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Navn på kandidat: Halvor Prytz

Effects of swim speed on growth and muscle development of Atlantic salmon post-smolt

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Abstract

Atlantic salmon (*Salmo salar*) is an important species in Norwegian aquaculture and with increased political pressure to improve sustainability and reduce impacts on local ecosystems, paired with pathogenic and parasitic presence; the technological development is pushed in the direction of closed systems. Fish farmers want to maximise production output, efficiency and product quality, making solutions enabling these improvements very valuable. Salmon were kept in closed cages for 8 weeks with either water velocity from pumping water through the cage alone (6 – 10 cms^{-1}), or with additional applied velocity from propellers in the cage (18 – 20 cms^{-1}). Growth was measured as weight and length, while nutritional quality was measured by analysis of crude lipid, crude nitrogen and water content. Muscle cellularity was also investigated by calculating fibre density, mean fibre diameter, small fibre frequency and probability distribution of muscle fibre diameters to determine difference in rates of hyperplasia.

Even with very modest speed increase and cost-efficient means the trial succeeded in increasing weight in the faster-swimming group by 12.8 % and length by 2.2 % compared to the control. The proximal composition was practically unaffected by the treatment as lipid levels only increased as a response to size of fish, and water content consequently decreased, while protein levels were very similar. No significant change was observed in muscle cellularity in general, neither among red nor white fibres, nor in rates of hyperplasia.

Water velocity below optimal speed is still capable of improving growth and production speed in closed cage systems and even rival that of traditional open systems, opening for a future of more efficient, sustainable and eco-friendly salmon production.

Acknowledgements

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Abbreviations

BLs^{-1}	Body-lengths per second. A relative measure of water velocity compared to the length of the fish.
HI	The sample group with the higher experienced speed (18 – 20 cms^{-1}).
HSI	Hepatosomatic index. A measure of relative liver weight, compared to body weight.
IPN	Infectious pancreatic necrosis. A deadly disease hitting
IQR	Interquartile range. The space between first (25^{th} percentile) and third (75^{th} percentile) quartile.
LO	The sample group with the lower experienced speed (6 – 10 cms^{-1}).
RVM	Relative ventricular mass. A measure of relative heart weight, compared to body weight.
SGR	Specific growth rate. A measure of the speed of growth in fish.
TGC	Thermal growth coefficient. A measure of growth rate in fish with regard to the water temperature.

Introduction

Norwegian Salmon farming

Farming of Atlantic salmon (*Salmo salar*) in Norway has been a large industry for the past years and is still growing. The production figures for 2016 are 1.23 million tonnes and the number of active concessions for fish production have increased by 20.9 % from 2005 – 2016, while sales and production have doubled in the same period. Production has seen numbers falling 5 % in 2016 from the year before, and a significant reason is sea lice (SSB, 2017). Concessions are limited by the Department of fisheries, and strict regulations are now implemented to ensure more sustainable development of future production (Forskrift om løyve til havbruk med matfisk, 2013).

Challenges with salmon farming in 2017

Salmon farming is not an industry without controversy, and farmed fish escaping into the wild are one of the primary concerns for both fish farmers and those opposed to farming. Escaped cultured salmon may negatively influence wild stocks by increasing competition for resources and breeding space (Jonsson & Jonsson, 2006), transmit diseases common in farmed populations (Madhun et al., 2015), transmit parasites or increase parasitic pressure (Naylor et al., 2005) and lead to genetic introgression after interbreeding (Bourret, O'Reilly, Carr, Berg, & Bernatchez, 2011; Glover et al., 2013). Cultured stocks are not adapted to wild conditions and interbreeding leads to reduced fitness and survivability for the offspring in the wild (Jonsson & Jonsson, 2006).

Environmental pollution and destruction of ecosystems is another great concern for the sustainability of fish farming. Spill feed and faeces put considerable strain on the benthic environment, lowering the biodiversity (Hall-Spencer & Bamber, 2007; Zhulay, Reiss, & Reiss, 2015) and have long-lasting negative effects on the ecosystems (Pereira, Black, McLusky, & Nickell, 2004), especially in close proximity to the farm (Mantzavrakos, Kornaros, Lyberatos, & Kaspiris, 2007). The effect is somewhat influenced by local hydrodynamic properties (Urbina, 2016), meaning greater current can distribute the organic matter over a greater area thus lowering the impact on a particular site. Feed in the water increases turbidity and leaching of nutrients to the water column is an additional issue where inorganic nitrogen and phosphorous can influence planktonic life (Mantzavrakos et al., 2007), both a possible detriment to water quality.

Parasites has been mentioned, and the primary one is Salmon lice (*Lepeophtheirus salmonis*); a naturally occurring marine crustacean parasite that feeds on skin mucus and blood of salmonids. The ectoparasite can cause severe lesions and sores, which open the host up for infection and disease, as well as reducing its overall growth and performance. Great exposure will eventually lead to death of the host. Salmon farming provide excellent breeding grounds for lice (and disease) due to the high density of fish, which can lead to large growth of lice populations (Torrissen et al., 2013). A serious concern regarding this is spreading to wild stocks of fish that pass through surrounding waters, potentially damaging them and their reproductive success (Krkošek, Lewis, & Volpe, 2005), but also leading to losses in production for farmed fish. Salmon lice larvae spread through the upper levels of the water, so currents play a significant role on their geographical spreading. They also require high-salinity water to thrive, making brackish water an unsuitable habitat (Torrissen et al., 2013). The Norwegian government has set limitations on salmon lice in fish farming to 0.5 adult female lice per salmon on average (Mattilsynet, 2017). Currently suggested or implemented countermeasures are chemical treatment (i.e. hydrogen peroxide or emamectin benzoate), cleaner fish (various species of wrasse), vaccination (Torrissen et al., 2013), scrubbing, brushing or heated water treatment. All these are treatment measures that bring different, but significant disadvantages (both ecological and to fish health) that will not be discussed further in detail here. Preventative measures that have been suggested are lice skirts (a permeable tarpaulin encasing upper levels of the sea cage), land-based facilities or closed farm technology.

"Green concessions" and Closed farm technology

The Norwegian department of Fisheries introduced so-called "Green concessions" in 2013, with the purpose of advancing technological solutions to the challenges of escapees and sea lice. After a reduction in newly appointed concessions in the face of ecological challenges, the green concessions were introduced to allow companies to test out new solutions (Forskrift om løyve til havbruk med matfisk, 2013).

Closed cage technology is one of the more recently applied strategies that aim to alleviate or remove some of these core challenges outlined above. Closed cages are in principle completely closed off from the surrounding water by a barrier, and water has to be pumped into the farm to bring in fresh seawater and remove waste (ammonia, spill feed and faeces). In this study, we examined the AkvaFuture facility in Brønnøysund, Norway. This is a floating platform, which collect water from a depth of 25 meters and have a waterproof tarpaulin

covering their pens completely blocking out any surrounding water. Oxygen levels are continuously monitored and additional oxygen is pumped into the water from land-based tanks to maintain optimal oxygen levels. There is fitted a pumping system to the bottom of the pens that remove dead fish, sediments and drain waste water (see Figure 1). The sludge water is pumped into a land-based facility for filtration of sludge, before release of effulent water into the sea, eliminating any sedimentation on the seafloor below the farms. The filtered solid wastes are then used for production of agricultural fertilisers and biogas (AkvaDesign, 2016).

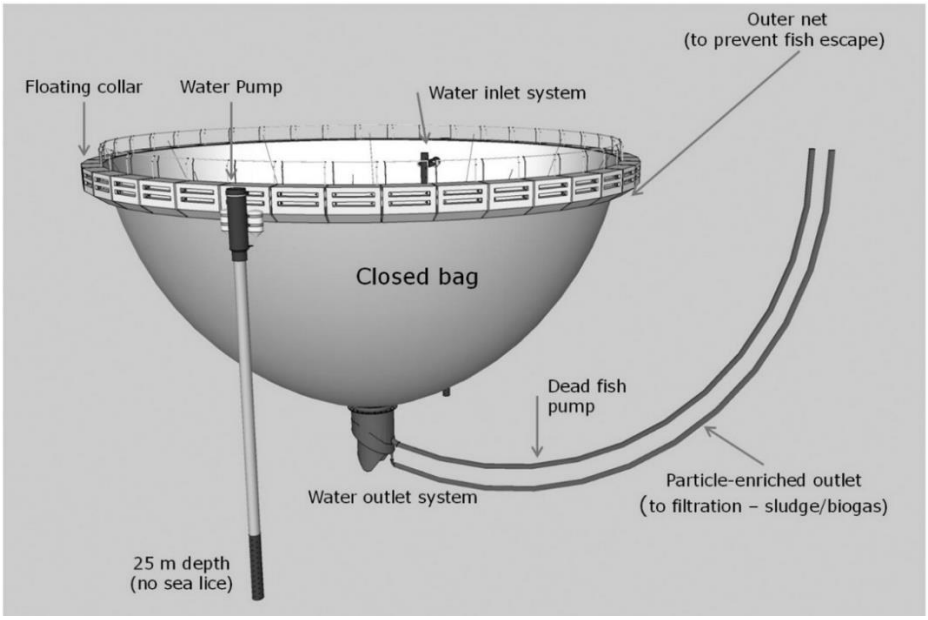

Figure 1: Overview of the closed net pen construction.

Closed farms are constructed differently than traditional open net pens, and therefore have other requirements and challenges. Nyhus, Ellingsen, and Aanondsen (2014) performed a life-cycle analysis on this facility, comparing it to a standard open sea cage and concluded that the closed farm facilities consume far more electricity, had a slightly lower (10 %) eutrophication of the surrounding water, and that the collection of sediments were promising regarding long-term ecological effects. The closed farms are far more expensive to construct than the open sea cages, requiring 10 – 30 times the investment (Rosten et al., 2011). Operation costs are also higher due to electricity costs, as mentioned, while the facility in question has had no instances of sea lice outbreaks in their farms for the entire production duration (Nilsen, Bergheim, & Iversen, 2014). The same report did however show that during cold winters, the frequency of cold sores on salmon increased in the closed pens when compared to deeper open cages, leading to increased mortality.

Growth and quality in fish

The growth of muscle is of key importance to aquaculture interests, and therefore important to understand. Fish muscles are built up from several layers of myotomes connected to each other by thin layers of tendons, or connective tissue, called myosepts or myocommata (Figure 2). The myotomes have a characteristic W-shape in most adult fish species, and consist of vast amounts of muscle fibres (Bone & Moore, 2008). Muscle fibres consist of muscle cells fused together, creating long fibres with several nuclei. Each fibre consist of numerous myofibrils; sets of actin and myosin filament proteins, joined by a Z-line at each end. These filaments are responsible for the contractive properties of the muscle fibres.

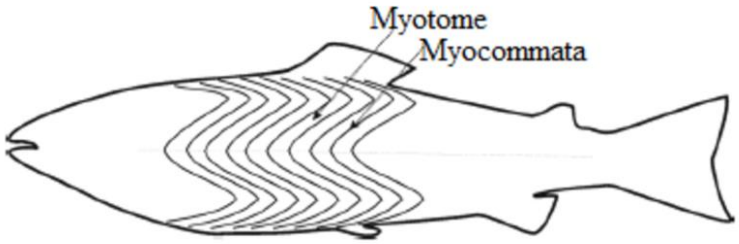

Figure 2: Schematic of fish muscle structure (Kiessling, Ruohonen, & Bjørnevik, 2006).

