The effects of different feed ratios on growth, welfare rating and maturation in juvenile Atlantic salmon (*Salmo salar*, L.)

Master of science in Biology - Aquaculture Biology

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Abstract

The world population is increasing day by day and has surpassed 8 billion, and there will continue to be high demands of more protein to feed the world. Salmon farming is a rapidly growing industry and has been for the past decades. The pressure for more protein and better operational results for the salmon producers, requires the salmon farmers to adapt their production method by increasing intensiveness in their rearing of the fish, which has resulted in more maturation of the salmon. The objectives of this study were to assess if there were a connection between different feed ratios and growth, welfare rating and early maturation of juvenile Atlantic salmon. Juvenile salmon (n = 450) were reared under 3 different feed rations (100%, 50% and 33%), producing nine experimental groups (3 tanks for each feed ratio) where 50% of fish was PIT-tagged individually in each tank. The criterions used to follow the development of growth were the weight, length, condition factor (K-factor) and specific growth rate (SGR). Number of fin wounds were used as the welfare indicator, and to inspect the development of maturation, the fish was euthanized, killed, and development of the gonads monitored by visual inspection. The fully fed group (100r) showed better growth and welfare rating compared to the lower fed groups (50r and 33r), however the growth of the lower fed groups was closer to the full fed groups, than expected. There was a significant difference found between the welfare rating of all the feed groups during the last sample, where the 100r showed the best rating and the 33r group the worst. Barely any difference was found in the maturation of the smolts, non at the female salmon and only in two different maturation stages for the males. Present findings indicate possible research avenues to help the salmon producers with their smolt strategies that can result in higher welfare rating and in lower maturation later in the production cycle. However, future research looking at other factors could make that clearer like temperature, light regimes, and utilization of the feed which could affect the producers feed strategies.

1. Introduction

1.1. History of aquaculture in Iceland

The history of aquaculture in Iceland stretches back more than a century (Halldór Halldórsson, 1992; Government, https://www.government.is/topics/business-and-industry/fisheries-in-iceland/aquaculture/) and the salmon farming industry in Iceland has been struggling to find its way forward until recently. The prospect of the aquaculture industry in Iceland was early on linked with the use of geothermal water to have the most suitable growth conditions, and to control the temperature of the water. In the Atlantic Ocean around Iceland, the natural conditions for open sea cages are good but there can be rough weathers and ice that has caused serious damage to the equipment in the past, and the cold seawater temperature can reach critically low levels during the winter months. That is most likely the reason why the progress of aquaculture has been relatively slower compared to our neighbouring countries like Norway. (Sigfusson et al., 2021).

Aquaculture is Norway's second most important industry after petroleum in terms of revenue and is a key focus of the country's research and development (Johansen et al., 2019). As with all big industries, they come with problems. The bigger the industry gets production increases and the number of farm-raised salmon with it. The farmed-raised salmon are a threat to the wild salmon stocks. Farm-raised Atlantic salmon (*Salmo salar*) escapees and sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus)* are those identified as the fastest growing threats to wild Atlantic salmon populations in Norway, which can affect wild salmon populations to the extent that they may become critically endangered or lost (Forseth et al. 2017). Sea lice are the parasites that have caused the largest problems in recent years. (Lekang et al., 2016).

1.1.1. Current aquaculture in Iceland

The Icelandic law has been very strict related to the aquaculture industry in open sea cages and have put in some preventors like the Risk assessment (Alþingi, 2019). The law states that the Marine and Freshwater Research Institute of Iceland will provide a risk assessment of genetic mixing of the wild salmon. In this risk assessment the max biomass of farmed salmon in each fjord is estimated to minimize the risk of genetic mixing. They consider for example, estimated number of escapees, the return ratio, effects of currents and distribution of fishes,

distance between rivers and salmon farms and the total population of salmon in those specific rivers. To add on that the law states also that the capacity load of each fjord shall be estimated by the Marine institute. The Marine and Freshwater Research Institute shall monitor the organic waste and stress to the natural environment and decide the max biomass of any farmed species in that fjord. (Alþingi, 2019).

The salmon production in the sea cage industry in Iceland comes with a benefit for the rural areas of Iceland. Iceland has only allowed salmon farming in open sea cages in specific parts of Iceland, mostly in the West-fjords and the East-fjords (Fig. 1) (Fiskistofa, 2004).

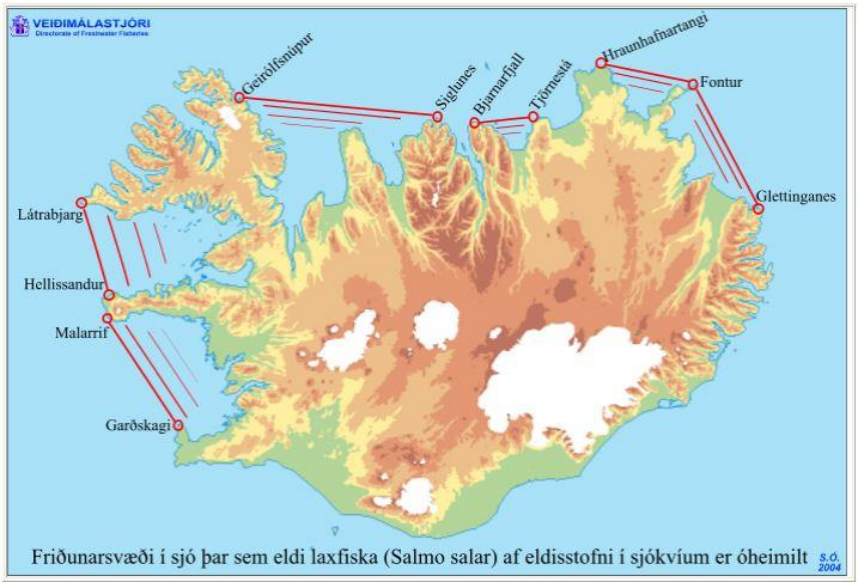

Figure 1: Red marked zones are where sea farming of Atlantic salmon (Salmo salar) is forbidden. (Fiskistofa, 2004).

The production from aquaculture has increased rapidly in the last years and was approx. 53 thousand tons in 2021 (Fig. 2). Which is an increase of 12,500 metric tons (MT) from the year 2020, but between the years 2019 and 2020 the increase was around 6,500 MT. (Statistics Iceland, 4.4.2022). After 2015 the growth started and took a big jump from 2018 to 2019. The increase is mostly related to salmon farming in open sea cages. Around 44.5 thousand MT of salmon was slaughtered from open sea cages in 2021, which is a record harvesting numbers in Icelandic history (Radar, https://radarinn.is/Fiskeldi/Eldi). Salmon production is the main product or around 90% of all aquaculture production in Iceland. The production of Arctic charr

(*Salvelinus alpinus*) and rainbow trout (*Oncorhynchus mykiss)* was around 6,300 mt total in 2021 (Statistics Iceland, 4.4.2022).

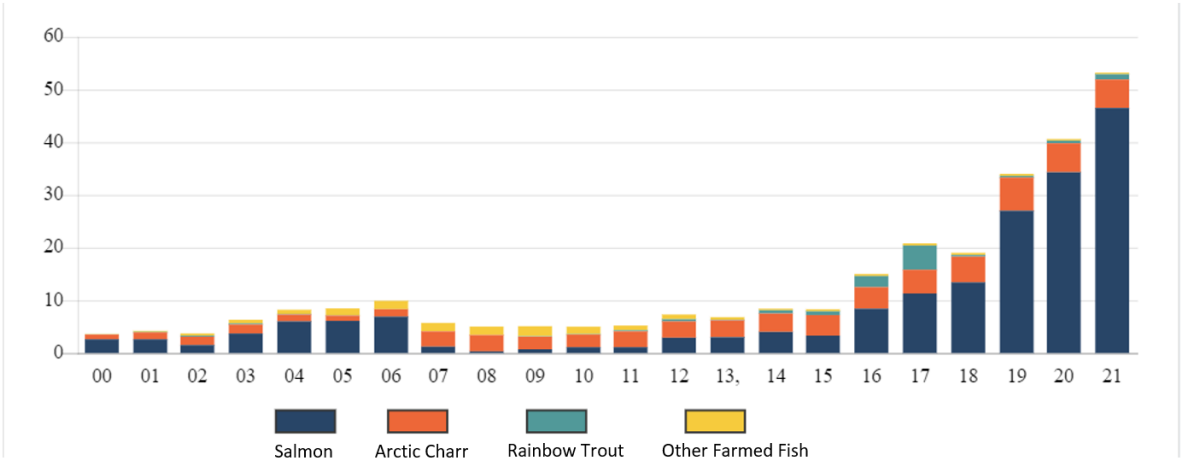

Figure 2: Fish farming production of different species in thousands of tons (Radar, https://radarinn.is/Fiskeldi/Eldi).

In the year 2020 Norway was the biggest producer of farmed salmon in Europe with 1.5 million tons a year (Fiskeridirektoratet, 2021). So, Iceland is still a very small producer worldwide. With an industry that is going through a growth phase it needs a lot of investments for further growth possibilities. Investments in the aquaculture industry has never been more than recent years in Iceland. The fixed investments in 2021 was more than 52 million euros and was around 49 million euros in the year 2020. From 2016 until end of 2022 the total fixed investments have been around 229 million euros (Radar, https://radarinn.is/Fiskeldi/Fjarfesting). To follow up on this rapid growth of producing salmon at sea there is a high demand of smolts to put at sea. A large emphasis on renovation and development has been on the production of smolts in recent years (Radar, https://radarinn.is/Fiskeldi/Eldi).

1.2. Atlantic salmon lifecycle

Like other salmonids, the Atlantic salmon is a diadromy species that starts the life cycle in freshwater where it hatches and migrates to the sea to gain weight and size before going back to the river to spawn in autumn (Fig. 3). After spawning fertilized roes are dug down in the gravel on the bottom of the river, after spawning where it completes oogenesis before the alevins hatch. Alevins have a yolk sac that provides them with all necessary nutritional needs, and they hide in the gravel and use the gravel as a support for their balance until they have absorbed all of the sac, over this period both morphological and physiological development continues before they are able to start hunting for feed as fry. Next step is that the fry develops into juvenile salmon parr, that has these parr marks that help them camouflage. They develop both vertical stripes and spots which are specific for the parr (Stefansson et al., 2002). Atlantic salmon and Arctic charr do not develop strong seawater tolerance until at a significantly later juvenile stage, most often the fish has reached 10–15 cm in body length. At this stage, environmental cues primarily photoperiod and temperature, initiate the smoltification process, preparing the fish for downstream migration and transition to the marine life-stage (Bjornsson et al., 2011).

The parr stays in the river for 2-6 years before they start migrating during spring and early summer (MFRI Iceland, 2022). This is depending on genetic factors and environmental conditions like growth rate, size, temperature, photoperiod, etc. before migrating into the North Atlantic Ocean during spring and early summer (Stefansson et al., 2002). Before the parr is ready to enter the saltwater they undergo a series of morphological, physiological, and behavioural changes, enabling it to survive the transfer to the ocean (Stefansson et al., 2002). These changes are known as smoltification and are the preadaptation to a totally new environment. After the smoltification and migration to sea, the salmon spends one to three years feeding and growing until it is ready to migrate back to the river it hatched from to spawn as a mature adult (Fig. 3) (Stefansson et al., 2002).

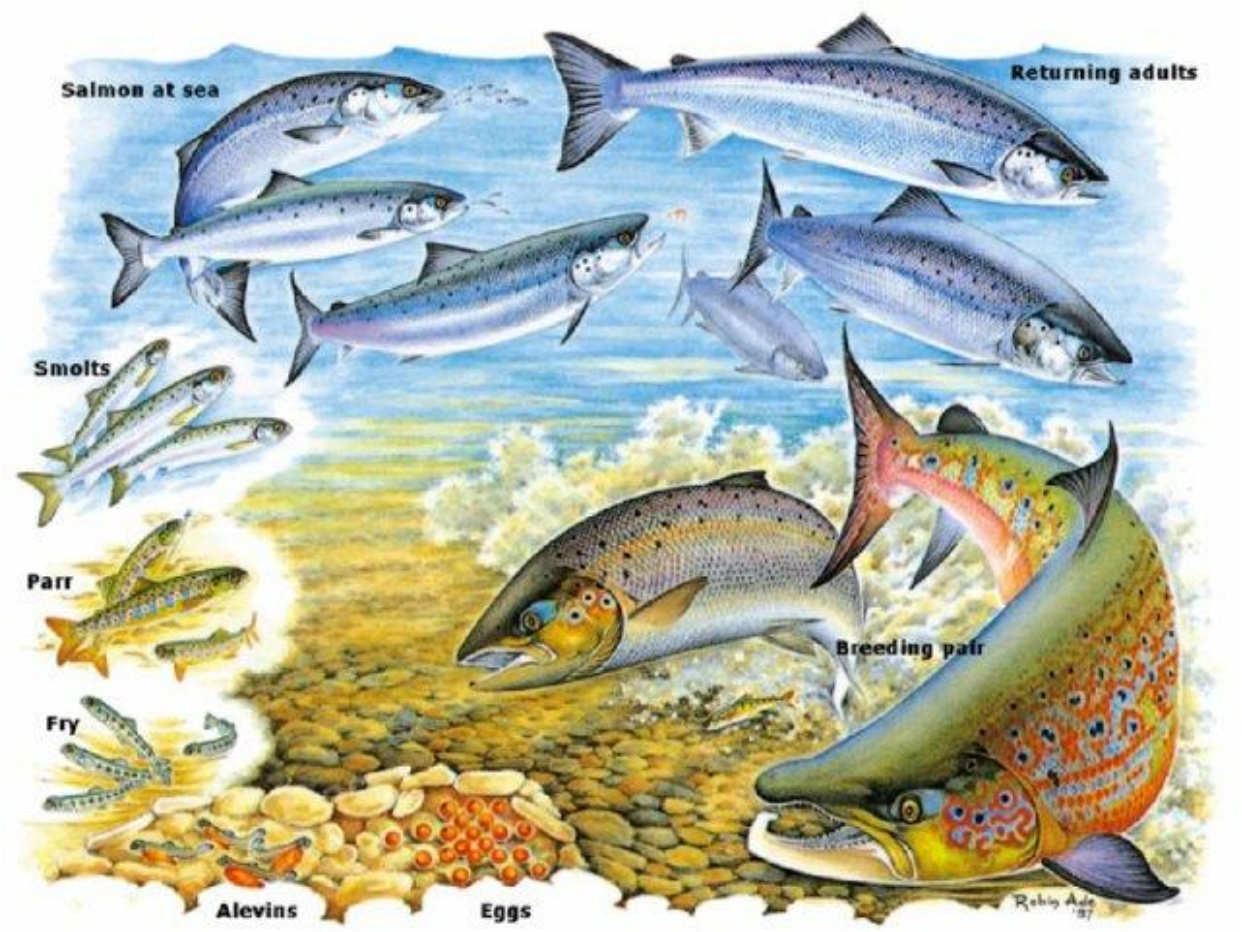

Figure 3: Atlantic salmon life cycle. Illustration courtesy of the Atlantic Salmon Trust and Robin Ade (Hagelin, 2019)

1.3 The hurdles with rearing salmon in open sea cages

1.3.1 Lice

The salmon louse is an ectoparasite of salmonids in the sea. Historically, salmon lice have been observed in moderate numbers on wild salmonids, because farmed Atlantic salmon act as hosts, open net cage farming has increased the pressure of salmon lice in many coastal areas. Even though salmon lice may only threaten population viability under strong infection pressures over several years, in combination with other impacts, salmon lice may have critical effects (Forseth et al., 2017).

There are different methods of preventing or treating lice on fish in open sea cages. Like putting up some physical lice barriers such as skirts or closed systems (Nilsen et al., 2017; Stien et al., 2018). Medical treatments may be applied through feeding or by bathing the fish with chemical therapeutants. Thermal or mechanical delousing are also used e.g., flush the

fish with seawater or warm water to remove the lice. Sometimes it is necessary to harvest the fish earlier (Eliasen et al., 2018).

The fight against lice is a significant expense for fish farmers in terms of direct costs, reduced growth, potential escapees, fish mortality which increases during delousing, and fish health. Early slaughter is the most welfare-friendly strategy, but it will often mean a big loss of profit (Eliasen et al., 2018). There is another big problem as the lice have developed resistance against all available chemotherapeutants (Coates et al., 2021), therefore the industry has needed to increasingly rely on non-medicinal alternatives to control salmon lice like cleaner fish, closed systems or to shorten the production time of the salmon.

Treatments against salmon lice with non-medical treatments have been discussed as one of the reasons for the increased mortality in the seawater phase of salmon production (Sviland et al., 2021). High mortality represents major economic losses and poor fish welfare (Oliveira et al., 2021).

1.3.2. Sea temperature in Iceland and output window

The difference from the coldest sea temperature and the warmest sea temperature in the Westfjords of Iceland are big. The coldest period is in February/March where the sea goes down to 0.5 degree Celsius in Patreks- and Tálknafjörður and down to 1 degree Celsius in Arnarfjörður, while the warmest period is in the end of August beginning of September, where the temperature is around 10.5-12 degrees Celsius in the Westfjords (Data from Arnarlax ehf.). The temperature rapidly decreases then in the coming autumn weeks. The window to put smolt out at sea in Iceland is short or only around 5 months a year, between May and October. This means that to fully utilize a salmon farming license is hard unless you have some variants of smolt sizes and can control the production time, by putting out large post smolts around >500 g and have a short production time until harvest, as well as putting out regular size smolts around 150 g and have a more normal production time of 20-24 months. A strategy like that can make a huge differential for the salmon farming producers in Iceland to have this as a possibility (Björn Hembre, CEO of Arnarlax, Iceland, pers. comm.).

