Intermittent fasting in ongrowing Atlantic salmon (*Salmo salar*) reared in sea cages: Effects on feed intake, appetite control and growth

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Thesis submitted in fulfilment of the requirements for the degree in Master of Master of Aquaculture and Seafood

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University of Bergen

November 2023

Acknowledgement

I would like to give a great thank you to my supervisor Professor Ivar Rønnestad, and co-supervisors Drs. Ole Folkedal and Floriana Lai for good follow-up throughout the master thesis period.

First of all, thank you Ole Folkedal for wanting to be my supervisor and suggest this topic/project for my master thesis. Further, I want to thank you for teaching me how to feed the salmon using an automatic feeding system, how to control the appetite and feed intake through cameras and echo sounder in the net pens, as well as general salmon behaviour. It has been very valuable to learn it myself for experience and further understanding. Throughout the period Ole has given detailed feedback and supervision on my writing and helped me to narrow it down to a focused master thesis, as well as always being available for questions and help. This has been very appreciated.

Secondly, I would like to give a big thank you to Floriana Lai for a good follow-up throughout the whole period and always being available. Floriana has also taught me how to collect the physiological samples during the experimental trial, as well as the different laboratory procedures and analyses in GraphPad Prism. I will deeply thank you both, Ole and Floriana for having patience with me and always being available when I needed assistance.

Then, a big thank you to Ivar Rønnestad for giving good feedback on important physiological topics that needed to be included in the thesis to support the experimental trial and discussions of the physiology.

This thesis was completed at the Institute of Marine Research (IMR) and at the Department of Biological Sciences (BIO) at the University in Bergen. The thesis was a part of the ongoing research project NoFood2Waste (NF2W; project no No. 317770), funded by the Research Council of Norway. Thanks to all at Solheim Cage Environment laboratory at the Matre Research Station, IMR, for daily operation at the facility, providing with necessary equipment and help during samplings. Also, a big thank you to the people at the BIO department at University of Bergen for helping with physiological samples.

Thanks to family for proofreading and general support.

Bergen

November 2023

Abstract

Farmed Atlantic salmon are normally fed at a daily basis and fasted before operational procedures and harvest. While the biological mechanisms and effects of short and long-term fasting are largely known, there is sparse information of how salmon respond to intermittent fasting. Less frequent meals are considered a measure to save feed under e.g., periods with food shortage or flesh quality improvement. This study investigates how large Atlantic salmon reared in sea cages adapt from being fed to satiation every day to restricted feeding with feeding to satiation every third day in an intermittent fasting regime (lasting for 43 days) followed by a period of refeeding (37 days with feeding to satiation every day). The experiment was conducted during spring 2023 and in research scale sea cages (12x12 m) at natural light and ~10 °C; ~33 ppt. Duplicate cage groups were either the intermittent fasting regime or the daily feeding (control). The response was measured at fish group level as daily appetite and periodic growth at cage level. In addition, individual fish was periodically sampled 7 hours after onset of the meal for gut fullness index and appetite assessment, and physiological indicators related to appetite control and energy storage (hepatosomatic- and gonadosomatic index).

During the first 30 days of intermittent fasting there was a progressive increase in feed intake and the total fed amount for the fasting period was estimated to 65% of the quantity fed in the control. Growth rate and biological feed conversion rate was respectively 52% and 141% of control levels, supporting that the intermitted fasted fish used a higher proportion of their feed intake on bodily maintenance. That was also showed by the fact that intermittently fasted group had 11% lower hepatosomatic index than the control after 43 days of intermittent fasting. During refeeding the fed amount for the previously fasted fish was ~120% of control level the initial 7 days and then remained elevated at ~110% for the subsequent 30 days of the study. The stomach fullness index increased in line with the fed amount during the intermittent fasting period and decreased during refeeding period, indicating adaption to the different feeding regimes. The gonadosomatic index showed early sign of maturation, however no difference was found between the treatment groups.

This study shows that intermittent fasting by feeding to satiation every third day will save feed, but hamper fish growth and feed utilization which the fish will require a considerable time to compensate lost growth from. In terms of fish welfare, no apparent negative effects from the fasting regime were found, suggesting the current regime as viable during periods of food shortage or flesh quality adjustment prior to harvest.

List of abbreviations

ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
AgRP	Agouti-related protein
BM	Body mass
CCK	Cholecystokinin
CG	Compensational growth
FCR	Feed conversion ratio
FSH	Follicle stimulating hormone
GFI	Gut fullness index
GH	Growth hormone
GnRH	Gonadotropin releasing hormone
GSI	Gonadosomatic index
HCl	Hydrochloric acid
HSI	Hepatosomatic index
H+	Hydrogen ion
IGF	Insulin-like growth factor
K-factor	Condition factor
LH	Luteinizing hormone
Na+	Sodium ion
NPY	Neuropeptide Y
SGR	Somatic growth rate
SFI	Stomach fullness index

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1. Introduction

1.1 Aquaculture in Norway

Atlantic salmon (*Salmo salar*) aquaculture started in Norway in the mid-60s when there was an increasing marked demand and marketing potential for salmon than rainbow trout (*Oncorhynchus mykiss*). Atlantic salmon was found suitable for cage rearing along Norway's long coastline and fjords, and local food resources was provided from the fisheries (Tilseth et al., 1991). In 1971 the first successful sea production of farmed salmon was harvested and since then the production volume has increased immensely to 1.54 million tonnes farmed salmon harvested in Norway in 2022 (Grefsrud et al., 2023). About 450 million individual farmed Atlantic salmon swam in sea cages along the Norwegian coast at the end of year 2022, which by far represents the largest livestock production in Norway (Grefsrud et al., 2023). In 2022 Norway exported 1.26 million tons of salmon with a value of 105.8 billion NOK (Grefsrud et al., 2023). Today Norway accounts for more than half of the world's farmed salmon production (Hamilton et al., 2016).

1.1.1 Feed use in aquaculture

The United Nations (UN) defines sustainable development as "development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (United Nations, 1987). In the Stortingsmelding 16 (2014-2015) the Norwegian government concludes that "environmentally sustainability must be used as the most important prerequisite for regulating further growth in the aquaculture industry"(Grefsrud et al., 2022).

The aquaculture industry is growing and contributes to food production, increased welfare and employment in Norway and the world (Shepherd et al., 2017). The fish farming industry seek to maximize growth and feed conversion rates, which is vital for the profitability along with development of more sustainable protein and fat resources in fish feed (Albrektsen et al., 2022). Economic motivation has led to substantial research for improved production performances, such as improved growth rate by selective breeding (Thodesen et al., 1999), and understanding of nutritional requirements and refinement of feed composition (Azevedo et al., 2017). Feed accounts for over 50% of the costs in finfish farming (Asche & Oglend, 2016) and minimizing feed waste and feed conversion ratio are great economic incentives. In 2021 the average economic feed conversion rate in Norway was 1.27 (Fiskeridirektoratet, 2022), which partly also includes a national average of ~15 % mortality rates in sea cage (Grefsrud et al., 2023). Other than mortalities, fish health status, nutritional value of feed and feeding control are key factors for the feed conversion rate which again affect the environment (Stead, 2002). To achieve maximum growth the fish must be fed until satiety on a daily basis, over- and under feeding will result in inefficiencies (Gomes et al., 2023); over feeding causing feed waste and under feeding lowered growth rates (Hasan et al., 2013). This can be improved by optimized feeding control systems, preferably by automatic and objective appetite proxies based on

pellets sinking depth and/or fish behaviour (Bjordal et al., 1993; Fernø et al., 1993; Føre et al., 2018; Folkedal et al., 2022). Fish farmers generally fear to underfeed their fish because it is believed to cause economic losses owing to forgone growth potential (Aunsmo et al., 2014). Consequently, to maximize fish growth, most farmers overfeed their fish. Additional feed is also lost due to insufficient feeding control such as adjusted feeding intensity by visually assessing the appetite between meals (Folkedal et al., 2022).

Fish appetite and feeding behavior needs to be closely monitored as a part of feeding control since internal and external factors strongly affect appetite of farmed salmon, causing large daily variations in feed intake (Folkedal et al., 2022). Appetite monitoring is predominately based on video streams from sub surface camera where fish feeding activity levels and depth of sinking pellets are the main proxies. When feeding intensity of the fish drops pellets will increasingly sink to a certain level below the main feeding zone. The feeding intensity is reduced or stopped as it is assumed that the fish have reduced appetite or are fully fed (Folkedal et al., 2022). Conservative estimates of feed waste are around 3-5% of the input (Otterå et al., 2009; Svåsand et al., 2016), while single farmers estimates both lower and much higher (Sigurd Handeland, UiB, pers. comm.). With almost 3 million tons of salmonids farmed in sea cages worldwide in 2021 (FAO, 2021), a spillage of 5% feed corresponds to 225 000 tons of feed, worth an economic loss of ~$US 500 million dollars per year (Iversen et al., 2020).

The pellets spreading outside the cages also attracts wild fish, which may affect wild species negatively if it's unsuitable for the species dietary requirements and thus health, and other species such as crustaceans are vulnerable to feed used in salmon lice treatment (Järnegren et al., 2022). The discharge of organic particles from finfish farming are high, as well as the impact on the seabed during the production, and has been considered as a comprehensive problem (Grefsrud et al., 2022). Feed waste and faeces from open sea cages releases directly to the environment and increase the concentration of nutrient salts in the coastal waters. This causes increased production of phytoplankton, leading to an increased amount of zooplankton and deposition of organic material sinking to the seabed, which effects the oxygen concentrations and ecosystems negatively (Grefsrud et al., 2023). The sedimentation of organic material can affect both species diversity and biomass (Grefsrud et al., 2022).

1.2 Temporal feed distribution for farmed salmon

Caged Atlantic salmon are commonly fed every day according to observed appetite to secure full utilization of their growth potential. The post-smolts are flexible in adapting to alternations in feeding intensity and can efficiently feed over short intensive meals (Talbot et al., 1999; Folkedal et al., 2022), and several studies show the same growth rate and feed utilization independent of whether feed is distributed over one or few short daily meals vs. multiple long meals or continuously over the day (Thomassen & Fjæra, 1996; Sveier & Lied, 1998; Johnsen et al., 2013). A high meal frequency or

continuous feeding is, however, common practice and beneficial during the preceding freshwater production stage, as well as over the first weeks after transfer to sea cages (Flood et al., 2012). The efficiency of feeding and feed intake in salmon post-smolts points towards that a meal frequency of ~24 hours is sufficient to maximize growth, and a 48-hour frequency has been shown adequate at cold temperatures (<5 °C) (Johnsen et al., 2013). This corresponds well with the temperature dependent stomach evacuation rate of post-smolts within the common temperature range of sea farming (6-18°C), where most (~55-95%) feed is evacuated 24 hours after ingestion, and little to no feed is left after 48-72 hours (Handeland et al., 2008; Aas et al., 2020). Another study of sea caged Atlantic salmon at 13°C, showed that the stomach was completely emptied after 12-24 hours and the mid- and hindgut was emptied after 48 hours (Aas et al., 2017).

Extension of meal frequency beyond that of stomach evacuation, should initialize fasting mechanisms by use of stored energy on expense of growth and stimulate appetite towards compensational feed intake. Naturally, the feed intake of post-smolts is negatively correlated with amount fed in the previous meal, and positively correlated with time since last meal (Juell et al., 1994; Gomes et al., 2023). There is, however, little information concerning the effects on growth and feed utilization during low frequent feeding in salmon. Such should be of relevance for strategies during periodic food shortage (break in supply chains etc.) and pre-harvest improvement of flesh quality when exposing the fish for long-term fasting is unwanted.

Restrictive feeding may be carried out by reduced daily rations, as tested for Atlantic salmon in several studies. Tank studies have shown that a restricted ration can trigger stress in small and intermediate sized fish (Cubitt et al., 2008) and contribute to skewed feed intake and growth among the individuals (Damsgård et al., 2004). This may not occur under restrictive feeding within the larger group sizes of salmon in sea cages where individual fish may be unable to monopolize food resources (Juell et al., 1994). However, using temporally restricted meals where fish groups are fed to satiation should allow all individual fish to feed and allow for natural fluctuations in appetite to be assessed and accounted for (Folkedal et al., 2022). Regimes of periodic feed withdrawal for days, also known as *intermittent fasting*, have been tested over weeks to months in several farmed fish species. Common for several studies is a progressive increase in feed intake over time which partially compensates for lost growth during days without feeding, often in line with increased stomach capacity which facilitates larger intake, and partial to full growth compensation after refeeding (Känkänen & Pirhonen, 2009; Mattila et al., 2009; Tunçelli & Pirhonen, 2021). Similarly, Atlantic salmon post-smolts that alternated between one week of feeding and one of fasting over two months compensated their daily intake during and after the regime and after months did not differ in size from that of daily fed control fish (Thorpe et al., 1990). Also, when caged Atlantic salmon given a temporal reduction in feed availability from several to one daily meal showed a temporal dip in feed intake which growth loss from was compensated for within 3 months (Johnsen et al., 2013). This may imply that conditioning

and learning is an important part of adapting to a novel feeding regime (Juell et al., 1994; Bermejo-Poza et al., 2015; Folkedal et al., 2022).

1.2.1 Adaptation to altered food availability.

How often animals feed in nature depends on the food the species are adapted to forage. Generalist species eat whatever is available nearby and develop search behaviour for a specific food type, while specialists' species seek out a food type and has a search behaviour for a particular prey. Foraging has its energy cost and animals usually have an optimal foraging strategy influenced by social behaviours, the density, size of and availability to prey, and a trade-offs with predation risk (Aarnes, 2004; Chapman & Hall, n.d.). In culture, farmers are concerned for the food availability of the fish they are rearing, which for Atlantic salmon in ongrowing systems results in many spatial and temporal feeding strategies that depends on cage and feeding technology, efficiency of pellet distribution and method of appetite observations used in feeding control (Folkedal et al., 2022).

The digestive system of different animals is evolutionary relatively well conserved within vertebras with many similarities, although the proportions of different organs and tissues varies between species (Stanger, 2015). The differences are caused by anatomical and physiological adaptations to the food the animals eat, including contents and compositions of carbohydrates, lipids and protein, and how easy it is to digest. The fish activity level and energy consumption also affects the design of the digestive organs (Bedin, 2021). Atlantic salmon has a digestive system that is shorter than their body length, which is common for carnivorous fish (Waagbø et al., 2001). Some carnivore animals can eat up to 30% of their body weight in one meal and may eat only once a week. One important feature is the capacity to increase stomach holding capacity although there is a maximal limit due to physical limitations in the peritoneal cavity. The stomach constitutes 60-70% of carnivore's digestive system, which allows the animal to store a large meal for later digestion. A carnivore diet also provides a high level of easily digested proteins and less carbohydrates with low digestibility. This also enables these animals to have a shorter intestine that requires less space in the peritoneal cavity (I. Rønnestad, pers. comm., Mills, n.d.).

Since Atlantic salmons are carnivore the food availability in nature may be scattered in time and space (Mattila et al., 2009). This contrast the situation for farmed salmon where food availability is highly predictable and also commonly available in surplus in the close vicinity of their preferred swimming volume within the sea cages. Wild Atlantic salmon eats when the food is available and will experience periods where it's difficult to catch food (Utne et al., 2021). Studies have documented that wild Atlantic salmon are moving over large distances up to several hundred kilometers, and it reasonable to assume they have periods of fasting (Rikardsen et al., 2021). During spawning season, the salmon have a voluntary fasting period 6-11 months where they use their energy storages (Fleming & Einum, 2011). Intermittent fasting periods also occur as a stress response in salmon, during poor environments

and diseases (Farrell & Munt, 1983). According to a digestive capacity study in predators most carnivorous fish in the wild eat once every two days (Armstrong & Schindler, 2011). In other words, the wildlife of Atlantic salmon gives us a clue about the biological limits and adaptations of the species, as well as how we can interpret animal welfare in aquaculture when it comes to feeding regimes and growth.

A study of Atlantic salmon after smolt transfer to seawater showed that one single meal per day resulted in lowered daily feed intake compared to a higher feed frequency consisting of eight meals per day (Flood et al., 2012). During a reduced meal frequency, the digestive tract needs time to adapt to an increased gut capacity to accommodate a larger feed intake at each meal. As long as the fish gets enough food one or several meals can give the same growth and feed conversion rate (Flood et al., 2012). However, several short intensive meals spread over the hours of daylight seems to be better than long meals as it provides a distinct signal of feed availability, higher access to feed during meals (less competition) and a possibility to feed when the fish is most hungry (Talbot et al., 1999; Folkedal et al., 2022). Adaptation to new feeding regimes takes time and can cause lowered appetite in the beginning (Flood et al., 2012), but will gradually adapt to increased appetite over time (Känkänen & Pirhonen, 2009).

Endothermic animals, such as virtually all mammals and birds, use a lot of energy to keep their body temperature stable, while ectotherm animals such as reptiles and fish do not need this (Bedin, 2021). Ectothermic animals have a lower metabolic rate than endothermic animals and tolerates food deprivation better. For example, small mammals and birds starves to death within days, while reptiles, amphibians and fish can survive months or years without food (Grably & Piery, 1981), as e.g. wild Atlantic salmon during their sexual maturation, spawning and subsequent recovery in the river habitat (Farrell & Munt, 1983). All biological processes are directly affected by temperature, that will in line with the metabolic rate and bodily maintenance requirements affect the feed intake and their growth potential (Johnston & Dunn, 1987; Volkoff & Rønnestad, 2020). In other words, the temperature dependent metabolic rate in fish determines their basal energy requirements and affect the fish's tolerance to duration of fasting (Hvas & Oppedal, 2019).

1.2.2 Growth

In finfish farming, growth in body size is an important parameter that defines the production efficiency, and high growth rates indicate a robust and healthy fish (Noble, Gismervik, et al., 2018). Growth is regulated by: i) abiotic factors such as water quality, temperature and light-regimes, ii) biotic factors as body size and acclimated temperatures, and iii) local factors as environment, management, nutrition, parasites and disease (Aunsmo et al., 2014).

An organism allocates the energy into vital functions as well as building energy storages. During stress, which in aquaculture may be caused by numerous factors such as high sea temperatures,

handling including de-lousing treatments, sickness and respiratory problems connected with gill health or poor oxygen levels, the energy demand increases along with reduced appetite and growth rate (Waagbø et al., 2001; Remen et al., 2013; Hvas et al., 2022). Growth in fish is commonly referred to in terms of body weight. However, growth in terms of body length is a "minimum factor" for weight gain, where the *condition factor* in fish accounts for the relationship between weight and length similar to the body mass index (BMI) in humans (Hvas et al., 2021). The condition factor changes between seasons and life stages, feed access, spawning seasons, and sickness, which makes it difficult to find a common lower limit value indicating reduced welfare. However, a condition factor under 0,9 indicates emaciation and a normal condition factor for harvest sized farmed salmon is 1,6 (Noble et al., 2018). Atlantic salmon continue their length growth, to some extent, even during fasting (Lie & Huse, 1992; Hvas et al., 2021), which next to weight loss also impact their condition factor. Naturally, lean salmon have a higher potential for weight gain than fish of higher condition, and the capacity to compensate for periods of poor growth or weight loss is high in farmed salmon. The condition factor increases during compensational growth after e.g. fasting periods or during the summer in salmon kept on a natural photo regime vs. continuous light that stimulates growth during the winter (Oppedal et al., 1999; Hvas et al., 2021).

1.2.3 Why intermittent fasting?

Fasting regimes in aquaculture may be motivated by saving feed under feed shortage, decreasing filet fat content for improved flesh and product quality, or save labor as e.g., keeping the fish of feed during the weekends. Fish feeds are composed of ingredients traded in a global market, and food shortage have previously occurred and was recently a risk during the COVID-19 pandemic. Scientific reports have recommended intermittent fasting as strategy, rather than reduced daily feed ration as a measure to save feed (O. Folkedal, pers. comm). During every day feeding using a restricted ration, it is difficult to assess the fish appetite and it may cause an uneven distribution of the feed among the individuals. This may enhance hierarchies in the cages, leading to the most dominant fish to eat most and create an uneven feed intake over time (Waagbø et al., 2001).

An intermittent fasting regime where salmon post-smolts are fully fed every third day has been recommended for saving of feed, as this is regarded to be sufficient for fulfilling the fish energy needs for bodily maintenance under most circumstances (O. Folkedal, pers. comm). Effects on feed use/saving and growth under such a regime are, however, largely unknown, but several studies of intermittent fasting in other species than Atlantic salmon have focused on a fasting period of 2-, 4 - days; more and less on 3 days (Mattila et al., 2009; Bermejo-Poza et al., 2015). A study of Pikeperch (*Sander lucioperca*) was fed every second, fourth and seventh day over eight weeks and showed no difference in growth rates compared to a daily fed group (Mattila et al., 2009). The stomach evacuation rate found in Handeland et al. (2008) showed that some of the fish still had food in their digestive tract for further absorption during all three days subsequent to their last meal. Another study

of long-term fasting (6 and 4 weeks) on Atlantic salmon post-smolt found that the gastrointestinal mass and enzymes was reduced by 20-50% after 21 days (Kalananthan et al., 2023). However, the reported stomach evacuation rates in Atlantic salmon post-smolts (Handeland et al., 2008) indicates that growth rate will be reduced if feeding is restricted to every third day.

1.2.4 Effect of fasting on energy reserves, body composition, and flesh quality

If fasted, Atlantic salmon as other animals will naturally lose weight as bodily maintenance requirements, including respiration and locomotion, will be fuelled by stored energy (Hvas et al., 2021). For bodily maintenance requirements, a temperature dependent amount of energy must be consumed, as reflected by both the resting and active metabolism of Atlantic salmon (Hvas et al., 2017). Smaller animals burn a lot of energy in relation to their body size and need to digest efficiently and eat often (Bedin, 2021). Mass specific metabolic rate decrease with size, implicating that larger fish can tolerate longer fasting periods than smaller fish (Killen et al., 2007). Energy utilization increases from zero, when in balance (intake = outtake), to maximum when feeding until satiety. In a study of Atlantic salmon post-smolts that fasted for 4 weeks, the metabolic rate at minimal swimming speed (resting metabolism) was found to be downregulated by 23.8% and 15.6% at 9°C and 18°C respectively, while the fish maintained their maximum metabolic rate during a critical swimming speed test (Hvas, 2022).

Aquaculture farmers aim to deliver a good product for the consumers and make a good profit, which means that the farmers under normal circumstances will not fast the fish if it compromises growth rates or flesh quality. Over time, fasting naturally leads to weigh loss and reduced condition factor in fish. Atlantic salmon do continue some length growth under long-term fasting (weeks to month), and the species show a strong capacity for compensational growth when refed (Hvas et al., 2021). During long term fasting the fish will reduce its metabolic rate to conserve energy, reduce their gastrointestinal tract, change their core microbiome composition and cellular mechanisms (Waagbø et al., 2001).