Fish have two primary types of muscle fibre, namely red (also called slow), and white (fast) fibres. Some species like Rainbow trout (*Oncorhynchus mykiss*) also have the pink (intermediate) fibres (Johnston, Ward, & Goldspink, 1975), but their properties are a mixture of red and white. Atlantic salmon (*Salmo salar*) do not possess these intermediate fibres, and exclusively grow red and white. These types of fibres are never mixed, but are divided into separate zones (

Figure 3). Fish has two primary mechanisms of muscle growth: growing existing muscle fibres by adding more nuclei and myofibrils to them (hypertrophy) or creating entirely new fibres (hyperplasia). Myogenic progenitor cells (MPC, also called myogenic satellite cells) are adaptable cells that differentiate into functional muscle fibre by either fusing with existing muscle fibre or forming new ones by fusing with each other. These cells are what contributes new nuclei and are important for both growth and repair of fibres, and consequently the fish continue to produce these cells as long as they grow (Koumans & Akster, 1995; Rowlerson & Veggetti, 2001).

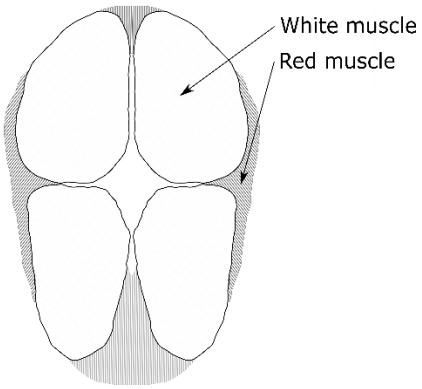

Figure 3: Cross section of salmonid fish, indicating red and white muscle fibre zones.

Several factors influence the growth and development of fish, and the most important ones are feed quality and availability (nutrition), seasonal changes which comprise variations in temperature, both light cycles and light intensity, and genetics. Lastly, the impact of exercise on fish will also be considered, as this is another area with potential gains for fish growth. Each topic will be covered briefly, as each one could fill a full review on its own.

Nutrients are what a fish grow from and are therefore important to its health and performance. Early feeding after hatching is a critical step for all fish as this defines its ability to survive the early stages of life and enable them to grow. The nutritional quality of this early feed is also very important to induce proper hyperplastic growth at the larval stage (Alami-Durante, Fauconneau, Rouel, Escaffre, & Bergot, 1997). Maintaining sufficient vitamin levels in the feed are also important to maintain growth and health for the fish, and deficiency can lead to supressed growth, immune system depression or organ failure (Pan et al., 2017; Wang, Kim, Bai, Huh, & Cho, 2003; Xu et al., 2016; Zhang et al., 2017). Feeding regime, feed intake and the energy content in this feed can also influence both growth and muscle structure (Johnsen et al., 2011). Lipid concentration and composition in feed can also significantly influence growth performance in Atlantic salmon (Hixson et al., 2017). Growth in fish has shown high plasticity as fish are able to (at least partially) make up for lost feeding after periods of starvation or feed deprivation by compensatory growth when feed becomes available again, as shown in two experiments with Rainbow trout (*Oncorhynchus mykiss*) (Azodi, Ebrahimi, Farhadian, Mahboobi-Soofiani, & Morshedi, 2015; Dobson & Holmes, 1984).

Seasonal variations determine maturation of fish and smoltification of salmonid juveniles, and maturation can be induced by light and heat-treatment as early as six weeks following smoltification (Fjelldal, Hansen, & Huang, 2011), so it appears likely that light plays an important role in controlling biochemical processes. Increased light exposure has produced

significantly increased growth in both mass and fibre count in Atlantic salmon, principally in the early parts of the seawater stages. Hyperplastic growth appear to be especially affected by increased light exposure and the proliferation of MPCs was observed to be depressed in low-light conditions, explaining the lower growth (Johnston et al., 2003). This study found no change in time of maturation between the light-exposed and the ambient light groups in seawater, indicating that perhaps a combination of temperature and light is required to induce sexual maturation, as per Fjelldal et al. (2011). Using an almost identical photoperiod as Johnston et al. (2003), Peterson and Harmon (2005) succeeded in greatly reducing maturation in Atlantic salmon from 50 % to 0.8 % for males, and 7 % to 0 % for females. Even periods of six weeks can be enough to stimulate enhanced growth (Endal, Taranger, Stefansson, & Hansen, 2000), but the timing of lighting appears to also be important to reduce maturation while maintaining growth (Taranger et al., 1999; Taranger et al., 1998).

Fish are poikilothermic and therefore subject to the temperature of their environment. Temperature is a strong influence on both hatching time, post-hatch fibre count and larval growth (Johnston et al., 2000a; Johnston, McLay, Abercromby, & Robins, 2000b). Increased temperatures lead to earlier hatching in Atlantic salmon, while lower (ambient) temperatures give rise to far greater levels of hyperplastic growth pre-hatching. Both groups showed similar cross sectional area, leaving the group reared at ambient temperature with a greater fibre density and lower average fibre diameter (Nathanailides, Stickland, & Lopez-Albors, 1995; Stickland, White, Mescall, Crook, & Thorpe, 1988). Higher temperature increase metabolic rates and feed ingestion, but only up to a certain point, where increasing temperature further will lead to a decrease in efficiency. This would also be linked to oxygen levels in the water which drops as temperatures rise, while oxygen consumption rises, limiting the total possible metabolic output. These factors combine to produce improved growth, but each species has a temperature optimum in which they grow best and increasing or decreasing temperatures beyond this is actually detrimental (Jobling, 1997). The increase in metabolic rates can actually lead to negative growth at higher temperatures if feeding rates are not adjusted to compensate (Sumpter, 1992).

Genetic variance between different strains or populations of the same species can have great impact on many morphological traits, as made evident by generations of selective breeding of plants and animals. Different strains can have significant variation in properties like fibre count and temperature adaptation (Johnston & McLay, 1997). Growth rates are also affected, as seen by variation of white muscle fibre recruitment in farmed trout (Fauconneau et al.,

1997). The big difference seen in total growth of wild Atlantic salmon compared to farmed strains also show that selective breeding can lead to significantly altered properties in muscle growth (Rowlerson & Veggetti, 2001). Rates of hypertrophic and hyperplastic growth can also vary through life stages between different strains, as demonstrated by Johnston et al. (2000a) who found differing priorities given to either hyperplastic or hypertrophic growth at different times, leading to differences in fibre count and mean fibre diameter during several stages of the growth of two strains of farmed salmon.

Just as humans exercise for improved fitness, so can fish. Increased swimming activity has shown to improve growth parameters in some, but not all species of fish. Training needs to be optimised with the correct current speed to maximise the growth output in order not to overexert or under-stimulate the fish. The species most responsive to swimming training are active swimmers, for instance salmonids, who respond positively to increased exercise. Speeds are measured in body lengths per second (BLs^{-1}) of water current (or distance travelled if the water was still). In this way, the size of the fish and its swimming capabilities is always taken into account (Davison & Herbert, 2014). Many different factors play into the growth of fish more actively swimming, compared to those not exposed to high levels of activity. Increased appetite, increased feed conversion efficiency, and increased oxygen demand are some of the physiological responses in swimming fish (Jobling, Baardvik, Christiansen, & Jørgensen, 1993).

Sustained swimming leads to both biochemical changes and also alterations of genetic expression in salmon after prolonged sustained swimming to cope with the increased load and metabolic demands (Morash, Vanderveken, & McClelland, 2014). Continuous swimming of 1-2 BLs^{-1} have shown promising results and appear to be the optimum range for several species of fish, such as qingbo (*Spinibarbus sinensis*) (Li, Yuan, Fu, & Zhang, 2016), Arctic charr (*Salvelinus alpinus*), brown- (*Salmo trutta*) and rainbow trout, and Atlantic salmon as summarised by Davison and Herbert (2014) and references therein. These results are somewhat contradicted by Solstorm et al. (2015), who found that sustained speeds of 1.5 BLs^{-1} decreased growth in both length and size when compared to lower speeds of 0.2 and 0.8 BLs^{-1} for Atlantic salmon. Exercise can however yield higher fibre density and fibre diameters in some species, while too high intensity is detrimental to the growth of the fish (Li et al., 2016). Studies have also shown slightly improved results for interval training compared to continuous training (Castro et al., 2011) although the results of interval training are

conflicting (Davison & Herbert, 2014). The effects of exercise can be seen at very early stages of life, demonstrated in gilthead sea bream fingerlings (*Sparus aurata*) (Blasco et al., 2015).

Listrat et al. (2016) define quality in four categories: hygienic quality, nutritional quality, organoleptic quality and serviceability. Hygienic quality (food safety) is the absence of pathogens, environmental toxins (pesticides and heavy metals), antibiotics and decomposing bacteria. Nutritional quality is the composition and quality of the contained nutrients, i.e. high content of essential amino acids and polyunsaturated fatty acids in fish. Organoleptic quality (or sensory quality) are everything relating to the senses, like taste, smell, colour, texture, and fillet gaping and water-binding capacity in the case of fish. Serviceability is defined as the price, availability and ease of use. Hygienic quality and serviceability will not be discussed further here. For fish or salmon in particular the most important quality factors are texture, colour, fillet gaping, taste and flavour (Kiessling et al., 2006). Several factors influence the quality of fish, and a general overview can be seen in Figure 4 (applied from Johnsen (2011), referring to Kestin and Warriss (2001)). Only those relevant to this experiment will be discussed here in further detail.

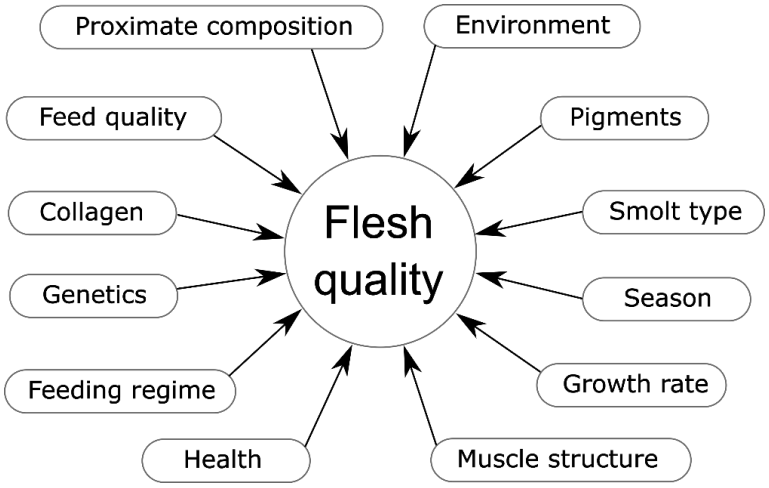

Figure 4: Factors influencing flesh quality in fish.

Feeding is the primary driving force for growth, and both diet composition and feeding regime are important for the total growth and nutritional and organoleptic quality of the flesh (Johnsen et al., 2013). Growth of farmed fish in itself is viewed as positive, but the rate of growth might influence the nutritional and organoleptic quality of the flesh. Bjørnevik, Karlsen, Johnston, and Kiessling (2003) found a negative correlation between growth rate and fillet texture in Atlantic cod (*Gadus morhua*). Growth has significant impact on proximal composition as lipid content in salmon is found to increase with body weight (Johnsen et al., 2011).