1.4 Are large post-smolts the answer?

A key factor in abating the current challenges of open sea cage farming is to reduce the open sea cage period. This reduction will reduce the exposure period to challenges such as sea lice and diseases. In addition, larger smolts are more resilient and capable of handling the transfer to open net pens in seawater (Øvrebø et al., 2022). The first few months after sea cage transfer, known as the early post-smolt phase, is considered the most vulnerable for the salmon lifecycle (Tang et al., 2022). Smolt size and high quality smolts are therefore an important factor in farming Atlantic salmon. Salmon farming companies have been experimenting by producing so called post-smolts. The Atlantic salmon is considered a post-smolt until it reaches weight of 1 kg (Øvrebø et al., 2022).

To produce a post-smolt the juvenile salmon is not put straight out into a sea cage after smoltification. Instead, the juveniles are raised longer in land-based systems at higher temperatures to be able to stocking post-smolts to sea cages all the year (Tang et al., 2022).

In Iceland it is not possible to stock the sea cages all year around and the suitable time period of transporting smolts to sea is only during May to October, so in Iceland we will focus on post-smolts in a way to have bigger and more robust smolts to put in sea over that period and manage the biomass accordingly so we are available to harvest fish all the year around (Björn Hembre, CEO of Arnarlax, Iceland, pers. comm.).

The aquaculture company Hiddenfjord which is located in Faroe Islands produces large smolt and that has been a success for the company. In their case they decided to produce large smolt as a preventive method to fight sea-lice. With larger smolts (500-700g) they have managed to shorten the rearing time in sea and thereby the overall biological risk (Jón Atlason, Hiddenfjord, Faroe Islands, pers. comm.). Larger smolts have also enabled the company to put out smolts in more exposed conditions in the sea (Jón Atlason, Hiddenfjord, Faroe Islands, pers. comm.).

However, producing large smolts does not only have benefits. Larger smolts require heavy investments, to have enough space on land (Jón Atlason, Hiddenfjord, Faroe Islands, pers. comm.). Large smolt also require more energy use. This means higher costs on land and higher emission of CO_2. Nesset et al. (2017) concluded that producing a harvest sized fish on land compared to a normal sea cages production, both in the capital requirements needed to

construct the stations and in the price per kilo of production. This results in the price per kilo of production from the farm-gate cost of production is up to 10 NOK higher in land-based (Nesset et al., 2017). We can then expect that longer grow outs on land making post-smolts would lead to some higher production cost. However earlier studies have looked at intensive rearing environments and have showed that the maturation is higher when the fish is reared at higher temperatures and continuous light (Fjelldal et al., 2011; Imsland et al., 2014).

1.5. Sexual maturation of Atlantic salmon

Sexual maturation refers to the process of reaching maturity, where the salmon will become capable of reproducing. This process typically occurs in the wild for over 1 to 9 years where the parr spends 1 to 6 years in the freshwater river before going to sea, where it spends 1 to 3 years to eat and grow before it migrates back to the river to finish the sexual maturation and starts the spawning (Fjelldal et al., 2018). When the fish goes through sexual maturation he goes through drastic changes in morphology, behaviour and physiology (Fjelldal et al., 2018).

During maturation, male and female salmon undergo morphological changes, such as the development of secondary sexual characteristics, such as the growth of a hook in males and the formation of a rounded belly in females. Hormonal changes also occur, including an increase in the levels of sex steroids, such as testosterone, which are responsible for the development of sexual characteristics (Mobley et al., 2021).

In all cases, sexual maturation is initiated by the activation of the Brain-Pituitary-Gonad (BPG) axis in response to various environmental and internal factors (Schulz et al., 2010; Zohar et al., 2010; Taranger et al., 2010; Fig. 4). This activation is characterized by increases in gonadotropin production in the pituitary, first of Fsh (Follicle-stimulating hormone) and later of Lh (Luteinizing hormone), as well as by increases in sex steroids like 11-KT (11-ketotestoterone). Other hormones related with the growth axis such as Igf-I (Insulin-growth factor-I) also seem to stimulate the activation of the BPG axis and to support 11-KT production during maturation. Together, gonadotropins, steroids and Igf-I regulate the process of testis maturation or spermatogenesis (Schulz et al., 2010; Martinez et al., 2022). The gonadotropins, LH and FSH, are the most important pituitary hormones regulating testicular physiology. Two points are of great relevance for their biological activity, the specificity, with which the

gonadotropins interact with their receptors and the cellular site(s) of receptor expression. However, information on FSH effects on fish spermatogenesis that is not related to steroidogenesis is not available so far (Schulz et al., 2010; Crespo et al., 2022). The sex steroids, progestogens, androgens, and estrogens are mainly produced in the gonads. Plasma levels of steroid hormones show important variations during male gonad maturation. In general, estrogens are considered 'female' hormones but are formed in male vertebrates as well (Schulz et al., 2010). Estrogens bind to nuclear receptors that act as ligand inducible transcription factors. Three estrogen receptor subtypes (alpha, beta1 and beta2) are expressed in fish and the male gonad is a major site of expression (Schulz et al., 2010).

Gonadotropin Releasing Hormone or GnRH, a neuropeptide hormone which is produced in the hypothalamus. It is released at the pituitary gland to manage gonadotropin secretion and is a key regulator of reproduction. Another important neurotransmitter is the amine Dopamine, DA. Dopamine has various functions like reward and motivation. It has an inhibitory effect on reproduction (Zohar et al., 2010). These are secreted to the pituitary where the Follicle-stimulating hormone, Fsh and Luteinizing hormone, Lh are released. The Fsh is a glycoprotein hormone which stimulates the early phase of gametogenesis or the spermatogenesis in males and oocytes development in females. The Lh is a gonadotropin that affects the late stage of maturation and functions through gonadal membrane receptors and stimulates steroidogenesis and gametogenesis (Zohar et al., 2010).

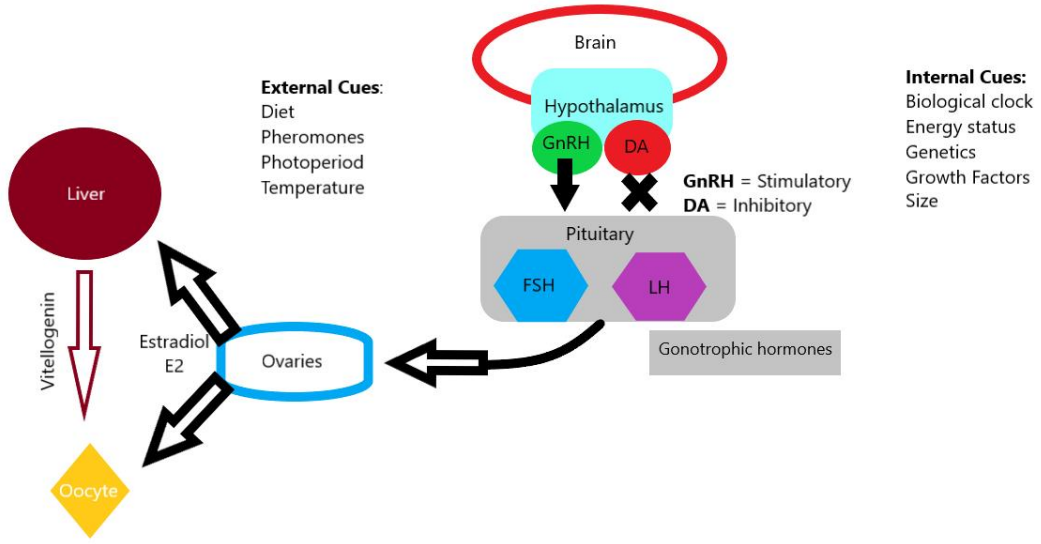

Figure 4: An overview picture of the hypothalamic–pituitary–gonadal axis (HPG axis), modified after Zohar et al 2010 and a lecture by Professor Tom Ole Nilsen, BIO, UIB,2022.

The testis is composed of two main compartments, the intertubular (or interstitial) and the tubular compartment. The intertubular compartment contains steroidogenic Leydig cells, blood/lymphatic vessels, macrophages and mast cells, neural and connective tissue cells, the latter being continuous with the tunica albuginea i.e., the testis organ wall (Schulz et al., 2010). Leydig cells are the site of androgen production in the testis. The principal and most important androgen produced by Leydig cells is testosterone. Testosterone biosynthesis is primarily under the control of the pituitary gonadotropin luteinizing hormone (LH) (Diemer et al., 2003). The main Sertoli cell functions are to support germ cell survival, development, and physiological functioning. Sertoli cells are also called cyst cells. Sertoli cells are the first somatic cell type to differentiate in the vertebrate testis and this cell type plays a crucial role in directing testis differentiation and development. In the cystic mode of spermatogenesis in fish, germ cell number and volume increase greatly per cyst during the spermatogenic process. The increases in cyst volume and Sertoli cell number per cyst both levels off during meiosis/start of spermiogenesis (Schulz et al., 2010). Spermatogenesis is a developmental process during which a small number of diploid spermatogonial stem cells produce many highly differentiated spermatozoa carrying a haploid, recombined genome (Schulz et al., 2010). Moreover, Sertoli cells secrete fluid that generates the tubular lumen, and they phagocytise apoptotic germ cells, residual bodies discarded by spermatids during spermiogenesis, and residual sperm. Therefore, the development of spermatogenic cells

strictly depends on their interaction with the somatic elements of the testis, amongst which Sertoli cells play a crucial role in animals in general (Schulz et al., 2010).

1.5.1 Vitellogenesis:

Estrogen secreted from the ovarian follicles triggers the synthesis of Vitellogenesis. Vitellogenesis is an antigen which will turn into egg yolk protein that is made in the liver of the female and secreted into the blood to be taken into the egg and will become egg yolk (Hara et al., 2016). In salmonids, oocyte maturation and ovulation are preceded by ovarian growth, vitellogenesis, that can happen over 6 months or more of the female reproductive cycle (King et al., 2007). As ovulation in salmonids happens over autumn and winter, vitellogenesis mostly takes place over the summer and early autumn, when natural water temperatures are high (King et al., 2007).

1.5.2 Early maturation

In nature, the occurrence of early maturation depends on the assessment of genetically determined size/growth/energy thresholds during specific time windows defined by photoperiod cues. Since salmon capacity for growth in nature is limited by seasonal variations in temperature, light, and access to feed among others, only a percentage of males normally undergo maturation early (Thorpe, 1994; Martinez et al., 2023).

The capacity for the wild salmon to grow in nature is limited, where there are seasonal changes in light, temperature, and access to feed. This affects that an early maturation is decided by genetics (Martinez et al., 2023). In aquaculture when a farmed salmon is kept for two years in the sea cage it may occur that the fish will sexually mature (Aksnes et al., 1986). Matured salmon is of poor meat quality. That is traced back to the reason that salmon uses the energy from the muscles to develop gonads, not from feed intake (Aksnes et al., 1986; Hendry et al., 1999; Frazer et al., 2023).

Earlier studies have looked at intensive rearing environments and have showed that the maturation is higher when the fish is reared at higher temperatures and continuous light, (Fjelldal et al., 2011; Imsland et al., 2014,) compared to - lower temperature and natural light regime. Those studies showed a clear connection between higher temperature and increased daylight resulted in earlier maturation (Martinez et al., 2023).

Early maturation occurs mostly in males due to the lower energetic investments required for testis development in comparison to female egg production (Martinez et al., 2023). In addition, some juvenile males, called precocious or mature parr, can reach sexual maturity without migrating to the sea (Saura et al., 2008).

Some earlier studies have suggested a connection between the age of maturation and feeding, where the male parr will either come mature after reaching a size threshold necessary for maturation or they become mature while they are still a parr (Berglund et al., 1995; Saura et al., 2008). Reduced access to feed is linked with slower growth (Åsgård et al., 1997). If the growth is reduced that leads to reduced energy stores, e.g., lipid stores or rate of lipid deposition. That has a clear effect on the weight and size of the fish which can delay the age of maturity (Taranger et al., 2010). In contrast, in intensive aquaculture facilities, growth of juvenile salmon is maximized by exposure to continuous light from first feeding, steady high temperature, and unlimited access to feed. These conditions represent a stimulatory environment for growth and development, allowing salmon to mature earlier (Martinez et al., 2023). The only photoperiodic cue that salmon under those conditions experience is a "winter signal" (some weeks under reduced photoperiod LD12:12) introduced to induce smoltification (Bjornsson et al., 2000; Fjelldal et al., 2011). Early maturation of Atlantic salmon male juveniles and post-smolts is undesirable in aquaculture due to its negative impact on growth, welfare, and seawater adaptation, however it is an increasing problem under intensive rearing conditions.

1.5.3 Control of maturation in aquaculture

Many different techniques are being used to minimize early maturation in the aquaculture industry (Taranger et al., 2010), but one of the most used is the use of photoperiod control to arrest or delay maturation. Photoperiod control, is done by putting lights in the sea cages over the winter to mimic the summer photoperiod and to therefore slow or minimize the sexual maturation (Hansen et al., 1992; Nordgarden et al., 2003). Oppedal et al. (2006) found that the sexual maturation was significally lower when continuous light was used superimposed on the natural photoperiod regime. This resulted in 0% maturation during the seawater-phase (Oppedal et al., 2006). Also, in the broodstock facilities photoperiod control

has been successfully applied to alter the phase of the annual sexual cycles to manipulate the spawning (Bromage et al., 2001; Taranger et al., 2015; Xu et al., 2023).

1.6 Lack of feeding and its effects on Atlantic Salmon

When animals go through a fasting period, it goes through three metabolic phases, first they use stored glucose, then they burn their fat and at last muscle proteins (Hvas et al., 2022). Fishes have low metabolic rates and can go through a long fasting period without suffering irreversible consequences (Hvas et al., 2022).

In the wild, Atlantic salmon face different periods where there is less access to food which results in lack of access to feeding. Many different factors influence those periods such as changes in the environment or migration (Hvas et al., 2020). The salmon is though capable of adapting to these changes by reducing their metabolic rate and use less energy to swim around, helps the salmon to survive during those periods (Cooke et al., 2000). In the early life stages of the salmon this has significant effects. The juvenile salmons rely heavily on getting all the energy needed to prepare them to grow and for smoltification. There is a powerful connection between surviving in the ocean and the size of the fish during smoltification (Nicieza and Metcalfe, 1997). Those changes can delay the maturation since the salmon requires energy to undergo the process of becoming mature.

Integral in the parr to smolt transformation and seawater adaptation are reductions in glycogen and changes in body lipids, including depletion of energy stores. Restricted feeding may, lead to a disruption of the smoltification process, resulting in reduced hypo-osmoregulatory ability (Imsland et al., 2011). So smolts are vulnerable to food-deprivation during the early post-smolt phase (Stefansson et al., 2009).

1.6.1 Starving periods, and feed restrictions, in salmon farming

The Atlantic salmon is starved over periods either voluntary or involuntary due to several factors. For example, to avoid poor water quality, feed withdrawal is done to empty the gut of the fish before the fish is handled (crowding, pumping, delousing, transportation, and harvest), suffering from a disease or over environmental conditions (temperature, hypoxia) (Hvas et al., 2022).

Feeding control, by putting the fish on starving periods will slower the growth (Åsgård et al., 1997), and if the growth is reduced it will affect the weight and size of the fish, and could delay timing of maturity (Taranger et al., 2010).

In the aquaculture industry if the fish has not sufficient access to feed it results in slower growth and longer production time. The fish will not be as big and robust to prepare for the smoltification and entering the seawater. This can result in lower quality flesh which has effect of the market value and the profitability of the company. It is very important for the managers of the company to monitor and manage the feeding to promote the health and growth of their fish populations.

There are many studies on how fasting or reduced access to feed affects Atlantic salmon. Hvas et al. (2021) investigated the effect of full starvation over four weeks and the results showed that Atlantic salmon maintain their full swimming capacity as well as their ability to respond and recover from stress during an extended period of food deprivation (Hvas et al., 2021). In another trial Atlantic salmon weight, length and condition factor did not change significantly during fasting period of four weeks and the fish immediately resumed eating upon refeeding. They concluded that starvation for up to four weeks have minor effects on the fish welfare (Hvas et al., 2020).

Different feeding ration have also been researched and Stefansson et al. (2009) found out that food-deprivation may result in significant osmotic disturbances, and ration levels significantly influenced growth rate and mean body size (Stefansson et al., 2009). Martinez et al. (2023) found out that reducing the feeding ration will not help reduce the maturation without significantly affecting growth (Martinez et al., 2023).

1.6.2 Welfare of the fish

Fish welfare is an important factor in modern aquaculture (Ashley, 2007, Cañon Jones et al., 2012). For welfare to have real meaning, the animal concerned must have the capacity for suffering, recent evidence suggests that external stressors and painful stimuli causes avoidance in fish (Turnbull et al., 2005). The welfare indicators presently used vary in include e.g. fin damage, morbidity, and mortality rate (Turnbull et al., 1998, Santurtun et al., 2017). There are many factors that may cause increased fin damage and fin wounds in a rearing cage, feed ratios being one of those (Cañon Jones et al., 2010). Aggression, as one form of social

interaction and has the potential to cause physical injury. Among the salmonid fishes, aggression has evolved as a behavioural strategy. It is used in the wild to obtain and defend territories, to gain preferential access to food and to maintain exclusive access to mates (Turnbull et al., 1998, Cañon Jones et al., 2010, Cañon Jones et al., 2017). Monitoring of fin damage is presently used as a welfare indicator in Icelandic salmonid culture (Kári Heiðar Árnason, Head of research station, Hólar University, pers. comm.) and will, therefore, be monitored in the present study.