During starvation fish uses three successive stages based of endogenous energy reserves to cover nutritional needs and metabolism (Hvas et al., 2022). The first stage the animal uses is glycogenolysis to break down glycogen to glucose in the liver as an energy source. When the glycogen depletes the energy is taken from oxidation of fatty acids in adipose tissues. When emptied, the body degrades muscle tissues and breaks down proteins. Entering the third stage the animal reduces physiological capacities leading to weakening and ultimately death (Bar, 2014). However, some fasting is positive for the animal. Atlantic salmon fasted for 35 days showed improved resistance to acute stress prior to slaughter that inhibited rigor mortis development and resulted in firmer flesh texture, more vibrant color and a lower liquid leakage from the muscle (Mørkøre et al., 2008). A long-term study in Atlantic salmon post-smolt concluded that reduced meal frequency was a promising tool for managing flesh

quality without compromising growth performance (Johnsen et al., 2013). Similarly a feeding frequency of every 2 days increased the fillet quality, but a 9-day fasting period reduced the filet quality in rainbow trout prior to slaughter (Bermejo-Poza et al., 2015). Short-term fasting results in immediate effect of re-filling and emptying the alimentary canal, including sensory and hormonal feedbacks, together with short-term changes in circulating nutrients (Hvas et al., 2021).

1.3 Intermittent fasting

In humans, food consumption frequency, variations in meal size and composition are largely based on social interactions and traditions, where it is believed that the "standard" meal regimes increase metabolic rate and gives proper regulation of blood sugar. The thermic effect of food (TEF), also called Standard Dynamic Action, reflects the amount of energy used for the body to process food, which in humans is found to increase by larger meal size, as well as affected by diet and meal frequency (Mansell et al., 1990). There are several myths linked to fasting, believing that fasting makes the body lower metabolic rate into saving mode, which is true during long-term fasting (>48 hours) where a study showed that the metabolic rate was reduced by 8% after 3 days of fasting (Mansell et al., 1990; Nair et al., 1987). However, during short-term fasting (<48 hours) a metabolic response study showed that resting metabolism increases with 3.6%. Concurrently, the body secretes adrenaline and noradrenaline that sharpen focus, which from an evolutionary perspective stimulate the need for finding food (Mansell et al., 1990). Intermittent fasting as alternate-day fasting in humans has been documented to have many health benefits, such as reduction of BMI, low-density lipoprotein cholesterol, triglycerides, fasting plasma glucose, fasting insulin, homeostatic assessment of insulin resistance and blood pressure in adults (Patikorn et al., 2021). Hvas et al. (2022) showed that Atlantic salmon had similar blood glucose and liver glycogen levels independent if fed regularly or starved for 22 days, indicating that energy is maintained readily available independent of dietary status (Hvas et al., 2022).

The Norwegian regulation of aquaculture (Akvakulturdriftsforskriften §27) regulates how farmers run an aquaculture facility and states that fish should normally be fed daily, unless it's not appropriate for the species or stage of development. If the fish cannot be fed due to reasons which negatively affect fish welfare, hygiene or quality, the fasting period should be as short as possible (Lovdata, 2008). In finfish farming, fasting periods occurs before handling operations, slaughter, transport, parasite treatments, harvesting and RAS-system cleansings (Farrell & Munt, 1983). Increased focus on animal welfare have raised concerns about ethical issues regarding fasting of farmed finfish, however, this legislation is based on a general approach about livestock that does not take fundamental fish biology into account.

1.3.1 Compensatory growth (CG)

Intermittent fasting is gaining interest due to acceleration of growth after a fasting period (Py et al., 2022). Compensatory growth is defined as "*a phase of accelerated growth when favourable conditions are restored after a period of growth depression*" (Hvas et al., 2022). Which is a response to hyperphagia that gives an abnormally increased appetite, better feed utilization, reduced energy demand for growth and metabolism (Waagbø et al., 2001; Tunçelli & Pirhonen, 2021; Hvas et al., 2022). The compensatory growth is affected by life-stage, diet, food availability, duration of fasting period and bodily status (Waagbø et al., 2001; Hvas et al., 2022). Periods of food deprivation induce changes in the storage reserves, particularly lipids in fish, where the somatic growth trajectories and lipid storages restores when food becomes sufficiently available (Hvas et al., 2022). After a period of growth depression farmed Atlantic salmon are highly capable of compensational growth as seen in groups given contrasting freshwater temperatures (Johnston et al., 2003; Frisk et al., 2020), stress levels (Vindas et al., 2017) or long term fasting as post-smolt, where fish fasted for 8 weeks showed a full growth compensation over the next 7 months (Hvas et al., 2021).

1.4 Physiological basis and proxies of growth and utilization

Fish gains energy and matter through food, where during catabolism the absorbed matter and energy are used for maintaining bodily needs, activity and building reproductive products (Brett & Groves, 1979). During the aerobic metabolism, 40-50% of the labile compounds provide the energy for driving endergonic processes such as biosynthesis, membrane transport, and are fuel for mechanical work in muscle tissues. Further chemical free energy is degraded to heat, where in fish the metabolic heat is lost fully to the animal (Brett & Groves, 1979).

For maintaining body mass, the absorbed energy must equal the loss during catabolism and for growth occurred from deposition of matter (proteins), where the feed intake must be larger than energy needed for catabolism. Surplus energy is stored as covalent bonds in proteins, fats, and carbohydrates. During insufficient dietary intake growth of some organs (e.g., liver and gonads) or body components (e.g., fat and muscle tissue) that was previously stored as endogenous sources will be reduced to cover catabolism needs (Brett & Groves, 1979).

1.4.1 Digestion, endocrine systems, and energy storages

The main role of the gastrointestinal tract is to degrade ingested food into absorbable units that are absorbed and distributed into the circulation while undigested matters are evacuated. Digestion combines mechanical and chemical degradation processes in different compartments of the gastrointestinal tract. The tract is divided into foregut (oesophagus and stomach), mid-gut, hindgut and rectum (Bone & Moore, 2008) (Figure 1). Muscle contractions in the stomach mechanically break down the food, mix enzymes and substrates it together, causing a chemical degradation process (Bone & Moore, 2008). A pyloric sphincter separates the stomach and mid-gut, controlling the amount of

food transferred from the stomach into the intestine and provides a stable supply of nutrients for further processing. Segmented muscle contractions in the intestine causes the food to move posteriorly downstream through the midgut and hindgut for continued degradation and absorption (Waagbø et al., 2001).

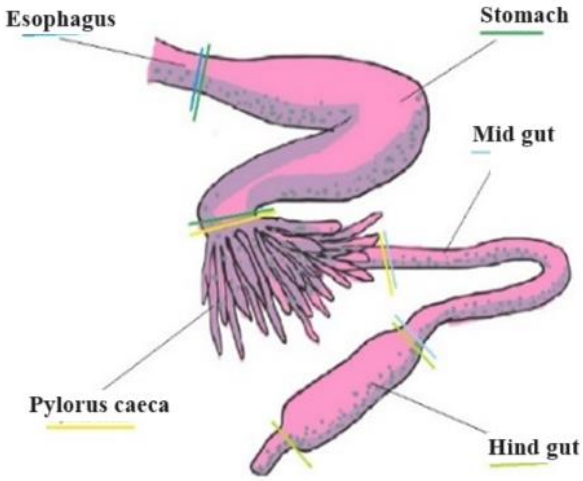

Figure 1 *- Overview of Atlantic salmon stomach and intestine (Olsen, 2023)*

Energy storages

1. Glucose

Glucose is absorbed from the intestine and transported by the portal vein to the liver, where the presence of insulin allows it to be absorbed over the cell surface. Glucose can either be used as energy through glycolysis, stored as glycogen, converted into fat through *de novo* synthesis, or enter other synthetic pathways (Waagbø et al., 2001). Glycolysis breaks down glucose in the liver by pyruvate enzyme that releases ATP-energy and pyruvate. The ATP-energy can be used in protein synthesis, muscle contractions and transporting substances, while pyruvate are further catabolised and releases more energy (Svihus, 2023). Gluconeogenesis is the revers process of glycolysis, and it is an energy demanding process where different metabolites are converted into new glucose molecules. This process occurs when the blood levels decline, for example during fasting and anaerobic muscle work. Glucose production depends on intake of carbohydrates, the glycaemic index, time since last meal, uptake of glucose into cells and consumption of glucose in the body. Glycolysis and glucogenesis gets stimulated by glucagon and the insulin hormone (Svihus, 2023). Further, the pancreas produce insulin that is released into circulation and serve it to increase glucose permeability, appetite and creates an anabolic state in the liver that's stimulates growth (Åsvold, 2023). The size of the liver, the hepatosomatic index (HSI) gives an indication of the fish's energy storages, and is typically low if the

fish have to compensate for less feed intake, and high if the fish has been overfed and can store more energy (Chellappa et al., 1995).

2. Lipid

Lipid digestion mainly starts in the anterior midgut after chyme from the stomach enters the intestine. Endocrine cells in the anterior parts of the digestive tract is then triggered to secrete cholecystokinin (CCK) causing the gallbladder to contract and secrete bile acid, and pancreas to release enzymes (Bone & Moore, 2008). Bile acid aids to digest fats and forms micelles that causes the fat molecules to be distributed in the chyme, which increases the surface area for digestive enzymes. Lipases then breaks down the ester bonds. Micelles dissolve when contact with water and the fatty acids passively diffuses across the intestinal cell membrane mostly in the cecum and midgut (Bone & Moore, 2008). Adipocyte cells produce leptin (Bone & Moore, 2008) that act as a lipostatic factor in a negative feedback loop between adipose tissue and the brain, to adequate fat mass reservoirs. Fat cells are found in the liver (HSI) around the organs, and in "fat fish" like salmon, fat are stored in muscle tissues (Rønnestad et al., 2017).

3. Protein

The digestion of proteins starts in the stomach where the glands secrete pepsinogen, that is converted into pepsin in contact with the acidic gastric juice digesting proteins to polypeptides. In the intestine hormones, mainly CCK stimulates pancreas to secrete trypsinogen and several other proteases, that further digest polypeptides to di and tripeptides and free amino acids that are subsequently transported via the enterocytes into circulation (Aabakken, 2020). These amino acids are transported to liver and other tissues, where cells select and absorb the amino acids needed to build new proteins (Waagbø et al., 2001). Proteins are important for growth and reparation, and also as an energy source. Lack of proteins, or specific limiting indispensable amino acids in the diet can reduce muscle tissue to maintain bodily functions (Merethe Kvam, 2023).

Endocrine system

The digestive system is considered as the largest endocrine organ. The hormones produced and secreted not only control digestive function but also appetite, feed intake and energy homeostasis; e.g. Ghrelin (Waagbø et al., 2001; Bone & Moore, 2008). However, the main factors for regulating growth in teleost fish are the growth hormone (GH) and insulin-like growth factors (IGFs). GH stimulate osmoregulation, adrenocorticotrophic activity, thyrotropic-, immunological- and reproductive effect, energy balance and metabolism in salmon. The opposite process, the liver secretes somatomedin that acts like insulin-like growth factors (IGF-1 and 2) and gives a negative feedback loop to supress GH. The amount of released GH depends on age, nutrition, blood sugar, IGF, stress and physical activity. Directly or through stimulation of IGF-1, GH stimulates the synthesis of proteins increasing muscle mass and cartilage, while reducing fat mass. During fasting GH increases (Berg, 2020) and in reverse

somatostatin (SS) inhibits the release of GH from the adenohypophysis (Bone & Moore, 2008). Different gastrointestinal peptides and catecholamines stimulate secretion of GH, such as neuropeptide Y (NPY) and ghrelin increases appetite, while gonadotropin releasing hormone (GnRH) and cholecystokinin (CCK) inhibits appetite (Bone & Moore, 2008).

In vertebras ghrelin from the gastrointestinal (GI) tract in the stomach regulates gut motility and stimulate GH release, feed intake and energy homeostasis. Ghrelin has receptors on NPY and Agouti-related protein (AgRP) neurons that increases appetite (Bone & Moore, 2008). A study on Atlantic salmon post-smolts showed that long-term fasting (40 days) reduced *ghrl1* after 6 weeks of fasting and no reduction of *ghrl2* throughout the trial (Kalananthan et al., 2023), supported by a second study showing that the *ghrl1* increased after 2 days of fasting indicating a higher appetite (Hevrøy et al., 2011). Another study of fasted Zebrafish (*Danio rerio*) showed that AgRP and NPY increased as a response to fasting and gave drive to feed more and enhance growth (Guillot et al., 2016).

Sexual maturation in teleost fish is regulated from the pituitary where the adenohypophysis produces peptide hormones, such as gonadotropin releasing hormone (GnRH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) for reproduction. The NPY further stimulate the food intake, reduces energy consumption and increases pituitary secretion of LH (Bone & Moore, 2008). Studies has shown that rapid growth during spring can lead to early maturation in Atlantic salmon (Taranger et al., 2010), as physical condition and energy reserves are used for building gonads during the summer months (Agarwal, 2008). During sexual maturation phase the salmon decreases the growth rate and uses the gained growth for building gonads leading to spawning in the fall (Taranger et al., 2010), which is undesirable in salmon farming, however, some studies has showed that fasting can inhibit sexual maturation in Atlantic salmon farming (Trombley, 2014; Hvas et al., 2021). The maturation stage can be measured by gonadosomatic index (GSI), where GSI > 0.06 - 0.1 is an early indication of maturation for both sexes in Atlantic salmon (Good et al., 2017).

1.5 Welfare, motivation, and adaption processes

Farming of animals in Norway, including of Atlantic salmon, are obligated to follow the legislation of animal welfare (*Lov Om Dyrevelferd*, 2009) and for aquaculture species the specific legislation for aquaculture management regulations (Lovdata, 2008). The purpose is to promote good animal welfare and respect for animals, where they must be treated well and protected against risk of unnecessary stress and strain (Lovdata, 2009). Both scientific and operational welfare indicators (WIs) can be divided into animal based or environmental based. Animal based WIs can be directly physically observable attributes like gill health, condition factor and presence of wounds, or fish behaviour like the response to feeding which signal the level of appetite in fish groups or individuals. Environmental based are indirect indicators like water temperature and oxygen levels, as interpreted on a background of knowledge about the species and life-stage specific needs and tolerance limits (Noble, Gismervik, et al., 2018). Animals need access to resources to gain enough energy to grow and reproduce, as well as protection against predators and harmful environments (Noble, Gismervik, et al., 2018). Fish have a good ability to learn and remember, they anticipate future events, sense time and place, map their surroundings, and are able to recognise other individuals and cooperate with them (Noble, Gismervik, et al., 2018).

According to the concept of allostasis (stability through change), "*good animal welfare is characterized by a broad predictive physiological and behavioral capacity to anticipate environmental challenges*" (Korte et al., 2007). In other words, the animal should benefit from updating its predictions by learning from its environment and own actions, and by adjusting its physiology according to environmental demands, provided that these are within the animal's tolerance limits. To fulfill bodily needs such as maintaining a healthy nutritional status or safety from e.g., predators, several motivational systems are continuously competing to control behavior, and will also affect physiological processes towards anticipated demand. The most urgent needs are most likely to be expressed, where e.g. a hungry fish will seek food and be more risk willing relative to predators than when satiated (Fernö et al., 2020). When stressed, fish tend to reduce their feed intake, which increases their metabolic scope towards dealing with an unpredictable environment (Schreck & Davis, 1997). Even after apparent physiological stress recovery farmed Atlantic salmon may show reduced anticipatory behavior for food, even though their level of feed intake may be normal (Folkedal et al., 2012).

Adaptational processes to a changing environment may occur at different levels and rates, where e.g. the fright response in Atlantic salmon towards an initial frightening, but in reality harmless, stimulus, may be down-regulated (habituated) over few repetitions/exposures (Folkedal et al., 2010). Recurring stimuli might also turn into signals of predictive value when paired with something of incentive value for the fish, such as food (i.e., classical conditioning) (Bratland et al., 2010). Other adaptational processes to environmental variations includes responses to e.g. altered temperature, where fish can

choose to leave in search for preferred temperature or stay and acclimate its physiology (Hvas et al., 2017).

Fish are vertebras that are considered to have sophisticated cognitive functions and capacity to experience distress (Sneddon, 2006; Sneddon et al., 2014). Long-term fasting may violate the fundamental ethics of animal welfare, that all animals should be free from hunger, thirst, and malnutrition. Good fish welfare is associated with thriving fish, that's stays healthy and has a good growth rate (Noble et al., 2018). Farmed Atlantic salmon are used to having excessive amount of feed available at a daily basis, and feed withdrawal may be stressful for the fish (Vindas et al., 2016). However, if the fish voluntarily start fasting due to health issues, the loss of appetite suggest that the fish welfare is not satisfactory. Feelings of hunger occur in Atlantic salmon based on endocrinologic signalling, such as ghrelin and leptin that's increasing during food withdrawal. However, based on the fasting periods in wild salmon, and measured levels of hunger signals in controlled fasting studies, we can assume that fish has adapted to supress their hunger feeling during long-term fasting (Hevrøy et al., 2012; Kullgren et al., 2013; Kalananthan et al., 2023).

Based on lack of knowledge of how intermittent fasting affect farmed Atlantic salmon, the present study investigates how large sea caged salmon respond to being fed every third day, followed by refeeding. The study is especially of interest for situations with feed shortage, and for gaining knowledge of salmon adaptive capacity when feeding becomes temporally restricted, and their capacity to recover from expected feed deprivation after return to daily feed ability (refeeding). This carried out by scrutinizing relevant physiological parameters for appetite, energy storage and sexual maturation, as well as how the present intermittent feeding regime impact highly relevant parameters of daily appetite, growth rate and feed utilization, all in a realistic farming situation. This thesis will include samples for indicating the hepatosomatic – and gonadosomatic indexes for energy storages, and adaption to a new feeding regime by measuring the gut content in the stomach, midgut, and hindgut.

1.6 Aim and hypothesis of this study.

The main aim of this thesis is to investigate how large sea caged Atlantic salmon, which are adapted to being fed to satiation every day, respond when feeding is restricted to every third day; a regime of intermittent fasting. Their response will be measured at fish group level as daily appetite and periodic growth, and for periodically sampled individual fish as gut filling for appetite assessment, and physiological indicators connected with appetite control and energy storage. A secondary aim is to investigate how return to a daily feeding regime (refeeding) affect the same parameters. The experiment is carried out by comparing fish in duplicate cage groups given either an intermittent fasting regime of feeding to satiety every third day (*intermittent fasting group*), or daily feeding to satiety (*control group*). To assess appetite, online camera observation of pellet sinking depth is used for feeding control per cage. Given at hypothesis that the fasting group will gradually adapt to increased appetite over consecutive days with feeding, the fasting regime will be kept for at least one month, and longer if the adaption persists, and similar time for recovery after return to daily feeding (refeeding).

More specifically, I will investigate:

1. How fast and how much the intermittent fasting group adapt their appetite, assessed as (daily fed amount) per sea cage, relative to the appetite of the control group, and how appetite is affected by return to daily feeding (refeeding).
2. Whether and how much the intermittent fasting regime reduce growth and feed utilization vs. that of the control group, and whether compensational effects will occur after refeeding.
3. Whether the intermittent fasting regime affect energy storage in terms of liver size relative to body mass (hepatosomatic index) and gonad size relative to body mass (gonadosomatic index).
4. Whether and how much the intermittent fasting regime will have effect on stomach-, midgut-, and hindgut fullness index based on measured wet gut content in sampled individual fish.

2. Materials and Methods

2.1 Experimental setting

The current experimental is a part of the NoFood2Waste project (NRC IPN 317770, 2020-2023) and was carried out at Solheim Cage Environment laboratory at the Matre Research Station (60.875 °N) of the Institute of Marine Research (IMR). The Department of Biological Sciences at the University of Bergen leads the research project and was in charge of sampling and analyses of fish samples from the trial. The trial and all handling were approved by Norwegian Food Authority (29794).

2.2 Fish and experimental design

Atlantic farmed salmon (Aquagen strain) were stocked as smolts (120g ~ 8500 fish per cage) over 4 square sea cages (12 x 12 m and 15 m deep) on 15 April 2022. Fish were reared according to standard rearing practise at IMR and under a natural photo period.

Upon start of the experiment on the 7th of March 2023, a sample per cage (n= 100 fish) was measured for weight (3363 ± 38 g, mean of all cages ± SD) and body length (62.3 ± 0.24 cm) (Table 1). For this and other sampling dates (Figure 2), the sampled fish (Figure 3) were caught by lowering a casting net (5 × 5 m, 5 m deep) into the sea cage and hauling it to surface. This captured ~200-300 fish which were crowded and sampled using a dip net.

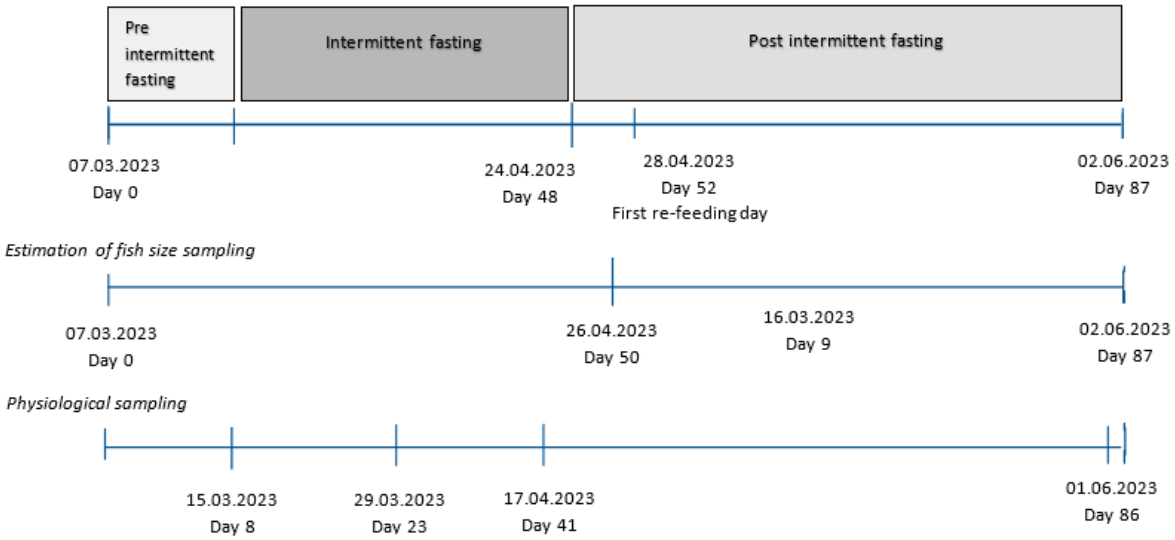

Figure 2 *– Overview of the samplings during the experimental trial.*

Table 1 *– Average of body weight, length, condition factor (± SD) and number of fish for each cage at the first sampling for estimation of fish size (07.03.23). n = 100.*

Cage	Weight (g)	Length (cm)	K-factor	Fish per cage
1	3275 ± 543	61.7 ± 3.39	1.37 ± 0.01	8939
2	3376 ± 627	62.0 ± 3.45	1.36 ± 0.01	8402
6	3321 ± 604	62.4 ± 3.61	1.35 ± 0.01	8758
7	3481 ± 576	63.0 ± 3.19	1.38 ± 0.01	8480

Figure 3 *– Experimental fish: Atlantic salmon from the first physiological sampling.*

The Solheim farming site has 10 sea cages and cages 1, 2, 6 and 7 were randomly chosen for use in this trail (Figure 4). Cages 1 and 6 were chosen as control cages (*Control* group) to be fed to satiation every day, and cages 2 and 7 for the experimental regime of intermittent fasting (*Intermittent fasting* group) where the fish were fed to satiation every third day and fasted the two following days. This feeding regime persisted over 43 days, before the fish were refed over 37 days before fasting prior to harvest.