Fillet texture and firmness varies along the rostral-caudal axis of the fish, where firmness increase caudally (Sigurgísladóttir, 2001). Texture and fillet gaping in salmon also have seasonal variations (Espe et al., 2004). Studies have shown positive correlation between fibre density and fillet firmness in salmonids (Bugeon, Lefevre, & Fauconneau, 2003; Hurling, Rodell, & Hunt, 1996; Johnston et al., 2000c), but others have failed to confirm this relationship (Sigurgisladottir, Sigurdardottir, Ingvarsdottir, Torrissen, & Hafsteinsson, 2001). Muscle fibre density has also been linked to gaping, both with a negative (Johnston, 2001) and a positive correlation (Bjørnevik, Espe, Beattie, Nortvedt, & Kiessling, 2004). Johnston et al. (2002) did however later contradict his previous findings of a negative correlation when it was found that a fibre density > 95 mm^{-2} significantly reduced gaping occurrence, indicating a possible threshold correlation, rather than a linear one.

How does swim speed affect growth and quality in fish?

Swimming speed, as mentioned previously, can increase growth performance in some fish species, primarily those accustomed to intense swimming in the wild, like salmon (Jørgensen & Jobling, 1993), while it may be ineffective to more inactive species, such as Atlantic cod (Bjørnevik et al., 2003).

Fish who exercise might possibly alter their body composition compared to inactive groups of the same species. However, in the words of Jobling et al. (1993): "*…no general consensus as to the effects of prolonged exercise on patterns of energy deposition and overall body composition in fish species…"*, a statement repeated and confirmed to still hold true by Rasmussen, López-Albors, and Alfnes (2014). Since feed intake and energy consumption often are able to compensate for each other in such scenarios, it might be challenging to observe effects of exercise alone in a meaningful way. Li et al. (2016) observed significantly increased firmness and hardness of flesh in juvenile qingbo (*Spinibarbus sinensis*) exposed to continuous currents, when compared to the slow-swimming control. There was also increased lipid deposition in the moderately swimming groups (1-2 BL sec^{-1}), while the high speed (4 BL sec^{-1}) one had no increase in lipid storage, compared to the control. The moderately swimming groups also exhibited an improved amino acid profile, while the high speed did not, and repeats the previously mentioned points of optimum swim speed. Continuous moderate exercise has been linked to increased hypertrophy in Atlantic salmon and several other strong swimming species, while no clear evidence of increased hyperplasia has been found (Bugeon et al., 2003; Ibarz et al., 2011; Li et al., 2016; Vélez et al., 2017). Signs of

increased fibre density in fast-swimming fish were found by Li et al. (2016), although this density increase is likely the effect of reduced growth rather than increased hyperplasia.

Inherent swimming performance among juvenile Atlantic salmon was found to significantly improve disease resistance, while exercising the same fish had no significant impact. A tendency towards improved survivability was observed for sustained medium intensity training of the fish who were poor swimmers to begin with, indicating a potential to improve health and disease resistance in some fish (Castro et al., 2013). The same team previously observed improved resistance to IPN for salmon smolts undergoing interval training, compared to fish undergoing continuous medium intensity or no training (Castro et al., 2011). Increased swimming efficiency, osmoregulatory ability and oxygen transportation was observed in exercised chinook salmon (*Oncorhynchus tshawytscha*), improving its ability to cope with external stressors (Gallaugher, Thorarensen, Kiessling, & Farrell, 2001). Enhanced bone formation has also observed in exercised Atlantic salmon by Ytteborg et al. (2013). These studies indicate the potential importance of the type and intensity of training, and its influence on overall health and performance in fish.

Active swimmers need to move as part of their natural behaviour, and enabling them to do so appears to be beneficial for their mental as well as physical health. Exercised groups of Altantic salmon and Arctic charr (*Salvelinus alpinus*) showed significantly decreased fin damage and bite marks when forced to swim rather than remain in static water (Jobling et al., 1993). These findings were corroborated by Solstorm et al. (2016) who also observed reduced aggression and interactions between fish in exercised groups. This reduced aggression could improve both product quality (visual) and fish health by avoiding infection of wounds, a view also shared by Huntingford and Kadri (2014).

The aim of the study was to investigate if additional applied water velocity could stimulate growth and quality improvements in salmon reared in commercial closed farms.

Materials and methods

Experiment

Atlantic salmon (*Salmo salar*, AquaGen) from Grytåga settefisk, 8860 Tjøtta, Norway. Fish were transferred to seawater in October 2016, and until January 2017 the fish were reared in commercial scale closed cages (6000 m^3 volume). Once deemed acclimated to seawater; salmon with an average weight of 300 g were transferred from large closed cages to smaller (40 m^3) closed cages (30-31.01.17). Six small cages were divided into two groups; high-water speed (HI, 18-20 cms^{-1}) and low water speed (LO, 6-10 cms^{-1}). To each cage were introduced 1200 salmon post-smolt (7200 total). Fish were moved by pumping after gathering close to surface with purse seine. Current speed was measured with two SD6000 current sensors (Nortek AS) mounted at 1 and 2 meters depth (see Figure 5). Mean oxygen saturation was regulated to 80 to 95 % in all cages, and each cage is set up with a control cabinet where data-collecting FDO 700 IQ SW, WTW/Xylem monitoring temperature and dissolved oxygen. All six net cages were left for three weeks at LO to acclimatise before increased speed was introduced to the HI group on the 24th of February. The water exchange was 250-275 L/min for each cage. The fish were fed to satiation every day with Intro 200 HH 50 mg Q 5 mm (Biomar AS, Myre, Norway), using Betten feeders S1-125 automatic feeding system (Betten Maskinstasjon AS, Vågland, Norway). Stocking density was between 10 kg/m^3 and 13 kg/m^3. Each cage was lit by an external light mounted on the floating ring supporting the tarpaulin bags (LED 2x50W 230V IP65, Etman Distribusjon AS, Egersund, Norway).

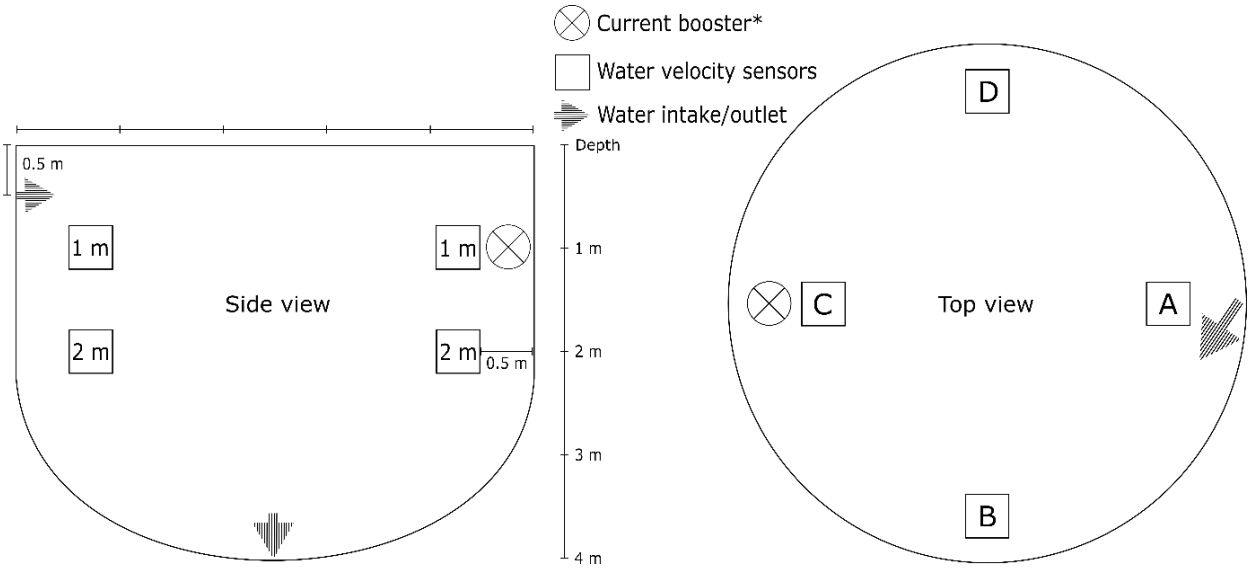

Figure 5: Position of water current sensors in pens.

Sampling procedures

The experiment lasted from 24.02.17 to 19.04.17, a total of 8 weeks. Fish were measured for weight and length at the start (22-23.02.17) and at the end of the experiment (19-20.04.17). A total of 150 fish from each net pen were gathered in the upper water levels by a purse seine, removed from net-pens by a dip net and anaesthetised with benzocaine (Benzoac 200 mg/ml, ACD Pharmaceuticals AS) 0.2-0.25 ml/L in a 50 L tank with seawater before weighing (±1,0 g) and measuring of fork length (± 0,5 cm). Of the 150 fish sampled from each net pen, 20 were sacrificed by a blow to the head, followed by cutting of gill arcs and bled in seawater before analyses of biometric data, chemical content and muscle structure, while the rest were returned to their cage.

Ten fish per net pen were kept as whole and packed in groups of 5 in Styrofoam boxes with ice for later analysis of chemical content in whole fish. Ten fish per cage were bled in seawater, gutted, and individually marked before removal of heart and liver. Gutted fish, heart and liver weight were measured (± 0.5 g) for calculation of hepatosomatic index (HSI) and relative ventricular mass (RVM). Five of these gutted fish from each cage had a 5 mm thick cross-sectional slice taken directly behind the dorsal fin of the fish and three blocks of 5 x 5 x 5 mm for histological analysis were collected from this (see Figure 6). Blocks were fixated with Shandon™ Cryomatrix™ (Thermo Fisher Scientific, MA, USA) and frozen in isopentane cooled to melting point in liquid nitrogen (- 160 °C) for 60 seconds, wrapped in aluminium foil and stored at -80 °C until preparation. The fish were individually packed in Styrofoam boxes with ice for later analysis of chemical content in fillet. All fish and liver samples were transported to Nord University, Bodø, where they were frozen at -40 °C.

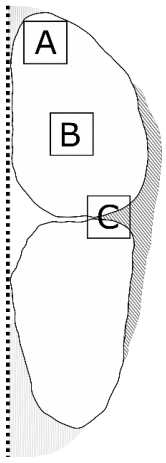

Figure 6: Sampling location of histological blocks from cross-sectional cut.

Proximate composition

Fish were later thawed for analysis of water, lipid and protein content. The whole fish were homogenised in groups of 5 and re-frozen, making two groups per net pen. The other fish were filleted and skinned before individual homogenisation. Homogenised samples were packed individually in sealed bags and re-frozen at - 40 °C. All analysis were done in parallels.

Water

Water content was measured by weighing out 5.0 g (± 0.5) of homogenised sample into pre-weighed aluminium vessels and dried in an oven at 105 °C for 20 hours. The vessel with dried sample was cooled to room temperature in a desiccator for 20 minutes, and weighed before lost liquid was calculated by weight lost from sample [(Wet sample-dried sample)/Wet sample].