1.7 Current study

The aim of this study was to investigate the effects of different feeding ratios on the growth, welfare rating and maturation development of Atlantic salmon juveniles. Specifically, this study assessed the impact of varying feeding ratios on growth rates and welfare rating (here measured as development of fin wounds) of the juveniles. By examining the relationship between feeding ratio and development of maturation in the parr, this study aimed to provide insight into optimal feeding practices for the production of healthy juveniles that will become well-developed Atlantic salmon smolts.

The experiment was based with these hypotheses:

H01: Raising Atlantic salmon parr and exposing them to different feeding ratios has no significant effect on body weight development.

HA1: Raising Atlantic salmon parr and exposing them to different feeding ratios has a significant effect on body weight development.

H02: Raising Atlantic salmon parr and exposing them to different feeding ratios has no significant effect on body length development.

HA2: Raising Atlantic salmon parr and exposing them to different feeding ratios has a significant effect on body length development.

H03: Raising Atlantic salmon parr and exposing them to different feeding ratios has no significant effect on development of maturity.

HA3: Raising Atlantic salmon parr and exposing them to different feeding ratios has a significant effect on development of maturity.

H04: Raising Atlantic salmon parr and exposing them to different feeding ratios has no significant effect on Condition factor (K-factor).

HA4: Raising Atlantic salmon parr and exposing them to different feeding ratios has a significant effect on Condition factor (K-factor).

H05: Raising Atlantic salmon parr and exposing them to different feeding ratios has no significant effect on the fish welfare rating.

HA5: Raising Atlantic salmon parr and exposing them to different feeding ratios has a significant effect on the fish welfare rating.

2. Materials and methods

2.1 The fish

Juvenile Atlantic salmon of the Saga strain was obtained from the aquaculture company Arctic Sea Farm. The roes were obtained from Benchmark genetics delivered to Norðurbotn in the bottom of Tálknafjörður in the Westfjords of Iceland, which is the smolt station for Arctic Sea Farm where the roes were hatched. In April 2022 the fish was delivered to the fish research station in Sauðárkrókur named Verið, which is run by the Hólar University where the fish was kept and reared until the experiment was finished in the end of January 2023. The fish was reared at average 10°C and the fish was fed by ECO 3.0 feed which is manufactured by Laxá (Akureyri, Iceland). The ECO 3.0 feed main ingredients are: Fish meal, fish oil, wheat, rapeseed meal, rapeseed oil, soy meal, shrimp meal, wheat gluten, vitamin, minerals and panaferd natural colouring. In the 3.0 feed the split of contents are in a table 1.

Table 1: Overview of content in ECO Feed 3.0 used during the experimental period.

Content %	Protein	49
	Fat	23
	Carbohydrate	13
	Ash	8
	Dry material	93
	Panaferd, mg/kg	50
	digestible energy (MJ/kg)	19.0
	Gross energy (MJ/kg)	22.2
Vitamines in kg of feed	Vitamin A IU	2,500
	Vitamin D3 IU	1,500
	Vitamin C (mg/kg)	250
	Vitamin E (mg/kg)	115

2.2 Design of the experiment

The juvenile salmon was brought from Norðurbotn to Verið in April 2022, and distributed randomly among 12, 2m^3 tanks the day of the arrival. Every tank was provided with a steady stream of fresh water of approximately 10°C. The oxygen saturation was kept above 83% (105% average) in all tanks throughout the experiment. The fish was kept at continuous light (LD24:0) through the whole experiment.

On 13th of August 2022 the fish was measured both by length and weight and 450 juvenile salmons with no deformations and no visible wounds was distributed equally among nine $2m^3$ tanks. 50 fish were put in each tank and 50% of the fish in each tank were individually tagged with a Passive Integrate Transporter (PIT-tag).

The nine tanks were set up three in line and three lines (3x3, Fig. 5). In each line the feeding was reduced in 2 of the three tanks over the whole experimental period. One tank in each line had a ratio of feeding 100R (feed every day), one tank had the ratio of feeding at 50R (every other day) and the third one has the ratio of feeding at 33R (feed every third day). The fish was measured four times over the period of the experiment, from 13th of August 2022 until 16th of January 2023. If the fish was not in an optimal state, had many wounds and was clearly unhealthy and lack of welfare (very small, open wounds with fin rays out) it was euthanized in a humane way. In those cases, the fish were put in a 40-litre tank which included an overdose of anaesthesia (Phenoxyethanol, 8-10ml, produced by Mjöll Frigg, Reykjavík Iceland) and in the end the fish then given a blunt force trauma to the head, and the gills cut.

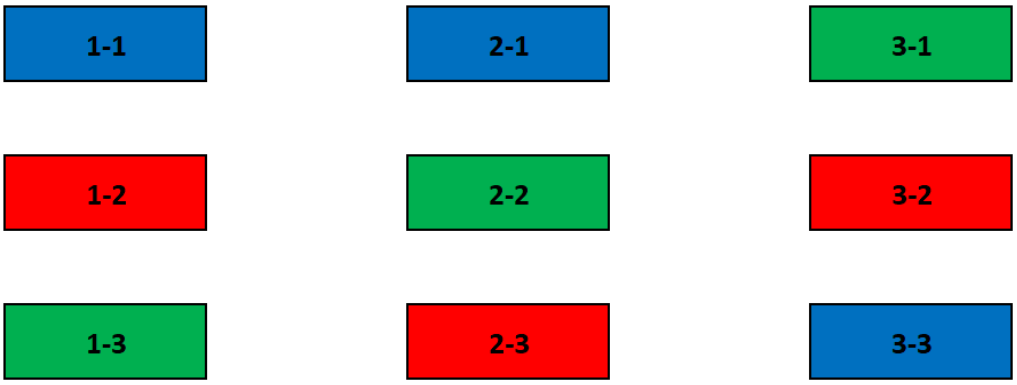

Figure 5: Overview of the tanks in the experience. Green = the 100R, Blue = the 50R and Red = the 33R

2.3 Sampling

The first follow up measurement was done 23rd of November 2022 or 3 months and 10 days later. All the fish was starved two days prior to measurement. Every fish in each tank was scanned for the PIT-tag, and the PIT-tag number, length (to nearest mm) and weight (to nearest 0.1 g) of the fish was measured and registered. After this measurement there were signs of wounds and some fungus growth (Fig. 6), so it was decided that the fish should be

treated with formalin bathing. Formalin bathing was done on the 30th of November 2022 where all the fish were treated with Formalin with the ratio 1:4000. The fish was starved one day prior to the treatment.

Figure 6: Wound on the Pectoral fins of the salmon, one of the reasons why a decision on bathing the fish in formalin was made.

During the third measurement which was done on the 13th of December 2023, the fish looked better. The fish was starved 2 days prior to sampling and the same sampling method as previously was performed, with two additions, 8 unmarked fish in each tank were euthanized and cut open. The development of maturation of the fish was rated according to Guðbergsson and Antonsson (1996) (See Fig. 7), and pictures taken of every fish (Figs. 8-9). The fish was also rated by welfare, the fish was rated from 0-5 on how many wounds he had (Kári Heiðar Árnason, Head of research station, Hólar University, pers. comm.), 0 as the best result and 5 the worst and the fish euthanized.

One day prior to the sampling in December an accident had happened where one of the tanks had an air bubble blocking the intake of water. All the fish in the tank had died, but as this

happened one day prior to planned sampling it was possible to rate the maturity of all fishes and include the data in the study.

The fourth and final measurement was performed on the 16th of January 2023, and all the same samplings were done the same as 13th of December. Except that now all the fish (tagged an untagged) were euthanized, cut open and development of maturity rated the same way as the sampling 13th of December, and picture taken of each individual and registered.

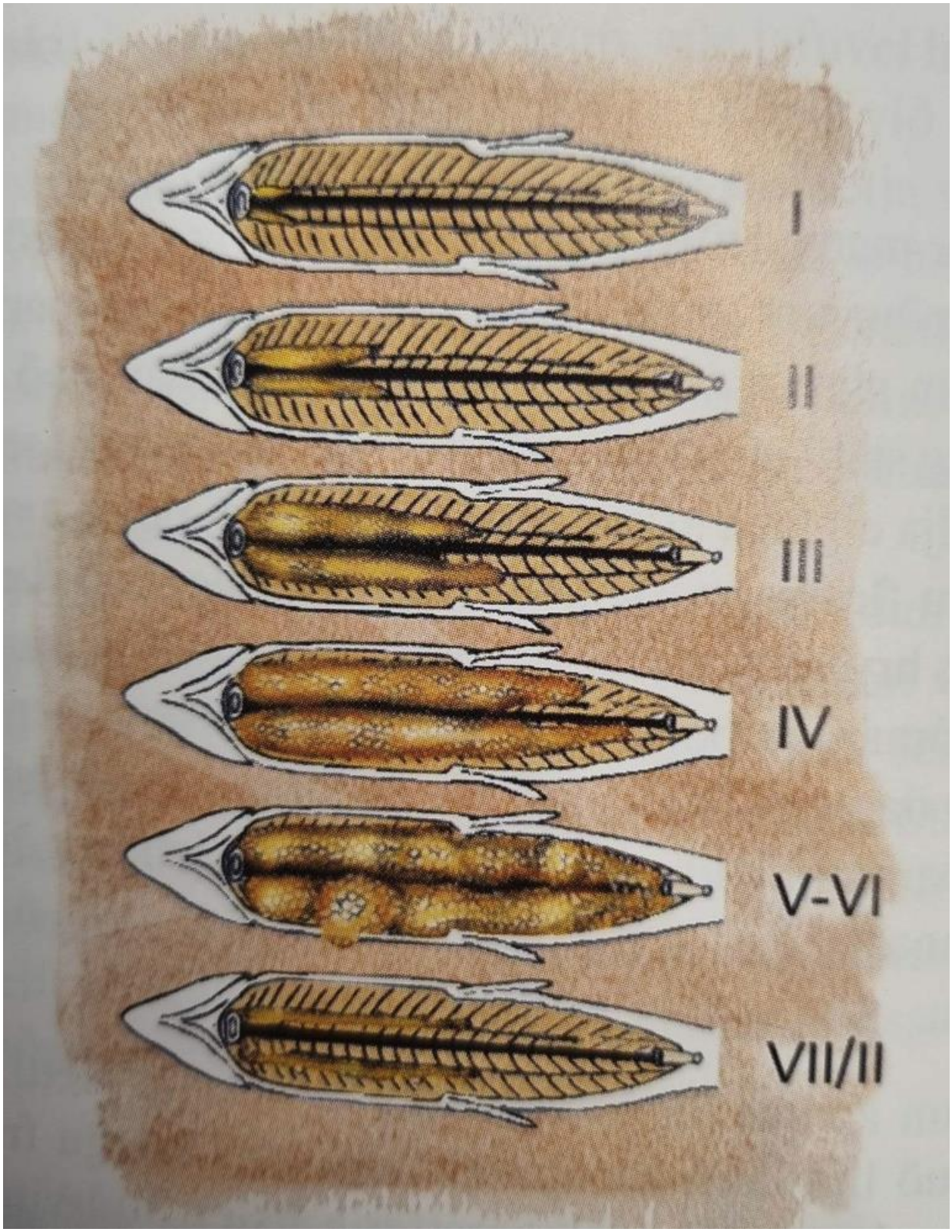

Figure 7: Scale for stages of maturity of Atlantic salmon (Guðbergsson & Antonsson, 1996)

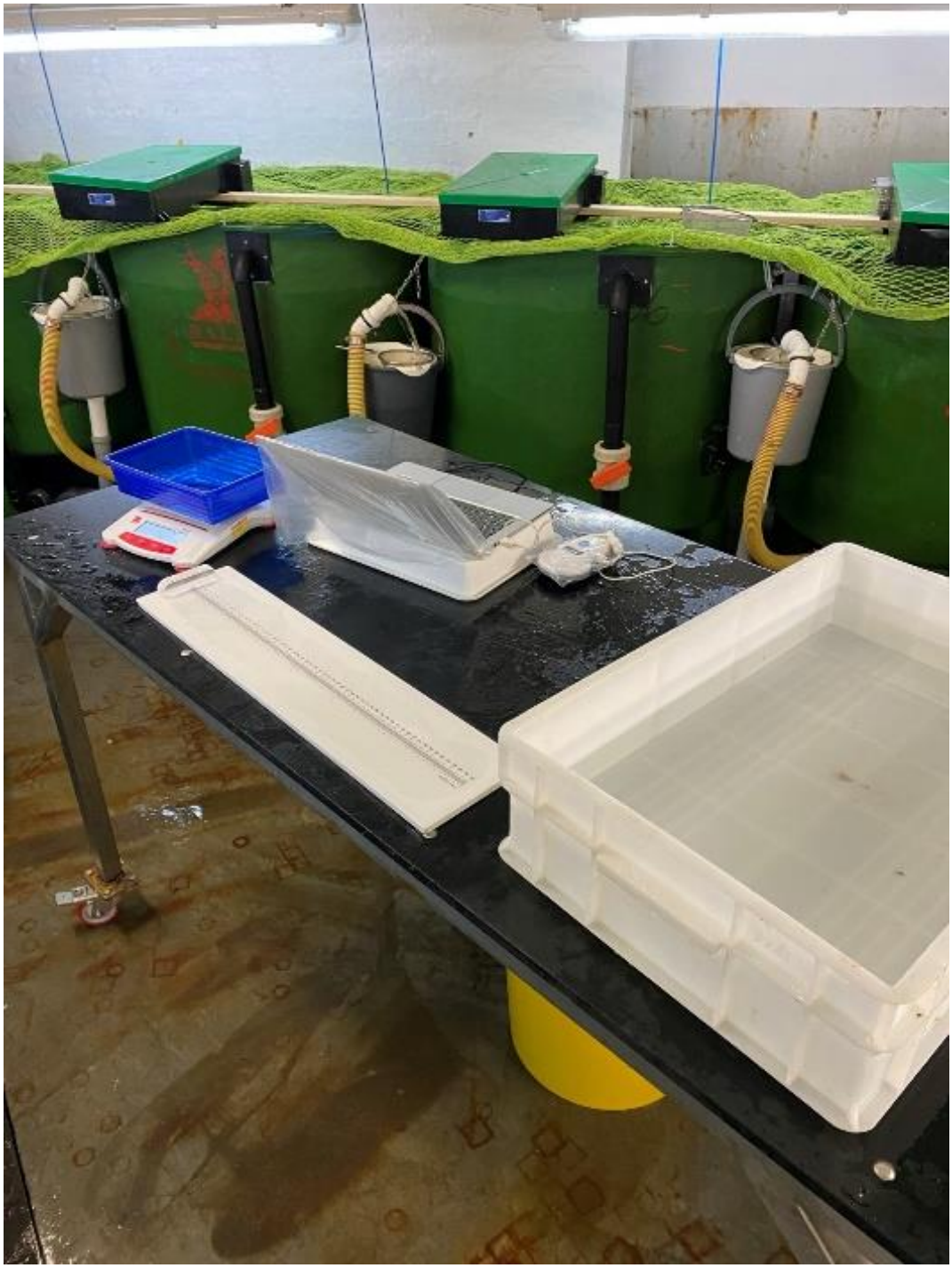

Figure 8: The measurement table, the scale, measurement board for length, anaesthesia tub, computer to register the results and a scanner to scan the internal PIT-tag.

Figure 9: Picture of how the setup was during the final sampling. Where the weight and length was measured, the registration in excel (yellow trousers), the gutting and rating sexual maturation (red trousers) and pictures were taken (White coat).

K factor

The condition factor (K-factor) was calculated between sampling and from first day of the experiment until the last using Fulton's condition factor formula (Mozsár et al., 2015):

Condition Factor (K) = 100W⁄ L^3

1. W[g] = Weight
2. L[cm] = Total length.

Specific growth rate (SGR)

SGR was calculated between samplings and for the whole period the experiment took using this formula (Dempster et al., 2008):

SGR = (Ln (Wt) - Ln(W0)) * 100 / t(d)

1. W0[g]= the weight in grams at the beginning of the period.
2. Wt [g]= the weight in grams at the end of the period.
3. t[d]= period, expressed in number of days.
4. Ln = natural logarithm.

Standard error of mean (SEM)

SEM was calculated for each criterion of the growth in the experiment (weight, length, K-factor, SGR)

$$SEM = \frac{SD}{\sqrt{n}}.$$

SD is standard deviation.

n is the sample size

2.4 Statistical methods

All statistical analysis on the collected data was done by using Microsoft Excel (Microsoft Corporation, 2018). All figures and tables were generated using Microsoft Excel (Microsoft Corporation, 2018).

The distribution of response variables (Body weight, body length, condition factor, specific growth rate) were checked for normality and homogeneity of variance using the Kolmogorov-Smirnov test and Levene test.

General Linear Models (two-way nested ANOVA with replication) was performed within each of the feed ratio group separately for the response variables. Two-factor nested ANOVA with replicate tanks (random effect) nested within feed ratio group (fixed effects), was used to determine if there are significant differences between response variables. In cases of significant differences, a Bonferroni correction post-hoc test was done based on each of the two-way models to identify where significant differences between groups occurred.

