69,59767	57	1,221012		

TABLE XXXVII. Test results from a one-way ANCOVA for the muscle fibre interval group 180-200 µm for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1,20607	1	1,206075	1,097724	0,299188
Length	0,53090	1	0,530899	0,483204	0,489799
Behandling-1	0,04074	1	0,040744	0,037084	0,847979
Error	62,62617	57	1,098705		

TABLE XXXVIII. Test results from a one-way ANCOVA for the muscle fibre interval group 200-220 µm for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	0,02149	1	0,021494	0,053513	0,817887
Length	0,10133	1	0,101330	0,252279	0,617410
Behandling-1	0,72134	1	0,721341	1,795903	0,185526
Error	22,89459	57	0,401659		

TABLE XXXIX. Test results from a one-way ANCOVA for the muscle fibre interval group 220- µm for sampling 2 (June29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	0,21290	1	0,212904	1,145627	0,288978
Length	0,13787	1	0,137866	0,741848	0,392678
Behandling-1	0,02768	1	0,027682	0,148954	0,700973
Error	10,59293	57	0,185841		

TABLE XL. Test results from a one-way ANCOVA for the muscle fibre interval group 0-20 µm for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	398,5968	1	398,5968	24,36491	0,000007
Length	210,9385	1	210,9385	12,89398	0,000687
Behandling-1	87,5259	1	87,5259	5,35017	0,024351
Error	932,4892	57	16,3595		

TABLE XLI. Test results from a one-way ANCOVA for the muscle fibre interval group 20-40 µm for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	351,797	1	351,7970	7,057771	0,010219
Length	8,999	1	8,9991	0,180540	0,672509
Behandling-1	285,434	1	285,4335	5,726384	0,020029
Error	2841,184	57	49,8453		

TABLE XLII. Test results from a one-way ANCOVA for the muscle fibre interval group 40-60 µm for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	82,755	1	82,7550	1,674604	0,200862
Length	50,602	1	50,6019	1,023965	0,315857
Behandling-1	115,626	1	115,6261	2,339772	0,131639
Error	2816,807	57	49,4177		

TABLE XLIII. Test results from a one-way ANCOVA for the muscle fibre interval group 60-80 µm for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	**p**
Intercept	0,519	1	0,5190	0,026550	0,871141
Length	95,031	1	95,0313	4,861587	0,031511
Behandling-1	107,291	1	107,2910	5,488763	0,022653
Error	1114,201	57	19,5474		

TABLE XLIV. Test results from a one-way ANCOVA for the muscle fibre interval group 80-100 µm for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	31,6861	1	31,68613	2,930417	0,092361
Length	4,4616	1	4,46163	0,412623	0,523217
Behandling-1	5,3563	1	5,35635	0,495369	0,484407
Error	616,3319	57	10,81284		

TABLE XLV. Test results from a one-way ANCOVA for the muscle fibre interval group 100-120 µm for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	30,8070	1	30,80697	4,992157	0,029400
Length	0,1020	1	0,10203	0,016533	0,898143
Behandling-1	7,6904	1	7,69040	1,246201	0,268963
Error	351,7512	57	6,17107		

TABLE XLVI. Test results from a one-way ANCOVA for the muscle fibre interval group 120-140 µm for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	10,3295	1	10,32955	1,282475	0,262181
Length	0,9330	1	0,93298	0,115835	0,734848
Behandling-1	0,1026	1	0,10262	0,012740	0,910527
Error	459,0999	57	8,05438		

TABLE XLVII. Test results from a one-way ANCOVA for the muscle fibre interval group 140-160 µm for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	0,2926	1	0,292591	0,053823	0,817371
Length	3,9888	1	3,988841	0,733752	0,395257
Behandling-1	4,5640	1	4,563960	0,839545	0,363387
Error	309,8650	57	5,436228		

TABLE XLVIII. Test results from a one-way ANCOVA for the muscle fibre interval group 160-180 µm for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	6,0404	1	6,040412	1,368800	0,246887
Length	0,9500	1	0,949984	0,215273	0,644432
Behandling-1	0,0289	1	0,028931	0,006556	0,935750
Error	251,5366	57	4,412924		