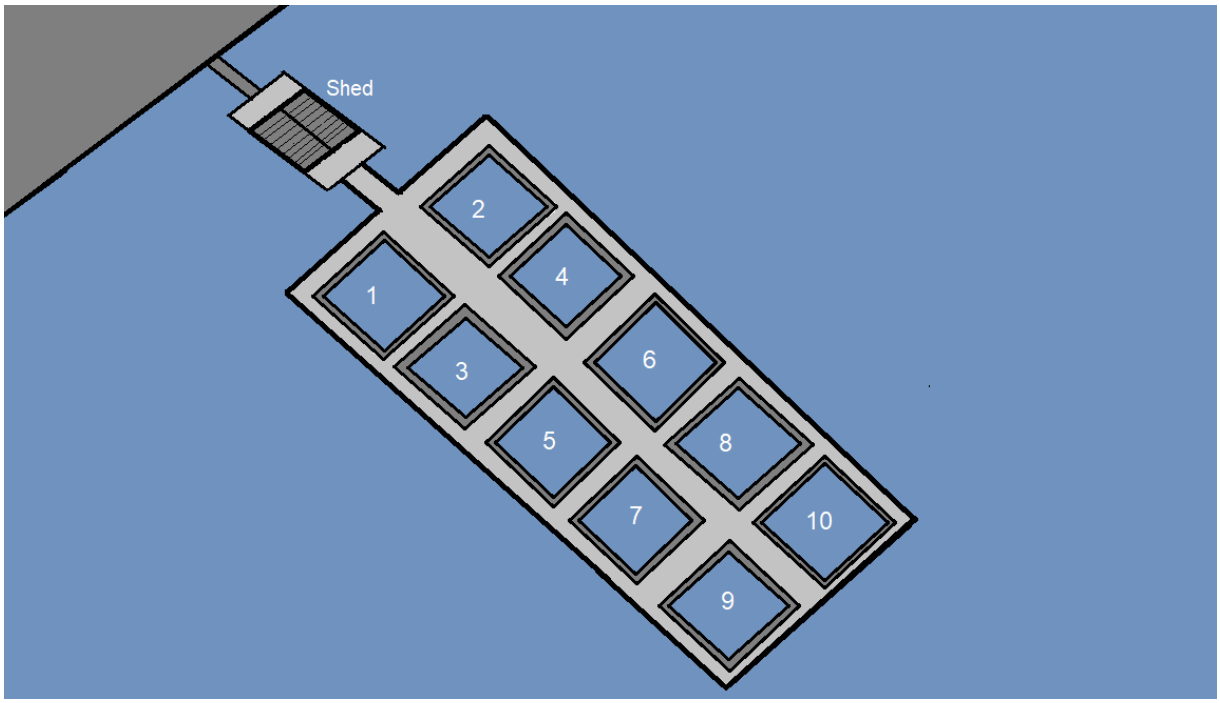

Figure 4 *- Outline of sea cages at the Solheim Cage Environment Laboratory. Cage numbers are indicated for each separate cage, where cages 1, 2, 6 and 7 were randomly chosen for the current trail.*

The experimental period started when the fish were measured on the 07.03.23 (experimental Day 0) whereafter all cages remained being fed to satiation at a daily basis, including Day 8, to obtain a data for baseline fed amount per cage. Shortly after the fish were fully fed at experimental Day 8, 10 fish per cage were sampled and sacrificed (overdose by 100 mg l^{-1} of Finquel Vet., Western Chemical Inc, Washington DC, USA) for physiological samples and measurements of gut content.

At experimental Day 9 (fasting day 1) the fasting regime was initiated, and physiological samples and measurement of gut content was again carried out at Day 23 (fasting day 15) and Day 41 (fasting day 33). Another sample for fish size estimation was carried out at Day 50 (fasting day 38) for each cage. The control group was also fasted for three days up front of this sampling, experimental days 48-50, to compare the following fed amount between the control and the fasting group. The intermittent fasting period lasted from Day 9 to Day 50.

During the post intermittent fasting period (refeeding for the intermittent group) all the cages were fed to satiation at a daily basis until Day 86. Then at Day 87 another fish size estimation sampling (n=100 fish per cage) was taken.

2.3 Experimental feeding

The experimental fish were fed a high-quality feed (9 mm pellets, Skretting ARMOR, Skretting AS, Stavanger, Norway). The ARMOR pellet contains ingredients that are supportive for skin health, healing of wounds and the fish immune system, including increased levels of omega-3, vitamins and minerals vs. in standard feed (Skretting AS, n.d.).

The feed was dispersed at surface in the cage centre by a pneumatic and automatic feeding system (Fluctus, Austevoll, Norway). Feeding was manually monitored in a video stream from a sub-surface pan and tilt camera in each cage (Gemini, Imenco AS, Aksdal, Norway) (Figure 5), and fish feeding activity and pellet sinking depth were used as proxies for fish appetite level and satiety. Each camera was positioned in the centre of each cage and mounted to a winch which allowed remote depth adjustment in software. Feeding was stopped for the day when pellets were observed sinking deeper than 10 m depth or pellets spillage was observed, indicating satiety.

The fish were administered two daily meals. The first meal was between 7:30 – 11:30, and the second meal from 12:30 – 14:00. The duration of the second meal was dependent on daily fish appetite, and when appetite was higher than predicted the second meal was elongated – until satiety was observed.

On experimental day 65 the feeding system was stopped, and feeding was discontinued by mistake after the weekly lice counting, resulting in lower quantity fed due to the feeding system turned on later that day.

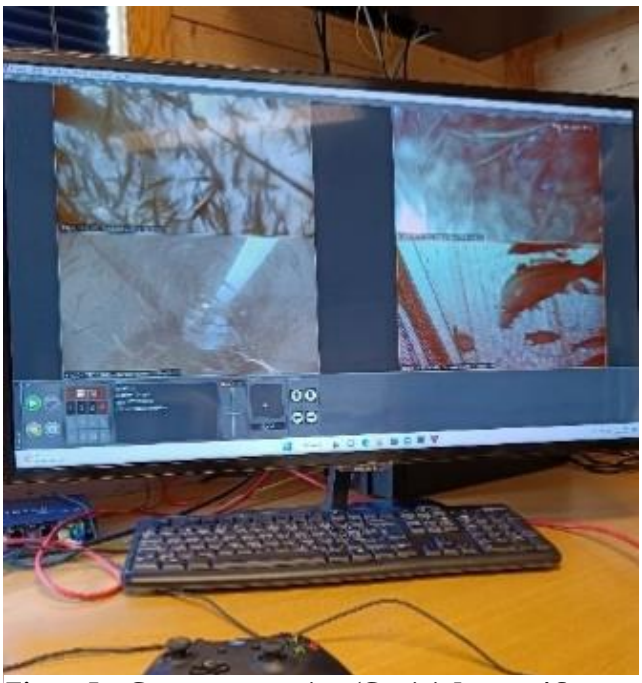

Figure 5 *– Camera screen view (Gemini, Imenco AS, Aksdal, Norway) of cage 5 and 6.*

2.4 Experimental environment

Water temperature, salinity and oxygen were recorded daily outside the cages between surface and 20 m depth by a profiling CTD connected to an automatic profiling buoy (APB5, SAIV AS, Norway) (Figure 6).

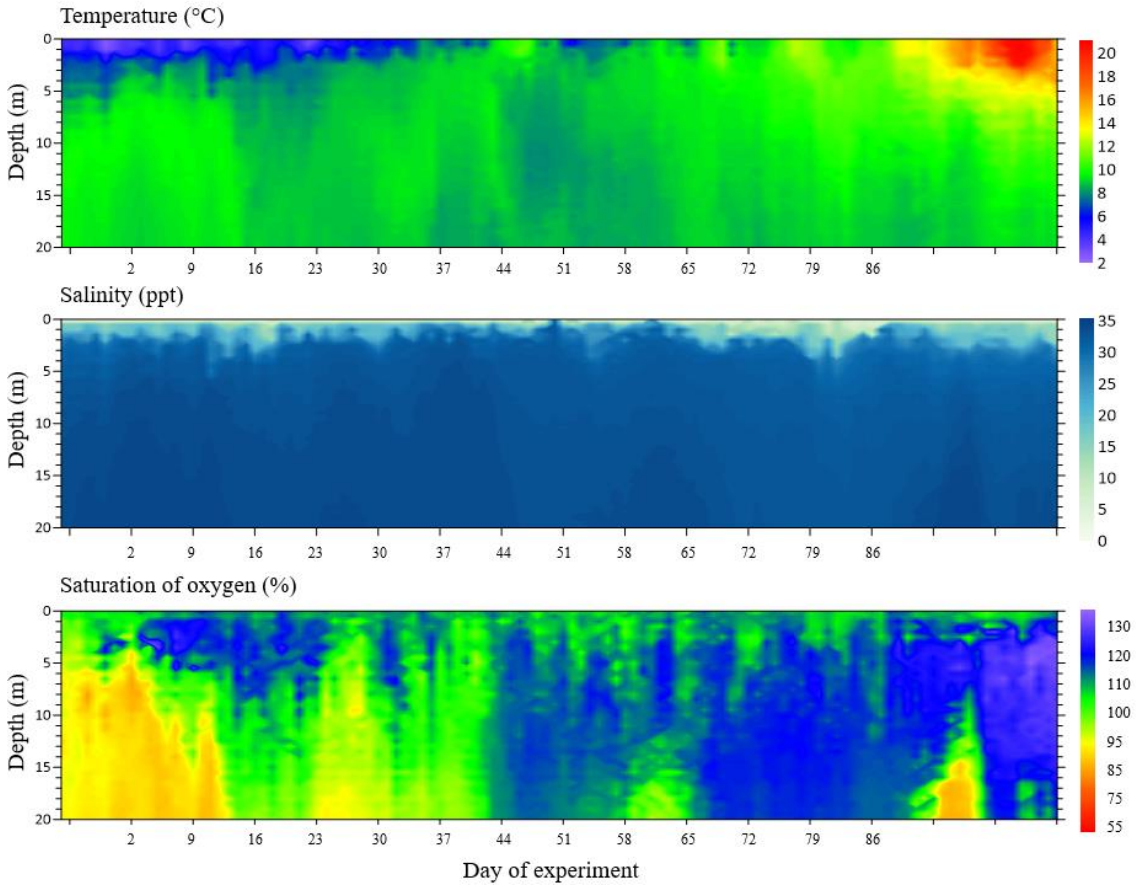

Figure 6 *– Environmental data collected over the experimental period. The upper pannel show water temperature (°C), middle pannel show salinity (ppt) and lower pannel show saturation of oxygen (%) in the sea over the study. The x-axis shows experimental day, and the y-axis on the left side shows the depth from 0 – 20 metres.*

2.4.1 Sampling procedure

Sampling procedure for fish size – Experimental day 0, 50 and 87

The 100 fish netted out from the casting net were sedated with anaesthesia for us to measure the weight and length (Figure 7 and 8). The measurements were written on a waterproof paper and later transferred to an Excel sheet (Olsen, 2023). Fish body length was measured as fork-length, measured from the tip of the snout anterior to the centre of a concave tail posterior. The length was measured in centimetres (cm) and the weight was measured in grams (g).

Harvest data isolated per cage was only available for Cage 1, as fish from the other cages were transported in a common well to the harvest facility.

***Figure 7** - Procedure collecting the fish.*

***Figure 8** - Anaesthesia tank on the work boat.*

Sampling procedure for physiology – Experimental day 8, 23, 41 and 86

The four physiological samplings at Day 8 (fasting day 0), Day 23 (fasting day 15), Day 41 (fasting day 33), and Day 86 (36 days after refeeding of intermittent group) was carried at the site by trained UiB personnel and assisted by IMR crew.

The sampling consisted of collecting tissues and gut content from 40 fishes (n=10 fish per cage). More specifically, after recording of fish weight and body length, liver, gonads, stomach-, midgut- and hindgut content was collected (Figure 9). Prior to the samplings the team labelled 120 plastic bags (120x170x0.05 mm, VWR International, Oslo, Norway 7 Eppendorf, Oslo, Norway) that were pre-

weighted, and labelled with fish number (Figure 10) and designated content of “stomach”, “midgut” or “hindgut”.

The fishes were fed to satiation with a~30-minute delay according to time for sampling per cage. The fish were sampled for both size and physiological parameters on a barge in close proximity to the cages. All samples were collected in chronologic order from start to end of the sampling. The stomach-, midgut- and hindgut content, liver and gonads were measured by a scale for weighing in grams (g) (3 decimals were used) and a ruler for fish length in centimetres (cm). The measurements were written on waterproof paper and later transferred to an Excel sheet.

The fish that were euthanized for the physiological sampling is not included in the mortality estimation (Table 4) but subtracted for the daily estimation of biomass.

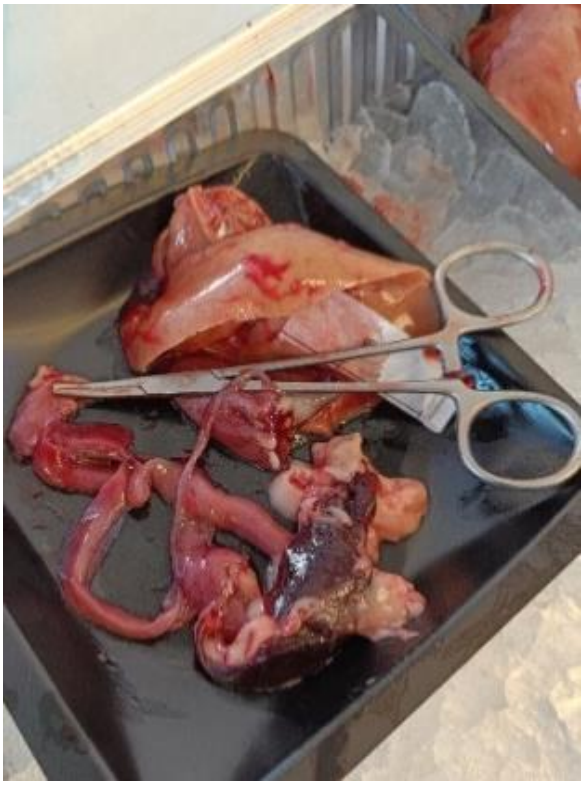

***Figure 9** - Collected tissue sample from 1/40 Atlantic Salmon (liver, heart, spleen, gall bladder and gonads).*

***Figure 10** – Sampled fish marked with each number, euthanised and ready for tissue sampling.*

2.4.2 Laboratory analysis

The gut content was stored in a freezer at -20° until further analysis (Table 2), the gut content was later thawed and weighted before bags were opened and placed on a drying rack in the drying machine. The drying machine was set to 74 °C and the timer to 48 hours to dry.

The dried bags with the gut content were weighted; and content calculated by subtraction. This procedure was repeated for all samplings. All the data collected were transferred to an Excel file for further analysis.

Table 2 *– Overview of the further processing of the gut content (stomach-, midgut- and hindgut fullness index).*

	Date:	
Gut content - sampling 1	20.03.2023	40 bags of gut content in the drying machine
	22.03.2023	40 bags of gut content in the drying machine
	25.03.2023	40 bags of gut content in the drying machine
	28.03.2023	Finished batch from sampling 1
Gut content - sampling 2	31.03.2023	80 bags of gut content in the drying machine
	03.04.2023	40 bags of gut content in the drying machine
	11.04.2023	Finished batch from sampling 2
Gut content - sampling 3	18.04.2023	80 bags of gut content in the drying machine
	20.04.2023	40 bags of gut content in the drying machine
	24.04.2023	Finished batch from sampling 3
Gut content - sampling 4	05.06.2023	80 bags of gut content in the drying machine
	07.06.2023	40 bags of gut content in the drying machine
	09.06.2023	Finished batch from sampling 4

2.5 Calculations

Daily feed intake as % of body mass (BM) was calculated as:

$$\%\ of\ body\ mass = \frac{Feed\ intake\ (kg)}{Body\ mass\ (g)} * 100\%$$

(Skretting AS, 2012).

Condition factor – k-factor

The condition factor (K) is a measurement used to determine health, energy allocation and well-being of the fish, ranging from excellent >1,6 to poor <0,9 (Froese, 2006).

$$K = \frac{100 * W}{L^3}.$$

Where *W* is the weight in gram (g) and L is the fork length in centimetres (cm).

Feed conversion ratios (FCR)

Feed conversion ratio (FCR) is a parameter expressing the relationship between amount feed given and how much the fish grow (Folkedal et al., 2022).

The FCR can be calculated as economic FCR, which includes the feed input and fish output (Folkedal et al., 2022). Economic FCR (eFCR) was calculated using the following formula:

$$\frac{Feed\ (kg)}{\Delta Biomass} = \frac{Feed\ T2 - Feed\ T1}{(W2 * number\ of\ fish\ at\ T2) - (W1 * number\ of\ fish\ at\ T1)}$$

Where *Feed* is the total fed amount between *T1* and *T2*, and *ΔBiomass* is the estimated biomass increase based on *W1* and *W2* times the number of fish alive at *T1* and *T2*.

The biological feed factor is the amount of feed used per kilogram of fish alive at *T2* (Folkedal et al., 2022). Biological FCR (bFCR) was calculated with the following formula:

$$\frac{Feed\ (kg)}{(Biomass\ T2 + Biomassmorts) - Biomass\ T1} = \frac{Feed\ T2 - Feed\ T1}{(Biomass\ T2 + Biomassmorts) - Biomass\ T1}$$

Where *Feed* is the total fed amount between *T1* and *T2*, and *Biomass T1* is the estimated biomass at start and *Biomass T2* is estimated biomass at the end of period. *Biomass morts* is the estimated total biomass of mortalities.

Specific growth rate (SGR)

Specific growth rate (SGR) is used to express growth by calculating the percentage increase in fish weight per day between size sampling time points (Mattila et al., 2009).

$$SGR = (ln(W_i) - \ln(W_0)) * \frac{100}{t(d)}$$

where W_0 is start weight, W_i is ending weight and t(d) is the number of days between start and end date.

Mortality rate

Mortality rate as percent of number of fish (Fish Farm Feeder, 2021).

$$\frac{Mortality\ (number\ of\ fish)}{Total\ number\ of\ fish} * 100\ \%$$

Hepatosomatic index (HSI)

HSI is the calculation of the liver mass as a proportion of the total body mass (Chellappa et al., 1995).

$$HSI\ = \left(\frac{Liver\ weight\ (g)}{Total\ body\ weigth\ (g)}\right) * 100$$

Gonadosomatic index (GSI)

GSI is the calculation of the gonad mass as a proportion of the total body mass (Martinez et al., 2021).

$$GSI\ = \left(\frac{Gonad\ weight\ (g)}{Total\ body\ weight\ (g)}\right) * 100$$

Stomach fullness index (SFI)

Gravimetric methods analyse the stomach content by measuring stomach filling (based on dry matter) relative to body size (Hyslop, 1980). Midgut fullness index (MFI) and hindgut fullness index (HFI) are calculated by the same approach. SFI, MFI and HFI was both calculated for dry weight and wet weight.

$$SFI\ (\%) = \frac{Stomach\ gut\ content\ (g)}{Total\ body\ weight\ (g) - \ (stomach + midgut + hindgut\ content\ (g))} * 100$$

$$MFI\ (\%) = \frac{Midgut\ content\ (g)}{Total\ body\ weight\ (g) - \ (stomach + midgut + hindgut\ content\ (g))} * 100$$

$$HFI\ (\%) = \frac{Hindgut\ content\ (g)}{Total\ body\ weight\ (g) - \ (stomach + midgut + hindgut\ content\ (g))} * 100$$

2.6 Statistical analysis

The statistical analyses were done using GraphPad Prism 9.1.0. (GraphPad Software, La Jolla, CA, United States) and Microsoft Excel version 18.2306.1061.0 (Microsoft 365 MSO, Redmond, WA, United States). Microsoft Excel was used to make the figures.

Before statistical evaluations all data was tested for normality and equal variance using the D'Agostino-Person test and F-test ratio, respectively. Grubb's outlier test was run prior to statistical evaluations. Comparisons between groups was carried out using two-way ANOVA test with time and feeding regime as explanatory variables. A post-hoc Sidak's multiple comparisons and paired t-test was used to analyse differences between the experimental groups. All values are presented with a ± standard derivation (SEM), and the level of significance was set to 0.05 ($* p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001$).

3. Results

3.1 Fed amount

During the pre-intermittent fasting period, from experimental day 0 – 9, cages were under the same feeding regime and showed very similar level of feed amount (% of daily estimated fish biomass) ($p = 0.5861$) (Figure 11A). During the intermittent fasting period thee fed amount of the intermittently fasted fish increased over time and peaked at Day 30 (experimental day 39) when they were fed 1.47 % of their estimated biomass. In comparison, the control group showed a stable average daily feed intake of ~0.6% of their biomass over the same period (Figure 11A) which was consistently lower than the intermittent fasting group ($p < 0.0001$). Following the intermittent fasting period, both treatment groups were fasted over 3 days (experimental Day 49-51) to compare appetite after fasting, where the intermittent group was fed 1.16 % which was significantly higher than the 0.73% of the control ($p < 0.0001$) (Figure 11A and B).

The following two days (Day 52-53), as the fasted group where give feed at a daily basis (refeeding), their mean fed amount was similar to control levels before it increased and persisted above 120% of control levels the subsequent 9 days and remained higher (~110%) until the end of the experimental period (Figure 11B). Different to the intermittent fasting period when the daily biomass was estimated from recorded weight before and after the period, the daily biomass for the refeeding period was based on recorded start weight and daily feed input as the final sampling for fish size showed unexpected high variation between cages (see section 3.2 below).