Graphically, various small letters were used to indicate significant differences between groups at each sample points. All statistical results generated are given in Appendix II. Data in all graphs are represented with the means of each group +- the Standard Error of Means (SEM).

Possible differences in maturity proportions and welfare rating between the experimental groups were tested with a Chi-squared test. A significance level of $\alpha = 0.05$ was used in all cases if not stated otherwise.

3. Results

3.1 Weight development

The two-factor nested ANOVA test for body weight showed significant difference between the three feed ratio groups ($p < 0.001$). Interactions between feed ratio and rearing tanks were not significant ($p > 0.05$). There was a significant difference (Bonferroni post-hoc test, $p<0.01$) in weight between the 33r group and the other groups (100r = 50.67 g, 50r = 50.67 g, and the 33r = 53.42 g) at the start of the experiment.

The difference in body weight under different feed ratio at each sampling were significant (Bonferroni post hoc test, $p <0.001$) after the experiment started.

All groups had a gradual increase in body weight (mean ± SEM) throughout the experiment, with individuals reared with the 100r displaying higher mean body weight than those reared at lower feed ratio of the 50r and the 33r (Bonferroni procedure post-hoc test, $p<0.001$, Fig. 10). Also, the 50r displayed higher body weight at all sampling points compared to the 33r (Bonferroni procedure post-hoc test, $p<0.001$, Fig. 10).

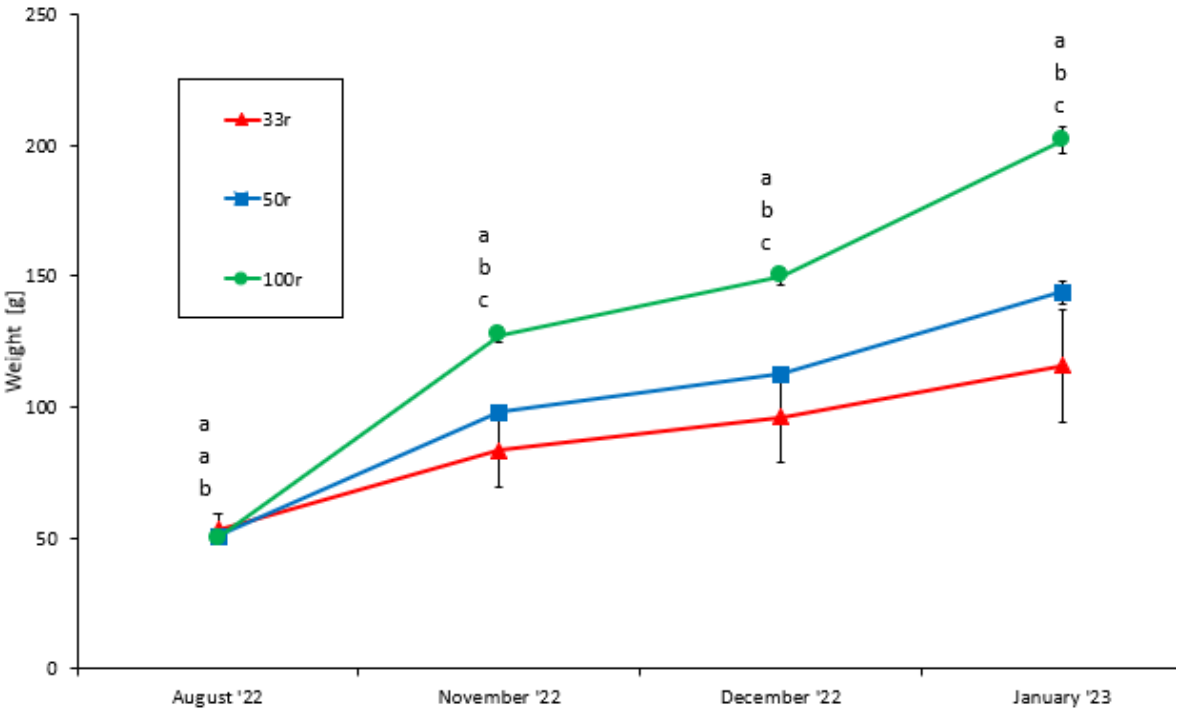

Figure 10: Mean weight of juvenile Atlantic salmon reared at different feeding ratios (100r, 50r, 33r). Vertical lines indicate SEM. Different letters indicate statistical difference (Bonferroni post-hoc test, $p<0.05$) between treatment groups at every sampling point, (a) being the 100r group.

3.2 Length development

The two-factor nested ANOVA test for body length showed significant difference between the three feed ratio groups ($p<0.01$). Interactions between feed ratio and rearing tanks were not significant ($p>0.05$). There was a significant difference (Bonferroni post-hoc test, $p<0.05$) in length between the 33r group and the other groups, (100r = 15.76 cm, 50r = 15.80 cm, and the 33r = 16.03 cm) at the start of the experiment.

The difference of body length between feed ratio at each sampling point were significant (Bonferroni post hoc test, $p<0.01$). The 33r group was significantly smaller compared to the two other feed ratio groups from August, onwards (Bonferroni procedure post-hoc test, $p<0.05$, Fig. 11).

All groups had a gradual increase in body length (mean ± SEM) throughout the experiment, with individuals reared with the 100r displaying longer mean lengths than those reared at lower feed ratio of the 50r and the 33r (Bonferroni procedure post-hoc test, $p<0.001$, Fig. 11). Also, the 50r group displayed higher body length at all sampling points compared to the 33r group (Bonferroni procedure post-hoc test, $p<0.01$, Fig. 10).

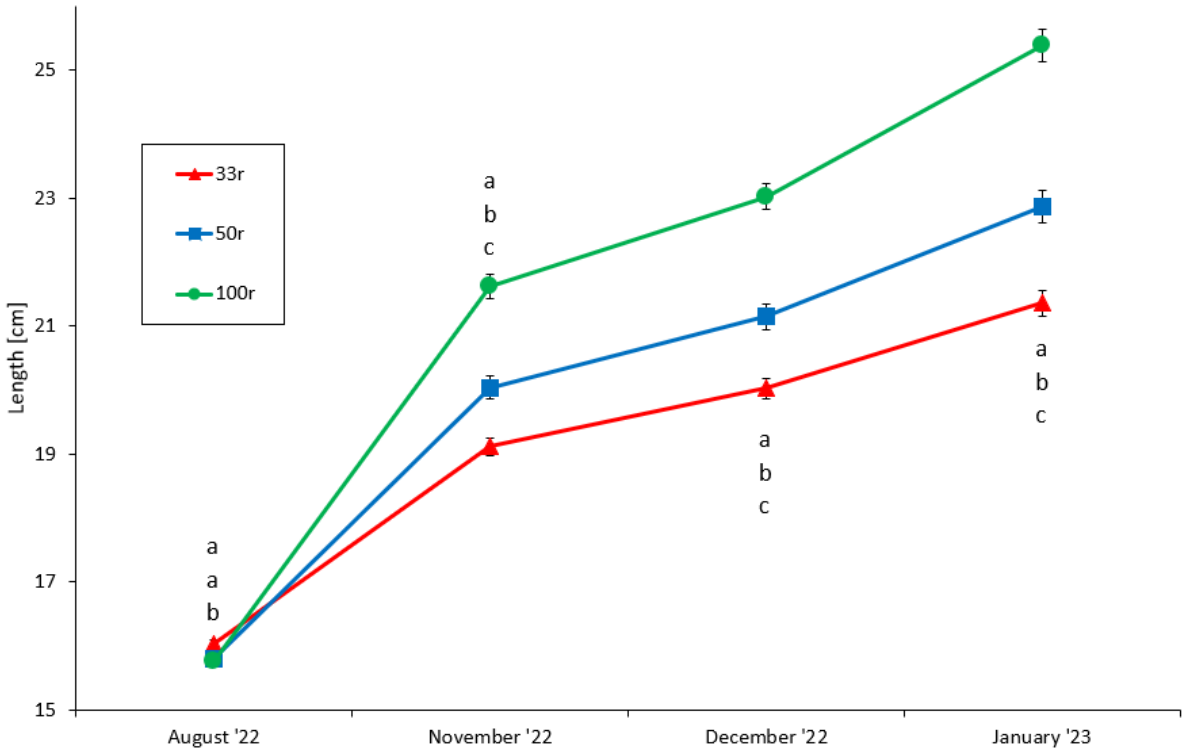

Figure 11: Mean length of juvenile Atlantic salmon reared at different feeding ratios (100r, 50r, 33r). Vertical lines display SEM. Different letters indicate statistical difference (Bonferroni procedure post hoc test, p<0,05) between treatment groups at every sampling point, (a) being the 100r group.

3.3 Condition factor (K-Factor)

The two-factor nested ANOVA test for Condition factor(K-factor) showed significant difference between feed ratio groups ($p < 0.001$). Interactions between feed ratio and rearing tanks were significant ($p<0.05$).

The difference of K-factor between the 100r feed ratio and other ratio at each sampling were significant (Bonferroni procedure post-hoc test, $p<0.05$, Fig. 12) after the experiment started.

All groups showed a decrease in K-factor during the experiment, with individuals reared with the 100r displaying less decline than those reared at lower feed ratio of the 50r and the 33r.

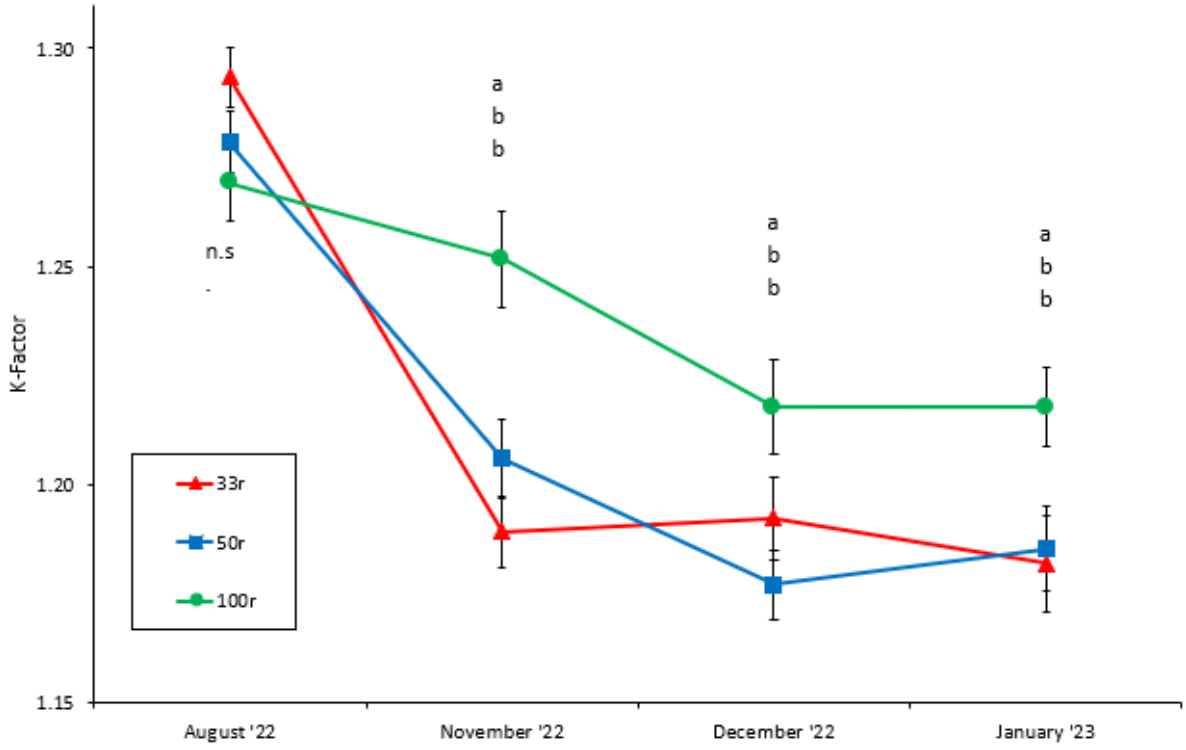

Figure 12: Mean K-factor of juvenile Atlantic salmon reared at different feeding ratios (100r, 50r, 33r). Vertical lines indicate SE. Different letters indicate statistical difference (Bonferroni post hoc test, p<0.05) between treatment group at every sampling point, (a) being the 100r group. n.s. = no significant difference.

3.4 Specific growth rate (SGR)

The two-factor nested ANOVA test for specific growth rate (SGR) showed significant difference between feed ratio of the experiment ($p < 0.001$). Interactions between feed ratio and rearing tanks were also significant ($p<0.001$).

Apart from the period between November and December there was found a significant difference between the SGR in all three experimental groups in all rearing periods as well as overall for the whole experimental period (Bonferroni post-hoc test, $p<0.001$, Fig. 13).

All groups had a steady SGR over the whole experiment, with individuals reared with the 100r displaying higher SGR than those reared at lower feed ratio of the 50r and the 33r (Bonferroni procedure post-hoc test, $p<0.001$, Fig. 13). Also, the 50r showed higher SGR than the 33r (Bonferroni procedure post-hoc test, $p<0.001$, Fig. 13).

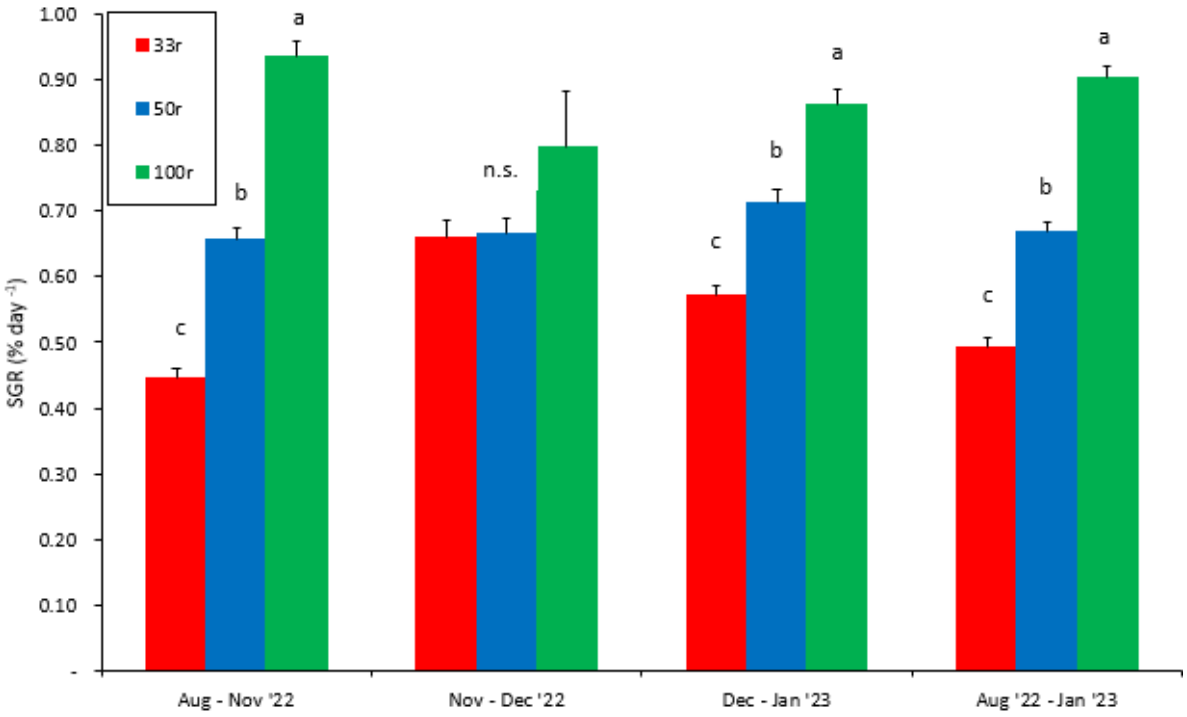

Figure 13: Mean SGR of juvenile Atlantic salmon reared at different feeding ratios (100r, 50r, 33r). Vertical lines display SE. Different letters indicate statistical difference (Bonferroni post hoc test, $p<0.05$) between treatment group at every sampling point, (a) being the 100r group.

3.5 Mortality

The mortality was very low in all the groups and there was no significant difference in mortality between the groups (Chi-squared test p> 0.25). In the 100r group 4 fish died or 2.67%, in the 50r group 6 fish died or 4.00%, and the 33r group had mortality of 3 fishes or 2.00%. Total combined mortality was 13 fishes or 2.89%

These mortality numbers are excluding the accident killing 48 fishes in one of the 33r tanks.

3.6 Welfare rating

A Chi-squared test for categories of data showed that there was significant difference in welfare rating of all the juveniles under different feeding ratio during the third sampling (Table 2) in December.

The 3rd sample showed much better welfare rating for feed ratio 100r than the other feed ratios, and better rating for the 50r than the 33r.

Comparing feed ratio 100r and the 50r showed significant difference in all welfare ratings except 4, there were no fish from these feed ratios rated 5. Welfare rating 0 and 2(Chi-squared test, $p<0.001$), welfare rating 3 (Chi-squared test, $p<0.01$) and welfare rating 1(Chi-squared test, $p<0.05$).

Comparing feed ratio 100r and the 33r showed significant difference in all welfare rating except 4 and 5. Welfare ratings 0, 2 and 3 (Chi-squared test, $p<0.001$), welfare rating 1 (Chi-square test, $p<0.05$).

Comparing feed ratio 50r and the 33r showed no significant difference in welfare ratings in all ratings (Chi-square test, $p>0.05$).

Table 2: Frequency table of each welfare stage under different feed ratio after the 3rd sampling. Superscript letters indicate significant differences between the experimental groups with a as the highest value.