Fat

Fat content was measured by the Norwegian standard procedure (NS-9402E, 1994). 10.0 g (± 0.5) of sample was weighted out, dried with 20 (±0.5) g of Na_2SO_4, followed by extraction while shaking with ethyl acetate for 60 minutes. The extract was filtered (using grade 41 filter paper, Whatman, UK) gravimetrically and filtrate (20.00 mL) was transferred to a pre-weighted vessel. The filtrate was evaporated over boiling water bath for 20 minutes, followed by drying for 20 minutes in an oven at 105 °C. The vessel with dried filtrate was cooled to room temperature in a desiccator for 20 minutes, and weighted before crude lipid content was calculated [Dried filtrate/Wet sample]. Liver samples were combined in groups of 5, as with whole fish, homogenised by hand before a 5.0 g (± 0.5) sample was analysed by the same procedure as above.

Protein

Protein content was measured using Kjeldahl standard procedure (N x 6.25). 1.00 g of sample was weighed into a nitrogen-free disposable vessel. To each sample was added 2 Kjeltabs (Cu) and H_2SO_4 (15.0 ml, conc.). These were heated to 420 °C for 60 minutes, cooled for 30 minutes in the heating manifold and another 30 minutes in room temperature until cooled (60 minutes total). Distilled water was added (75.0 mL) before crude protein was measured by using a Kjeltec™ 8400 (FOSS, Denmark).

NIR

Proximate composition of fillet homogenates was measured by Near Infrared Spectroscopy using a DA 7200 Diode Array High Speed analyser (Perten Instruments AB, Hägersten, Sweden), operating in the wavelength range 950-1650 nm. A representative selection of 28, 30 and 18 fish from the April sampling was used for calibration of NIR data to chemical data of water (six factors, R^2=0.984), fat (five factors, R^2=0.978) and protein (nine factors, R^2=0.976) respectively, using PLS regression in the Unscrambler® X software (version 10.4, CAMO Software AS, Oslo, Norway). The three separate PLS models was configured with full cross-validation and SD^{-1} weighting of the Y-variable, further used for prediction of individual content of water, fat and protein of all 120 fish, according to a previously described method (Solberg, 1992, 1997).

Muscle fibre growth

Samples were acclimated in cryostat (CryoStar NX50, Thermo Fisher Scientific, MA, USA) for 20 minutes before slicing with a thickness of 8 µm at -23 °C. Slides were stained with hematoxylin solution (Papanicolaou's solution 1a Harris' hematoxylin solution, Merck, Germany) for 8 minutes and afterwards flushed with gently running water for 8 minutes before covering with glycerol and a covering glass.

Slides were examined with light microscopy (Axioskop 2 mot plus, Zeiss, Germany) and photographed with a digital camera (Axiocam HRc, Zeiss, Germany) mounted directly on the microscope with 10x magnification. Using Axiovision 4.8.2/4.9.1 (Zeiss, Germany) the circumference of a minimum of 450 fast cells were measured for each fish, and diameter, fibre area was and fibre density calculated. Fibres with a diameter < 20 µm were viewed as an indication of hyperplasia (Johnston et al., 2000a).

Statistical analysis

All statistical analysis was performed with R (3.3.1). Data are presented graphically as median within first and third quartile, or as means ± standard deviation (SD) in Appendix A. All data were checked for normality by the Shapiro-Wilk test and homogeneity of variance by an F-test. Effect of water velocity on growth performance, proximate composition and biometric parameters for sampled fish were analysed using one-way ANOVA. A nonparametric Mann-Whitney U test was used when the statistical assumption were not met. In addition, a general linear model (GLM) for ANCOVA was used to investigate the effect of

water velocity on lipid content, with gutted weight as covariate and water velocity as fixed factor (Johnsen et al., 2011).

Distribution of muscle fibre diameter was evaluated using smooth nonparametric distributions where 450 measurements of fast fibre diameter were fitted using a kernel function (Bowman & Azzalini, 1997) as applied to muscle fibre diameters by Johnston, Strugnell, McCracken, and Johnstone (1999). Groups compared had a similar body mass and length (n = 13 HI, n = 9 LO). Fish were selected by removing any fish outside the standard deviation area around the median length, and any fish with extremely altered muscle fibre composition compared to the rest. A Kolmogorov–Smirnov two-sample test was used to test the null hypothesis that the probability density functions (PDFs) of groups were equal over all diameters. Density curves for each treatment were also compared graphically by constructing a variability band around the density estimate for the combined populations using the mean smoothing parameter h, varying between 0.17 and 0.19 for the different groups. This can be used to distinguish underlying structure in the distributions from random variation providing an indicator of which part(s) of the distribution of diameters contributed to any significant differences. The same analysis was performed with 150 red/slow fibres for the April sampling as well.

Results

All plots present data with the median as a solid black line, and the first and third quartiles as coloured area (aka. Interquartile range or IQR for short). The whiskers are all values within 1.5 × IQR, and dots indicate outlying points outside this range (McGill, Tukey, & Larsen, 1978).

Mortality

Mortality was recorded to 2.3 % (HI) and 1.9 % (LO), with the primary cause of death (91 %) being ulcers and fin damages. The main cause of these injuries was the netting and pumping of fish into the research cages.

Growth

Mean values with standard deviation can be found in Table A - 1, Appendix A. Both groups were similar at the beginning in both weight and length ($p > 0.39$). The salmon in HI-group grew significantly faster than the LO-group, both in weight and in length ($p < 0.01$). HI increased from a mean weight of 337 g to 485 g, while LO increased from 330 g to 430 g. In length, HI increased from 30.0 cm to 33.8 cm, while LO increased from 29.9 cm to 33.0 cm. HI ended up 12.8 % heavier and 2.2 % longer with an SGR of 0.67 (HI) vs 0.49 (LO), and a TGC of 2.71 (HI) vs 1.92 (LO). Condition factor in LO decreased from 1.20 to 1.14, a 4.5 % decrease ($p < 0.01$). HI increased from 1.18 to 1.19, or 1.0 % ($p < 0.05$). The HI-group ended with a 4.4 % higher condition factor ($p < 0.01$) than LO during the course of the experiment (Figure 7). HI displayed greater variance in length ($p < 0.05$) and weight ($p < 0.01$) than LO.

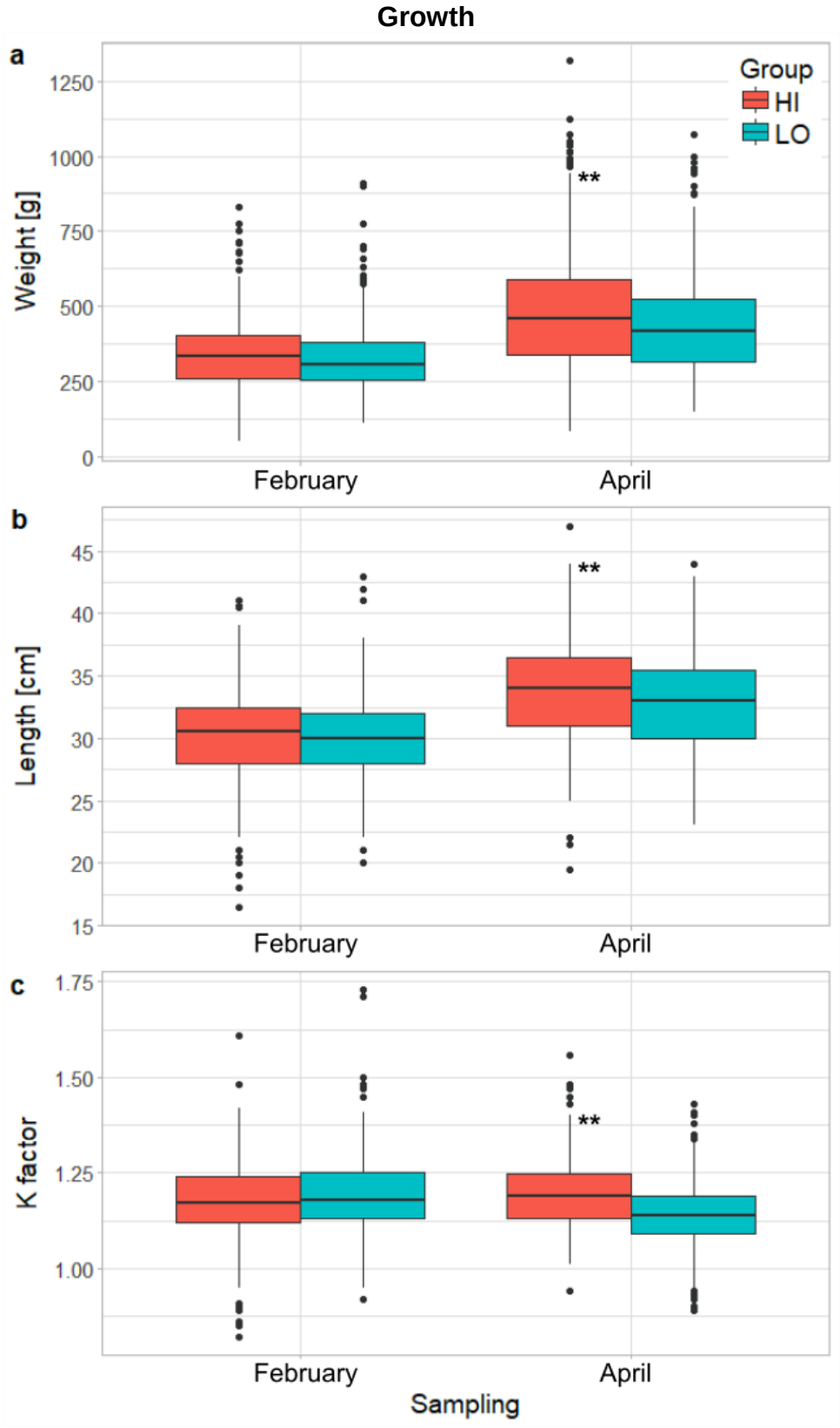

Figure 7: Weight (a), length (b) and condition factor (c) for the HI-speed LO-speed group.
**** indicates significant difference (p < 0.01)**

RVM was compared in both samplings and there was no difference between groups in either instance (Figure 8), nor did the RVM change over the course of the experiment in either group ($p > 0.30$). HSI was not significantly different between groups in either sampling ($p > 0.14$), and while there was no significant change in HI, the LO-group had an 11.0 % decrease ($p < 0.01$). Mean values and SD can be seen in Table A - 1, Appendix A.