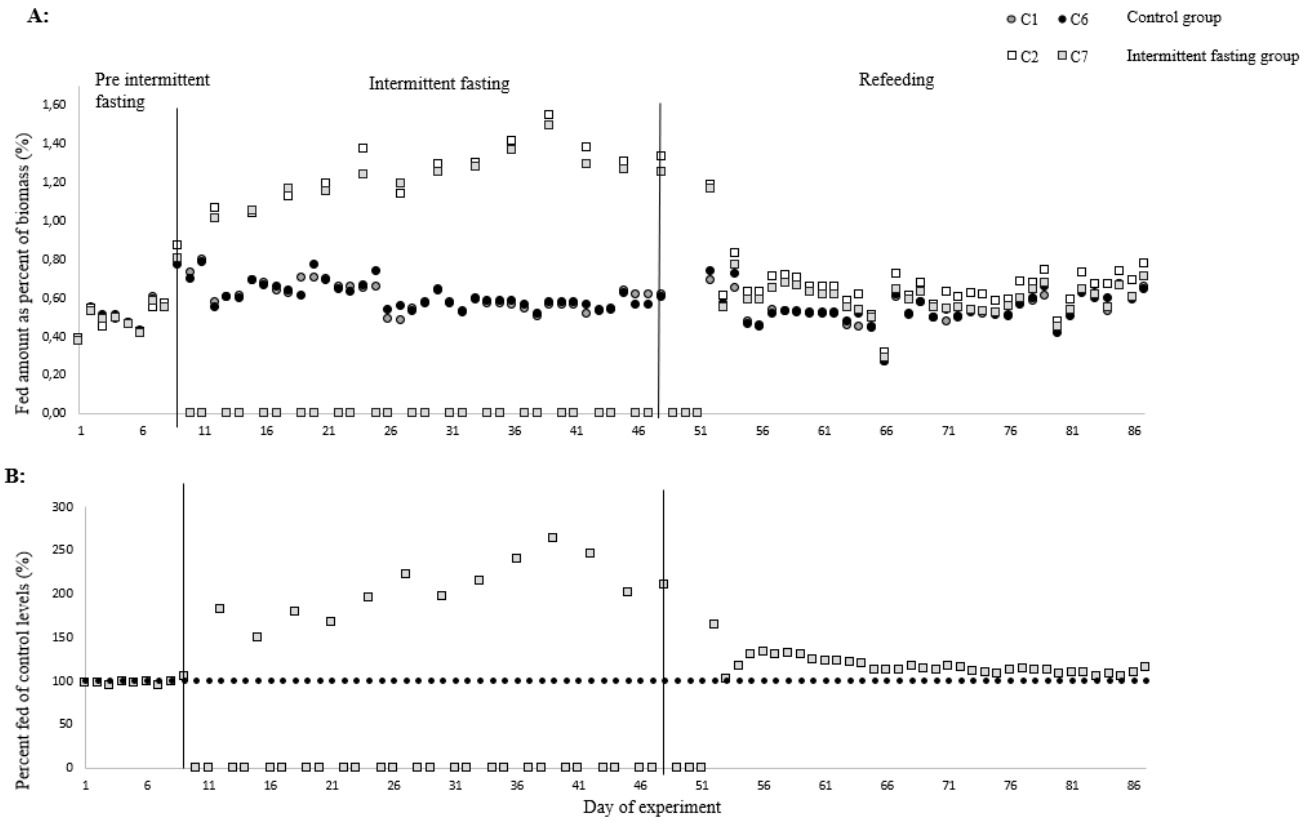

Figure 11 -

A: *Daily fed amount as percent of biomass (%) for the intermittent fasting group (cages 2 and 7: C2 and C7) and the control group (cages 1 and 6: C1 and C6), over the whole experimental period. All cages were fully fed from experimental day 0 – 9, followed by intermittent fasting treatment from experimental day 10 – 48, before all cages were fully fed from experimental days 52 – 87. At experimental days 49-51 all cages were fasted to give a comparison of appetite at Day 52.*

B: *Percentage relationship between fed amount (% biomass per day) for mean values of control cages and mean of intermittent cages over the whole experimental trial.*

Relative to the fed amount to the control group summed up over three days, corresponding to the two fasting days and day with feeding in the fasted group, the fed amount for the fasted fish was ~50% of control levels for their first feeding day under the intermittent regime (Figure 12). Using this parameter, the fasting group showed a linear increase relative to the control fed amount over the first month of intermittent fasting (multiple R^2=0.94, p<0.0001) (Figure 12). After the peak at 92 % found at intermittent fasting day 30 (experimental day 39), the levels decreased and was on average similar to the control levels after 21 days of the fasting regime (~75% of the control) (Figure 12).

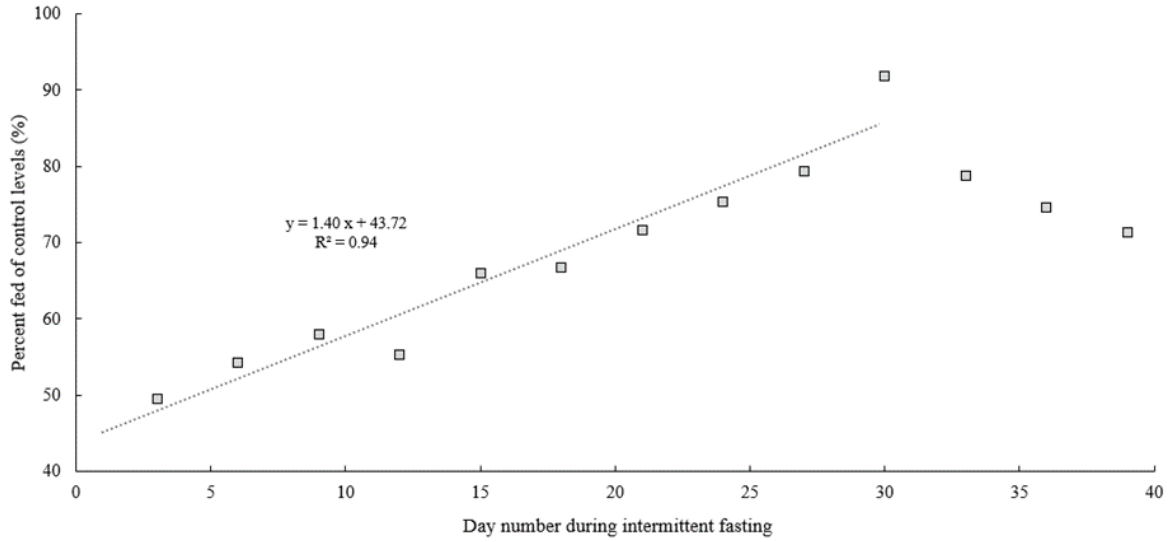

Figure 12 - *Fed amount according to appetite in intermittently fasted salmon (fed every third day) relative to sum of periodically fed amount in control salmon fed daily (sum over 3 days, including the two previous days and day of feeding for the fasted group).*

Between the two first size samplings, including the intermittent fasting period, the accumulated fed amount per cage biomass was 73% of control levels in the fasted group, and 68% when only accounting for the 43 days of intermittent fasting (Figure 13). As the cages showed some difference in biomass at the initial sampling (both size and n fish per cage (Tabel 1)) and the fasted group showed a much lower SGR than the control during fasting (see section 3.2 below), the absolute quantity (kg) of reduced feed use from intermittent fasting cannot be directly drawn from between group difference in kg fed. However, by applying the mean SGR of the control group to the estimated start biomass of the intermittent fasting group, the quantity of feed required to obtain the mean bFCR of the control group was calculated. This allows for estimation of feed use if the intermittent fasting group was fully fed, in line with the control.

Accumulated between the two first size samplings, the mean recorded feed use for the intermittently fasted cages was 68.5% of the estimated if the fish were fully fed. When only accounting for the intermittent fasting period, the recorded feed use was 65% of the estimated for fully fed fish, implying that the current fasting regime in total reduced the fed quantity used by estimated 35%.

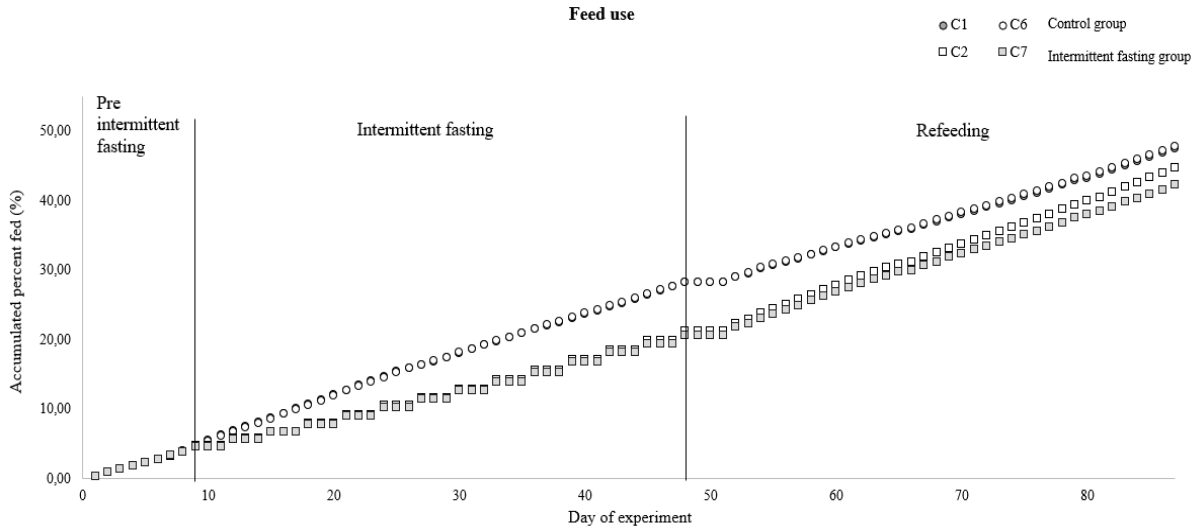

Figure 13 *– Accumulated precent fed of estimated biomass in 4 salmon sea cages, where the control group (cages 1 and 6: C1 and C6) were fully fed at a daily basis while a treatment of intermittent fasting (cages 2 and 7: C2 and C7) was given between experimental days 10 and 48 when feeding was temporally restricted to feeding to satiation every third day. All cages were fasted between day 49 and 51 for comparison of fed amount, and otherwise fully fed at a daily basis.*

3.2 Growth and development

Length (cm)

At Day 0 both groups had approximately the same length ($p = 0.5925$) and showed increased length over time ($p < 0.0001$). After the intermittent fasting period there was significant difference in length between the two groups ($p = 0.0565$), however after the refeeding period at the last fish size sampling the groups had similar lengths ($p = 0.9515$) (Table 3 and Figure 14).

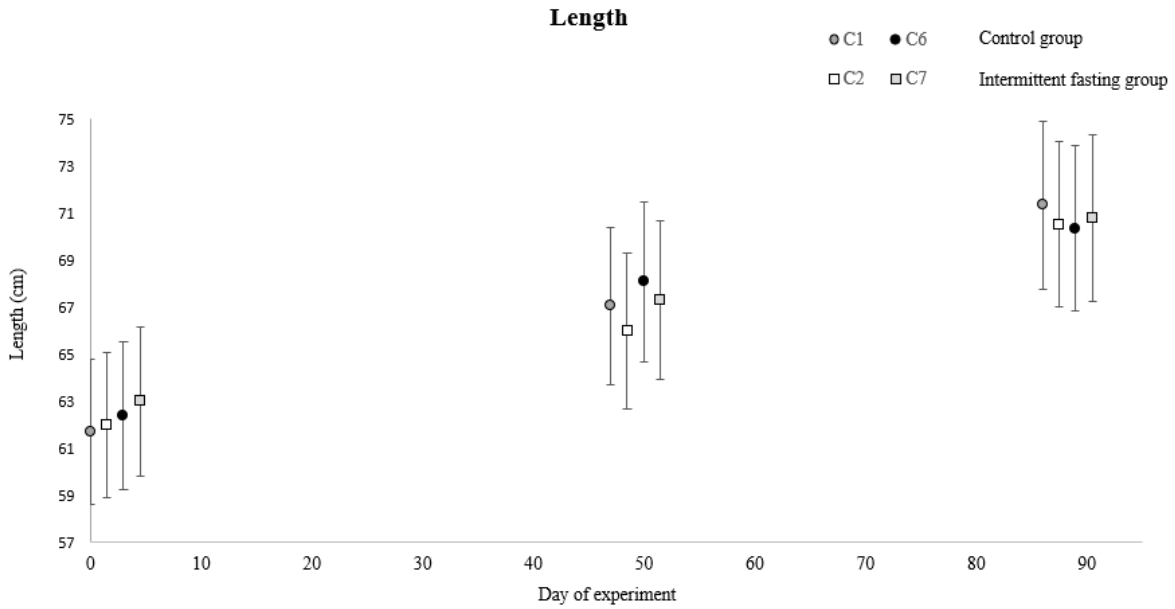

Figure 14 – *Atlantic salmon body length (cm) of the control group (cages 1 and 6: C1 and C6) that were fully daily fed, and a group that were given a regime of intermittent fasting (fully fed every third day, cages 2 and 7: C2 and C7) between days 10 to 48. The x-axis indicates experimental day number. A sample for estimation of fish size per cage (n=100 fish per cage) was collected on experimental day 0, 50 (fasting day 38) and 87 (re-feeding day 37). Made with SEM error bars.*

Weight (g)

At Day 0, both groups had approximately the same weight (p = 0.1214) (Table 3 and Figure 15) and showed increased weight over time (p<0.0001). After the intermittent fasting period the control had gained 28% of their initial weight before fasting, while the fasted group showed significantly less weight gain by 13% (p<0.0001). The weight gain between before and after 37 days of refeeding was 14% of the initial value for the control and 21% for the previously fasted group (p < 0.0001) (Table 3 and Figure 15).

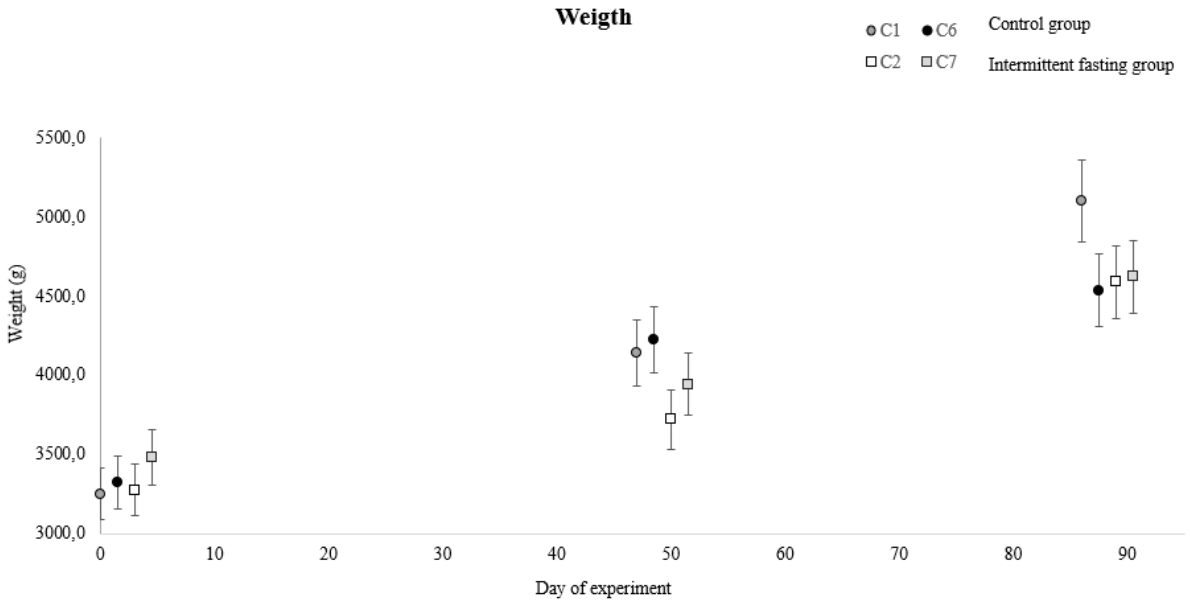

Figure 15 – *Atlantic salmon body weight (g) of the control group (Cage 1 and 6) that were fully daily fed, and a group that were given a regime of intermittent fasting (fully fed every third day, Cage 2 and 7) between days 10 to 48. The x-axis indicates experimental day number. A sample for estimation of fish size per cage (n=100 fish per cage) was collected on experimental day 0, 50 (fasting day 38) and 87 (re-feeding day 37). Made with SEM error bars.*

K-factor

At Day 0, the two groups had similar condition factor (p = 0.5515) (Table 3 and Figure 16). After the intermittent fasting period the control had lost 2% of their initial K-factor before fasting, while the fasted group showed significantly less by 7% (p<0.0001). The k-factor between before and after 37 days of refeeding was 1% of the initial value for the control and 2% for the previously fasted group (p < 0.0001) (Table 3 and Figure 16).

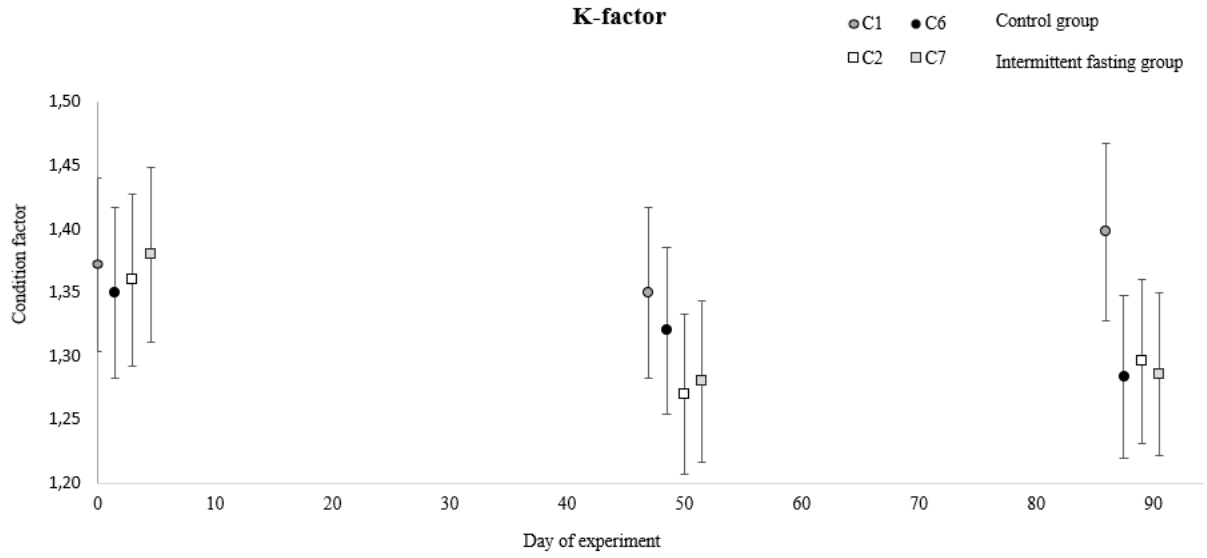

Figure 16 – *Atlantic salmon condition factor of th*e control group (cages *1 and 6: C1 and C6) that were fully daily fed, and a group that were given a regime of intermittent fasting (fully fed every third day, cages 2 and 7: C2 and C7) between days 10 to 48. The x-axis indicates experimental day number. A sample for estimation of fish size per cage (n=100 fish per cage) was collected on experimental day 0, 50 (*fasting day 38) *and 87 (re-feeding day 37). Made with SEM error bars.*

Table 3 *– Estimated fish size and growth (economical and biological FCR, and SGR) (mean ± SE) in 4 salmon sea cages at and between sampling points relative to a period of intermittent fasting (day 10 to 48 in cages 2 and 7 (C2 and C7), where cages 1 and 6 (C1 and C6) were fed at a daily basis except between days 49 and 51. Weight, length, and k-factor used to estimate the economic feed conversion ratio (eFCR), the biological feed conversion ratio (bFCR) and the specific growth rate (SGR) for the four different cages. Cage 1 and 6 was the control group and cage 2 and 7 was the intermittent fasting group. The eFCR, bFCR and SGR was calculated for the period between the first and second fish size sampling from experimental day 0 – 50, and between the second and third fish size sampling from experimental day 50 – 87. The last bottom last column shows the values for the whole experimental period, experimental day 0 – 87.*

	Control group		**Intermittent fasting group**	
Experimental day 0	C1	C6	**C2**	**C7**
Weight (g)	3251 ± 54	3321 ± 60	**3276 ± 63**	**3481 ± 58**
Length (cm)	61.7 ± 0.3	62.4 ± 0.4	**62.0 ± 0.4**	**63.0 ± 0.3**
Condition factor	1.37 ± 0.01	1.35 ± 0.01	**1.36 ± 0.01**	**1.38 ± 0.01**
Experimental day 50 (fasting day 38)				
Weight (g)	4140 ± 91	4224 ± 78	**3722 ± 85**	**3942 ± 80**
Length (cm)	67.1 ± 0.5	68.1 ± 0.4	**66.0 ± 0.5**	**67.3 ± 0.4**
Condition factor	1.34 ± 0.01	1.32 ± 0.01	**1.27 ± 0.01**	**1.28 ± 0.01**
SGR% (Day 0-50)	0.50	0.50	**0.27**	**0.26**
eFCR (Day 0-50)	1.21	1.21	**1.79**	**1.79**
bFCR (Day 0-50)	1.17	1.18	**1.65**	**1.66**
Experimental day 87 (re-feeding day 37)				
Weight (g)	5100 ± 92	4536 ± 98	**4590 ± 86**	**4650 ± 98**
Length (cm)	71.3 ± 0.5	70.4 ± 0.5	**70.5 ± 0.4**	**70.8 ± 0.4**
Condition factor	1.40 ± 0.02	1.29 ± 0.01	**1.30 ± 0.01**	**1.30 ± 0.01**
SGR% (Day 50-87)	0.56	0.20	**0.57**	**0.45**
eFCR (Day 50-87)	0.92	3.00	**1.14**	**1.40**
bFCR (Day 50-87)	0.90	2.85	**1.12**	**1.38**
Total experimental period (Day 0-87)				
SGR%	0.52	0.36	**0.39**	**0.33**
eFCR	1.08	1.71	**1.39**	**1.59**
bFCR	1.06	1.65	**1.34**	**1.53**

Economic feed conversion rate (eFCR)

The intermittent fasting group showed 9% higher eFCR than the control group over the full experimental period ($p = 0.0156$). The effect was highest during the period which included intermittent fasting (Days 0-50) when the fasted group had 32% higher eFCR than the control ($p > 0.0001$), and no significant difference during the refeeding period (Days 50-87) ($p = 0.5887$) (Table 3).

Biological feed conversion rate (bFCR)

The intermittent fasting group showed 6% higher bFCR than the control group over time (day 0-87) ($p=0.0374$). However, the intermittent fasting group showed a significant higher value (29%) during the intermittent fasting period (Days 0-50) ($p=0.0024$), and no significant difference during the refeeding period (Days 50-87) ($p=0.5951$) (Table 3).

The sampled mean fish weight at the end of the intermittent fasting period was for all cages highly consistent with modelled growth based on start weight, fed amount and table FCR for the fish size (Skretting AS, 2012), while the final sampling was not, which supports that the two first samplings to be much more reliable than the final sampling pre harvest. Harvest data of mean gutted and trimmed weight as isolated to Cage 1 was obtained for comparison, which was the only fish group kept isolated throughout transport and harvest. Given high precision of fish number (8837 in estimate vs. 8732 harvested with unknown n dead fish after wellboat pickup), a measured (harvest facility data) round to gutted weight and trimmed fish ratio of 1:0.83 and estimated weight loss over 8 days pre harvest fasting (-0.63% day^{-1}, (Einen et al., 1998)), the mean individual fish weight after their last meal (Day 86) is estimated to 4900 g, which is very similar to the modelled estimate based on sampled weight and fed amount for Cage 1 (4906 g).