Welfare rating (0-5)	**33r**	**50r**	**100r**	**Grand Total**
0	27^{b}	32^{b}	75^{a}	134
1	65^{a}	62^{a}	48^{b}	175
2	30^{a}	29^{a}	16^{b}	75
3	21^{a}	15^{a}	8^{b}	44
4	4	3	2	9
5	1	0	0	1
Grand Total	148	141	149	438

A Chi-squared test for categories of data showed that there was significant difference in welfare rating of all the parr under different feeding ratio during the 4th and last sampling in January (Table 3).

The fourth and last sample showed much better welfare rating for feed ratio 100r than the other feed ratios, also the 50r showed better welfare rating than the 33r.

Comparing feed ratio 100r and the 50r showed significant difference in all welfare ratings except 4 and 5. Welfare rating 2 (Chi-squared test, $p<0.001$), welfare rating 0 (Chi-squared test, $p<0.01$), and welfare rating 1 and 3(Chi-squared test, $p<0.05$). There was no difference in welfare rating 5 (Chi-squared test, $p>0.05$) since there was only 1 fish from these feed ratios in that category.

Comparing feed ratio 100r and the 33r showed significant difference in all welfare ratings (Chi-squared test, $p<0.001$), except welfare rating 5 (Chi-squared test, $p>0.05$),

Comparing feed ratio 50r and the 33r showed significant difference in welfare ratings in category 0 and 4(Chi-square test, $p<0.001$), but not in 1, 2 and 5(Chi-squared test, $p>0.05$).

Table 3: Frequency table of each welfare stage under different feed ratio after the last sampling. Superscript letters indicate significant differences between the experimental groups with a as the highest value.

Welfare rating (0-5)	**33r**	**50r**	**100r**	**Grand Total**
0	5^{c}	18^{b}	35^{a}	58
1	24^{b}	51^{b}	70^{a}	145
2	28^{a}	38^{a}	11^{b}	77
3	19^{a}	12^{b}	6^{c}	37
4	5^{a}	1^{b}	0^{b}	6
5	2	0	1	3
Grand Total	83	120	123	326

3.7 Sexual maturation

A Chi-squared test for categories of data showed that there was minor connection between feed ratio and the development of maturation of all the juveniles in the present study (Tables 4-5).

There was significant difference between the feeding ratio groups and between the maturity in males in stage 4 (Chi-squared test, $p<0.05$, Table 4), but no significant difference between the females (Chi-squared test, $p>0.05$, Table 5)

There was significant difference between feed ratio 33r and the 50r at the males in maturity stages 0 (Chi-squared test, $p<0.05$, Table 4).

When looking at the combined numbers of males in stages 2-5 (Table 4) there was an overall trend towards lower numbers in the 33r group (Chi-squared test, $p=0.06$) compared to the 100r group.

*Table 4: Frequency table of male individuals in each maturation stage under different feed ratio in January 2023. *An accident happened in one of the tanks and part of those numbers are from the 3rd sampling, one month before (see appendix II for split). Superscript letters indicate significant differences between the experimental groups with a as the highest value.*

Maturity stage (0-5)	33r - M	50r - M	100r - M	Grand Total - M
0	3^{a}	1^{b}	2^{ab}	6
1	70*	59	59	188
2	0	1	3	4
3	4*	2	4	11
4	0^{b}	3^{a}	1^{b}	4
5	0	0	2	2
Grand total	78	66	71	215

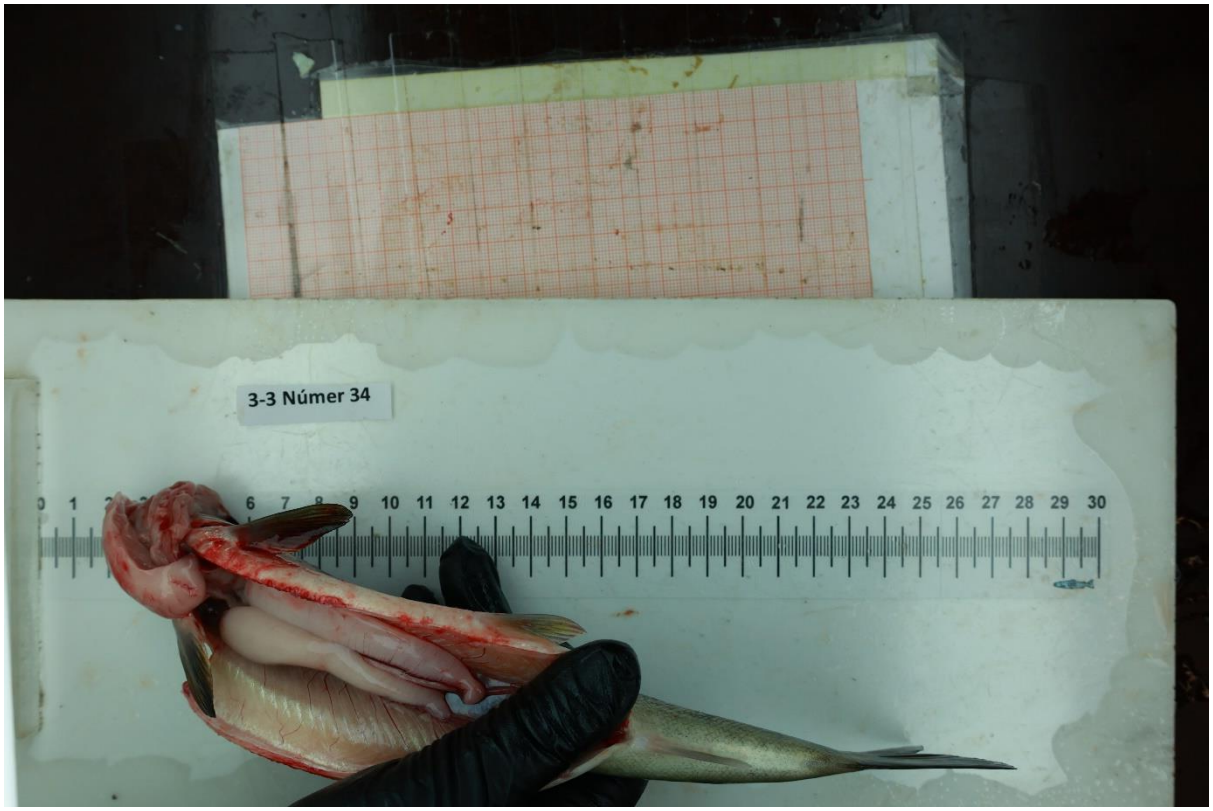

Figure 14: Fish nr. 34 in tank 3-3, 50r feed ratio, male, maturity rate 4

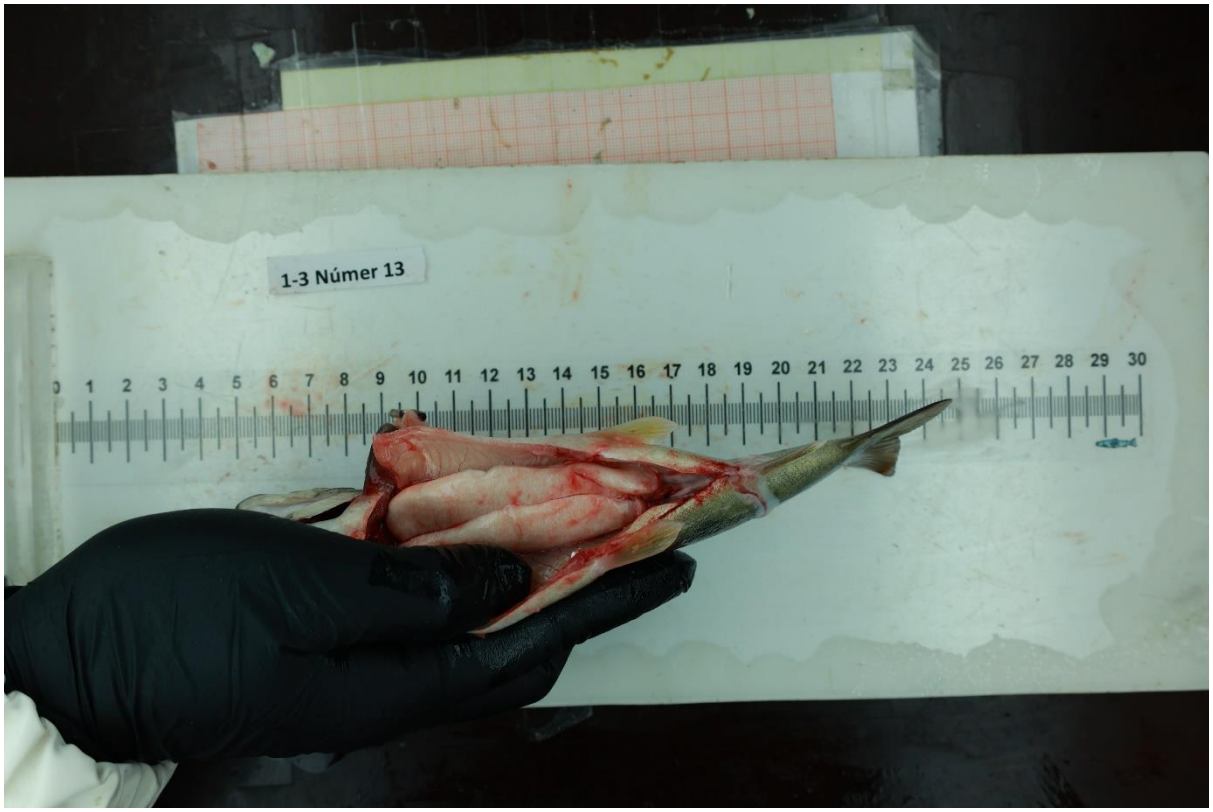

Figure 15: Fish nr in tank 1-3, 100r feed ratio, male, maturity rate 5, typical precocious male

There were no significant differences between the feed ratio groups and maturity stages in the females (Chi-squared test, p>0.05, Table 5, Fig. 16)

*Table 5: Frequency table of female individuals in each maturation stage under different feed ratio in January 2023. *An accident happened in one of the tanks and part of those numbers are from the 3rd sampling, one month before (see appendix II for split). Superscript letters indicate significant differences between the experimental groups with a as the highest value.*

Maturity stage (0-5)	33r - F	50r - F	100r - F	Grand Total - F
0	0	0	1	1
1	69*	78	71	219
2	1	0	1	2
3	0	0	0	0
4	0	0	0	0
5	0	0	1	1
Grand total	70	78	75	223

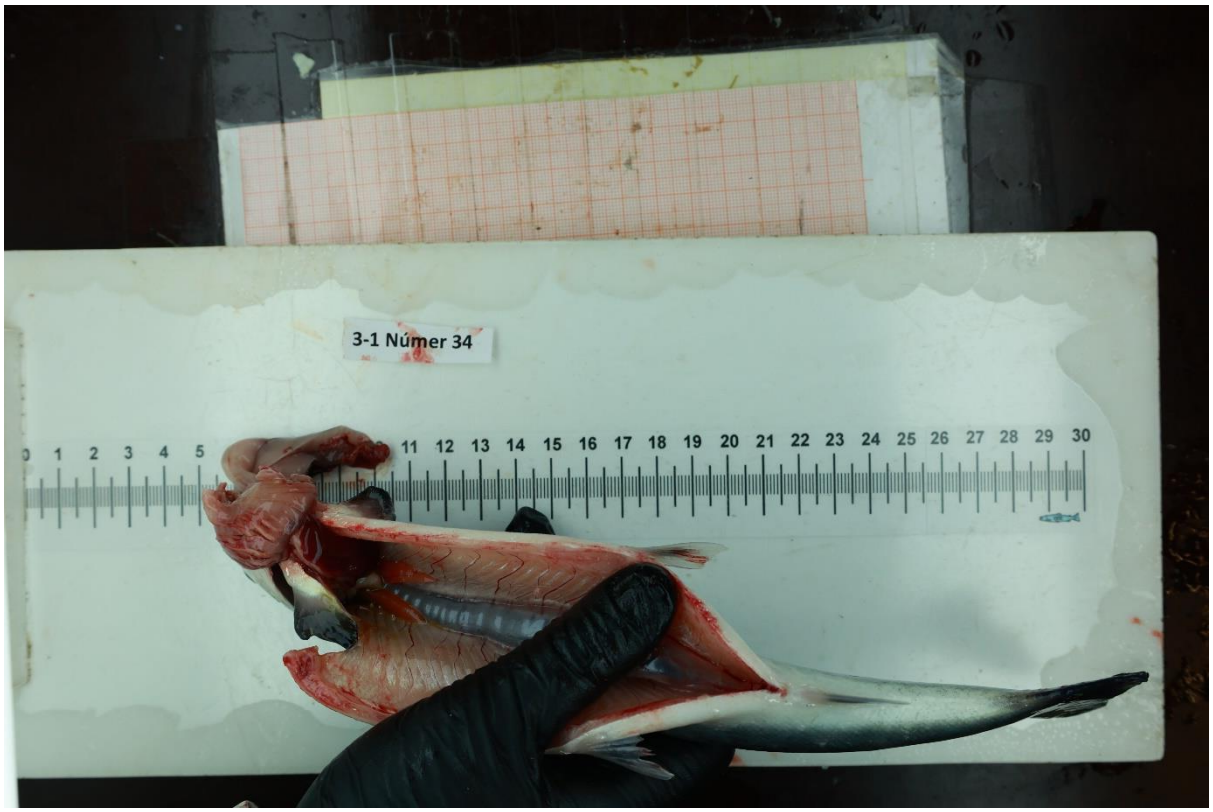

Figure 16: Fish nr. 34 in tank 3-1, 100r feed ratio, female, maturity rate 1

4. Discussion

The current study has demonstrated significant variations in essential metrics, such as weight, length, condition factor, welfare rating, and specific growth rate among the Atlantic salmon juveniles reared under different feeding ratios. While this study detected relatively smaller differences in maturation development among the juveniles, this finding should not be overlooked. Even subtle variations in maturation can have effects on how the salmon industry will continue its on-growing production of large juveniles and post-smolts, emphasizing the need for continued monitoring and research in this area.

4.1 Growth

As expected, growth, length, condition factor, and specific growth rate results were better when the feed ratio is 100r compared to other feed ratios. The difference between the 50r and 33r groups were more subtle. This was in line with what Åsgard et al. (1997) and Stefansson et al. (2009) stated that by reducing access to feed will result in slower growth of the salmon (Åsgård et al., 1997; Stefansson et al., 2009).

There was a gradual increase in the growth factors (weight and length) except in the condition factor where there was a decrease, which is expected since it is characteristic during smoltification (van Rijn et al., 2021). However, the fish was fed with different ratio 100%, 50% and 33%, but surprisingly the difference in the measured growth variables was not matching those percentage. The difference between the 100r group and the 50r group in weight was that the 50r group was only 29% lighter in weight and 10% shorter.

The same can be said when measuring the 50r group and the 33r group, an assumption of the difference would be that the 33r group would have the results of being 33% smaller than the 50r group but the results showed that the 33r group was only 20% lighter in weight and 7% shorter. One possible explanation is that the salmon that had less feed, utilised the feed better and slowed down other metabolic rates. In the nature Atlantic salmon face periods where there is less access to food which results in lack of access to feeding. Cooke et al. (2000) stated the salmon can adapt to changes by reducing their metabolic rate and for example use less energy to swimming, this helps to survive during harsh periods (Cooke et al., 2000). But current trial did not measure the feed utilization, but that would need to be looked into in future research.

Martinez et al. (2023) found that reducing the feeding ratio will not help reduce the maturation of juvenile Atlantic salmon without significantly affecting growth (Martinez et al., 2023). However, the growth in the present trial was significantly less in the lower fed groups (50r and 33r), although it is not as affected by the lack of feeding as expected, so maybe 90r or 80r groups could show less maturation with a full lifecycle experience and not that significantly smaller growth. Further studies with different feeding ratios are needed to fully enlighten the topic of feeding ratios and its effect on the maturation process in juvenile and post-smolt Atlantic salmon.

Effect of different feeding ratios in juvenile Atlantic salmon has been studied previously and Stefansson et al. (2009) and Imsland et al. (2011) concluded that smolts are vulnerable to lack of feed during the early post-smolt phase resulting in reduced hypo-osmoregulatory ability (Stefansson et al., 2009; Imsland et al., 2011). So, if present trial would have continued to sea, we might possibly have seen increased mortality or other negative results for the salmon who had less feed.

4.2 Welfare rating

The problem of how to assess the welfare status of fish is an ongoing debate and no consensus has been reached on definitions or assessment methodology (Stien et al., 2013). According to Santurtun et al. (2018) welfare outcome indicators, such as fin damage, morbidity, and mortality rate, should be used in standards and laws relating to salmon welfare. In the present trial the welfare rating was decided on fin condition, or amount of wounded fins, since fin damage is increasingly being used as a potential indicator of the welfare of farmed fish (Cañon Jones et al., 2010; Stien et al., 2013; Noble et al., 2018).

There was significant difference in welfare rating between all the feed ratios groups where the 100r group was rated with best welfare rating, then the 50r group and last the 33r group. The mortality was low in all the tanks and did not vary between the feeding ratio groups, apart from the unfortunate incident in one of the 33r tanks. If looked closer there were no difference between the 50r group and the 33r group during the 3rd sample but it increased significantly in the last sampling, there could be various reasons for that, the fish was getting bigger and maybe the aggressiveness increased the last month, since the fish felt more need

for feed, or that the fish was weaker and had come worse out of the fungi infection and treatment, however, that was not specifically looked at in this research.