TABLE XLIX. Test results from a one-way ANCOVA for the muscle fibre interval group 180-200 µm for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	3,2632	1	3,263219	1,759782	0,189942
Length	1,2493	1	1,249291	0,673715	0,415179
Behandling-1	0,2279	1	0,227908	0,122906	0,727196
Error	105,6969	57	1,854331		

TABLE L. Test results from a one-way ANCOVA for the muscle fibre interval group 200-220 µm for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	4,92357	1	4,923574	3,243409	0,077002
Length	3,43453	1	3,434532	2,262501	0,138061
Behandling-1	2,79821	1	2,798213	1,843326	0,179913
Error	86,52739	57	1,518024		

TABLE LI. Test results from a one-way ANCOVA for the muscle fibre interval group 220- µm for sampling 3 (August 29/30th), during the post-smolt phase.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1,04356	1	1,043559	2,724728	0,104305
Length	0,62013	1	0,620133	1,619164	0,208375
Behandling-1	0,03538	1	0,035376	0,092368	0,762295
Error	21,83075	57	0,382996		

Heart

TABLE LII. Test results from a Kruskal-Wallis ANOVA for the relative expression levels of MEF2C between control and freshwater.

Depend.: MEF2C	**FW R:41,233**	Control R:15,407
FW		0,000000
Control	0,000000	

TABLE LIII. Test results from a Kruskal-Wallis ANOVA for the relative expression levels of MEF2C between Preline and freshwater.

Depend.: MEF2C	**FW R:39,833**	Preline R:19,828
FW		0,000008
Preline	0,000008	

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TABLE LIV. Test results from a Kruskal-Wallis ANOVA for the relative expression levels of MEF2C between control and Preline.

Depend.: MEF2C	**Control R:22,074**	Preline R:34,483
Control		0,004442
Preline	0,004442	

TABLE LV. Test results from a Kruskal-Wallis ANOVA for the relative expression levels of GATA4 between control and freshwater.

Depend.: GATA4	**FW R:34,367**	Control R:15,773
FW		0,000012
Control	0,000012	

TABLE LVI. Test results from a Kruskal-Wallis ANOVA for the relative expression levels of GATA4 between Preline and freshwater.

Depend.: GATA4	**FW R:37,567**	Preline R:22,172
FW		0,000578
Preline	0,000578	

TABLE LVII. Test results from a Kruskal-Wallis ANOVA for the relative expression levels of GATA4 between control and Preline.

Depend.: GATA4	**Control R:20,682**	Preline R:30,034
Control		0,026069
Preline	0,026069	

TABLE LVIII. Test results from a Kruskal-Wallis ANOVA for the relative expression levels of VEGF between control and freshwater.

Depend.: VEGF	**FW R:34,200**	Control R:16,000
FW		0,000019
Control	0,000019	

TABLE LIX. Test results from a Kruskal-Wallis ANOVA for the relative expression levels of VEGF between Preline and freshwater.

Depend.: VEGF	**FW R:38,500**	Preline R:21,207
FW		0,000110
Preline	0,000110	

TABLE LX. Test results from a Kruskal-Wallis ANOVA for the relative expression levels of VEGF between control and Preline.

Depend.: VEGF	**Control R:22,091**	Preline R:28,966
Control		0,101923
Preline	0,101923	

SAV test

TABLE LXI. Test results from a One-way ANOVA for the muscle fibre interval group 0-20 µm for within the control group, between SAV positive and SAV negative fish.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	548,9348	1	548,9348	61,09972	0,000000
Behandling	0,3868	1	0,3868	0,04305	0,837129
Error	251,5588	28	8,9842		

TABLE LXII. Test results from a One-way ANOVA for the muscle fibre interval group 20-40 µm for within the control group, between SAV positive and SAV negative fish.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	9201,638	1	9201,638	177,4916	0,000000
Behandling	0,026	1	0,026	0,0005	0,982404
Error	1451,594	28	51,843		