Specific growth rate (SGR)

The intermittent fasting group showed a lower SGR over time (day 0-87) ($p = 0.7740$). The effect was highest during the intermittent fasting period (Days 0-50) where the SGR of the fasted fish was 25% higher than the control ($p = 0.0138$). There was no significant difference between the two groups during the refeeding period (day 50 – 87) ($p = 0.3556$) (Table 3).

Mortality

There was no significant difference in mortality rate between the two groups during the trial (day 0-87) ($p = 0.2292$).

Table 4 *– Accumulated mortality in Cage 1, 2, 6 and 7 within and over the full experimental period. The mortality is shown as the percentage of the total number of fish at Day 0. The fish euthanized for the physiological samplings is not included as mortalities.*

	Control group		Intermittent fasting group	
Mortality %	**C1**	**C6**	**C2**	**C7**
Experimental day 0 - 50	0.4	0.3	0.6	0.6
Experimental day 50- 87	0.2	0.3	0.2	0.1
Total experimental period	0.6	0.6	0.8	0.7

3.3 Metabolic state

Hepatosomatic index (HSI%)

On Day 8, the intermittently fasted and the control group showed no significant difference ($p = 0.9798$) (Figure 17). However, the intermittent fasted group showed a significant decrease in HSI over time ($p < 0.0001$) and had 11% lower HSI than the control group at Day 41 (fasting day 33) ($p = 0.0228$) (Figure 17). At the final physiological sampling there was no significant difference between the two groups ($p = 0.8927$) (Figure 17).

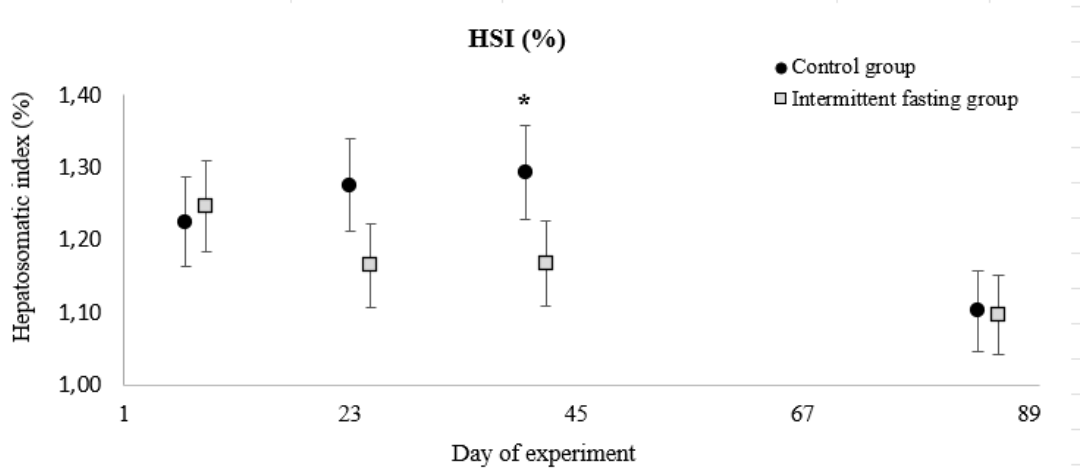

Figure 17 *– Atlantic salmon HSI of the control group (Cage 1 and 6) that were fully daily fed, and a group that were given a regime of intermittent fasting (fully fed every third day, Cage 2 and 7) between days 9 to 50. The x-axis indicates the experimental day number. A sample for physiology (n = 20 fish per group) was taken on experimental day 8, 23 (fasting day 23), 41 (fasting day 33) and 86 (re-feeding day 37). Made with SEM error bars.*

Gonadosomatic index (GSI)

On Day 8, the two female groups had approximately the same GSI (p = 0.4228) (Figure 18 A). During the trial the females showed no difference between the two groups (p = 0.5126), however both groups showed a GSI between values between 0.9 and 0.15, which is an early sign of maturation.

On Day 8, the two male groups had approximately the same GSI (p = 0.3348) (Figure 18 B) and showed no differences along the trial (p = 0.7802), however both groups showed a GSI between 0.06 and 0.1, which is an early sign of maturation.

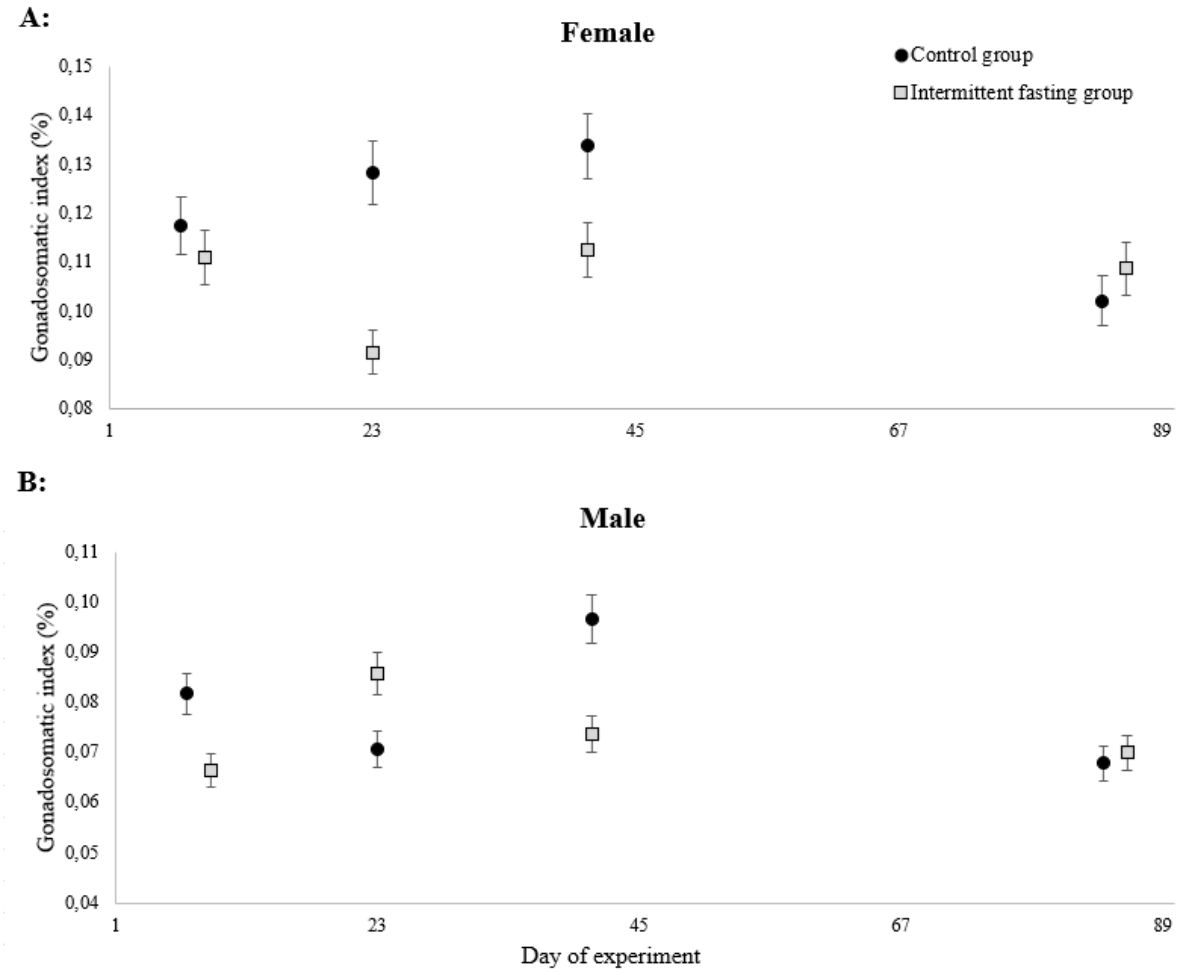

Figure 18 *– Atlantic salmon GSI of the control group (Cage 1 and 6) that were fully daily fed, and a group that were given a regime of intermittent fasting (fully fed every third day, Cage 2 and 7) between days 9 to 50. The x-axis indicates the experimental day number. Made with SEM error bars.*

A: *GSI for the female Atlantic salmon. Number of fish from the control group at Day 8 (fasting day 0) was n = 4, Day 23 (fasting day 15) n = 6, Day 41 (fasting day 33) n = 8 and Day 86 (re-feeding day 36), n = 8. Number of fish from the intermittent fasting group at Day 8 (fasting day 0) is n = 9, Day 23 (fasting day 15) n = 7, Day 41 (fasting day 33) n = 7 and Day 86 (re-feeding day 36) n = 11. The x-axis indicates the experimental day number.*

B: *GSI for the male Atlantic salmon. Number of fish from the control group at Day 8 (fasting day 0) was n = 16, Day 23 (fasting day 15) n = 14, Day 41 (fasting day 33) n = 12 and Day 86 (re-feeding day 36), n = 12. Number of fish from the intermittent fasting group at Day 8 (fasting day 0) is n = 11, Day 23 (fasting day 15) n = 13, Day 41 (fasting day 33) n = 12 and Day 86 (re-feeding day 36) n = 9. The x-axis indicates the experimental day number.*

3.4 Gastro-intestinal tract content as % of body mass

Stomach fullness index (SFI)

On Day 8, there was no significant difference in wet stomach content between the two groups (p = 0.8937) (Figure 19). The intermittently fasted group showed a significant increase in wet stomach content over time (p < 0.0001), where at Day 23 (fasting day 15) the fasted group had 34% higher SFI than the control group (p < 0.0001). This corresponds to the fed amount as percent of biomass (Figure 11A) that was 0.70%, 1.19%, 0.69% and 1.15% of cage 1, 2, 6 and 7 respectively. Further at Day 41 the SFI had stabilised were the fasted group had a SFI 41% higher than the control (p<0.0001), matching the fed amount for the cages (Figure 11A); 0.51%, 1.38%, 0.56% and 1.29% for cage 1, 2, 6 and 7 respectively. At the final physiological sampling there were no significant difference between the two groups (p = 0.4063) (Figure 19).

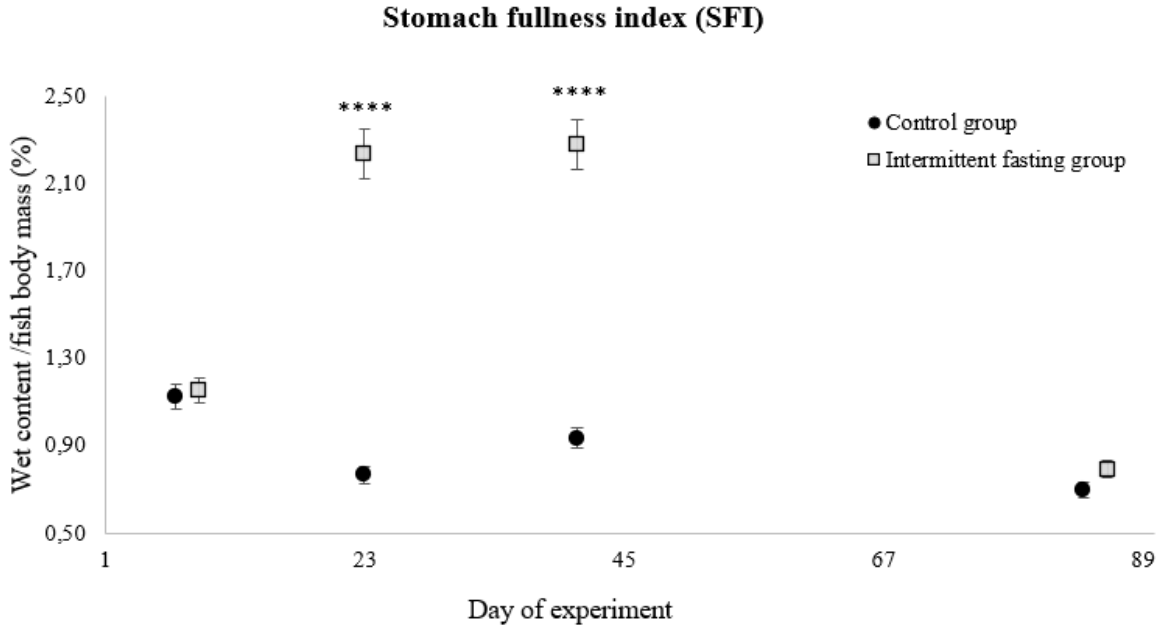

Figure 19 *– Atlantic salmon wet stomach content as percent of body mass of the control group (Cage 1 and 6) that were fully daily fed, and a group that were given a regime of intermittent fasting (fully fed every third day, Cage 2 and 7) between days 9 to 50. The x-axis indicates the experimental day number. A sample for physiology (n = 20 fish per group) was collected on experimental day 8, 23 (fasting day 15), 41 (fasting day 33) and 86 (re-feeding day 37). Made with SEM error bars.*

Midgut fullness index (MFI)

On Day 8, there was no significant difference in wet midgut content between the two groups (p = 0.8889) (Figure 20), and no significant differences along the trial (p = 0.1030). Two weeks into the intermittent fasting period, the intermittently fasted group had 10% lower midgut content than the control group (p = 0.9253) and 25% lower at Day 41 (fasting day 33) (p = 0.1665). At the final physiological sampling there were still no significant difference between the groups (p = 0.9863) (Figure 20).

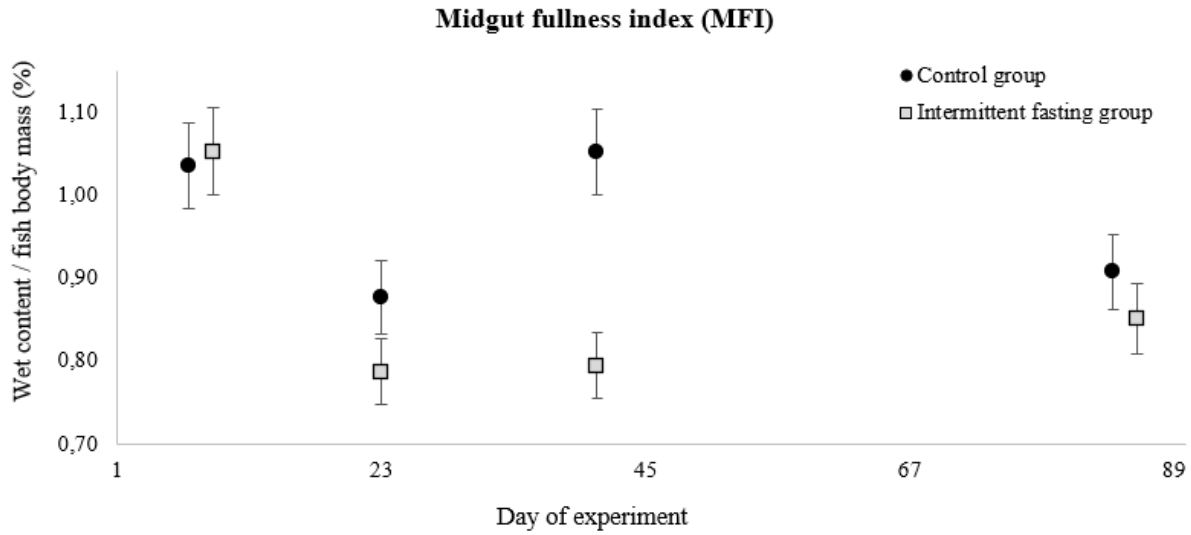

Figure 20 *– Atlantic salmon wet midgut content as percent of body mass of the control group (Cage 1 and 6) that were fully daily fed, and a group that were given a regime of intermittent fasting (fully fed every third day, Cage 2 and 7) between days 9 to 50. The x-axis indicates the experimental day number. A sample for physiology (n = 20 fish per group) was collected on experimental day 8, 23 (fasting day 15), 41 (fasting day 33) and 86 (re-feeding day 37).*

Hindgut fullness index (HFI)

On Day 8, there was no significant difference in hindgut content between the two groups (p = 0.6929) (Figure 21) and no significant difference over time (p = 0.2260). Two weeks into the intermittent fasting period (Day 23), the intermittently fasted group had 35% lower midgut content than the control group (p = 0.0569) and 46% lower at Day 41 (fasting day 33) (p = 0.0002) (Figure 21). At the final physiological sampling there was no significant difference (p = 0.8605) (Figure 21).

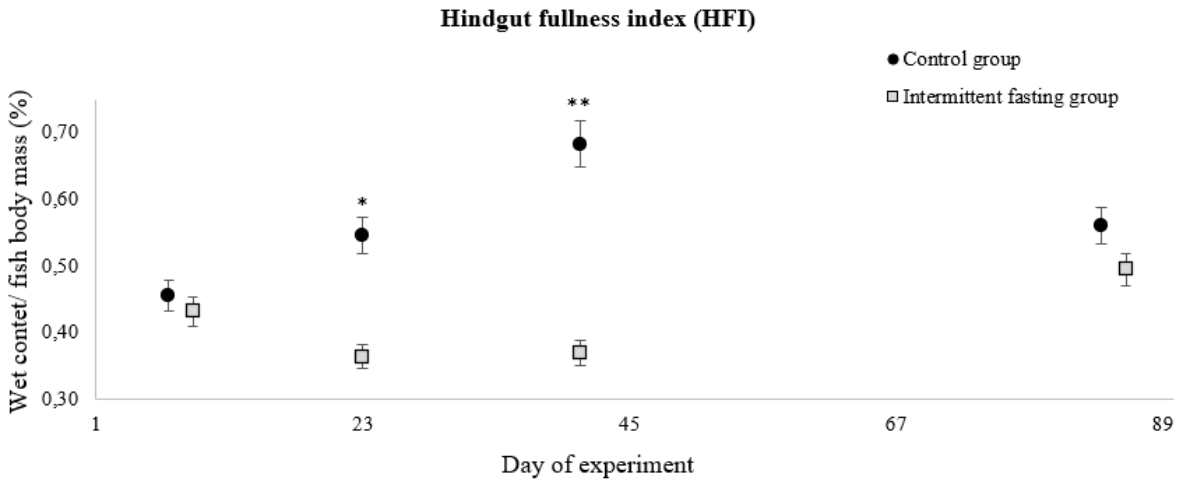

Figure 21 *– Atlantic salmon wet hindgut content as percent of body mass of the control group (Cage 1 and 6) that were fully daily fed, and a group that were given a regime of intermittent fasting (fully fed every third day, Cage 2 and 7) between days 9 to 50. The x-axis indicates the experimental day number. A sample for physiology (n = 20 fish per group) was collected on experimental day 8, 23 (fasting day 15), 41 (fasting day 33) and 86 (re-feeding day 37). Made with SEM error bars.*

4. Discussion

4.1 Discussion of materials and methods

4.1.1 Experimental design

This study compared two different feeding regimes in sea caged Atlantic salmon, a control group consisting of two cages with a daily feeding regime and one group consisting of two cages with an intermittent fasting regime. Because of a parallel experiment running at Solheim Cage Environment laboratory, there were only available four cages for this experiment. Originally it was planned to use triplicate cages for each group to obtain higher statistical power. Although this limitation hampered preferred statistical power the intermittent fasting regime was tested in a highly realistic farming situation.

Considering that the experimental trial was conducted from early spring to early summer, higher sea temperatures and longer day lengths due to seasonal changes can stimulate sexual maturation. At this time the Atlantic salmon tend to increase their growth to build energy storages that's later used for building gonads when they enter an anorectic phase (e.g. Juell et al., 1994; Taranger et al., 2010; Pino Martinez et al., 2021). The mean GSI values during this trial was 1.1 for females and 0.08 for males (Figure 18 A and B), which is an early sign of maturation when the limit is set to > 0.06 - 0.1. There were no significant differences between the control- and the intermittent fasting group, and no increase in GSI over the full study period. Some studies has shown that fasting can inhibit sexual maturation when applied during the rapid growth phase, prior to the anorectic phase (Trombley, 2014). However, this cannot be ensured from this study.

To achieve an independent variable for feed intake the experiment should ideally have been conducted under stable environmental condition (Handeland et al., 2008; Remen et al., 2013), whereas sea cage trials are prone to be affected by environmental changes and fluctuations, including effects on feeding control precision by e.g. shifting water current conditions which affect risk of pellets drift. However, experimental trials conducted in sea cages do provide a realistic approach towards identifying expected outcomes in commercial scale farming.

Food availability for the farmed Atlantic salmon depends on the cage size, feeding technology, efficiency of pellet distribution and appetite observations from the feeding control (Folkedal et al., 2022). Feeding control based on the view from a single camera is inherently difficult as the full cage volume cannot be monitored at any time, and especially when appetite in several cages are assessed simultaneously (Folkedal et al., 2022). The infrastructure for pellet distribution is key for how efficient the fish can be fed, where the currently used feeding system was rather inefficient as it severed fish in 10 cages using one single feeding line. This implies that if cages are to be fed simultaneously in meals, they are fed in batches where the fed amount per batch is dependent on number of cages to be fed within the same scheduled time slot. Here it was key to adapt the feeding schedule to attain the highest

possible batch frequency and thus temporal distribution which resulted in batch sizes that the fish easily could consume (Flood et al., 2012).

4.1.2 Samplings

For estimation of fish size distribution within groups a sample size of 100 fish per cage was used, giving n = 200 for each treatment group. Representative size sampling of caged Atlantic salmon can be difficult, as shown for the effect of sample size on sample accuracy and precision for fish weight and condition factor; estimates may be wrong even when sampling a large proportion of the fish group (Nilsson & Folkedal, 2019). Pragmatically, hundred fish is commonly sampled in sea cage studies, where Nilsson and Folkedal (2019) in their example of large, caged salmon showed that the mean weight of the hundred first sampled fish did not deviate much from the true mean of all 5600 individuals, but that the condition factor of the hundred first was significantly higher than the true mean.

From own experience working as production planner in Seaborn AS, the fish farmers estimation of pre harvest Atlantic salmon size and quantity at cage level often deviate from true figures reported by harvest facilities. Under harvest of fish groups from commercial cages, the mean fish size and distribution commonly stabilises after approximately 20 tonnes slaughtered (approximately 4% of the total number of fish from an average large sea cage), implying a sampling bias also at commercial scale (T. Olsen, Seaborn AS, pers. comm.). Studies have shown that commercial salmon production needs to improve the precision and accuracy in stock estimations, based on common errors in number estimation resulting in inverse errors in mean weight (Aunsmo et al., 2013).

The sampled mean fish weight at the end of the intermittent fasting period was highly consistent with modelled growth based on start weight, fed amount and table FCR for the fish size, while the final sampling was not. Between the first and second fish size sampling the measured growth and recorded feed use for the control group gave FCR which is close to the FCR value for the size span in the Skretting growth table (Skretting, 2012), and the cage groups given intermittent fasting were similar in growth and condition factor (Table 3). This supports that the two first samplings were reliable, while the last sampling was not. The cause for this apparent inconsistency between samplings is largely unknown, and it may be speculated that size or environmental driven vertical size segregation of the fish changed during the last month of the experiment. Strong vertical size segregation has been shown for large salmon in the very same cages as used in this study (Folkedal et al., 2012; Nilsson et al., 2013), and the current casting net hauls may have been biased by such.