This supports that fin damage may reflect aggressive behaviour within the rearing unit (Cañon Jones et al., 2010; Stien et al., 2013). There are many indicators that may cause increased fin wounds on the salmon, feeding ratios being one of those (Cañon Jones et al., 2010). Overall, there was a very good relationship between the feeding ratio and the increase in fin wounds in the present trial supporting the idea that reduction in offered feed may lead to more aggressive behaviour in the fish e.g., fin biting, thereby effecting the measured welfare rating. However, unlike Cañon Jones et al. (2010, 2012, 2017) no social network analysis was done during this experience.

Density was not high in the tanks during the research, or at maximum 5 kg per cubic meter. Fish stocking densities have been implicated in the occurrence of fin damage in Atlantic salmon. Higher fin damage has been described at high fish stocking densities (Ashley, 2007; Stian et al., 2013; Calabrese et al., 2017). However, as Cañon Jones et al. (2010) state so does low fish stocking densities in Atlantic salmon in hatcheries as well.

Damage to the fins of salmonids is, more often caused by chronic infection with biofilm forming bacteria that progressively necrotize the fin edges (Stian et al., 2013). Poor fin condition is coupled with a high stocking density, poor water quality, decreased condition factor (K-factor), and increased plasma glucose and cortisol levels (Stian et al., 2013; Virtanen et al., 2023). However, in this trial there were no measurements on plasma glucose and cortisol levels, so it is not possible to deduce the possible causal relationship in relation to those variables. Water quality did not differ between the feeding groups and the stocking density was low (< 5 kg/m^3) in all groups. The condition factor did decline in all groups, but this was more apparent in the 50r and the 33r groups possibly indicating a relationship between the poorer fin condition seen in those groups and lower K-factor.

If the juveniles in the present trial would have been adapted to sea water and put to sea, the performance of the fish could be questioned, since the fish already had open fin wounds would be more vulnerable to external pathogens since open wounds could be a route for pathogenic infection as they disrupt the epidermal barrier (Solstorm et al., 2016; Noble et al., 2018).

4.3 Sexual maturation

As the fish in the study was small it was expected to find only minor differences in maturation development of the fish studied in the present trial. However, a reduced growth can lead to less energy stores, e.g., lipid stores, needed for the sexual maturation process (Taranger et al., 2010). Such reduced growth and lowered energy stores can in theory delay the maturation of the fish (Taranger et al., 2010). But, in the present trial, there were only found minor differences in maturation development between the different feed ratios among the juvenile Atlantic salmon. This could be traced back to that the experimental period was quite short (approx. 5 months) or from August to January and the fact that the fish in all groups were small (max 332.7 g). If the experiment would have lasted longer and the fish put in and followed to the sea cages, maybe more difference in sexual maturation development could have been seen. Then it could have been investigated further in line with what Aksnes et al. (1986) stated that when a farmed Atlantic salmon is kept for two years in the sea cage it may occur that the fish will sexually mature and that the salmon uses energy from the muscles to develop gonads not from feed intake, the growth is less due to that energy stores are mainly used for sexual development, and that will result in that fillet will become more watery and pale (Aksnes et al., 1986 ; Hendry et al., 1999; Frazer et al., 2023).

During the present trial period there were only found minor connection between feed ratio and the development of maturation of all the juveniles, and the minor connection was only found in males, in addition no female surpassed maturity stage 2. The subtle findings found were for the earliest stage (0) where the 100r differed from the 33r group, and the later stages (4) where the 100r differed from the 50r group. This is in line with what Martinez et al. (2023) states that early maturation occurs mostly in males due to the lower energetic investments required for testis development in comparison to female egg production (Martinez et al., 2023). It is suggested that the relative low maturity found in the males could be related to a short trial time, few fishes and that the fish was still relatively small and had not started full development of maturation. However, if we would combine the maturity stages of the males from 2-5 (since 0 and 1 rating equal barely started development of gonads), there was almost a significant difference between the 100r group and the 33r group (Chi-squared test 3.66, p=0.06). This difference, although subtle, might have been further enhanced if the trial period had been longer.

Some juvenile Atlantic salmon males, called precocious or mature parr, will reach sexual maturity without migrating to the sea (Saura et al., 2008; Frazer et al., 2023). Two precocious males were found in the present trial, both found in the 100r group. That could have caused a small deviation in the present trial since precocious male development is not directly linked to feeding but is also related to genetics (Martinez et al., 2023).

4.4 Conclusion

The groups with 100% feed ratio (the 100r) showed better growth and welfare overall compared to the lower fed groups (the 50r and the 33r). There was found a small connection between different feed ratios and development of gonads. Although findings were subtle due to limited time frame of the trial the findings offer a foundation for future investigation into the relationship of feeding ratio and development of sexual maturation in rearing of juvenile Atlantic salmon. This knowledge when further explored can help the salmon producers with their smolt strategies and how they rear their juveniles and post-smolts.

These hypotheses were accepted or rejected in the present study:

H01: Raising Atlantic salmon parr and exposing them to different feeding ratios has no significant effect on body weight development, is rejected. Present study found a significant difference on body weight development, so **HA1** is accepted.

H02: Raising Atlantic salmon parr and exposing them to different feeding ratios has no significant effect on body length development, is rejected. Present study found a significant difference on body length development, so **HA2** is accepted.

H03: Raising Atlantic salmon parr and exposing them to different feeding ratios has no significant effect on development of maturity. As present study found relatively minor significant difference on development of maturity **H03** is not rejected.

In hindsight a different H03 hypothesis for males and females should have been formulated. If hypothesis “H03” would have been split up H03A (for males) and H03B (for females) the HO3A would be partly rejected (based on the possible emerging trend between 33r and 100r males) but not H03B.

H04: Raising Atlantic salmon parr and exposing them to different feeding ratios has no significant effect on Condition factor is rejected. Present study found a significant difference on Condition factor, so **HA4** is accepted.

H05: Raising Atlantic salmon parr and exposing them to different feeding ratios has no significant effect on the fish welfare rating is rejected. Present study found a significant difference in welfare rating between the different feeding ratios, so **HA5** is accepted.

4.5 Discussion of Materials and Methods

The experiment took a long time to start, the fish was stored at in Verið at Hólar University from April to August without starting the trial. The fish was relatively large when the experience started with different feed ratios or approximately 50 g. The experiment was ongoing for 5 months which is quite a short duration when one of the hypotheses was to look at different maturation development. The fish was 115-202 g when the experience ended, and all the salmon were euthanized. To be able to look at the maturation development the fish would have to be followed longer and the experience should have lasted for 1-2 years, to get a proper picture if different feed ratio is affecting the development of maturation. However, in the time frame of a master study this is not possible, so the experimental set-up is a compromise between the optimal study duration and the reality of finalizing the study within a given (short) time frame of M.Sc. study.

During the experience the condition for the fish was good, even though the fish had been on hold at Hólar University from April until the start of the research in August the fish was in good shape and the mortality rates had been low in all feed ratio groups during the whole experiment, except for one unfortunate accident in one of the 33r tanks (see Materials and Methods page 21).

More sampling points with more regular interval could have been conducted. The rearing station at Hólar University is in the northern part of Iceland in Sauðárkrókur, which is a 4-hour drive from the capital area, that made it impossible for me to follow the fish closely on day-to-day basis, and the rearing of the fish was taken care of by the employees of Hólar University. This resulted in that fewer sampling points were taken when the researcher was in the area. The experience started on the 16th of August 2022, then there were no measures

until 23rd of November or for 103 days, then again 13th of December 20 days later and 16th of January 2023 with 34 days between measurement. This may partly explain why the SGR was not significant in the measurement between November and December, but significant in the measurement between December to January.

Between November and December only 20 days passed between measurements, and the fish had to go through formalin treatment during that period due to fungi growth in the tanks and after the treatment the fish looked much better. This though resulted in 2 extra days of starving (one prior to treatment, the other day of the treatment). Bath exposure of fish to chemicals such as formalin, chlorine compounds and detergents is a common treatment and prophylactic method for external bacterial, parasitic, and fungal diseases in commercial salmonid aquaculture (Speare et al., 1997; Leal et al., 2016). The fish might have taken some time to start eating properly after that treatment, since usually treatments like this cause some stress in the fish (Madaro et al., 2015), and stress negatively affects the appetite and growth of Atlantic salmon (Walde et al., 2022).

Unfortunately, a whole tank of the 33r fish had an accident in December, where an air bubble got stuck in the inflow pipe and blocked the flow of new water the day before the 3rd sampling on 13th of December killing all the fish in the tank. This reduced the 33r sample for the last sample by approximately 33% but the results from the 3rd sampling from that tank were used in the research. This was very inconvenient as the sample size of this feeding ratio group was already relatively small.

Foraging behaviour is one of the widest and most complex areas of investigation in the aquaculture industry (Toni et al.,2019). It could be questioned that the welfare of the fish was not adequate since the 33r group only got fed every 3rd day, however fishes have low metabolic rates and are more than capable of going through a long fasting period without suffering irreversible consequences (Hvas et al., 2022). As Hvas et al. (2022) found out that full 8-week fasting period did not reduce fish welfare status neither in the short or in the long term as documented by scoring of external morphology traits (Hvas et al., 2022). The fish in this study were of course not fully starved and the SGR of the lowest fed group (the 33r) never went below 0.

Since the only variation between groups during this research was feed given 100%, 50% and 33%, the only possible results from this research were if growth, welfare rating, and maturation are directly connected to only feed intake. There were no other variations in this trial like earlier studies have done, with for example higher temperature and light (Fjelldal et al., 2011; Imsland et al., 2014). Those previous studies have shown a clear connection between higher temperature and more light causing an earlier maturation Atlantic salmon (Martinez et al., 2023). However, in the present study all groups were reared on same temperature and photoperiod regime so amount of feed was the only limiting factor on early sexual development of Atlantic salmon.

There were some notable differences of the fish in 33r which had more parr signs compared to 50r and 100r which had all turned more silver. Parr has marks on the side that disappears, with time and the scales and skin becomes more silver. The coloration changes on the tails, dorsal and pectoral fins and go from dark to light with black margins. Conditional factor decreases and the length/weight ratio decreases (lower condition factor) (van Rijn et al., 2021). That the smolts had gone further into the smoltification process could explain the gap between the condition factor of the 100r group compared to the others were less during later sampling points (December and January), but this was not investigated further.

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Appendix I - Supplementary Introduction

I-I. Growth of aquaculture in the Westfjords of Iceland

Rural areas in Iceland have been under threat of depopulation. The total population in the south part of West-fjords dropped by 32% in the year 1994-2011, people under 20 years old dropped by 50% and people between the age 20-39 dropped by 40% (Þorgrímsdóttir, 2012). The same pattern is in the north part of the Westfjords where the total population dropped by 23% in the same period and most of them were under 39 years old (Þorgrímsdóttir, 2012). Aquaculture is one of the main growing possibilities in the Westfjords and is the sustainable build-up of the industry. On south part of the West-fjords there have been created approximately 300 new jobs in the last years related to the aquaculture industry and it creates a lot of possibilities to march forward (Finnbogadóttir, 2021). As of 1st of May 2022 the population of people in Vesturbyggð was 1,153, which is an increase of 133 people from 1st of December 2019 (12% increase). In Tálknafjarðarhreppur the population has increased by 8 people over the same period. These are the main communities in the south part of the West-fjords (Þjóðskrá, May 2022).

I.II Challenges in salmon farming in the Westfjords: Rearing through the second winter in sea

During the first couple of months in 2020, a high mortality was measured in Hringsdalur in Arnarfjörður, one of Arnarlax sites. The high mortality was mainly due to winter wounds in the area during the first weeks of the year 2020. Also, there was mortality a little later in the winter at another site called Eyri in Patreksfjörður, where a high mortality was recorded in March and April 2020 resulting in loss of 419 MT due to winter wounds. Increasing mortality due to winter wounds is an inherent biological risk in the operation of fish farming. The main factors for this incident were cold temperatures, density, and winter storm (Icelandic Salmon, 2020).

The 19th of January 2022 Arctic Sea Farm announced to the food and veterinary agency of Iceland that they were experiencing increased mortality at one of their sites. Their fish health manager said that they had seen less appetite of the fish earlier in the month and at the same time the sea temperature dropped. Unfortunately, there were many days in a row with bad

weather. After the agency had investigated the matter, they concluded that this was due to combination of several factors; the fish was big and the biomass was high, the sea temperature was low, and weather and sea was rough. The only solution was to call for Norwegian Gannet to harvest the fish and solve this issue. The mortality over that period was 2.498 tons of salmon(Matvælastofnun, 2022). The Norwegian Gannet is a transportable harvest plant for farmed fish built in 2018 and is the first of its kind (Corvusenergy, https://corvusenergy.com/projects/norwegian-gannet/).

If we look at Figure 17, we can see that the mortality spikes every year over the winter or from January to April, the average weight of the dead fish shows us that this is the fish that is most likely his second winter in sea.

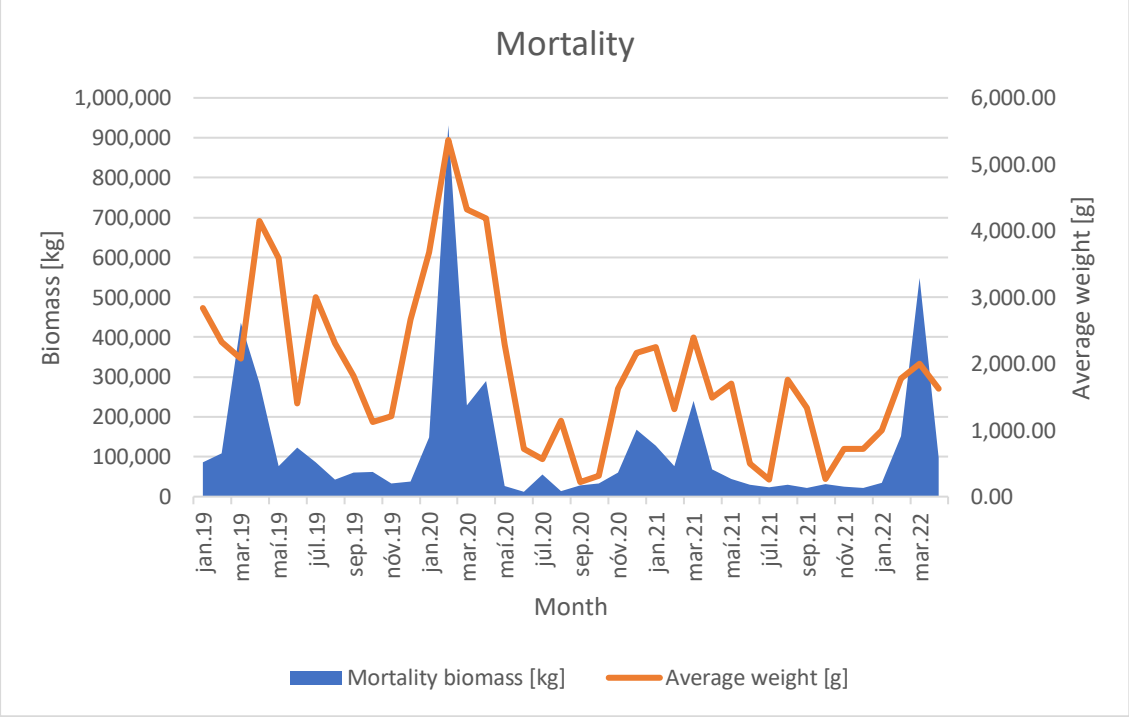

Figure 17: Mortality biomass [kg], shows that the highest biomass is lost in the winter (Arnarlax, Fishtalk)

I-III Is land-based aquaculture the future of salmon farming?

The earth is 509,600,000 square kilometres, and the area of land is 148,326,000 square kilometres, or approximately 29% of the earth's surface. The area that is covered with water is 361,740,000 square kilometres and 97% of that is salt water (Earth how, 2023). Land-based facilities use a lot of water and by moving all salmon production up on land could lead to additional pressure on freshwater resources for food production. Freshwater is in some cases

used partly or wholly during the final growing phase because of lack of access to seawater or because of the need to run the production with a lower salinity for physiological reasons (ISAF, 2016). Land is not an unlimited resource as is stated above and even though it would be unlimited and with unlimited access to water, moving all salmon production in Canada alone on land would require 28,000 football fields, 33,719 acres, or 136 square kilometres of land to grow fish in appropriate densities and water depths in land-based facilities. This number could be multiplied by tens in Norway where plans were announced to produce 20,000 tons of salmon in land-based facilities by 2018 (ISAF, 2016).

In the modern world almost everything, in the end, comes down to cost. Land-based facilities are more expensive. Both in the capital requirements needed to construct the stations and in the price per kilo of production. If we compare the cost of production down to price per kilo, we can expect that farm-gate cost of production is higher in land-based than in sea-based grow-out, or up to 10 NOK higher per kilo (Nesset et al., 2017).The Conservation Fund(The Conservation Fund's Freshwater Institute Shepherdstown, West Virginia, USA) compared a model of both land-based RAS farm and net pen farm that produced 3.300 metric tons of HOG (head on gutted) Atlantic salmon (Liu et al., 2016). Estimated cost of building to produce 3.300 metric tons of HOG in land-based RAS farm is 54 million US$, but the net pen farm is 30 million US$. This is almost half the price of an RAS farm (Liu et al., 2016). They then summarized the cost down to Capital expenditures (CAPEX), Operating expenses (OPEX) and return of investment (ROI). Land-based RAS farms have greater capital cost per unit of annual production and slightly higher operating cost than the net pen farm. The ROI is double from the net pen than the land-based RAS farm (Liu et al., 2016). To counter that cost, the land-based facilities would focus on select sites with cheap power in close proximity to key markets (ISAF, 2016). For example, the most important single markets for Mowi are North America and the United States (McGinley, 2019). Which means that this does not seem like a good future development strategy for more remote areas such as Norway or Iceland.