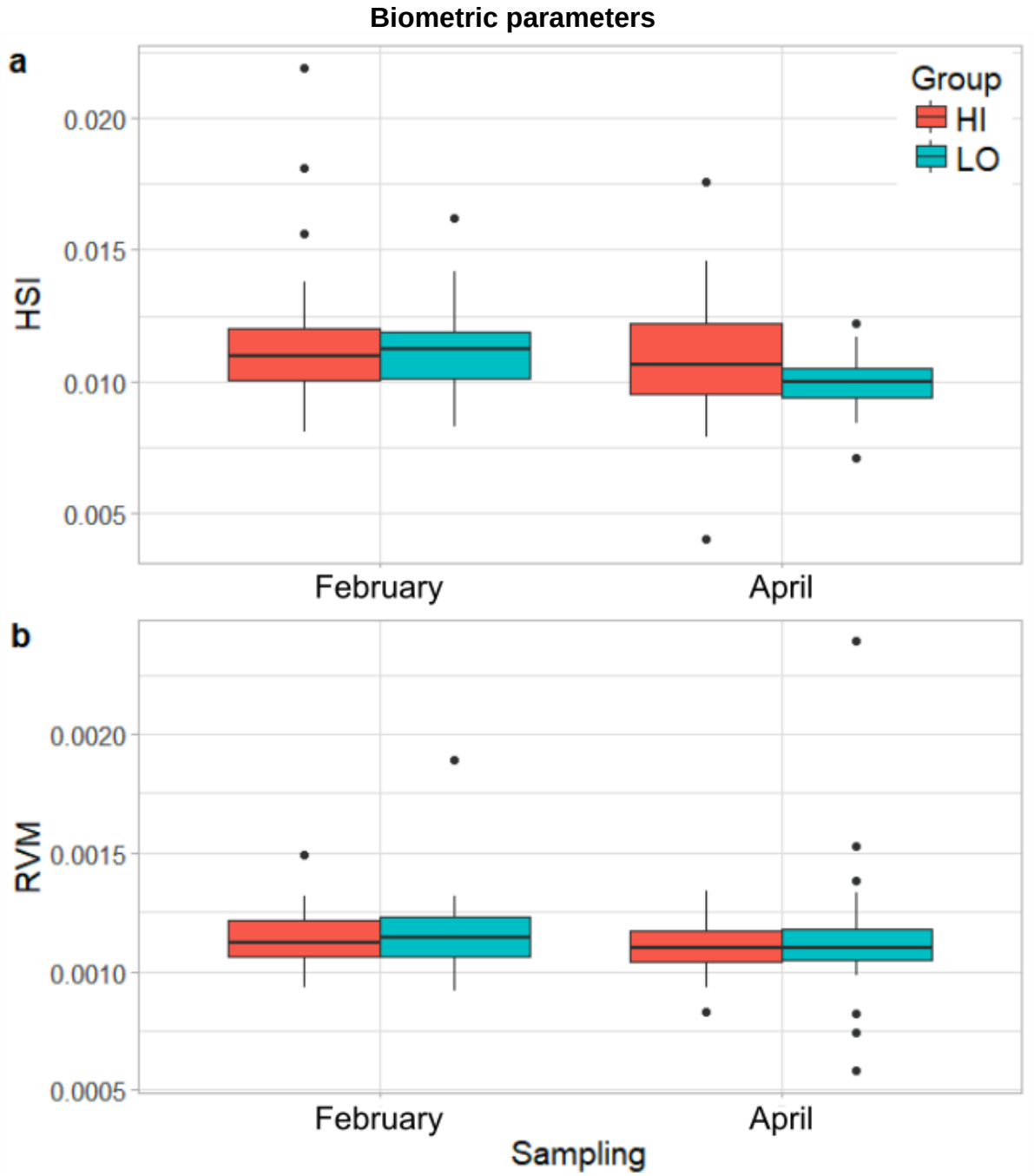

Figure 8: Hepatosomatic index (HSI) (a) and relative ventricular mass (RVM) (b) for the HI- and LO-speed groups.

Proximate analysis

Flesh

Mean values and SD can be seen in Table A - 1, Appendix A. Lipid content was non-significantly higher in the HI-group compared to LO (11.26 % vs 10. 57 %) at the start ($p = 0.068$), and this difference increased significantly as lipid content dropped more in LO than HI (9.37 % vs 10.43 %) (Figure 9) over the course of the experiment ($p < 0.05$). Correlation between lipid content, size and speed was investigated (Figure 10), finding no significant correlation between speed and lipid content ($p = 0.21$), while size showed a positive correlation ($p < 0.01$). Water content increased in both groups, but LO had significantly higher levels (69.58 % vs 68.55 %) at the end ($p < 0.05$). Protein content was non-significantly higher at the start in LO (19.27 % vs 18.98 % respectively), but became significantly higher (20.48 % in LO vs 20.30 % in HI) as the experiment progressed ($p < 0.01$).

Whole fish

Lipid levels in whole fish were 13.99 % (LO) and 14.42 % (HI) at the start, and 14.68 % and 14.32 % respectively at the end. Protein content remained virtually unchanged at 17.7 – 17.5 % for both groups. There was no significant difference between lipid and protein levels between the groups (Figure 11), but water content was significantly higher in the LO-group (66.30 % in LO vs 65.80 % in HI) both in the beginning ($p < 0.05$) and the end (66.36 % vs 65.67 %) of the trial ($p < 0.01$). Mean values and SD can be seen in Table A - 2, Appendix A.

Liver

Lipid content in liver was only measured at the end of the experiment, and was not significantly different ($p = 0.42$), 6.08 % (LO) and 6.53 % (HI) respectively. Mean values and SD can be seen in Table A - 3, Appendix A.

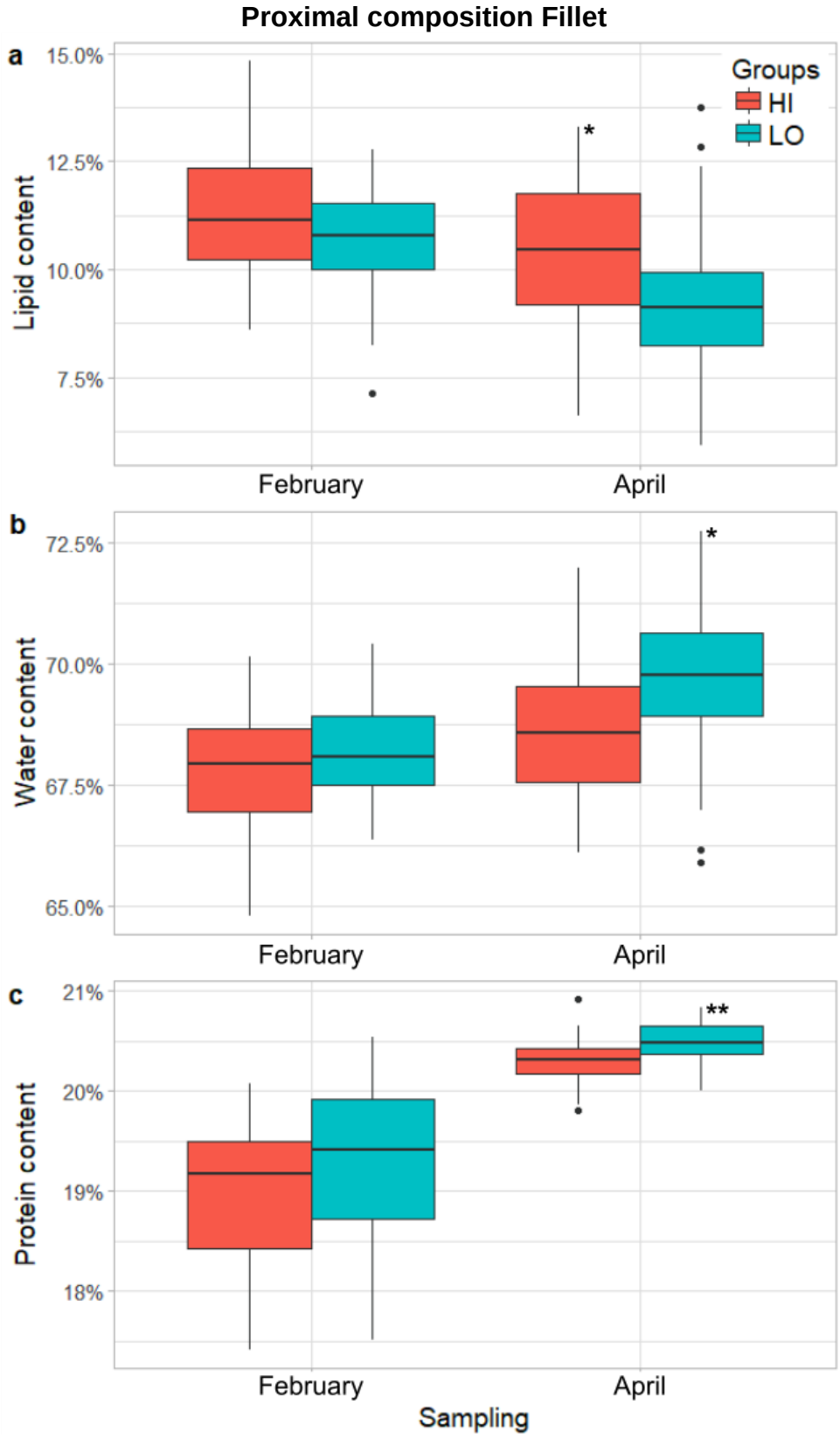

Figure 9: Lipid (a), water (b) and protein content (c) of salmon fillet for the HI- and LO-speed groups.
**** indicates significant difference ($p < 0.01$), * for ($p < 0.05$).**

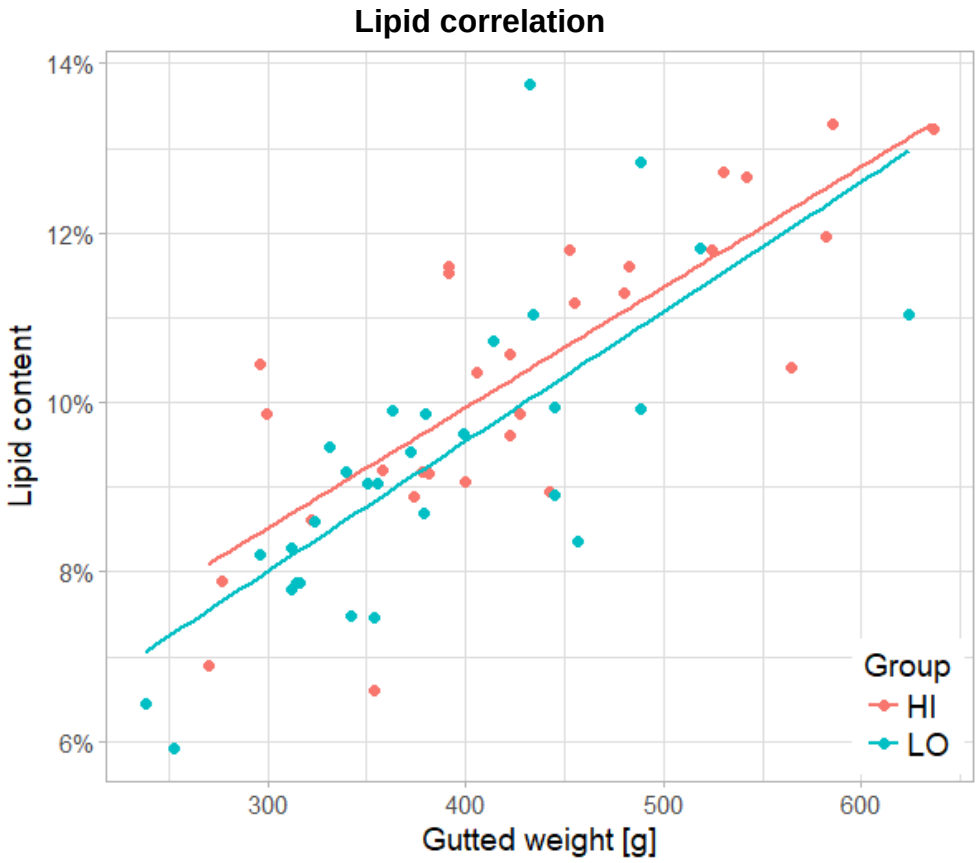

Figure 10: Relation between lipid in flesh and gutted weight for the HI- and LO-speed groups during April sampling. Lines show linear regression of the model. R^2 = 0.60 for the linear model.