Using the estimated mean final weight for Cage 1 (4900 g) its SGR value is 0.39 which corresponds to the SGR estimated for the whole experimental period for Cage 2 and 7 (Table 3). Looking at the SGR for Cage 6 during the refeeding period, the last sampling was probably underestimated based on the much lower SGR compared to Cage 2 and 7, and the SGR for Cage 1 based on harvest data. The

inequality can be caused by skewed sampling for condition factor, due to low sample sizes and cannot fully be trusted (Nilsson & Folkedal, 2019).

For the physiological sampling, a higher sample size than the currently used n=10 fish per cage would naturally have increased the statistical power. The physiological samplings were labour and time consuming due to all the different recorded parameters needed in the experiment (blood, brain, pituitary, liver, heart, spleen, gallbladder, tissue from stomach, midgut and hindgut, gut content). The sampling required eight people from UiB and at least three people from the IMR to conduct the sampling carefully and efficiently. The decision for 20 individuals per group was made with regard to available labour, money, and time available.

The selection of individuals for the sampling was achieved by lowering a casting net into the cage, before pulling it and carefully crowding the caught fish into one side of the cage. Aiming to catch a representative selection from the haul, individuals were randomly taken out using a dip net. By performing a normality test of the data collected it showed that the number of individuals selected were representative for the whole group, as its variation in size was similar to that found in the large sample size for size estimation. However, the data would have been more valid if it was possible to increase the sampled number.

Loss of gut content did occur with some digestive tracts that were fully loaded with content. When separating the stomach, midgut, and hindgut it was sometimes difficult not to spill any gut content or when trying to lift the intestine for collecting the content, it could rip. However, the collection of gut content required concentration which made it easier to see which part of the intestine the spilled content occurred from. The leaked spillage was collected with the scalpel and placed into the correct bags.

4.1.3 Selection of data

The gut content consists of feed and gastric acid, however the wet content was highly correlated to the dry content. Furthermore, wet feed weight is also what is used in aquaculture growth tables. Gravimetric measurement gives a reasonable estimate of bulk of wet gut content and are easier to perform than volumetric techniques (Hyslop, 1980). Therefore, the dry gut content was not shown as a result (It is included in the appendix for reference purposes).

4.2 Discussion of the results

4.2.1 Fed amount

A central aim of the study was to investigate whether adaptation to intermittent fasting occurred over time with regards to fish appetite. A linear increase in fed amount over time until a peak of 1.47% of biomass on day 30 of the regime was found, while the control group showed stable appetite with a daily fed amounts of ~0.6% of their biomass (Figure 11A). The gradual increase is in line with

previous studies on intermittent fasting in other farmed fish species (Johnsen et al., 2013; Mattila et al., 2009; Tunçelli & Pirhonen, 2021), where the current study is the first demonstration such in Atlantic salmon. Tuncelli and Pirhonen (2021) fasted rainbow trout during the weekends, which resulted in increased feed intake after two weeks and compensational growth after four weeks. The same response is showed in Mattila et al., (2009), where the intermittently fasted juvenile pikeperch compensated for less feeding by increased feed intake over time. This study lasted over eight weeks, where one group were fed each second day, one group each fourth day and one group each seventh day. All treatments groups combined showed a significant linear relationship between the two variables ($R^2 = 0.995$) (Mattila et al., 2009), corresponding to the linear increase found in our study ($R^2 = 0.94$, Figure 12). The current intermittent regime has previously been recommended for use in the industry to save feed during periodic feed shortage, but its outcome for feed saving has largely been unknown. The presently observed adaption rate to intermittent fasting and the level of feed intake is an important finding, showing that large salmon under the current temperature reduced their feed use by ~40% over the first 21 days of the feeding regime, and after 43 days when presumably fully adapted to the regime, as a reduction of fed quantity (~35%) was found relative to the control group (Figure 12, 13 and 14). In salmon aquaculture, fish feed is the highest production expense (Iversen et al., 2020) and finding a way to reduce feed quantity, maintain fish growth and good fish welfare will be attractive for the farmers. Further reduced feed quantity can possibly reduce the discharges of organic matter around the net pens (E. L. Grefsrud et al., 2023), however, underfeeding is not sustainable either. Amount of feed waste as effect of feeding regime was, however, not registered for in the present study.

The level of compensation/adaption to intermittent fasting will naturally vary with the frequency of meals, where 3 days as currently tested is apparently to seldom for large salmon to fully adapt a full feed intake under; as shown, the fed amount levelled at about 75% of the control levels accumulated over 3 days. More frequent feeding as e.g. every second day, as previously been shown as sufficient to maintain control growth rates in caged Atlantic salmon during winter (Johnsen et al., 2013), would most probably given a higher total fed amount if applied in the current study, and perhaps a faster adaption rate. Other than the temporal distribution of meals, factors such as fish size, water temperature (metabolic rate) and thus gut evacuation rate should also be considered for the efficiency which fish feed under intermittent regimes.

At refeeding (daily feeding in all cages) starting on Day 52, both groups had been given a three-day fasting period (days 49-51) to enable comparison between the group experienced with fasting and the control, similar to the test used by Känkanen, et al. (2009) on juvenile whitefish (*Coregonus lavaretus*). The study fed one group every second day and one group only during weekdays, compared to a daily fed control group. After the intermittent fasting period all groups were fasted 3 days before daily refeeding, where the two fasted group ate significantly more (1.16% of their body mass)

compared to the control (0.73% of their body mass) (Figure 11A). (Känkänen & Pirhonen, 2009). In the present trial the fasted group showed a much higher appetite than the control (>150% of control level) (Figure 11B), which demonstrate the effect of adaption to temporally restricted feeding in the intermittent fasted group. The following two days the mean fed amount of the fasted group was like that of the control, which indicate maximum feed intake in both groups. After this the fed amount of the fasted group persisted above 120% of control levels the subsequent 9 days and remained higher (~110%) until the end of the experimental period (Figure 11B). This suggest a long-term compensational effect on growth as previously observed after long-term fasting in Atlantic salmon (Hvas et al., 2021).

4.2.2 Fish growth

In line with lower fed amount to the intermittently fish, they grew significantly less than the control as fed to satiation on a daily basis. While the intermittently fed fish were between the two first size sampling points fed 69.5% of their estimated feed use to obtain the same SGR and bFCR as recorded in the control, their SGR was only 0.52% of the control level. This is reflected in the much higher calculated FCR in the fasting group (bFCR 1.65) during this period and should imply that a larger proportion of the consumed feed was used for bodily maintenance in the fasting group than in the control. In terms of production efficiency and farming economy, this FCR would over time be detrimental. The bFCR for the control between the two first size sampling was 1.17 and 1.18, for the two respective cages growing from mean sample fish weight of 3275 to 4139 g and 3481 to 4223 g during the period, where expected FCR is 1.06 to 1.14 over this weight span (Skretting, 2012). This indicates some overfeeding in the control group, which should also apply to the intermittently fasted fish. However, it suggests that the fish were not systematically underfed under the present feeding control.

The mean eFCR in 2021 in Norway over full sea production is estimated to 1.27 (Fiskeridirektoratet, 2022), suggesting that the present FCR, as based on a short period for large fish, were better for the control group and worse for the intermittent fasting group. FCR is an important production measure since feed costs are of great importance for the production costs. The lower the FCR, the lower the feed costs, and more fish can be produced using the same amount of feed. If the post intermittent fasting period (compensatory growth period) had continued for a longer time, the feed conversion ratios may have been more equal between the two groups.

The condition factor in healthy farmed Atlantic salmon is between 0.9 – to 1.6 where 0.9 indicates emaciation and 1.6 is normal around slaughter weight (Froese, 2006). At the end of the intermittent fasting period the condition factor (experimental day 50) for the control group was 5% higher than the intermittently fasted group. The condition factor for the intermittent fasting group dropped severely in comparison to the control group. Atlantic salmon farmers are gaining interest in intermittent fasting

due to acceleration of growth after a fasting period (Py et al., 2022), such were indicated by the higher fed amount in the intermittent fasting group when refed. However, the compensational effects cannot fully be trusted due to the apparent sampling bias for the final size sampling.

Harvest data of mean gutted and trimmed weight as isolated to Cage 1 was obtained for comparison, which was the only fish group kept isolated throughout transport and harvest. Given high precision of fish number (8837 in estimate vs. 8732 harvested with unknown n dead fish after well-boat pickup), a measured (harvest facility data) round to gutted and trimmed fish ratio of 1:0.83 and estimated weight loss over 8 days pre harvest fasting (-0.63% day^{-1}, (Einen et al., 1998)), the mean individual fish weight after their last meal is estimated to 4900 g, which is very similar to what estimated for Cage 1 (4906 g). This renders a true comparison in size and growth parameters dubious based on the final size sample but suggests the previous size estimation to be fair for calculations of fed amount during the refeeding period.

4.2.3 Metabolic state

An aim was to investigate whether the intermittent fasting regime affect the energy storage in terms of liver size relative to body mass (HSI) and gonad size relative to body mass (GSI). After 14 days into the intermittent fasting period, the intermittent fasting group had a lower HSI compared to the control group (Figure 17) and at experimental day 41 (fasting day 33), the intermittently fasted group had 11% lower HSI compared to the control group (Figure 17). This corresponds well with the food deprivation as apparent from amount fed (Chellappa et al., 1995; Bar, 2014; Hvas, 2022) and poorer FCR compared with the control. At the last physiological sampling the HSI in was similar for both groups, indicating that the energy loss was compensated after returning to daily feeding regime.

In this study there were no significant differences in GSI values between the two groups, however both sexes showed sign of early maturation with GSI > 0.06 - 0.1 (Figure 18A and B). Rapid growth during spring in Atlantic salmon can indicate maturation, where the salmon reserves energy before entering an anorectic phase where energy storages are used for building gonads during the summer months (Hevrøy et al., 2012; Good et al., 2017). The gonads will then start to increase in size, which did not occur during this trial where the GSI was stable for the whole experimental period.

4.2.4 Gut fullness index (GFI)

One of the aims were to investigate whether and how much the fasting group adapt their feed intake, measured as individual fish gut fullness, and how such is affected by return to daily feeding. Both groups started the experimental trial with similar amounts of wet stomach content at the first physiological sampling (experimental Day 8), and at Day 23 and Day 41 there were significant differences between the two groups (Figure 19). Even though the average stomach fullness index (SFI) was similar between the groups, the SFI varied between sampled individuals. The highest SFI at sampling Day 23 (fasting day 15) was 3.94% and the lowest was 0.99%. At Day 41 (fasting day 33)

the highest was 6.65% and the lowest was 0.77% in the fasted group, where one of the sampled fish also had an empty stomach. These observed maximum values highlights the capacity which salmon have for feed intake during a single meal. At the last sampling (Day 86) the highest SFI was estimated to 1.49% and lowest at 0.35%, which was similar to the control group (Figure 20), however 3 of the individuals had an empty stomach in the fasted group and none in the control group. One of the sampled fish with no gut content had very pale colour at the flesh and organs, inflamed tissue, and clear plasma, indicating it was sick. However, the 2 other sampled fish with no stomach content looked healthy and supports the theory that these individuals had been lower ranked in the hierarchy due to more feeding competition in the group.

There was a correlation between fed amount (Figure 11A) and SFI (Figure 19), where the SFI increased corresponding to an increased fed amount during the intermittent fasting period (1.47% of their body mass), and decreased amount during the refeeding (0.66% of their body mass) (Figure 11A). This corresponds to the biology for carnivore fish with a stomach capacity that enables to expand if needed to eat larger meals, and their natural instinct to eat when the feed is available (Flood et al., 2012; Mills, n.d.). Several studies of intermittent fasting on different species has showed a progressive increase in feed intake over time to compensate for lost growth during days without feeding, often in line with increased stomach capacity facilitating larger intake and growth compensation after refeeding (Känkänen & Pirhonen, 2009; Mattila et al., 2009; Tunçelli & Pirhonen, 2021).

At the final physiological sampling (experimental day 86), the control group had 0.09 (in % of body weight) lower for gut filling value than the intermittent fasting group, indicating that the intermittent fasting group had adapted to the daily feeding regime (Figure 19). The compensational effects on weight gain were not fully accounted for in this study, but the intake indicates long term compensation. The wet stomach content samples during the intermittent fasting periods indicates an adaption in feed availability of stomach size to the temporally restricted feed availability, even though stomach size adaption is not accounted for in this study. An intriguing question is whether learning to consume feed when available is a vital part of this adaption, but difficult to answer from the parameters recorded in this study.

There was no significant difference in the midgut wet content observed between the control and intermittent fasting group along the trial, however, 33 days into the intermittent fasting period the control group had 25% lower value than the intermittent fasted group (Figure 20). The hindgut fullness index was after 14 days (experimental day 23) and 33 days into the intermittent fasting period (experimental day 41) significant lower in the fasted group (35% and 46%) compared to the control (Figure 21). Since the control group were fed daily, they presumably had mid- and hindgut content from yesterdays and the same day's feeding, when the intermittent fasted group mid- and hindgut

content was less present. According to Handeland et al. (2008) the mid- and hindgut are evacuated after 2 days (48 hours) at 13°C. This means that the mid- and hindgut content in the fasted group was processed feed from the same day.

4.2.5 Fish welfare

Based on the fed amount (Figure 11) and the GFI (Figure 19, 20 and 21), the salmon showed adaption by adjusting their feed intake to increased (intermittent fasting regime) and decreased (re-feeding regime) interval between meals, reflecting the species adaption to their as their natural environment with temporal variability in feeding opportunity (Flood et al., 2012; Mills, n.d.). Farmed Atlantic salmon are used to having excessive amount of feed available at a daily basis and may be stressed by feed withdrawal (Vindas et al., 2016). On the other hand, wild Atlantic salmon are predators that eats when the food is available and will experience periods where it's difficult to locate or catch food (Utne et al., 2021). The currently observed flexibility in feed intake of the fish along with normal growth rate over the full experimental period support that the treatment was within the scope of what Atlantic salmon is adapted to cope with, and that fish welfare was not compromised.

A sensation of hunger seems to occur in Atlantic salmon as indicated by ghrelin and leptin increase during food withdrawal (Kullgren et al., 2013; Kalananthan et al., 2023). Studies have shown that *ghrl1* decreases during long-term fasting (weeks) indicating that the salmon adapt their feeling of hunger (Hevrøy et al., 2012; Guillot et al., 2016). Such should be beneficial for fish welfare during long-term fasting periods (Hevrøy et al., 2012) by easier facilitating priority of other motivational systems than hunger in order to guide behaviour and ultimately promote survival, as e.g. by avoiding predators and optimizing environmental conditions. For the much shorter time scale of fasting in the present study, increased appetite was evidently advantageous to maximize feed intake, when possible, although a feeling of hunger was apparently present. It is, however, highly difficult to interpret whether a poikilotherm animal as an Atlantic salmon finds this highly aversive or not. Besides the physiological adaptations which occur under long-term fasting, Atlantic salmon may require weeks to recover appetite after refeeding (Hvas et al., 2021), which signals a rather high cost, and was not observed during refeeding in the present study.

As shown by a decreasing HSI value, the fasted fish consumed parts of their stored energy but did apparently not deplete their energy storage (Figure 17). Even though the intermittent fasted group may have felt hunger, the experimental fish where during the 6 weeks of intermittent fasting able to cover their daily energetic needs and compensated by eating more when the feed was available. Although they grew less than the control, I assume that the fish welfare was not compromised.

The mortality rate was low for the whole experimental period (experimental day 0-87) and were similar between all the cages, also during the intermittent fasting period (Table 4). the low mortality

rate indicates that the fasting regime did neither compromise welfare among the individuals in the worst possible extent (Noble, Nilsson, et al., 2018).

5. Conclusion

During the first 30 days of intermittent fasting there was a progressive increase in feed intake and the total fed amount for the fasting period was estimated to 65% of the quantity fed in the control. SGR and bFCR was respectively 52% and 141% of control levels, supporting that the intermitted fasted fish used a higher proportion of their feed intake on bodily maintenance. That was also showed by the fact that intermittently fasted group had 11% lower HSI than the control after 43 days of intermittent fasting. During refeeding the fed amount for the previously fasted fish was ~120% of control level the initial 7 days and then remained elevated at ~110% for the subsequent 30 days of the study. The SFI increased in line with the fed amount during the intermittent fasting period and decreased during refeeding period, indicating adaption to the different feeding regimes. The GSI showed early sign of maturation, however no difference was found between the treatment groups. This study shows that a regime of intermittent fasting by full feeding every third day will save feed, but hamper fish growth and feed utilization which the fish will require a considerable time to compensate from. In terms of fish welfare, no apparent negative effects from the fasting regime were found, suggesting the current regime as viable during periods of food shortage or flesh quality adjustment prior to harvest.

6. References

Aabakken. (2020). Fordøyelsessystemet. *Store Norske Leksikon*. https://sml.snl.no/ford%C3%B8yelsessystemet

Aarnes, H. (2004). *Atferdsbiologi*.

Aas, T. S., Sixten, H. J., Hillestad, M., Sveier, H., Ytrestøyl, T., Hatlen, B., & Åsgård, T. (2017). Measurement of gastrointestinal passage rate in Atlantic salmon (Salmo salar) fed dry or soaked feed. *Aquaculture Reports*, *8*, 49–57. https://doi.org/10.1016/j.aqrep.2017.10.001

Aas, T. S., Sixten, H. J., Hillestad, M., Ytrestøyl, T., Sveier, H., & Åsgård, T. (2020). Feed intake, nutrient digestibility and nutrient retention in Atlantic salmon (Salmo salar L.) fed diets with different physical pellet quality. *Journal of Fisheries*, *8*(2), 768–776. https://doi.org/10.17017/j.fish.133

Agarwal, N. K. (2008). Fish reproduction. *APH Publishing.*

Albrektsen, S., Kortet, R., Skov, P. V., Ytteborg, E., Gitlesen, S., Kleinegris, D., Mydland, L., Hansen, J., Lock, E., Mørkøre, T., James, P., Wang, X., Whitaker, R. D., Vang, B., Hatlen, B., Daneshvar, E., Bhatnagar, A., Jensen, L. B., & Øverland, M. (2022). Future feed resources in sustainable salmonid production: A review. *Reviews in Aquaculture*, *14*(4), 1790–1812. https://doi.org/10.1111/raq.12673

Alfredo I., & Stanger, B. Z. (2015). Organ-Size Regulation in Mammals. *Cold Spring Harbor Perspectives in Biology*, *7*(9), a019240. https://doi.org/10.1101/cshperspect.a019240

Armstrong, J. B., & Schindler, D. E. (2011). Excess digestive capacity in predators reflects a life of feast and famine. *Nature*, *476*(7358), 84–87. https://doi.org/10.1038/nature10240

Asche, F., & Oglend, A. (2016). The relationship between input-factor and output prices in commodity industries: The case of Norwegian salmon aquaculture. *Journal of Commodity Markets*, *1*(1), 35–47. https://doi.org/10.1016/j.jcomm.2015.11.001

Åsvold, B. O. (2023). *Insulin* [Leksikon]. Store Norske Leksikon. https://sml.snl.no/insulin

Aunsmo, A., Krontveit, R., Valle, P. S., & Bohlin, J. (2014). Field validation of growth models used in Atlantic salmon farming. *Aquaculture*, *428–429*, 249–257. https://doi.org/10.1016/j.aquaculture.2014.03.007

Aunsmo, A., Skjerve, E., & Midtlyng, P. J. (2013). Accuracy and precision of harvest stock estimation in Atlantic salmon farming. *Aquaculture, 396–399*, 113–118. https://doi.org/10.1016/j.aquaculture.2013.03.001

Azevedo, P. A., Bureau, D. P., Leesons, S., & Cho, C. Y. (2017). *Estimated content of nutrients and energy in feed spill and faeces in Norwegian salmon culture*.

Bar, D. N. (2014). Physiological and hormonal changes during prolonged starvation in fish. *2014*. https://doi.org/10.1139/cjfas-2013-0175

Baumann, H. (2023). *Glukoneogenese*. https://sml.snl.no/glukoneogenese

Bedin, T. (2021). *Næringsopptak og fordøyelse hos dyr*. https://ndla.no/nb/subject:1:83ce68bc-19c9-4f2b-8dba-caf401428f21/topic:65689269-acc2-466b-95ee-ef09d6a25e40/resource:62f2c2f4-715a-458a-b41d-160d846742df

Berg, J. P. (2020). *Veksthormon* [Leksikon]. Store Norske Leksikon. https://sml.snl.no/veksthormon

Bermejo-Poza, R., De La Fuente, J., Pérez, C., Lauzurica, S., González, E., Diaz, M. T., & Villarroel, M. (2015). The effect of intermittent feeding on the pre-slaughter fasting response in rainbow trout. *Aquaculture, 443*, 24–30. https://doi.org/10.1016/j.aquaculture.2015.03.007

Bjordal, Å., EJuell, J., Lindem, T., & Fernô, A. (1993). *Hydroacoustic monitoring and feeding control in cage rearing of Atlantic salmon (Salmo salar L.).* Fish Farming Technology.

Bone, Q., & Moore, R. H. (2008). *Biology of fishes* (Third edition). Taylor & Francis Group.

Bratland, S., Stien, L. H., Braithwaite, V. A., Juell, J., Folkedal, O., Nilsson, J., Oppedal, F., Fosseidengen, J. E., & Kristiansen, T. S. (2010). From fright to anticipation: Using aversive light stimuli to investigate reward conditioning in large groups of Atlantic salmon (Salmo salar). *Aquaculture International*, *18*(6), 991–1001. https://doi.org/10.1007/s10499-009-9317-8

Brett, J. R., & Groves, T. D. D. (1979). Physiological energetics. In *Fish physiology* (p. 281). Academic Press.

Chapman & Hall. (n.d.). Functions of shoaling behaviour in teleosts, in; Pitcher, T.J. (Ed.), Behaviour of teleost fishes. *London*, 363–439.

Chellappa, S., Huntingford, F. A., Strang, R. H. C., & Thomson, R. Y. (1995). Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. *Journal of Fish Biology*, *47*(5), 775–787. https://doi.org/10.1111/j.1095-8649.1995.tb06002.x

Cubitt, K. F., Winberg, S., Huntingford, F. A., Kadri, S., Crampton, V. O., & Øverli, Ø. (2008). Social hierarchies, growth and brain serotonin metabolism in Atlantic salmon (Salmo salar) kept under commercial rearing conditions. *Physiology & Behavior*, *94*(4), 529–535. https://doi.org/10.1016/j.physbeh.2008.03.009

Damsgård, B., Sørum, U., Ugelstad, I., Eliassen, R. A., & Mortensen, A. (2004). Effects of feeding regime on susceptibility of Atlantic salmon (Salmo salar) to cold water vibriosis. *Aquaculture*, *239*(1–4), 37–46. https://doi.org/10.1016/j.aquaculture.2004.05.037

Einen, O., Waagan, B., & Thomassen, M. S. (1998). *I. Effects on weight loss, body shape, slaughter- and fillet-yield, proximate and fatty acid composition*.