But open net pens can have negative environmental consequences and cages are directly exposed to the open environment. Two of the main problems in recent years that come from the environment around net pens in the sea are the control of diseases as well as parasites and the escape of fish. The sea lice are the parasites that have caused the largest problems in

recent years. (Lekang et al. 2016). Therefore, moving the fish to land-based facilities is one suggested solution to the problems with escapes and sea lice. A land-based facility has the possibility to have greater security against environmentally caused cage failures (e.g., waves and water currents). In land-based facilities it is also possible to add double security by building a wall around the tank area (requirement for smolt farms in Norway). If a structural failure occurs in a land-based tank, the salmon will have less possibility to escape (Lekang et al. 2016). A salmon grown in land-based operations does not experience the same stresses of fluctuating environmental conditions e.g., sea lice, algae blooms, and jellyfish (Gísli Jónsson, Senior Veterinary Officer for Aquaculture Animals at Matvælastofnun, pers. Comm.)

A land-based facility is though not 100% safe from not being affected by any environmental affects and putting all the fish up to land can affect the fish welfare in another way. A normal net pen station raises fish at a density of maximum 25 kg per m^3 at their peak size. For land-based facility to be profitable it is suggested that salmon needs to be produced at a density of 80 kg per m^3 (ISAF, 2016). With such high density this also increases the risk of problems with removing introduced pathogens from the facility unless the facility is fully depopulated, and all the biological filters are cleaned and disinfected (ISAF, 2016). If a more serious contagious virus gets into the station like BKD (Bacterial kidney disease caused by *Renibacterium salmoninarum*) then nothing can be done except empty the station and let it stay empty for 4-6 weeks. With the ever more common RAS stations, serious infections can be terribly hard to eradicate and regain control of the station. RAS-stations in foreign countries have felt this on their own skin (Gísli Jónsson, Senior Veterinary Officer for Aquaculture Animals at Matvælastofnun, pers. Comm.). There are several documented cases of fish health problems that have caused a complete loss of the fish due to pathogens in closed containment systems (ISAF, 2016). There are some counter measures to reduce the risk of spreading the diseases like ultraviolet light and ozone. Ultraviolet and ozone sterilizing units can help reduce overall pathogen numbers in a system, but they will not prevent the spread of pathogens within a system unit (e.g., tank or vat). Sterilizers need to be accessible and changed regularly but poor husbandry for example. by not dipping nets in sterilizers when using them between tanks will negate any benefits of these sterilizing systems. Also, ozone can be dangerous to fish and humans (Yanong, 2004).

I.IV Smoltification

In their natural habitat, to be able to adjust to the salt water the juvenile salmon needs to go through a complicated process that is driven by the endocrine system. It consists of many different but coordinated developmental changes that have a high energetic cost for the fish. These changes can be seen in the behaviour, biochemistry, morphological and physiological changes of the juveniles (Morera et al., 2021). The main environmental cue responsible for indicating for the parr to start the smoltification is the photoperiod changes during different seasons (Porter et al., 1998).

I.IV.I Endocrine control of smoltification

Salinity tolerance is the most important physiological change during smoltification, the smolt quality is evaluated by salinity tolerance (Berge et al., 1995). Many hormones are involved when it comes to smoltification and the changes in endocrinology.

The key hormones are Melatonin that controls the biological clock and changes during daylength. Thyroxin or T4 that Increases early and either has a direct effect on specific changes or indirect effects on other hormones. Growth hormone increases steadily during smoltification and is important for hypo osmoregulatory ability during the transformation (Berge et al., 1995). Insulin growth factor increases, and cortisol has many effects on salinity tolerance and metabolic changes (Clarke et al., 1996). All these hormones make sure that the smolt development occurs and the fish goes into the smolt window, which is the period where the smolts can migrate or be transferred to saltwater without any problems.

I.IV.II Osmoregulatory physiology

Osmoregulation is the key factor for adaptation to saltwater, if that is not sufficient, all the other factors become irrelevant because the fish will dry out and die. When parr starts the smoltification there are changes in gill ionocytes, Na+, K+ and ATPase and intestinal fluid uptake. The filtration rate in the kidney is lower and the reabsorption of ions is less. Before getting ready to enter salt water the filtration over the gills change to get ready to lose water instead of gaining it, they are altering their capacity to produce urine and become hypo-osmotic (Clarke et al., 1996). If the smolts are transferred to saltwater in the smolt window the changes remain and the salmon will become marine fish, if they are kept in freshwater these traits will be lost and they will go through de-smoltification where the critical

physiological smolt characters will be lost (Morera et al., 2021). During the smoltification the gill Na+, K+ and ATPase enzyme activity (NKA) increases and reaches its peak in freshwater before the fish enters the smolt window where the enzymes have high pumping capacity which is needed in the saltwater.

The increase of the ATPase activity happens as a result of the smoltification; therefore, it can be used to see whether the fish is ready for saltwater and there is a possibility to test its capacity to regulate ions before transferring it to saltwater. Higher temperature results in a shorter smolt window with elevated gill ATPase activity. There is an interaction between light and temperature and possible effect of rapid growth during smoltification. In aquaculture to get smolts bigger and ready quicker they are kept in constant light which makes measuring the gill ATPase activity tricky.

I.IV.III The morphological and behavioural changes during smoltification

Parr has marks on the side that disappears, there is silvering of skin and scales, and they become very loose. That results in the smolts are vulnerable to handling, losing scales opens way for pathogens to get in (Solstorm et al., 2016; Noble et al., 2018). The coloration changes on the tails, dorsal and pectoral fins and go from dark to light with black margins (Stefansson et al., 2002). Conditional factor decreases and the length/weight ratio decreases (lower condition factor), the smolts are longer compared to their weight because of the mobilization of lipids and growth (van Rijn et al., 2021). In aquaculture you get the silvery morphological appearance even though you do not expect your fish to be smolt because you are producing larger smolts, there is less decrease in condition factor in aquaculture smolts than in nature because in aquaculture you have high growth, high feed so the condition factor will be higher.

Parr is often oriented to the currents and is very territorial. Smolts often show a reduced rheotaxis so they are not as oriented to the currents as the parr, they lose their territoriality and start schooling, (schooling happens in aquaculture because of high density) and they are more synchronised in movement.

I.IV.IV When the fish is ready for seawater?

Predicting the future performance of smolts in seawater is always difficult but there are ways to test if the fish can grow and survive. Many factors can influence smolt quality. It can be

connected to genetics, seawater tolerance, the size (80-300g), documentation on the smolts health status, the vaccination and how the skin status is. The most important thing is the seawater tolerance, and that is always tested (Berge et al., 1995). Testing the gill Na+, K+-ATPase activity, where samples are collected from the gills and put in a buffer, sent to a lab that measures the enzyme activity or the salmon probe, this test measures are on a genetic level. To start the smoltification period the salmon is tricked by “winter signals” light regime (photoperiod LD12:12) since that is one of the main environmental cues to initiate the start of smoltification (Stefansson et al., 2002; Bjornsson et al., 2011).

Appendix II – Data and Statistical analysis

II.I Growth measurements of the PIT-Tagged fish

Table 6: The weight results of the PIT-tagged fish were expressed as mean weight ± standard error (SE) for each group.

Sample:	Mean weight (g)			Standard deviation (SE)		
	33r	50r	100r	33r	50r	100r
August '22	53.42	50.67	49.97	0.72	0.79	0.81
November '22	83.42	97.89	127.55	1.46	2.30	2.90
December '22	96.05	112.58	149.90	1.77	2.89	3.49
January '23	115.83	143.90	201.86	2.88	4.24	5.27

Table 7: The length results of the PIT-tagged fish were expressed as mean length ± standard error (SE) for each group.

Sample:	Mean lenght (cm)			Standard deviation (SE)		
	33r	50r	100r	33r	50r	100r
August '22	16.03	15.80	15.76	0.07	0.08	0.08
November '22	19.11	20.04	21.62	0.14	0.18	0.19
December '22	20.02	21.14	23.02	0.15	0.20	0.21
January '23	21.36	22.87	25.39	0.20	0.26	0.25

Table 8: The condition factor results of the PIT-tagged fish was calculated and expressed as mean K-factor ± standard error (SE) for each group.

Sample:	Mean Condition factor (K-factor)			Standard deviation (SE)		
	33r	50r	100r	33r	50r	100r
August '22	1.29	1.28	1.27	0.01	0.01	0.01
November '22	1.19	1.21	1.25	0.01	0.01	0.01
December '22	1.19	1.18	1.22	0.01	0.01	0.01
January '23	1.18	1.19	1.22	0.01	0.01	0.01

Table 9: The Specific growth rate results for the PIT-tagged fish was calculated and expressed as mean SGR ± standard error (SE) for each group.

Sample:	Mean Specific growth rate (SGR)			Standard deviation (SE)		
	33r	50r	100r	33r	50r	100r
August '22	0.44	0.66	0.94	0.02	0.02	0.02
November '22	0.66	0.67	0.80	0.03	0.02	0.09
December '22	0.57	0.71	0.86	0.02	0.02	0.02
January '23	0.49	0.67	0.90	0.02	0.02	0.02

Table 10: Weight, Anova: Two-Factor with replication and Bonferroni corrected post-hoc test. Cell coloured green are significant difference (P<0.05).

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	451512.9347	2	225756.4673	92.69921091	0.0000	3.009067124
Columns	132015.3272	74	1783.990908	0.732535158	0.9531	1.306569435
Interaction	342241.9753	148	2312.445779	0.949527168	0.6456	1.225540807
Within	1643871.765	675	2435.365578			
Total	2569642.002	899				

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Tanks	474,257.30	222	2,136.29	0.88	0.8780

POST-HOC TEST - Bonferroni corrected		Alpha	0.050
		Bonferroni corrected	**0.017**
W1			
100r v 80r	0.2582	No	a
80r v 50r	0.0024	yes	a
100r v 50r	0.0026	yes	b
W2			
100r v 80r	0.0000	Yes	a
80r v 50r	0.0000	Yes	b
100r v 50r	0.0000	Yes	c
W3			
100r v 80r	0.0000	Yes	a
80r v 50r	0.0000	Yes	b
100r v 50r	0.0000	Yes	c
W4			
100r v 80r	0.0000	Yes	a
80r v 50r	0.0004	Yes	b
100r v 50r	0.0000	Yes	c

Table 11. Length, Anova: Two-Factor with replication and Bonferroni corrected post-hoc test. Cell coloured green are significant difference (P<0.05).

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	2582.98142	2	1291.49071	55.3492153	5.5101E-23	3.00906712
Columns	1536.21816	74	20.7597048	0.88969542	0.73147946	1.30656944
Interaction	4102.06691	148	27.7166683	1.18784892	0.08196249	1.22554081
Within	15750.11	675	23.3334963			
Total	23971.3765	899				

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Tanks	5,638.29	222	25.40	1.09	0.2124

POST-HOC TEST - Bonferroni corrected		Alpha	0.050
		Bonferroni corrected	**0.017**
L1			
100r v 80r	0.3832	No	a
80r v 50r	0.0114	Yes	a
100r v 50r	0.0161	Yes	b
L2			
100r v 80r	0.0000	Yes	a
80r v 50r	0.0000	Yes	b
100r v 50r	0.0000	Yes	c
L3			
100r v 80r	0.0000	Yes	a
80r v 50r	0.0000	Yes	b
100r v 50r	0.0000	Yes	c
L4			
100r v 80r	0.0000	Yes	a
80r v 50r	0.0042	Yes	b
100r v 50r	0.0000	Yes	c

Table 12: Condition Factor (K-Factor), Anova: Two-Factor with replication and Bonferroni corrected post-hoc test. Cell coloured green are significant difference (P<0.05).

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	2.47961853	2	1.23980926	20.4811496	2.3152E-09	3.00906712
Columns	6.08850797	74	0.08227713	1.35918512	0.02933989	1.30656944
Interaction	10.1309334	148	0.06845225	1.13080364	0.15941555	1.22554081
Within	40.8605607	675	0.06053416			
Total	59.5596206	899				

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Tanks	16.22	222	0.07	1.21	0.0390

POST-HOC TEST - Bonferroni corrected		Alpha	0.050
		Bonferroni corrected	**0.017**
August			
100r v 80r	0.2119	No	n.s.
80r v 50r	0.0519	no	
100r v 50r	0.0210	no	
Nov			
100r v 80r	0.0004	Yes	a
80r v 50r	0.0805	no	b
100r v 50r	0.0000	Yes	b
Dec			
100r v 80r	0.0026	Yes	a
80r v 50r	0.0835	no	b
100r v 50r	0.0416	no	b
Januar			
100r v 80r	0.0160	Yes	a
80r v 50r	0.2633	no	b
100r v 50r	0.0044	Yes	b

Table 13: Specific growth rate (SGR), Anova: Two-Factor with replication and Bonferroni corrected post-hoc test. Cell coloured green are significant difference (P<0.05).

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	21.8840641	2	10.9420321	151.884602	3.431E-55	3.00906712
Columns	8.90981284	74	0.12040288	1.67129312	0.00064272	1.30656944
Interaction	20.6408726	148	0.13946536	1.93589635	1.6627E-08	1.22554081
Within	48.6281792	675	0.07204175			
Total	100.062929	899				

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Tanks	29.55	222	0.13	1.85	0.0000

POST-HOC TEST - Bonferroni corrected		Alpha	0.050
		Bonferroni c	**0.017**
100r v 80r	0.0000	yes	a
80r v 50r	0.0000	Yes	b
100r v 50r	0.0000	Yes	c
Nov - Dec			
100r v 80r	0.1246	no	n.s
80r v 50r	0.4433	no	n.s
100r v 50r	0.0671	no	n.s
Dec - January			
100r v 80r	0.0000	Yes	a
80r v 50r	0.0010	Yes	b
100r v 50r	0.0000	Yes	c
August -January			
100r v 80r	0.0000	Yes	a
80r v 50r	0.0000	Yes	b
100r v 50r	0.0000	Yes	c

II.II Welfare and Maturation measurements of all the fish

Table 14. Welfare rating comparison between different feed groups, after the 3rd sample. Cells are Red if p<0.001, yellow if p<0.01 and green if p<0.05. Ratio is used to equal the number of fish during the Chi-squared test. The degree of freedom was in all cases equal to 1.

	Ratio	0.99			
	Observed	Expected	Welfare		
Welfare (0-5)	33r	100r	O-E	(O-E)^2	$(O-E)^2 / E$
0	27	74	-47	2,256	30.28
1	65	48	17	300	6.29
2	30	16	14	199	12.52
3	21	8	13	170	21.44
4	4	2	2	4	2.04
5	1	-	1	1	#DIV/0!
Grand Total	148	148			

	Ratio	0.95			
	Observed	Expected	Welfare		
Welfare (0-5)	50r	100r	O-E	(O-E)^2	$(O-E)^2 / E$
0	32	71	-39	1,519	21.40
1	62	45	17	275	6.05
2	29	15	14	192	12.69
3	15	8	7	55	7.29
4	3	2	1	1	0.65
5	-	-	-	-	#DIV/0!
Grand Total	141	141			

	Ratio	1.05			
	Observed	Expected	Welfare		
Welfare (0-5)	33r	50r	O-E	(O-E)^2	$(O-E)^2 / E$
0	27	34	- 7	43	1.29
1	65	65	- 0	0	0.00
2	30	30	- 0	0	0.01
3	21	16	5	28	1.75
4	4	3	1	1	0.23
5	1	-	1	1	#DIV/0!
Grand Total	148	148			

Table 15. Welfare rating comparison between different feed groups, after the last sample. Cells are Red if p<0.001, yellow if p<0.01, and green if p<0.05. Ratio is used to equal the number of fish during the Chi-squared test. The degree of freedom in all cases was equal to 1.

	Ratio	0.67			
	Observed	Expected	Welfare		
Welfare (0-5)	33r	100r	O-E	(O-E)^2	(O – E)² / E
0	5	24	-19	347	15
1	24	47	-23	540	11.43
2	28	7	21	423	57.04
3	19	4	15	224	55.21
4	5	-	5	25	#DIV/0!
5	2	1	1	2	2.60
Grand Total	83	83			

	Ratio	0.98			
	Observed	Expected	Welfare		
Welfare (0-5)	50r	100r	O-E	(O-E)^2	(O – E)² / E
0	18	34	-16	261	7.63
1	51	68	-17	299	4.38
2	38	11	27	744	69.29
3	12	6	6	38	6.45
4	1	-	1	1	#DIV/0!
5	-	1	- 1	1	0.98
Grand Total	120	120			

	Ratio	0.69			
	Observed	Expected	Welfare		
Welfare (0-5)	33r	50r	O-E	(O-E)^2	(O – E)² / E
0	5	12	- 7	56	4.46
1	24	35	-11	127	3.60
2	28	26	2	3	0.11
3	19	8	11	114	13.79
4	5	1	4	19	26.84
5	2	-	2	4	#DIV/0!
Grand Total	83	83			

Table 16: Maturity comparison between different feed groups of all the fish, after the last sample, where measurements from the 3rd sample has been added. Cells are Red if p<0.001, yellow if p<0.01 and green if p<0.05. Ratio is used to equal the number of fish during the Chi-squared test. The degree of freedom was in all cases equal to 1.