TABLE LXIII. Test results from a One-way ANOVA for the muscle fibre interval group 40-60 µm for within the control group, between SAV positive and SAV negative fish.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	15903,71	1	15903,71	201,3343	0,000000
Behandling	5,09	1	5,09	0,0644	0,801476
Error	2211,76	28	78,99		

TABLE LXIV. Test results from a One-way ANOVA for the muscle fibre interval group 60-80 µm for within the control group, between SAV positive and SAV negative fish.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	7992,224	1	7992,224	229,5234	0,000000
Behandling	26,068	1	26,068	0,7486	0,394261
Error	974,987	28	34,821		

TABLE LXV. Test results from a One-way ANOVA for the muscle fibre interval group 80-100 µm for within the control group, between SAV positive and SAV negative fish.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	3354,587	1	3354,587	266,6982	0,000000
Behandling	0,315	1	0,315	0,0250	0,875381
Error	352,190	28	12,578		

TABLE LXVI. Test results from a One-way ANOVA for the muscle fibre interval group 100-120 µm for within the control group, between SAV positive and SAV negative fish.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	2000,062	1	2000,062	262,1003	0,000000
Behandling	3,565	1	3,565	0,4672	0,499913
Error	213,665	28	7,631		

TABLE LXVII. Test results from a One-way ANOVA for the muscle fibre interval group 120-140 µm for within the control group, between SAV positive and SAV negative fish.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	873,6115	1	873,6115	80,52800	0,000000
Behandling	0,2748	1	0,2748	0,02533	0,874680
Error	303,7592	28	10,8485		

TABLE LXVIII. Test results from a One-way ANOVA for the muscle fibre interval group 140-160 µm for within the control group, between SAV positive and SAV negative fish.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	294,6290	1	294,6290	44,41233	0,000000
Behandling	0,0010	1	0,0010	0,00015	0,990447
Error	185,7505	28	6,6339		

TABLE LXIX. Test results from a One-way ANOVA for the muscle fibre interval group 160-180 µm for within the control group, between SAV positive and SAV negative fish.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	94,3758	1	94,37578	14,07306	0,000815
Behandling	1,2189	1	1,21892	0,18176	0,673123
Error	187,7717	28	6,70613		

TABLE LXX. Test results from a One-way ANOVA for the muscle fibre interval group 180-200 µm for within the control group, between SAV positive and SAV negative fish.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	16,65887	1	16,65887	5,959179	0,021219
Behandling	2,57451	1	2,57451	0,920950	0,345440
Error	78,27394	28	2,79550		

TABLE LXXI. Test results from a One-way ANOVA for the muscle fibre interval group 200-220 µm for within the control group, between SAV positive and SAV negative fish.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	7,02049	1	7,020487	2,705835	0,111164
Behandling	1,11026	1	1,110255	0,427914	0,518351
Error	72,64805	28	2,594573		

TABLE LXXII. Test results from a One-way ANOVA for the muscle fibre interval group >220 µm for within the control group, between SAV positive and SAV negative fish.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1,75286	1	1,752859	4,252658	0,048578
Behandling	0,06840	1	0,068398	0,165942	0,686841
Error	11,54103	28	0,412180		

TABLE LXXIII. Test results from a Kruskal-Wallis ANOVA expressed mRNA levels of MEF2C within the control group, between SAV positive and SAV negative fish.

Depend.: MEF2C	**PD R:13,600**	NoPD R:14,091
PD		0,900652
NoPD	0,900652	

TABLE LXXIV. Test results from a Kruskal-Wallis ANOVA expressed mRNA levels of GATA4 within the control group, between SAV positive and SAV negative fish.

Depend.: GATA4	**PD R:22,200**	NoPD R:12,136
PD		0,010492
NoPD	0,010492	

TABLE LXXV. Test results from a Kruskal-Wallis ANOVA expressed mRNA levels of VEGF within the control group, between SAV positive and SAV negative fish.

Depend.: VEGF	**PD R:25,000**	NoPD R:11,500
PD		0,000597
NoPD	0,000597	