FAO. (2021). FishStatJ - Software for Fishery and Aquaculture Statistical Time Series. *Version 4.01.7*. https://www.fao.org/fishery/statistics/software/fishstatj/en.

Farrell, A. P., & Munt, B. (1983). Cholesterol levels in the blood of Atlantic salmonids. *Comparative Biochemistry and Physiology Part A: Physiology*, *75*(2), 239–242. https://doi.org/10.1016/0300-9629(83)90076-2

Fernø, Ejuell, Lindem, & Bjordal. (1993). *Fish Farming Technology* (1st Edition). CRC Press.

Fernö, Pavlidis, van de Vis, & Kristiansen. (2020). *The Welfare of Fish*. Springer.

Fiskeridirektoratet. (2022). *Lønnsomhetsundersøkelse for produksjon av laks og regnbueørret—Matfiskproduksjon*. Fiskeridirektoratet.

Fleming, I. A., & Einum, S. (2011). Reproductive Ecology: A Tale of Two Sexes. In Ø. Aas, S. Einum, A. Klemetsen, & J. Skurdal (Eds.), *Atlantic Salmon Ecology* (pp. 33–65). Wiley-Blackwell. https://doi.org/10.1002/9781444327755.ch2

Flood, M. J., John Purser, G., & Carter, C. G. (2012). The effects of changing feeding frequency simultaneously with seawater transfer in Atlantic salmon Salmo salar L. smolt. *Aquaculture International*, *20*(1), 29–40. https://doi.org/10.1007/s10499-011-9439-7

Folkedal, O., Macaulay, G., Fosseidengen, J. E., Mikkelsen, G., Myrland, J., Søvegjarto, B., Klepaker, T. O., Fernö, A., Dempster, T., Oppedal, F., & Stien, L. H. (2022). Deployment of hydroacoustic feeding control in salmon sea-cages; biological and technical considerations. *Aquaculture*, *561*, 738700. https://doi.org/10.1016/j.aquaculture.2022.738700

Folkedal, O., Stien, L. H., Torgersen, T., Oppedal, F., Olsen, R. E., Fosseidengen, J. E., Braithwaite, V. A., & Kristiansen, T. S. (2012). Food anticipatory behaviour as an indicator of stress response and recovery in Atlantic salmon post-smolt after exposure to acute temperature fluctuation. *Physiology & Behavior*, *105*(2), 350–356. https://doi.org/10.1016/j.physbeh.2011.08.008

Folkedal, O., Torgersen, T., Nilsson, J., & Oppedal, F. (2010). Habituation rate and capacity of Atlantic salmon (Salmo salar) parr to sudden transitions from darkness to light. *Aquaculture*, *307*(1–2), 170–172. https://doi.org/10.1016/j.aquaculture.2010.06.001

Føre, M., Frank, K., Norton, T., Svendsen, E., Alfredsen, J. A., Dempster, T., Eguiraun, H., Watson, W., Stahl, A., Sunde, L. M., Schellewald, C., Skøien, K. R., Alver, M. O., & Berckmans, D. (2018). Precision fish farming: A new framework to improve production in aquaculture. *Biosystems Engineering*, *173*, 176–193. https://doi.org/10.1016/j.biosystemseng.2017.10.014

Frisk, M., Høyland, M., Zhang, L., Vindas, M. A., Øverli, Ø., & Johansen, I. B. (2020). Intensive smolt production is associated with deviating cardiac morphology in Atlantic salmon (Salmo salar L.). *Aquaculture*, *529*, 735615. https://doi.org/10.1016/j.aquaculture.2020.735615

Froese, R. (2006). Cube law, condition factor and weight-length relationships: History, meta-analysis and recommendations. *Journal of Applied Ichthyology*, *22*(4), 241–253. https://doi.org/10.1111/j.1439-0426.2006.00805.x

Gomes, A. S., Zimmermann, F., Hevrøy, E. M., Søyland, M. A. L., Hansen, T. J., Nilsen, T. O., & Rønnestad, I. (2023). Statistical modelling of voluntary feed intake in individual Atlantic salmon (Salmo salar L.). *Frontiers in Marine Science*, *10*, 1127519. https://doi.org/10.3389/fmars.2023.1127519

Good, C., Davidson, J., Iwanowicz, L., Meyer, M., Dietze, J., Kolpin, D. W., Marancik, D., Birkett, J., Williams, C., & Summerfelt, S. (2017). Investigating the influence of nitrate nitrogen on post-

smolt Atlantic salmon Salmo salar reproductive physiology in freshwater recirculation aquaculture systems. *Aquacultural Engineering*, *78*, 2–8. https://doi.org/10.1016/j.aquaeng.2016.09.003

Grably, S., & Piery, Y. (1981). Weight and tissue changes in long term starved frogs Rana esculenta. *Comparative Biochemistry and Physiology Part A: Physiology*, *69*(4), 683–688. https://doi.org/10.1016/0300-9629(81)90156-0

Grefsrud, E. L., Andersen, L. B., Grøsvik, B. E., Kvamme, B. O., Hansen, P. K., & Husa, V. (2023). *RISIKORAPPORT NORSK FISKEOPPDRETT 2023*.

Grefsrud, E. S., Andersen, L. B., Bjørn, P. A., Einar, B., Hansen, P. K., Husa, V., Karlsen, Ø., Olav, B., Samuelsen, O., Sandlund, N., & Stien, H. (2022). *RISIKORAPPORT NORSK FISKEOPPDRETT 2022—RISIKOVURDERING*. *2022–12*.

Guillot, R., Cortés, R., Navarro, S., Mischitelli, M., García-Herranz, V., Sánchez, E., Cal, L., Navarro, J. C., Míguez, J. M., Afanasyev, S., Krasnov, A., Cone, R. D., Rotllant, J., & Cerdá-Reverter, J. M. (2016). Behind melanocortin antagonist overexpression in the zebrafish brain: A behavioral and transcriptomic approach. *Hormones and Behavior*, *82*, 87–100. https://doi.org/10.1016/j.yhbeh.2016.04.011

Hamilton, H. A., Brod, E., Hanserud, O. S., Gracey, E. O., Vestrum, M. I., Bøen, A., Steinhoff, F. S., Müller, D. B., & Brattebø, H. (2016). Investigating Cross-Sectoral Synergies through Integrated Aquaculture, Fisheries, and Agriculture Phosphorus Assessments: A Case Study of Norway: Cross-Sectoral Synergies in P Analysis. *Journal of Industrial Ecology*, *20*(4), 867–881. https://doi.org/10.1111/jiec.12324

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*(1–4), 36–42. https://doi.org/10.1016/j.aquaculture.2008.06.042

Hasan, M. R., New, M. B., & Food and Agriculture Organization of the United Nations (Eds.). (2013). *On-farm feeding and feed management in aquaculture*. Food and Agriculture Organization of the United Nations.

Hevrøy, E. M., Azpeleta, C., Shimizu, M., Lanzén, A., Kaiya, H., Espe, M., & Olsvik, P. A. (2011). Effects of short-term starvation on ghrelin, GH-IGF system, and IGF-binding proteins in Atlantic salmon. *Fish Physiology and Biochemistry*, *37*(1), 217–232. https://doi.org/10.1007/s10695-010-9434-3

Hevrøy, E. M., Waagbø, R., Torstensen, B. E., Takle, H., Stubhaug, I., Jørgensen, S. M., Torgersen, T., Tvenning, L., Susort, S., Breck, O., & Hansen, T. (2012). Ghrelin is involved in voluntary anorexia in Atlantic salmon raised at elevated sea temperatures. *General and Comparative Endocrinology*, *175*(1), 118–134. https://doi.org/10.1016/j.ygcen.2011.10.007

Hvas, M. (2022). Swimming energetics of Atlantic salmon in relation to extended fasting at different temperatures. *Conservation Physiology*, *10*(1), coac037. https://doi.org/10.1093/conphys/coac037

Hvas, M., Folkedal, O., Imsland, A., & Oppedal, F. (2017). The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon *Salmo salar*. *Journal of Experimental Biology*, jeb.154021. https://doi.org/10.1242/jeb.154021

Hvas, M., Folkedal, O., & Oppedal, F. (2021). Fish welfare in offshore salmon aquaculture. *Reviews in Aquaculture*, *13*(2), 836–852. https://doi.org/10.1111/raq.12501

Hvas, M., Nilsson, J., Vågseth, T., Nola, V., Fjelldal, P. G., Hansen, T. J., Oppedal, F., Stien, L. H., & Folkedal, O. (2022). *Full compensatory growth before harvest and no impact on fish welfare in Atlantic salmon after an 8-week fasting period*. https://doi.org/10.1016/j.aquaculture.2021.737415

Hvas, M., & Oppedal, F. (2019). Influence of experimental set-up and methodology for measurements of metabolic rates and critical swimming speed in Atlantic salmon *Salmo salar*. *Journal of Fish Biology*, jfb.14087. https://doi.org/10.1111/jfb.14087

Hyslop, E. J. (1980). Stomach contents analysis-a review of methods and their application. *Journal of Fish Biology*, *17*(4), 411–429. https://doi.org/10.1111/j.1095-8649.1980.tb02775.x

Iversen, A., Asche, F., Hermansen, Ø., & Nystøyl, R. (2020). Production cost and competitiveness in major salmon farming countries 2003–2018. *Aquaculture*, *522*, 735089. https://doi.org/10.1016/j.aquaculture.2020.735089

Johanna Järnegren, Nina Bloecher, & Ingebrigt Uglem. (2022). *Effekter av organiskt utslipp fra havbruk i Norge – en kunnskapsoppsummering*.

Johnsen, C. A., Hagen, Ø., Solberg, C., Björnsson, B. T. H., Jönsson, E., Johansen, S. J. S., & Bendiksen, E. Å. (2013). Seasonal changes in muscle structure and flesh quality of 0+ and 1+ Atlantic salmon (*Salmo salar* L.): Impact of feeding regime and possible roles of ghrelin. *Aquaculture Nutrition*, *19*(1), 15–34. https://doi.org/10.1111/j.1365-2095.2011.00927.x

Johnston, I. A., & Dunn, J. (1987). *Temperature acclimationand metabolism in ectotherms with particular reference to teleost in fish*. Society for Experimental Biology.

Johnston, I. A., Manthri, S., Alderson, R., Smart, A., Campbell, P., Nickell, D., Robertson, B., Paxton, C. G. M., & Burt, M. L. (2003). Freshwater environment affects growth rate and muscle fibre recruitment in seawater stages of Atlantic salmon (*Salmo salar* L.). *Journal of Experimental Biology*, *206*(8), 1337–1351. https://doi.org/10.1242/jeb.00262

Juell, J.-E., Bjordal, Å., Fernö, A., & Huse, I. (1994). Effect of feeding intensity on food intake and growth of Atlantic salmon, Salmo salar L., in sea cages. *Aquaculture Research*, *25*(4), 453–464. https://doi.org/10.1111/j.1365-2109.1994.tb00710.x

Kalananthan, T., Folkedal, O., Gomes, A. S., Lai, F., Handeland, S. O., Tolås, I., Gelebart, V., & Rønnestad, I. (2023). Impact of long-term fasting on the stomach-hypothalamus appetite regulating genes in Atlantic salmon postsmolts. *Aquaculture*, *563*, 738917. https://doi.org/10.1016/j.aquaculture.2022.738917

Känkänen, M., & Pirhonen, J. (2009). The effect of intermittent feeding on feed intake and compensatory growth of whitefish Coregonus lavaretus L. *Aquaculture*, *288*(1–2), 92–97. https://doi.org/10.1016/j.aquaculture.2008.11.029

Killen, S. S., Brown, J. A., & Gamperl, A. K. (2007). The effect of prey density on foraging mode selection in juvenile lumpfish: Balancing food intake with the metabolic cost of foraging. *Journal of Animal Ecology*, *76*(4), 814–825. https://doi.org/10.1111/j.1365-2656.2007.01237.x

Korte, S. M., Olivier, B., & Koolhaas, J. M. (2007). A new animal welfare concept based on allostasis. *Physiology & Behavior*, *92*(3), 422–428. https://doi.org/10.1016/j.physbeh.2006.10.018

Kullgren, A., Jutfelt, F., Fontanillas, R., Sundell, K., Samuelsson, L., Wiklander, K., Kling, P., Koppe, W., Larsson, D. G. J., Björnsson, B. T., & Jönsson, E. (2013). The impact of temperature on the metabolome and endocrine metabolic signals in Atlantic salmon (Salmo salar). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *164*(1), 44–53. https://doi.org/10.1016/j.cbpa.2012.10.005

Lie & Huse. (1992). The effect of starvation on the composition of Atlantic salmon (Salmo salar). *Fisk Dir Skr, Ernæring 5: 11-16*.

Lov om dyrevelferd. (2009). Lovdata. https://lovdata.no/dokument/NL/lov/2009-06-19-97

Lovdata. (2008). *Forskrift om drift av aquakulturanlegg*. Lovdata. https://lovdata.no/dokument/SF/forskrift/2008-06-17-822

Mansell, P. I., Fellows, I. W., & Macdonald, I. A. (1990). Enhanced thermogenic response to epinephrine after 48-h starvation in humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *258*(1), R87–R93. https://doi.org/10.1152/ajpregu.1990.258.1.R87

Martinez, E. P., Balseiro, P., Pedrosa, C., Haugen, T. S., Fleming, M. S., & Handeland, S. O. (2021). The effect of photoperiod manipulation on Atlantic salmon growth, smoltification and sexual maturation: A case study of a commercial RAS. *Aquaculture Research*, *52*(6), 2593–2608. https://doi.org/10.1111/are.15107

Mattila, J., Koskela, J., & Pirhonen, J. (2009). The effect of the length of repeated feed deprivation between single meals on compensatory growth of pikeperch Sander lucioperca. *Aquaculture*, *296*(1–2), 65–70. https://doi.org/10.1016/j.aquaculture.2009.07.024

Merethe Kvam. (2023, February 2). *Protein*. https://nhi.no/kosthold/ernaring/protein/?page=1

Mills, M. R. (n.d.). *The Comparative Anatomy of Eating*.

Mørkøre, T., Mazo T., P. I., Tahirovic, V., & Einen, O. (2008). Impact of starvation and handling stress on rigor development and quality of Atlantic salmon (Salmon salar L). *Aquaculture*, *277*(3–4), 231–238. https://doi.org/10.1016/j.aquaculture.2008.02.036

Nair, S., Paul D. Woolf, Stephen L. Welle, & Dwight E Matthews. (1987). *Leucine, glucose and energy metabolism after 3 days of fasting in healthy human subjects*. Original Reasearch Comminications - general.

Nilsson, J., & Folkedal, O. (2019). Sampling of Atlantic salmon Salmo salar from tanks and sea cages is size-biased. *Aquaculture*, *502*, 272–279. https://doi.org/10.1016/j.aquaculture.2018.12.053

Noble, C., Nilsson, J., Stien, L. H., Iversen, M. H., Kolarevic, J., & Gismervik, K. (2018). *Velferdsindikatorer for oppdrettslaks*. Nofima.

Noble, Gismervik, K., Iversen, M. H., Kolarevic, J., Nilsson, J., Stien, L. H., & Turnbull, J. F. (2018). *Welfare Indicators for farmed Atlantic salmon—Tools for assessing fish welfare*.

Olsen, T. S. (2023). *DATA SAMLING - MASTEROPPGAVE* (18.2306.1061.0) [Microsoft Excel].

Otterå, H., Karlsen, Ø., Slinde, E., & Olsen, R. E. (2009). Quality of wild-captured saithe (*Pollachius virens* L.) fed formulated diets for 8 months. *Aquaculture Research*, *40*(11), 1310–1319. https://doi.org/10.1111/j.1365-2109.2009.02230.x

Patikorn, C., Roubal, K., Veettil, S. K., Chandran, V., Pham, T., Lee, Y. Y., Giovannucci, E. L., Varady, K. A., & Chaiyakunapruk, N. (2021). Intermittent Fasting and Obesity-Related Health Outcomes: An Umbrella Review of Meta-analyses of Randomized Clinical Trials. *JAMA Network Open*, *4*(12), e2139558. https://doi.org/10.1001/jamanetworkopen.2021.39558

Py, C., Elizondo-González, R., & Peña-Rodríguez, A. (2022). Compensatory growth: Fitness cost in farmed fish and crustaceans. *Reviews in Aquaculture*, *14*(3), 1389–1417. https://doi.org/10.1111/raq.12656

Remen, M., Oppedal, F., Imsland, A. K., Olsen, R. E., & Torgersen, T. (2013). Hypoxia tolerance thresholds for post-smolt Atlantic salmon: Dependency of temperature and hypoxia acclimation. *Aquaculture*, *416–417*, 41–47. https://doi.org/10.1016/j.aquaculture.2013.08.024

Rikardsen, A. H., Righton, D., Strøm, J. F., Thorstad, E. B., Gargan, P., Sheehan, T., Økland, F., Chittenden, C. M., Hedger, R. D., Næsje, T. F., Renkawitz, M., Sturlaugsson, J., Caballero, P., Baktoft, H., Davidsen, J. G., Halttunen, E., Wright, S., Finstad, B., & Aarestrup, K. (2021). Redefining the oceanic distribution of Atlantic salmon. *Scientific Reports*, *11*(1), 12266. https://doi.org/10.1038/s41598-021-91137-y

Rønnestad, I., Gomes, A. S., Murashita, K., Angotzi, R., Jönsson, E., & Volkoff, H. (2017). Appetite-Controlling Endocrine Systems in Teleosts. *Frontiers in Endocrinology*, *8*. https://doi.org/10.3389/fendo.2017.00073

Schreck, C. B., & Davis, L. E. (1997). The energetic response to handling stress in juvenile coho salmon. *Transactions of the American Fisheries Society*.

Shepherd, C. J., Monroig, O., & Tocher, D. R. (2017). Future availability of raw materials for salmon feeds and supply chain implications: The case of Scottish farmed salmon. *Aquaculture*, *467*, 49–62. https://doi.org/10.1016/j.aquaculture.2016.08.021

Skretting AS. (n.d.). *Skretting*. Armor. Retrieved 17 March 2023, from https://www.skretting.com/no/for-til-havbruksnaeringen/armor-90/?utm_source=website&utm_medium=banner&utm_campaign=lansering_armor

Skretting AS. (2012). *Skretting*. https://www.skrettingguidelines.com/readimage.aspx?pubid=cd8a45bd-0e6e-409c-a2ee-1da2b7d19b0

Sneddon, L. U. (2006). *Ethics and Welfare: Pain Perception in Fish*.

Sneddon, L. U., Elwood, R. W., Adamo, S. A., & Leach, M. C. (2014). Defining and assessing animal pain. *Animal Behaviour*, *97*, 201–212. https://doi.org/10.1016/j.anbehav.2014.09.007

Stead, C. M. (2002). *Handbook of salmon farming.* Praxis Publishing.

Svåsand, Karlsen, Kvamme, Stien, Taranger, & Boxaspen. (2016). *Risikovurdering norsk fiskeoppdrett 2016*. Havforskningsinstituttet, NINA, Fiskeridirektoratet, NOFIMA.

Sveier, H., & Lied, E. (1998). *The effect of feeding regime on growth, feed utilisation and weight dispersion in large Atlantic salmon ž Salmo salar/ reared in seawater*.

Talbot, C., Corneillie, S., & Korsøen, Ø. (1999). Pattern of feed intake in four species of fish under commercial farming conditions: Implications for feeding management. *Aquaculture Research*, *30*, 509–518.

Taranger, G. L., Carrillo, M., Schulz, R. W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F.-A., Dufour, S., Karlsen, Ø., Norberg, B., Andersson, E., & Hansen, T. (2010). Control of puberty in farmed fish. *General and Comparative Endocrinology*, *165*(3), 483–515. https://doi.org/10.1016/j.ygcen.2009.05.004

Thodesen, J., Grisdale-Helland, B., & Gjerde, B. (1999). *Feed intake, growth and feed utilization of offspring from wild and selected Atlantic salmon (Salmo salar)*.

Thomassen, J. M., & Fjæra, S. O. (1996). Studies of feeding frequency for atlantic salmon (Salmo salar). *Aquacultural Engineering*, *15*(2), 149–157. https://doi.org/10.1016/0144-8609(95)00010-0

Thorpe, J. E., Talbot, C., Miles, M. S., & Keay, D. S. (1990). Control of Maturation in Cultured Atlantic Salmon, Salmo salur, in Pumped Seawater Tanks, by Restricting Food Intake. *Aquaculture*, *86*, 315–326.

Tilseth, S., Hansen, T., & Møller, D. (1991). Historical Development of Salmon Culture. *Elsevicr Science Publishers B.V., Amsterdam*.

Trombley, S. (2014). *Regulation of the seasonal leptin and leptin receptor expression profile during early sexual maturation and feed restriction in male Atlantic salmon, Salmo salar L., parr*.

Tunçelli, G., & Pirhonen, J. (2021). Effects of weekend starvation and the duration of daily feeding on production parameters of rainbow trout Oncorhynchus mykiss. *Aquaculture*, *543*, 737028. https://doi.org/10.1016/j.aquaculture.2021.737028

United Nations. (1987). *Report of the World Commission on Environment and Development: Our Common Future*.

Utne, K. R., Pauli, B. D., Haugland, M., Jacobsen, J. A., Maoileidigh, N., Melle, W., Broms, C. T., Nøttestad, L., Holm, M., Thomas, K., & Wennevik, V. (2021). Poor feeding opportunities and reduced condition factor for salmon post-smolts in the Northeast Atlantic Ocean. *ICES Journal of Marine Science*, *78*(8), 2844–2857. https://doi.org/10.1093/icesjms/fsab163

Vindas, M. A., Johansen, I. B., Folkedal, O., Höglund, E., Gorissen, M., Flik, G., Kristiansen, T. S., & Øverli, Ø. (2016). Brain serotonergic activation in growth-stunted farmed salmon: Adaption versus pathology. *Royal Society Open Science*, *3*(5), 160030. https://doi.org/10.1098/rsos.160030

Vindas, M. A., Madaro, A., Fraser, T. W. K., Höglund, E., Olsen, R. E., Kristiansen, T. S., & Øverli, Ø. (2017). Uncontrollable chronic stress reduces growth disparities in farmed Atlantic salmon. *Physiology & Behavior*, *179*, 246–252. https://doi.org/10.1016/j.physbeh.2017.06.012

Volkoff, H., & Rønnestad, I. (2020). Effects of temperature on feeding and digestive processes in fish. *Temperature*, *7*(4), 307–320. https://doi.org/10.1080/23328940.2020.1765950

Waagbø, R., Espe, M., Hamre, K., & Lie, Ø. (2001). *Fiskeernæring*. Kystnæringen Forlag & bokklubb AS.