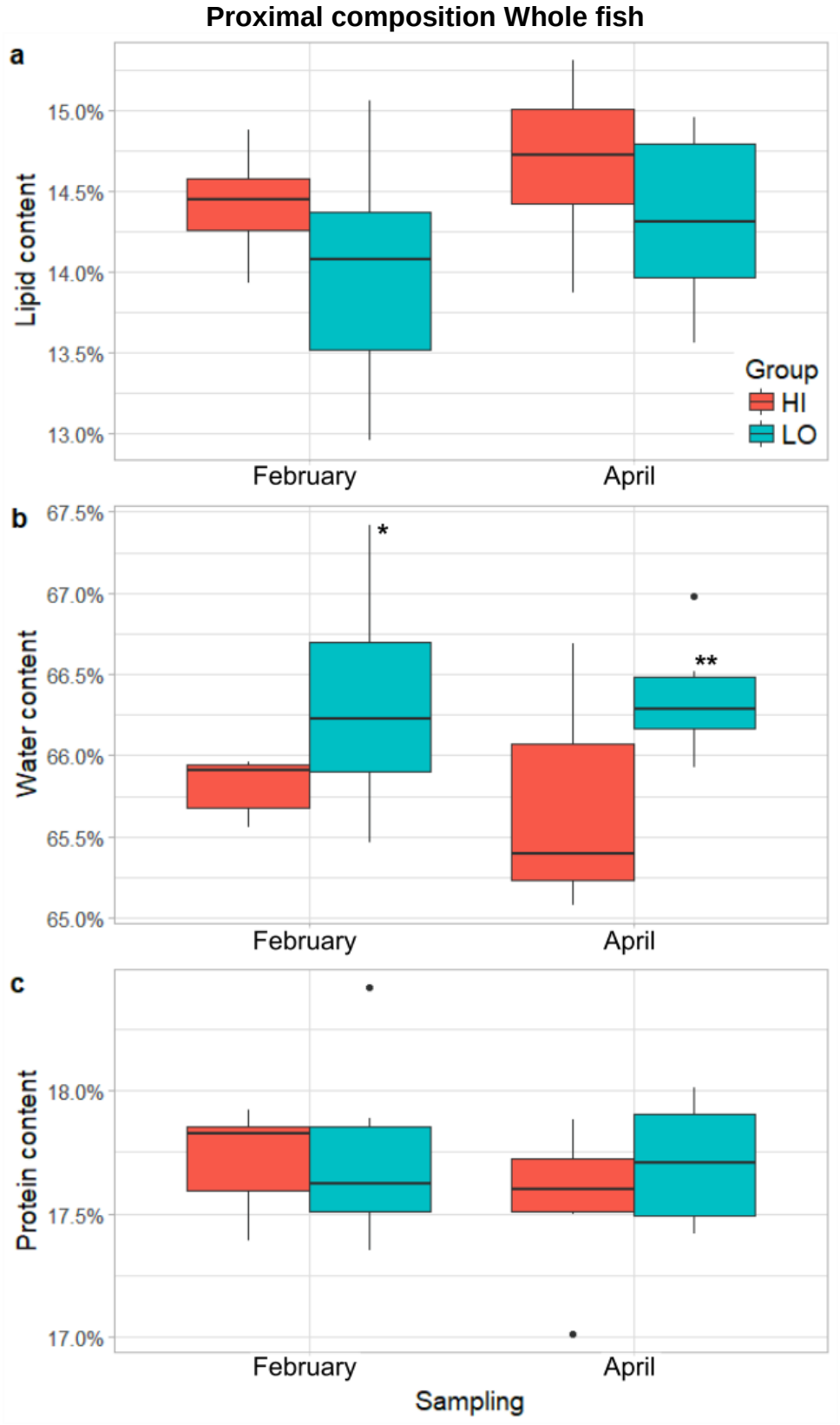

Figure 11: Lipid (a), water (b) and protein content (c) of whole salmon for the HI- and LO-speed groups.
**** indicates significant difference ($p < 0.01$), * for ($p < 0.05$).**

Muscle fibre growth

White muscle fibre diameter distribution was significantly different between HI- and LO-groups at the end of the trial according to a Kruskal-Wallis test ($p < 0.01$). This despite no deviation from the probability density area observable in the plot (Figure 12). No significant difference was observed at the start between groups ($p = 0.87$). Difference in both fibre frequency ($p = 0.11$) and percent fibre frequency ($p = 0.15$) in the < 20 µm diameter category was non-significant between groups (Table A - 4, Appendix A). No difference was found in red muscle fibre distribution ($p = 0.52$) in April sampling (Figure B - 1, Appendix B).

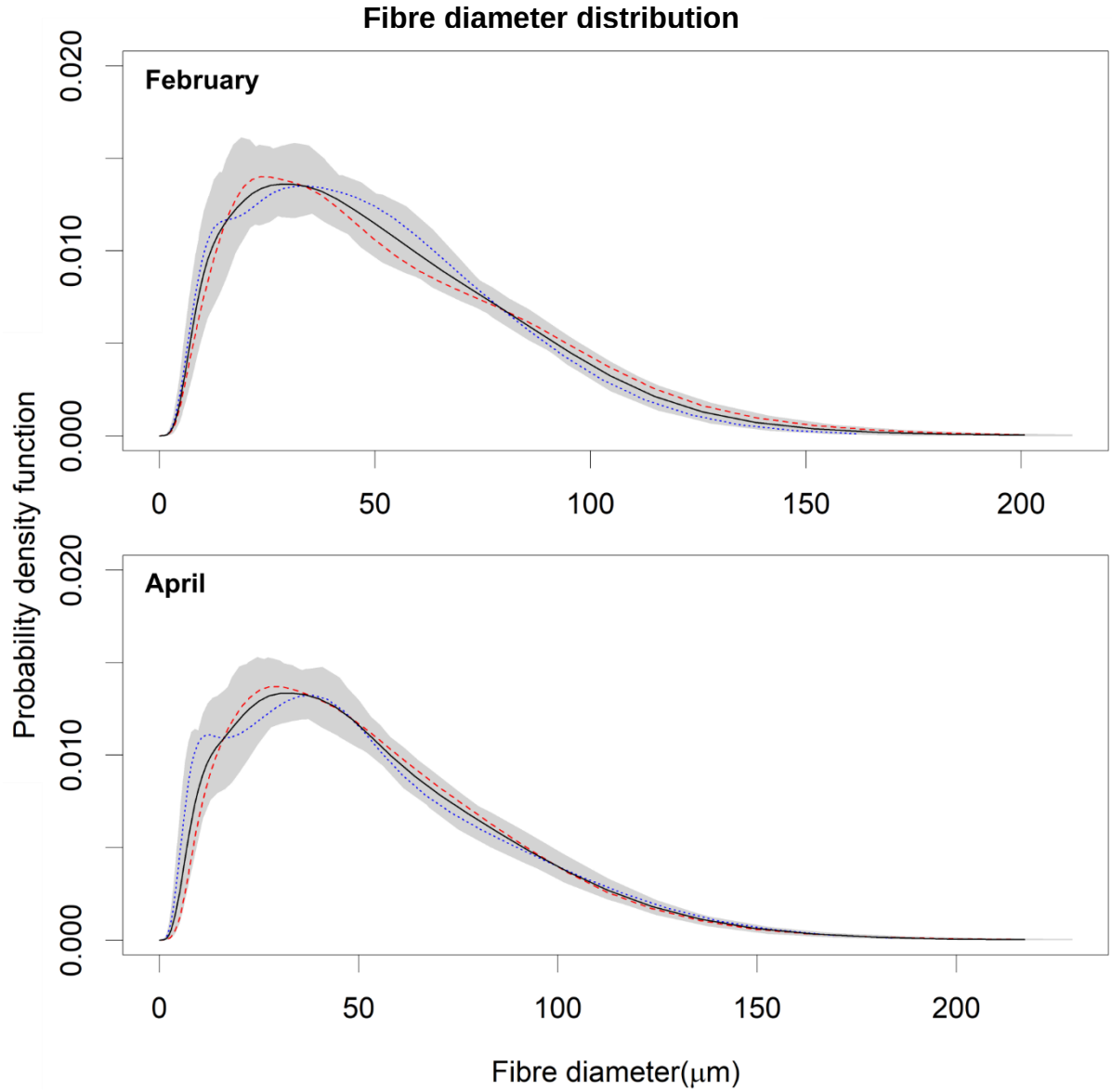

Figure 12: Probability density distribution of white muscle fibre diameters for HI- (Red, dashed) and LO-speed groups (Blue, dotted) in February and April sampling.

Mean values and SD shown in Table A - 4, Appendix A. Fibre density (fibres/mm^2) started out lower in HI ($p = 0.052$), but evened out towards the end ($p = 0.83$) as it dropped significantly in LO ($p < 0.05$, Figure 13). Mean fibre diameter remained steady at 54.4 – 54.7 µm for the HI-group ($p = 0.88$), while increasing (non-significantly) from 50.2 µm to 53.7 µm the LO-group ($p = 0.059$), correlating with decreasing fibre density. Fibre diameters were not significantly different between groups in neither start nor end ($p > 0.24$)