7. Appendix

7.1 Statistical analysis

7.1.1 Fed amount

Table 5: *Output from a 2-way ANOVA of the "Feed intake as % of biomass", Figure 11A.*

Table Analyzed	**Feed intake as % of biomass**				
Two-way ANOVA	**Ordinary**				
Alpha	0,05				
Source of Variation	**% of total variation**	**P value**	**P value summar**	**Significant?**	
Row Factor	58,05	<0,0001	****	Yes	
Column Factor	14,19	<0,0001	****	Yes	
ANOVA table	**SS (Type III)**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Row Factor	8,763	86	0,1019	F (86, 202) = 5,679	P<0,0001
Column Factor	2,142	1	2,142	F (1, 202) = 119,4	P<0,0001
Residual	3,624	202	0,01794		
Difference between column means					
Predicted (LS) mean of Control group	0,5411				
Predicted (LS) mean of Intermittent fasting group	0,7333				
Difference between predicted means	-0,1922				
SE of difference	0,01759				
95% CI of difference	-0,2268 to -0,1575				
Data summary					
Number of columns (Column Factor)	2				
Number of rows (Row Factor)	87				
Number of values	290				

Table 6*: Output from a 2-way ANOVA of the "Percent fed of control levels (%), Figure 11B.*

Table Analyzed	**Percent fed of control levels (%)**				
Two-way ANOVA	**Ordinary**				
Alpha	0,05				
Source of Variation	**% of total variation**	**P value**	**P value summary**	**Significant?**	
Row Factor	53,23	0,0117	*	Yes	
Column Factor	17,77	<0,0001	****	Yes	
ANOVA table	**SS (Type III)**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Row Factor	14,82	57	0,2601	F (57, 57) = 1,836	P=0,0117
Column Factor	4,95	1	4,95	F (1, 57) = 34,94	P<0,0001
Residual	8,074	57	0,1417		
Difference between column means					
Predicted (LS) mean of Control	1,105				
Predicted (LS) mean of Intermittent	1,519				
Difference between predicted means	-0,4131				
SE of difference	0,06989				
95% CI of difference	-0,5531 to -0,2732				
Data summary					
Number of columns (Column Factor)	2				
Number of rows (Row Factor)	58				
Number of values	116				

Table 7: *Output from a 2-way ANOVA of the "Percent fed of control levels (%)", Figure 12.*

Table Analyzed	**Percent fed of control levels %**				
Two-way ANOVA	**Ordinary**				
Alpha	0,05				
Source of Variation	**% of total variation**	**P value**	**P value summary**	**Significant?**	
Row Factor	2,914	0,9967	ns	No	
Column Factor	81,32	<0,0001	****	Yes	
ANOVA table	**SS (Type III)**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Row Factor	0,08018	12	0,006682	F (12, 12) = 0,1848	P=0,9967
Column Factor	2,238	1	2,238	F (1, 12) = 61,89	P<0,0001
Residual	0,4338	12	0,03615		
Difference between column means					
Predicted (LS) mean of Fasted	1,238				
Predicted (LS) mean of Control	1,824				
Difference between predicted means	-0,5867				
SE of difference	0,07458				
95% CI of difference	-0,7492 to -0,4242				
Data summary					
Number of columns (Column Factor)	2				
Number of rows (Row Factor)	13				
Number of values	26				

Table 8: *Output from a 2-way ANOVA of the "Accelerated percent fed", Figure 13.*

Table Analyzed	**Percent fed of control levels (%)**				
Two-way ANOVA	**Ordinary**				
Alpha	0,05				
Source of Variation	**% of total variation**	**P value**	**P value summary**	**Significant?**	
Row Factor	53,23	0,0117	*	Yes	
Column Factor	17,77	<0,0001	****	Yes	
ANOVA table	**SS (Type III)**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Row Factor	14,82	57	0,2601	F (57, 57) = 1,836	P=0,0117
Column Factor	4,95	1	4,95	F (1, 57) = 34,94	P<0,0001
Residual	8,074	57	0,1417		
Difference between column means					
Predicted (LS) mean of Control	1,105				
Predicted (LS) mean of Intermittent	1,519				
Difference between predicted means	-0,4131				
SE of difference	0,06989				
95% CI of difference	-0,5531 to -0,2732				
Data summary					
Number of columns (Column Factor)	2				
Number of rows (Row Factor)	58				
Number of values	116				

7.1.2 Growth and development

Table 9: *Output from a 2-way ANOVA of "Length (cm)", Figure 14.*

Table Analyzed	**Length (cm)**				
Two-way ANOVA	**Ordinary**				
Alpha	0,05				
Source of Variation	**% of total variation**	**P value**	**P value summary**	**Significant?**	
Interaction	0,296	0,0489	*	Yes	
Row Factor	41,34	<0,0001	****	Yes	
Column Factor	0,04653	0,3297	ns	No	
ANOVA table	**SS (Type III)**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Interaction	103,5	2	51,76	F (2, 1192) = 3,025	P=0,0489
Row Factor	14459	2	7229	F (2, 1192) = 422,5	P<0,0001
Column Factor	16,27	1	16,27	F (1, 1192) = 0,9510	P=0,3297
Residual	20394	1192	17,11		
Difference between column means					
Predicted (LS) mean of Control group	66,85				
Predicted (LS) mean of Intermittent fasting	66,61				
Difference between predicted means	0,2331				
SE of difference	0,239				
95% CI of difference	-0,2358 to 0,7020				
Data summary					
Number of columns (Column Factor)	2				
Number of rows (Row Factor)	3				
Number of values	1198				

Table 10: *Output from a 2-way ANOVA of "Weight (g)", Figure 15.*

Table Analyzed	**Weight (g)**				
Two-way ANOVA	**Ordinary**				
Alpha	0,05				
Source of Variation	**% of total variatio**	**P value**	**P value summar**	**Significant?**	
Interaction	2,003	<0,0001	****	Yes	
Row Factor	32,84	<0,0001	****	Yes	
Column Factor	1,146	0,0001	***	Yes	
ANOVA table	**SS (Type III)**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Interaction	23659766	6	3943294	F (6, 1186) = 6,176	P<0,0001
Row Factor	387971471	2	193985736	F (2, 1186) = 303,8	P<0,0001
Column Factor	13536509	3	4512170	F (3, 1186) = 7,066	P=0,0001
Residual	757305764	1186	638538		
Data summary					
Number of columns (Column Factor)	4				
Number of rows (Row Factor)	3				
Number of values	1198				

Table 11: *Output from a 2-way ANOVA of the "K-factor", Figure 16.*

Table Analyzed	**K-factor**				
Two-way ANOVA	**Ordinary**				
Alpha	0,05				
Source of Variation	**% of total variatio**	**P value**	**P value summar**	**Significant?**	
Interaction	2,755	<0,0001	****	Yes	
Row Factor	4,365	<0,0001	****	Yes	
Column Factor	3,715	<0,0001	****	Yes	
ANOVA table	**SS (Type III)**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Interaction	0,5295	6	0,08825	F (6, 1186) = 6,108	P<0,0001
Row Factor	0,8388	2	0,4194	F (2, 1186) = 29,03	P<0,0001
Column Factor	0,7139	3	0,238	F (3, 1186) = 16,47	P<0,0001
Residual	17,14	1186	0,01445		
Data summary					
Number of columns (Column Factor)	4				
Number of rows (Row Factor)	3				
Number of values	1198				

Table 12: *Output from a ratio paired T-test of the "SGR" in Table 3.*

Table Analyzed	**SGR**
Column A	Control group
vs.	vs,
Column B	Intermittent fasting group
Test details	
Test name	Ratio paired t test
Variance assumption	Individual variance for each group
Multiple comparisons	Set P value threshold
Method	Holm-Šídák method
Alpha	0,05
Number of tests performed	3
Number of rows omitted	0

Table 13: *Output from a ratio paired T-test of the "eFCR" in Table 3.*

Table Analyzed	**eFCR**
Column A	Control group
vs.	vs,
Column B	Intermittent fasting group
Test details	
Test name	Ratio paired t test
Variance assumption	Individual variance for each group
Multiple comparisons	Set P value threshold
Method	Holm-Šídák method
Alpha	0,05
Number of tests performed	2
Number of rows omitted	1
Number of rows with incomplete data	0

Table 14: *Output from a ratio paired T-test of the "bFCR" in Table 3.*

Table Analyzed	**bFCR**
Column A	Control group
vs.	vs,
Column B	Intermittent fasting group
Test details	
Test name	Ratio paired t test
Variance assumption	Individual variance for each group
Multiple comparisons	Set P value threshold
Method	Holm-Šídák method
Alpha	0,05
Number of tests performed	3
Number of rows omitted	0
Number of rows with incomplete data	0

Table 15: *Output from a ratio paired T-test of the "Mortality" in Table 4.*

Table Analyzed	**Mortality**
Column A	control group
vs.	vs,
Column B	intermittent fasting group
Test details	
Test name	Ratio paired t test
Variance assumption	Individual variance for each group
Multiple comparisons	Set P value threshold
Method	Holm-Šídák method
Alpha	0,05
Number of tests performed	2
Number of rows omitted	1
Number of rows with incomplete data	0

7.1.3 Metabolic state

Table 16: *Output from a 2-way ANOVA of the "HSI (%)", Figure 17.*

Table Analyzed	**HSI %**				
Two-way ANOVA	**Ordinary**				
Alpha	0,05				
Source of Variation	**% of total variation**	**P value**	**P value summary**	**Significant?**	
Interaction	5,596	0,3025	ns	No	
Row Factor	15,91	<0,0001	****	Yes	
Column Factor	4,024	0,0561	ns	No	
ANOVA table	**SS (Type III)**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Interaction	0,2201	9	0,02446	F (9, 143) = 1,195	P=0,3025
Row Factor	0,626	3	0,2087	F (3, 143) = 10,20	P<0,0001
Column Factor	0,1583	3	0,05276	F (3, 143) = 2,578	P=0,0561
Residual	2,927	143	0,02047		
Data summary					
Number of columns (Column Factor)	4				
Number of rows (Row Factor)	4				
Number of values	159				

Table 17: *Output from a 2-way ANOVA of the "GSI (%) – Male", Figure 18A.*

Table Analyzed	**GSI % - Male**				
Two-way ANOVA	**Ordinary**				
Alpha	0,05				
Source of Variation	**% of total variation**	**P value**	**P value summary**	**Significant?**	
Interaction	7,32	0,6489	ns	No	
Row Factor	1,157	0,7802	ns	No	
Column Factor	2,193	0,5622	ns	No	
ANOVA table	**SS (Type III)**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Interaction	0,01812	9	0,002013	F (9, 83) = 0,7649	P=0,6489
Row Factor	0,002863	3	0,0009544	F (3, 83) = 0,3626	P=0,7802
Column Factor	0,005429	3	0,00181	F (3, 83) = 0,6875	P=0,5622
Residual	0,2185	83	0,002632		
Data summary					
Number of columns (Column Factor)	4				
Number of rows (Row Factor)	4				
Number of values	99				

Table 18: *Output from a 2-way ANOVA of the "GSI (%) – Female", Figure 18B.*

Table Analyzed	**GSI % - Female**				
Two-way ANOVA	**Ordinary**				
Alpha	0,05				
Source of Variation	**% of total variation**	**P value**	**P value summary**	**Significant?**	
Interaction	14,14	0,5253	ns	No	
Row Factor	4,029	0,5126	ns	No	
Column Factor	4,708	0,4444	ns	No	
ANOVA table	**SS (Type III)**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Interaction	0,008615	9	0,0009572	F (9, 44) = 0,9100	P=0,5253
Row Factor	0,002455	3	0,0008182	F (3, 44) = 0,7779	P=0,5126
Column Factor	0,002869	3	0,0009562	F (3, 44) = 0,9091	P=0,4444
Residual	0,04628	44	0,001052		
Data summary					
Number of columns (Column Factor)	4				
Number of rows (Row Factor)	4				
Number of values	60				

Table 19: *Output from a 2-way ANOVA of the "Wet stomach content", Figure 19.*

Table Analyzed	**Stomach**				
Two-way ANOVA	**Ordinary**				
Alpha	0,05				
Source of Variation	**% of total variation**	**P value**	**P value summary**	**Significant?**	
Interaction	13,89	<0,0001	****	Yes	
Row Factor	13,48	<0,0001	****	Yes	
Column Factor	16,31	<0,0001	****	Yes	
ANOVA table	**SS (Type III)**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Interaction	17,84	3	5,947	F (3, 148) = 12,22	P<0,0001
Row Factor	17,31	3	5,771	F (3, 148) = 11,86	P<0,0001
Column Factor	20,94	1	20,94	F (1, 148) = 43,03	P<0,0001
Residual	72,03	148	0,4867		
Difference between column means					
Predicted (LS) mean of Control group	0,8826				
Predicted (LS) mean of Intermittent fasting group	1,617				
Difference between predicted means	-0,7339				
SE of difference	0,1119				
95% CI of difference	-0,9550 to -0,5128				
Data summary					
Number of columns (Column Factor)	2				
Number of rows (Row Factor)	4				
Number of values	156				

Table 20: *Output from a 2-way ANOVA of the "Wet midgut content", Figure 20.*

Table Analyzed	**Midgut**			
Two-way ANOVA	**Ordinary**			
Alpha	0,05			
Source of Variation	**% of total variation**	**P value**	**P value summary**	**Significant?**
Interaction	1,56	0,478	ns	No
Row Factor	3,93	0,103	ns	No
Column Factor	1,469	0,1273	ns	No
ANOVA table	**SS (Type III)**	**DF**	**MS**	**F (DFn, DFd)**
Interaction	0,3985	3	0,1328	F (3, 149) = 0,832
Row Factor	1,004	3	0,3346	F (3, 149) = 2,097
Column Factor	0,3752	1	0,3752	F (1, 149) = 2,352
Residual	23,77	149	0,1596	
Difference between column means				
Predicted (LS) mean of Control group	0,9686			
Predicted (LS) mean of intermittent fasting group	0,8708			
Difference between predicted means	0,09784			
SE of difference	0,0638			
95% CI of difference	-0,02823 to 0,2239			
Data summary				
Number of columns (Column Factor)	2			
Number of rows (Row Factor)	4			
Number of values	157			

Table 21: *Output from a 2-way ANOVA of the "Wet hindgut content", Figure 21.*

Table Analyzed	Hindgut			
Two-way ANOVA	**Ordinary**			
Alpha	0,05			
Source of Variation	**% of total variation**	**P value**	**P value summary**	**Significant?**
Interaction	5,206	0,0286	*	Yes
Row Factor	2,463	0,226	ns	No
Column Factor	8,729	0,0001	***	Yes
ANOVA table	**SS (Type III)**	**DF**	**MS**	**F (DFn, DFd)**
Interaction	0,4984	3	0,1661	F (3, 149) = 3,100
Row Factor	0,2358	3	0,07861	F (3, 149) = 1,467
Column Factor	0,8357	1	0,8357	F (1, 149) = 15,59
Residual	7,986	149	0,0536	
Difference between column means				
Predicted (LS) mean of control group	0,5611			
Predicted (LS) mean of intermittent fasting group	0,4151			
Difference between predicted means	0,146			
SE of difference	0,03698			
95% CI of difference	0,07295 to 0,2191			

7.1.4 Gut fullness index (%) estimated with dry gut content.

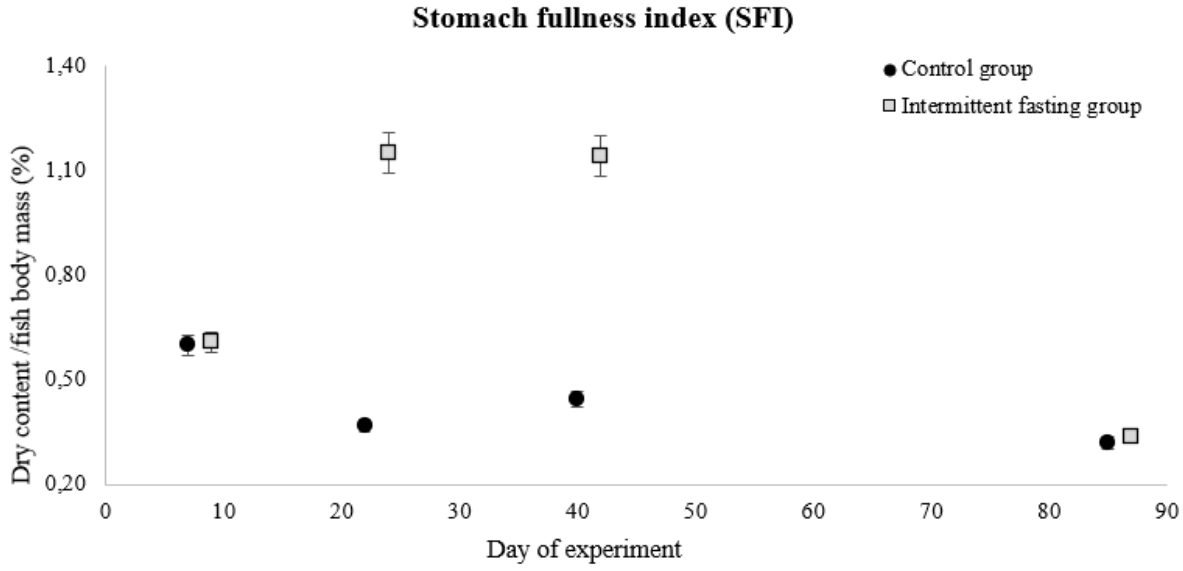

Figure 23 - *Atlantic salmon dry stomach content as percent of body mass of the control group (Cage 1 and 6) that were fully daily fed, and a group that were given a regime of intermittent fasting (fully fed every third day, Cage 2 and 7) between days 9 to 50. The x-axis indicates the experimental day number. A sample for physiology (n = 20 fish per group) was collected on experimental day 8, 23 (fasting day 15), 41 (fasting day 33) and 86 (re-feeding day 37). Made with SEM error bars.*

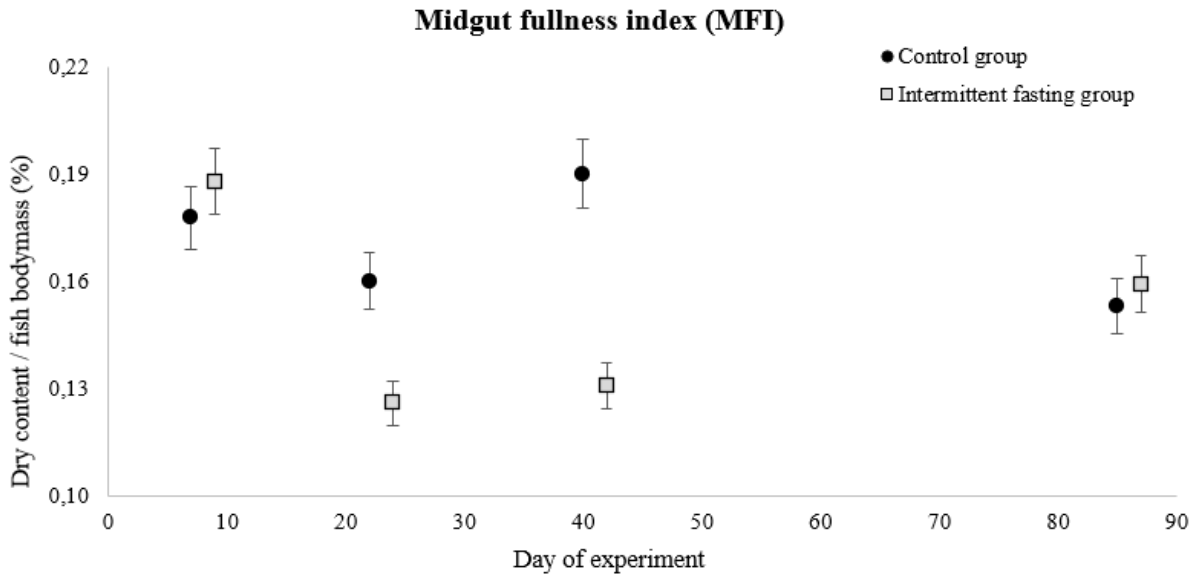

Figure 24 - *Atlantic salmon dry hindgut content as percent of body mass of the control group (Cage 1 and 6) that were fully daily fed, and a group that were given a regime of intermittent fasting (fully fed every third day, Cage 2 and 7) between days 9 to 50. The x-axis indicates the experimental day number. A sample for physiology (n = 20 fish per group) was collected on experimental day 8, 23 (fasting day 15), 41 (fasting day 33) and 86 (re-feeding day 37). Made with SEM error bars.*

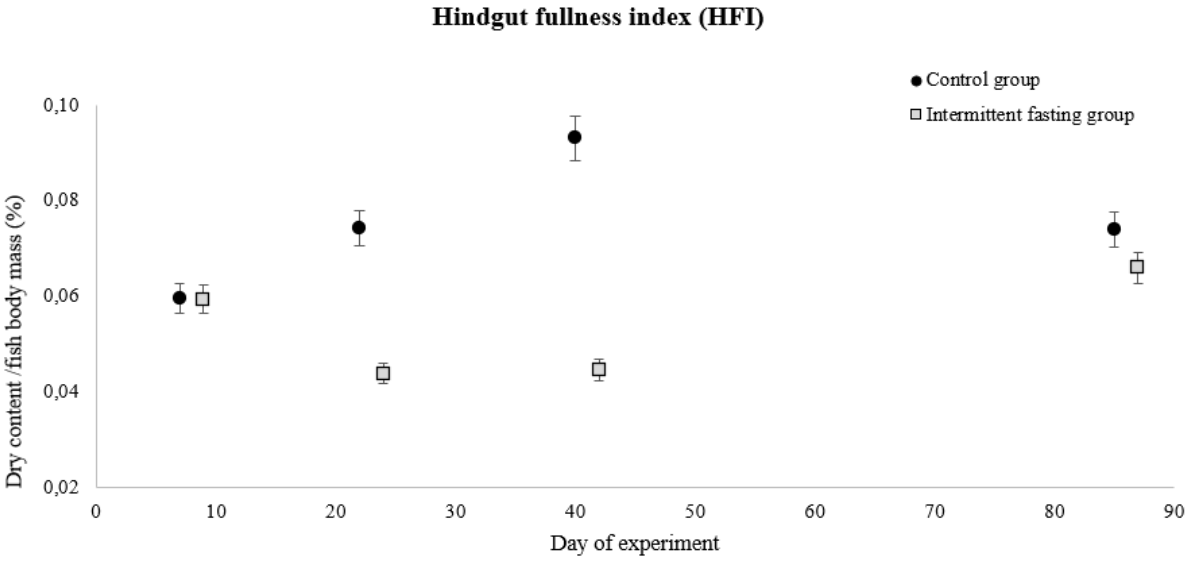

Figure 25 - *Atlantic salmon dry hindgut content as percent of body mass of the control group (Cage 1 and 6) that were fully daily fed, and a group that were given a regime of intermittent fasting (fully fed every third day, Cage 2 and 7) between days 9 to 50. The x-axis indicates the experimental day number. A sample for physiology (n = 20 fish per group) was collected on experimental day 8, 23 (fasting day 15), 41 (fasting day 33) and 86 (re-feeding day 37). Made with SEM error bars.*