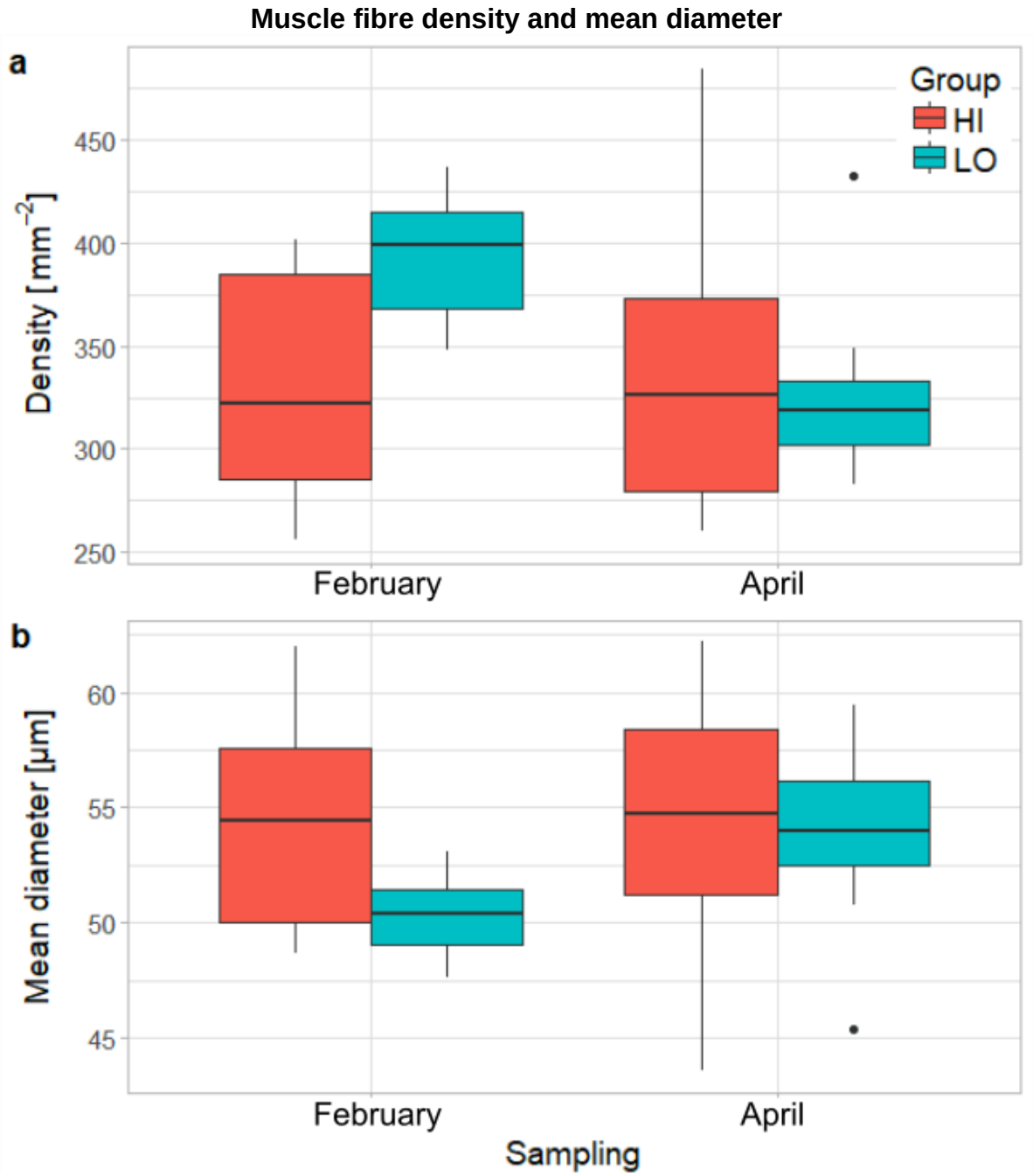

Figure 13: Mean white muscle fibre density and fibre diameter for the HI- and LO-speed groups.

Discussion

Growth and proximate composition

As the experiment started the measured water velocity in the pens were approximately 0.6 – 0.67 BLs^{-1} (HI) and 0.2 – 0.33 BLs^{-1} (LO) based on mean length, which is low to moderate intensity when compared to other studies (Castro et al., 2011; Li et al., 2016; Solstorm et al., 2016), but still enough to show significant effects. At the end of the experiment these velocities had dropped to 0.53 – 0.59 $BLIs^{-1}$ (HI) and 0.18-0.3 BLs^{-1} as the fish had grown and velocity remained constant. The findings of a growth increase above 12 % for such low speeds are of industrial interest as even simple and cost-effective measures to apply a slightly higher water velocity in closed containment systems can yield significantly improved production rates and results. Swimming at elevated water velocities have shown increased feed intake and feed conversion efficiency in salmon (Davison, 1997; Jobling et al., 1993; Jørgensen & Jobling, 1993). Skov, Lund, and Pargana (2015) found no bioenergetic advantage in swimming trout fed restrictively suggesting that increased feed intake could be the primary reason of the enhanced growth, or that the fish at least needs its increased metabolic demands to be met. Studies summarised by Davison (1997) still indicate improved feed conversion efficiency, meaning a better utilisation of feed for the producer and more cost-effective production. The potential for aquaculture production is large, as even a 10 % increase in production mass could lead to significant economic gains, despite a partial offset in increased feed costs. The Nasdaq Salmon index for the Norwegian export market has varied between 45 – 75 kr/kg in 2017 (NASDAQ, 2018), meaning a 10 % production increase on a biomass of 200 tonnes could yield 1 – 1.5 million kr increase in production value (not accounting for increased feed cost). Less aggressive interactions and injuries is positive for fish welfare, health and possibly mortality in the long term, which carry both ethical and economic implications for the producer.

A higher variation in weight was observed in HI compared to LO in April. A possible explanation is that fish of small size at the beginning of the experiment experienced higher relative water velocity than the average fish, and thus may have experienced speeds higher than their optimum swim speed, thus reducing growth (Li et al., 2016; Solstorm et al., 2015). It is also possible that small fish were of poor health to begin with and therefore were never able to grow much, regardless. Jobling et al. (1993) observed a more uniform feed intake and growth in salmon exposed to increased water velocity, which is conflicting with our observations.

SGR for both groups is low compared to the projected 1 % daily growth (SGR ≈ 1.0) for farmed salmon in the 300 – 400 g range, with a mean temperature of 7 °C (Skretting, 2012). The fish were transferred from large commercial cages to small research cages, and the environmental change in itself might have led to suppressed growth, which might help explain the lower growth rates compared to commercial production tables. TGC is more comparable to previously observed production levels for closed systems for the HI group, while LO is far below expected (Thorarensen & Farrell, 2011), suggesting that a certain level of speed may be required to maintain current growth projections in the industry.

In this study there was no difference in HSI and RVM, contrary to findings by Solstorm et al. (2015) where RVM increased in high-speed groups, albeit much higher speed than this experiment. Castro et al. (2011) found no change in RVM as a result of exercise with similar speeds as Solstorm et al. (2015). It is possible that this trial did not go on for long enough to observe any differences here, or that the water velocity was insufficient to promote this morphological change. Exercise of this intensity appears not to influence relative heart size, but may yield improved circulation or aerobic metabolism as Gallaugher et al. (2001) observed in Chinook salmon.

Even though protein content was statistically significantly higher in LO, the actual difference is so small (0.18 % units) that its nutritional significance can be called into question. Houlihan and Laurent (1987) observed increased protein synthesis efficiency in exercised Rainbow trout (*Oncorhynchus mykiss*), but this lead only to improved growth and not any significant change in relative protein content, just as observed in this trial. Lipid levels were different between groups, but ANCOVA uncovered that the reason was solely larger fish in HI and not the treatment itself. This corroborates the findings of Johnsen et al. (2011) who found a positive correlation between growth and lipid content. Outliers were removed from the calculation, but two very large individuals (~1000 g, one from each group) had similar lipid levels to the largest ones in Figure 10, suggesting a lipid plateau of 13 ± 1 % for the current feed and feeding regime. Mean lipid levels in fillet decreased during the course of the experiment, despite increased size, implying a more complex situation than purely greater weight = higher lipid levels. Nordgarden, Ørnsrud, Hansen, and Hemre (2003) observed reduced lipid retention during early spring in Atlantic salmon, despite growth, although an increase in lipid retention occurred later in the year. This seasonal variation might explain why lipid levels decreased, despite growing fish. A different reason might be depletion of

lipid reserves for energy to maintain protein retention and muscle growth during a stressful period like after the transfer to small pens.

The observed difference at the start in water and lipid content in whole fish is harder to explain, as there is no difference in flesh, HSI or liver lipid content. There is a trend of a negative relationship between lipid and water content in whole fish. The lipid levels were approaching 13 % as the salmon grew, well within normal salmon production standards, which vary between 7.5 – 17.5 % for commercially available salmon, with a mean lipid content of 10 – 12.5 % (Henriques, Dick, Tocher, & Bell, 2014).

Histology

Fibre diameter correlates strongly with fish length (Rowlerson & Veggetti, 2001) and hyperplastic growth is continuous until it appears to cease at approximately 44 % of total growth potential (Weatherley, 1990; Weatherley, Gill, & Lobo, 1988), so to eliminate size factors only fish of similar length were chosen.

The observed difference in fibre density between HI and LO in the February sampling is curious, as the fibre diameter distribution was very similar (Figure 12) and there appeared to be no difference to speak of. No apparent difference in hyperplasia was discovered between the two groups in April as fibre frequency and fibre ratio of small (< 20 µm) fibres were similar, supporting the conclusion of the review by Vélez et al. (2017) that exercise currently has no observable effect on hyperplastic growth, only hypertrophic. There was a slightly higher mean fibre density in HI, compared to LO, but the difference was non-significant, while LO had a (non-significantly) higher proportion of small fibres. This increased ratio in LO appears to be dominated by a single individual (Figure B - 3, Appendix B) which might skew the mean frequency of small fibres.

Due to time constraints, only 450 fibres per fish were used in the bootstrapping models which is half of what Johnston et al. (2000a) used in the same analysis. Smaller fibre counts give a weaker bootstrapping model, but 450 were deemed sufficient for this experiment. It is however more exposed to sampling bias as sample size is smaller.

Experiment

Several issues arose during the course of the experiment, causing early termination of the trial. Water velocity was variable in the different cages, with especially large variance in the LO-group. One LO-cage started out at 5 cms^{-1} while another started at 12 cms^{-1}, and while changes in speed are not that great, the relative difference over 100 %. One HI-cage also

started 12 cms^{-1}, making it no different from the fastest LO cage, but speeds were adjusted accordingly as errors were discovered. HI-group was otherwise very stable in speed during the experiment. In circular systems, the water velocity is not homogenous in the entire cage as water along the edge move up to three times the speed of that in the centre (Duarte, Reig, Masaló, Blanco, & Oca, 2011). This could lead to inconsistency in results if fish prefer to stay close to the slower centre, rather than the faster edge, especially since velocity measurements were performed close to the edge (Figure 5). Small eddies and pockets of still water can occur, especially around submerged sensors and other protruding equipment if these are present.

Some fin damage and lesions were observed in April sampling, probably caused by the transfer from the commercial sized cage to the smaller research cages, and most had begun healing. Difference in mortality is low, and might not be caused by treatment effects, although the fish of weaker innate swimming ability might have experienced increased metabolic demands in the HI-group and suffered more from injuries due to potentially weaker immune systems (Castro et al., 2013).

Concluding remarks

The primary findings in this experiment is the increased growth of salmonids exposed to increased water velocities in agreement with earlier studies (Bugeon et al., 2003; Castro et al., 2011; Jobling et al., 1993; Jørgensen & Jobling, 1993; Morash et al., 2014), even over short timespans and with the moderate speed-increase possible in commercial systems. No other significant effects (proximate or cellular) were observed from the treatment. We therefore conclude that even modest applied water velocity is able to enhance growth rates in Atlantic salmon, but not influence nutritional quality of salmon fillet.

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Appendix A: Tables of results

Table A - 1: Total growth parameters, biometric parameters and proximate composition of fillet at the start (S. 1) and end (S. 2) of the experiment.

		LO		HI			
Parameter	Sampling	Mean	SD	Mean	SD	Difference	p-value
Weight [g]	1	330	±121	337	±127	7	0.501
	2	430	±161	485	±198	55	< 0.01**
Length [cm]	1	29.9	±3.6	30.1	±4.1	0.2	0.388
	2	33.0	±3.9	33.8	±4.3	0.8	< 0.01 **
C. factor	1	1.20	±0.11	1.18	±0.13	0.02	0.153
	2	1.14	±0.082	1.19	±0.085	0.05	< 0.01 **
HSI [%]	1	1.12	±0.170	1.16	±0.279	0.04	0.988
	2	0.996	±0.114	1.08	±0.248	0.08	0.146
RVM [%]	1	0.117	±0.0172	0.113	±0.0126	0.004	0.446
	2	0.114	±0.0321	0.110	±0.0120	0.004	0.630
Lipid [%]	1	10.57	±1.30	11.26	±1.58	0.69	0.0684
	2	9.37	±1.81	10.43	±1.78	1.06	0.0252 *
Water [%]	1	68.21	±1.00	67.77	±1.32	0.44	0.156
	2	69.58	±1.62	68.55	±1.45	1.03	0.0121 *
Protein [%]	1	19.27	±0.87	18.98	±0.77	0.29	0.178
	2	20.48	±0.22	20.30	±0.23	0.18	< 0.01 **

Table A - 2: Proximate composition of whole fish at the start (S. 1) and end (S. 2) of the experiment.

		LO		HI			
Parameter	Sampling	Mean	SD	Mean	SD	Difference	p-value
Lipid [%]	1	13.99	± 0.73	14.42	± 0.33	0.43	0.0767
	2	14.68	± 0.51	14.32	± 0.55	0.36	0.114
Water [%]	1	66.33	± 0.64	65.80	± 0.19	0.53	0.0148*
	2	66.36	± 0.39	65.67	± 0.68	0.59	< 0.01 **
Protein [%]	1	17.74	± 0.37	17.72	± 0.21	0.02	0.925
	2	17.71	± 0.27	17.55	± 0.31	0.16	0.443

Table A - 3: Lipid content in liver at the end (S. 2) of the experiment.

		LO		HI			
Parameter	Sampling	Mean	SD	Mean	SD	Difference	p-value
Lipid [%]	1	-	-	-	-	-	-
	2	6.08	±1.30	6.53	±1.12	0.45	0.416

Table A - 4: Fibre diameter, fibre density and frequency of small muscle fibres at the start (S. 1) and end (S. 2) of the experiment.

Parameter	Sampling	LO		HI		Difference	p-value
		Mean	SD	Mean	SD		
Fibre diameter [µm]	1	50.22	±26.93	54.71	±31.94	4.49	0.240
	2	53.71	±32.37	54.36	±31.10	0.65	0.840
Fibre density [mm^{-2}]	1	393.35	±34.50	329.95	±61.40	63.4	0.052
	2	328.86	±46.27	334.82	±68.17	5.96	0.834
% fibres < 20 µm	1	-	-	-	-	-	-
	2	15.01	±4.72	11.61	±4.84	3.40	0.15

Appendix B: Additional fibre distribution plots

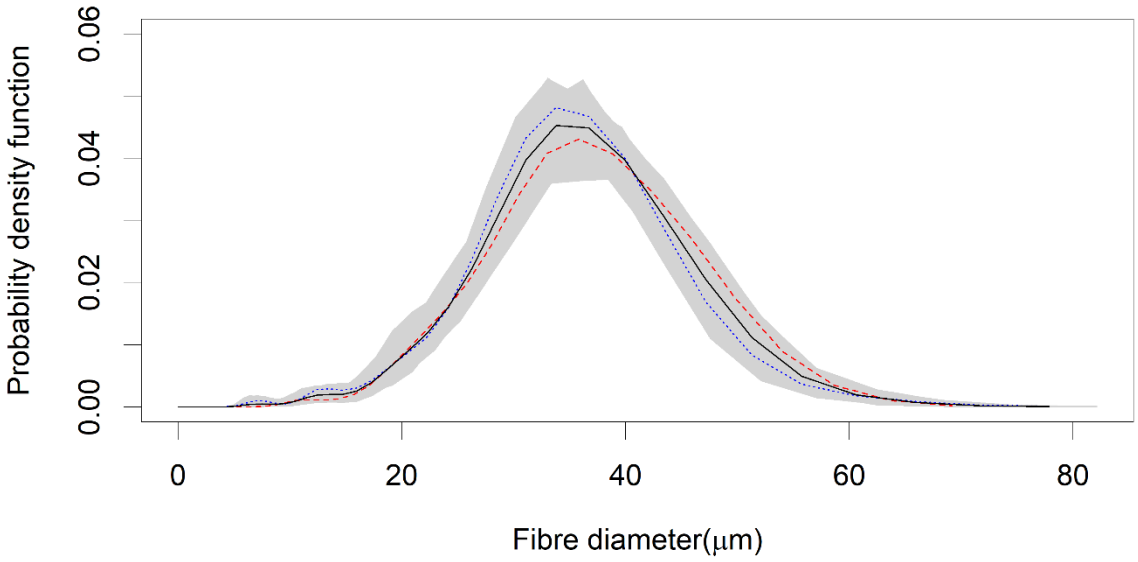

Figure B - 1: Red muscle fibre diameter distribution with probability density distribution. Mean (black, solid), HI (red, dashed) and LO (blue, dotted).

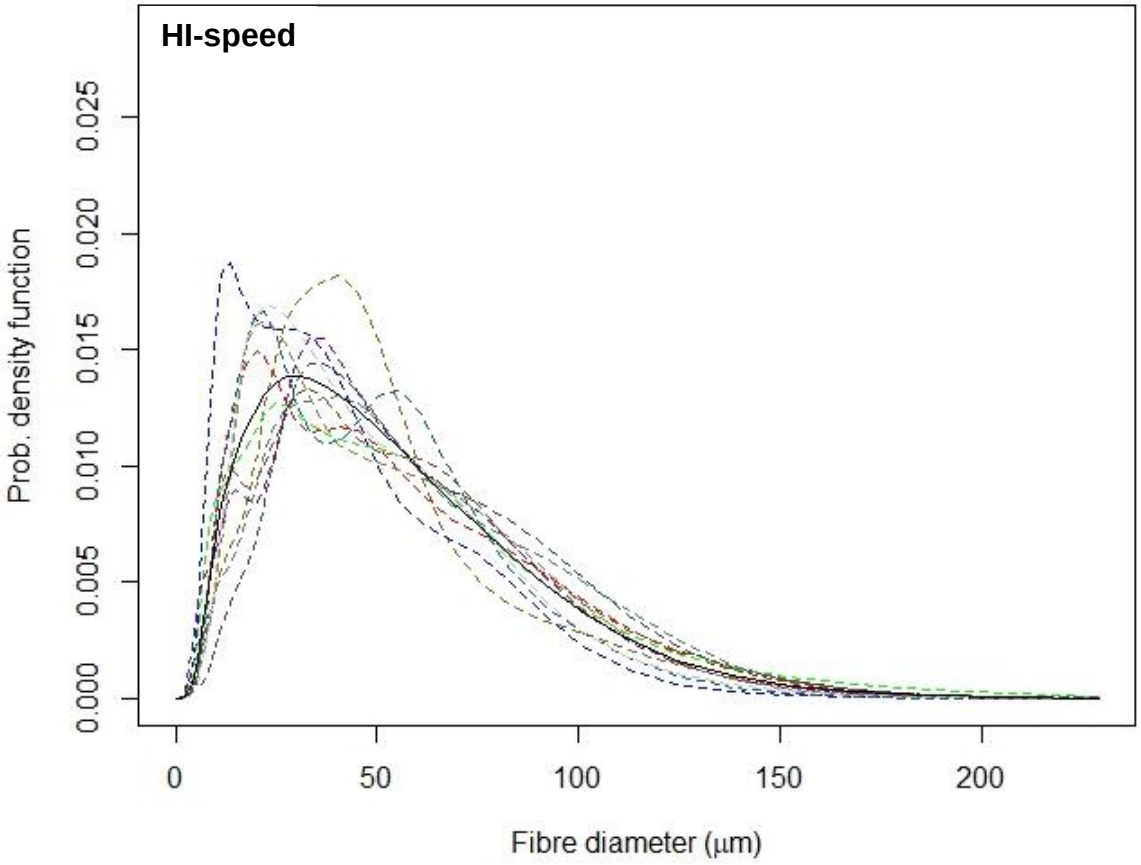

Figure B - 2: Fibre diameter distribution of individual fish in HI-group during April sampling. Dashed lines signifies individuals, while the solid line is the mean.

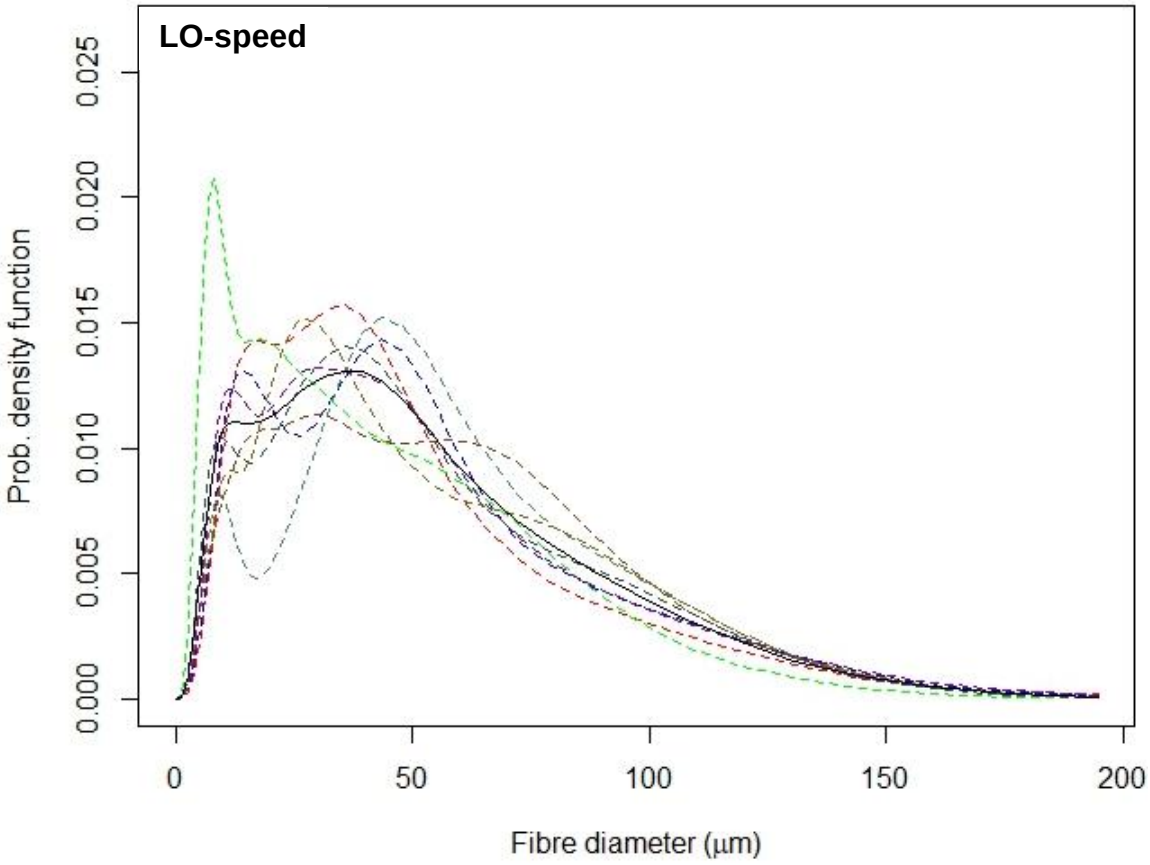

Figure B - 3: Fibre diameter distribution of individual fish in LO-group during April sampling. Dashed lines signifies individuals, while the solid line is the mean.