	Observed		Expected	
			Ratio	1.01
Maturity stage (0-5)	33r (M)	33r (F)	100r (M)	100r (F)
0	3	-	2	1
1	70	69	59	72
2	-	1	3	1
3	4	-	4	-
4	-	-	1	-
5	-	-	2	1
Grand Total	77	70	71	76

M			F		
O-E	(O-E)^2	(O – E)² / E	O-E	(O-E)^3	(O – E)² / E
0.99	0.97	0.48	- 1.01	1.01	1.01
10.60	112.27	1.89	- 3.49	12.20	0.17
- 3.02	9.12	3.02	- 0.01	0.00	0.00
- 0.03	0.00	0.00	-	-	#DIV/0!
- 1.01	1.01	1.01	-	-	#DIV/0!
- 2.01	4.05	2.01	- 1.01	1.01	1.01

	Observed		Expected	
			Ratio	0.99
Maturity stage (0-5)	50r (M)	50r (F)	100r (M)	100r (F)
0	1	-	2	1
1	59	78	58	71
2	1	-	3	1
3	2	-	4	-
4	3	-	1	-
5	-	-	2	1
Grand Total	66	78	70	74

M			F		
O-E	(O-E)^2	(O – E)² / E	O-E	(O-E)^3	(O – E)² / E
- 0.97	0.95	0.48	- 0.99	0.97	0.99
0.81	0.65	0.01	6.99	48.81	0.69
- 1.96	3.84	1.30	- 0.99	0.97	0.99
- 1.95	3.78	0.96	-	-	#DIV/0!
2.01	4.05	4.11	-	-	#DIV/0!
- 1.97	3.89	1.97	- 0.99	0.97	0.99

	Observed		Expected	
			Ratio	1.02
Maturity stage (0-5)	33r (M)	33r (F)	50r (M)	50r (F)
0	3	-	1	-
1	70	69	60	80
2	-	1	1	-
3	4	-	2	-
4	-	-	3	-
5	-	-	-	-
Grand Total	77	70	67	80

M			F		
O-E	(O-E)^2	(O – E)² / E	O-E	(O-E)^2	(O – E)² / E
1.98	3.92	3.84	-	-	#DIV/0!
9.77	95.47	1.59	- 10.63	112.89	1.42
- 1.02	1.04	1.02	1.00	1.00	#DIV/0!
1.96	3.84	1.88	-	-	#DIV/0!
- 3.06	9.38	3.06	-	-	#DIV/0!
-	-	#DIV/0!	-	-	#DIV/0!

II-III. Welfare rating and sexual maturation of the PIT-Tagged fish

II-III-I. Welfare rating of the PIT-tagged fish:

A Chi-squared test for categories of data showed that there was significant difference in welfare rating of the PIT-tagged smolts in the 3rd sample (Table 17).

In December there was a significant difference between feed ratio 100r and 33r in welfare rating 0 (Chi-squared test, $p<0.001$) and welfare rating 2 there were significantly fewer in feed ratio 100r than 33r (Chi-squared test, $p<0.01$).

There was also a significant difference between feed ratio 100r and 50r in welfare rating 0 and 3 (Chi-squared test, $p<0.05$) and welfare rating 2 (Chi-squared test, $p<0.01$, Table 17).

In other welfare rating there was no significant difference between the feed ratios (Chi-squared test, $p>0.05$)

Table 17: Frequency table made in Excel of the PIT-tagged fish after the third sampling (13.12.2022). Different Count of individuals in each welfare stage under different feed ratio. Superscript letters indicate significant differences between the experimental groups with a as the highest value.

Welfare rating (0-5)	**33r**	**50r**	**100r**	**Grand Total**
0	11^{b}	18^{b}	32^{a}	61
1	36	28	29	93
2	16^{a}	15^{a}	8^{b}	39
3	8^{ab}	10^{a}	5^{b}	23
4	2	0	1	3
5	1	0	0	1
Grand Total	74	71	75	220

In January (Table 18), a notable disparity in welfare ratings was observed, with feed ratio 100r showing significantly lower welfare ratings compared to the other feed ratios.

Comparing feed ratio 100r with 33r, significant differences were detected in welfare ratings 0, 1, (Chi-squared test, $p<0.01$) and 2 and 3 (Chi-squared test, $p<0.001$).

By comparing feed ratio 100r with 50r, there were significant differences in welfare rating 1 (Chi-squared test, $p<0.01$) and 2 and 3 (Chi-squared test, $p<0.001$).

Additionally, a significant difference in welfare ratings was noted between feed ratio 50r and 33r, specifically in welfare rating 0 (Chi-squared test, $p<0.01$). However, for other welfare ratings, no significant differences were found among the feeding ratios (Chi-squared test, $p>0.05$).

Table 18: Frequency table made in Excel of the PIT-tagged fish after the last sampling (16.1.2023). Different Count of individuals in each welfare stage under different feed ratio. Superscript letters indicate significant differences between the experimental groups with a as the highest value.

Welfare rating (0-5)	**33r**	**50r**	**100r**	**Grand Total**
0	1^{b}	13^{a}	17^{a}	31
1	16^{b}	28^{b}	48^{a}	92
2	14^{a}	21^{a}	4^{b}	39
3	12^{a}	11^{a}	3^{b}	26
4	4^{a}	0^{b}	0^{b}	4
5	1	0	1	2
Grand Total	48	73	73	194

II-III-II. Maturity stage of the PIT-tagged fish:

A Chi-squared test for categories of data showed that there was minor connection between feed ratio and the development of maturation in the PIT-Tagged juveniles in the present study (Tables 19-20).

There was a significant difference between feed ratio 100r and 33r in maturity stage 1 for female (Chi-squared test, $p<0.05$, Table 19) and in maturity stage 1 for the males (Chi-squared test, $p<0.01$, Table 20).

In all other feed ratios, there were no significant differences between the maturity stages and sex (Chi-squared test, $p>0.05$)

*Table 19: Frequency table made in Excel of the female PIT-tagged fish after the last sampling. Different Count of individuals in each maturation stage under different feed ratio. *An accident happened in one of the tanks and part of those numbers are from the 3rd sampling, one month before.*

Maturity Stage (0-5)	33r (F)	50r (F)	100r (F)	Grand Total
0	-	-	-	**0**
1	29[b]	39[ab]	43[a]	**111**
2	1	0	0	**1**
3	0	0	0	**0**
4	0	0	0	**0**
5	0	0	0	**0**
Grand Total	**30**	**39**	**43**	**112**

*Table 20: Frequency table made in Excel of the male PIT-tagged fish after the last sampling. Different Count of individuals in each maturation stage under different feed ratio. *An accident happened in one of the tanks and part of those numbers are from the 3rd sampling, one month before*

Maturity Stage (0-5)	33r (M)	50r (M)	100r (M)	Grand Total
0	2	1	3	**6**
1	38[a]	29[ab]	24[b]	**91**
2	0	1	1	**2**
3	1	1	0	**2**
4	0	2	1	**3**
5	0	0	1	**1**
Grand Total	**41**	**34**	**30**	**105**

II-IV. Data analysation of the maturation and welfare rating of the PIT-tagged fish

Table 21: Maturity comparison between different feed groups of the PIT-tagged fish, after the last sample. Cells are Red if p<0.001, yellow if p<0.01 and green if p<0.05. Ratio is used to equal the number of fish during the Chi-squared test. The degree of freedom was in all cases equal to 1.

	Observed		Expected	
			Ratio	0.97
Maturity Stage (0-5)	33r (F)	33r (M)	100r (F)	100r (M)
0	-	2	-	3
1	29	38	42	23
2	1	-	-	1
3	-	1	-	-
4	-	-	-	1
5	-	-	-	1
Grand Total	30	41	42	29

F			M		
O-E	(O-E)^2	$(O-E)^2 / E$	O-E	(O-E)^3	$(O-E)^2 / E$
#VALUE!	#VALUE!	#VALUE!	- 0.92	0.84	0.29
- 12.82	164.40	3.93	14.66	214.84	9.20
1.00	1.00	#DIV/0!	-	-	-
-	-	-	1.00	1.00	#DIV/0!
-	-	-	-	-	-
-	-	-	-	-	-

	Observed		Expected	
			Ratio	1.00
Maturity Stage (0-5)	50r (F)	50r (M)	100r (F)	100r (M)
0	-	1	-	3
1	39	29	43	24
2	-	1	-	1
3	-	1	-	-
4	-	2	-	1
5	-	-	-	1
Grand Total	39	34	43	30

F			M		
O-E	(O-E)^2	$(O-E)^2 / E$	O-E	(O-E)^3	$(O-E)^2 / E$
#VALUE!	#VALUE!	#VALUE!	- 2.00	4.00	1.33
- 4.00	16.00	0.37	5.00	25.00	1.04
#VALUE!	#VALUE!	#VALUE!	-	-	-
-	-	-	1.00	1.00	#DIV/0!
-	-	-	-	-	-
-	-	-	-	-	-

	Observed		Expected	
			Ratio	0.97
Maturity Stage (0-5)	33r (F)	33r (M)	50r (F)	50r (M)
0	-	2	-	1
1	29	38	38	28
2	1	-	-	1
3	-	1	-	1
4	-	-	-	2
5	-	-	-	-
Grand Total	30	41	39	34

F			M		
O-E	(O-E)^2	$(O-E)^2 / E$	O-E	(O-E)^3	$(O-E)^2 / E$
#VALUE!	#VALUE!	#VALUE!	1.03	1.06	1.09
- 8.93	79.77	2.10	9.79	95.93	3.40
1.00	1.00	#DIV/0!	-	-	-
-	-	-	0.03	0.00	0.00
-	-	-	-	-	-
-	-	-	-	-	#DIV/0!

Table 22: Welfare rating comparison between different feed groups of the PIT-tagged fish, after the last sample. Cells are Red if p<0.001, yellow if p<0.01 and green if p<0.05. Ratio is used to equal the number of fish during the Chi-squared test. The degree of freedom was in all cases equal to 1.

	Ratio	0.66				
	Observed	Expected	Welfare			
Welfare (0-5)	33r	100r	O-E	(O-E)^2	(O – E)² / E	
0	1	11	-10.18	103.59	9.27	
1	16	32	-15.56	242.16	7.67	
2	14	3	11.37	129.27	49.15	
3	12	2	10.03	100.55	50.97	
4	4	-	4.00	16.00	#DIV/0!	
5	1	-	1.00	1.00	#DIV/0!	
Grand Total	48	47				

	Ratio	1.00				
	Observed	Expected	Welfare			
Welfare (0-5)	50r	100r	O-E	(O-E)^2	(O – E)² / E	
0	13	17	-4.00	16.00	0.94	
1	28	48	-20.00	400.00	8.33	
2	21	4	17.00	289.00	72.25	
3	11	3	8.00	64.00	21.33	
4	-	-	-	-	#DIV/0!	
5	-	1	-1.00	1.00	1.00	
Grand Total	73	73				

	Ratio	0.66				
	Observed	Expected	Welfare			
Welfare (0-5)	33r	50r	O-E	(O-E)^2	(O – E)² / E	
0	1	9	-7.55	56.97	6.66	
1	16	18	-2.41	5.81	0.32	
2	14	14	0.19	0.04	0.00	
3	12	7	4.77	22.73	3.14	
4	4	-	4.00	16.00	#DIV/0!	
5	1	-	1.00	1.00	#DIV/0!	
Grand Total	48	48				

Table 23. Welfare rating comparison between different feed groups of the PIT-tagged fish, after the 3rd sample. Cells are Red if p<0.001, yellow if p<0.01 and green if p<0.05. Ratio is used to equal the number of fish during the Chi-squared test. The degree of freedom was in all cases equal to 1.

	Ratio	0.987			
	Observed	Expected	Welfare		
Welfare (0-5)	50r	100r	O-E	$(O-E)^2$	$(O-E)^2 / E$
0	11	32	-20.57	423.26	13.41
1	36	29	7.39	54.56	1.91
2	16	8	8.11	65.72	8.33
3	8	5	3.07	9.40	1.91
4	2	1	1.01	1.03	1.04
5	1	-	1.00	1.00	#DIV/0!
Grand Total	74	74			

	Ratio	0.947			
	Observed	Expected	Welfare		
Welfare (0-5)	80r	100r	O-E	$(O-E)^2$	$(O-E)^2 / E$
0	18	30	-12.29	151.13	4.99
1	28	27	0.55	0.30	0.01
2	15	8	7.43	55.16	7.28
3	10	5	5.27	27.74	5.86
4	-	1	-1.00	1.00	1.06
5	-	-	#VALUE!	#VALUE!	#VALUE!
Grand Total	71	71			

	Ratio	1.042			
	Observed	Expected	Welfare		
Welfare (0-5)	50r	80r	O-E	$(O-E)^2$	$(O-E)^2 / E$
0	11	19	-7.76	60.23	3.21
1	36	29	6.82	46.47	1.59
2	16	16	0.37	0.13	0.01
3	8	10	-2.42	5.87	0.56
4	2	-	2.00	4.00	#DIV/0!
5	1	-	1.00	1.00	#DIV/0!
Grand Total	74	74			

Appendix III - Statistical methods

III.I – Levene's test of different growth factors

III.I.I – Weight Levene's test

Table 24: Anova: Single factor results from the 1st Weight sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is not less than 0.05, we fail to reject the null hypothesis. In other words, we don't have sufficient evidence to say that the variance between the three groups is different.

W1

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - w1	75	413.296	5.510613333	18.71642457
80r - w1	75	427.896	5.70528	13.25141499
50r - w1	75	386.5173333	5.153564444	11.53574974

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11.74422686	2	5.87211343	0.404939927	0.667508545	3.036523693
Within Groups	3219.265608	222	14.50119643			
Total	3231.009835	224				

Table 25: Anova: Single factor results from the 2nd Weight sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is less than 0.05, we reject the null hypothesis. In other words, we do have sufficient evidence to say that the variance between the three groups is different.

W2

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - w2	75	1419.2	18.92266667	267.0207856
80r - w2	75	1392.632877	18.56843836	286.4066308
50r - w2	75	708.048	9.44064	69.53641256

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4333.775256	2	2166.887628	10.43505671	4.66292E-05	3.036523693
Within Groups	46099.32334	222	207.6546096			
Total	50433.0986	224				

Table 26: Anova: Single factor results from the 3rd Weight sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is less than 0.05, we reject the null hypothesis. In other words, we do have sufficient evidence to say that the variance between the three groups is different.

W3

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - w3	75	1753.490667	23.37987556	359.2956256
80r - w3	75	1806.334722	24.08446296	503.1824074
50r - w3	75	981.6797297	13.08906306	180.2397063

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5682.402103	2	2841.201052	8.174410805	0.000375436	3.036523693
Within Groups	77161.11271	222	347.5725798			
Total	82843.51481	224				

Table 27: Anova: Single factor results from the last weight sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is less than 0.05, we reject the null hypothesis. In other words, we do have sufficient evidence to say that the variance between the three groups is different.

W4

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - w4	75	2860.542466	38.14056621	1602.500234
80r - w4	75	2433.471233	32.44628311	768.9681996
50r - w4	50	973.0875	19.46175	544.329274

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10615.00435	2	5307.502173	5.172011269	0.006468574	3.04175303
Within Groups	202160.7985	197	1026.196947			
Total	212775.8029	199				

III.I.II – Length Levene's test

Table 28: Anova: Single factor results from the 1st length sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is less than 0.05, we reject the null hypothesis. In other words, we do have sufficient evidence to say that the variance between the three groups is different.

L1

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - L1	75	1182.3	15.764	0.488010811
80r - L1	75	1184.8	15.79733333	0.454587387
50r - L1	75	1201.9	16.02533333	0.376511712

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.034755556	2	1.517377778	3.450912846	0.033428176	3.036523693
Within Groups	97.61413333	222	0.439703303			
Total	100.6488889	224				

Table 29: Anova: Single factor results from the 2nd length sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is less than 0.05, we reject the null hypothesis. In other words, we do have sufficient evidence to say that the variance between the three groups is different.

L2

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - L2	75	1621.7	21.62266667	2.586100901
80r - L2	73	1462.8	20.03835616	2.491563927
50r - L2	75	1433.5	19.11333333	1.391711712

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	241.4640621	2	120.7320311	56.06544711	2.10E-20	3.036897906
Within Groups	473.7507361	220	2.153412437			
Total	715.2147982	222				

Table 30: Anova: Single factor results from the 3rd length sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is less than 0.05, we reject the null hypothesis. In other words, we do have sufficient evidence to say that the variance between the three groups is different.

L3

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - L3	75	1726.8	23.024	3.276983784
80r - L3	72	1522.3	21.14305556	2.966148279
50r - L3	74	1481.6	20.02162162	1.700621992

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	343.1436198	2	171.5718099	64.79581567	8.23E-23	3.037279048
Within Groups	577.2387332	218	2.647884097			
Total	920.3823529	220				

Table 31: Anova: Single factor results from the last length sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is less than 0.05, we reject the null hypothesis. In other words, we do have sufficient evidence to say that the variance between the three groups is different.

L4

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - L4	73	1853.2	25.38630137	4.636476408
80r - L4	73	1669.3	22.86712329	4.793626332
50r - L4	48	1025.2	21.35833333	1.976950355

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	508.4877402	2	254.2438701	62.9117525	1.02E-21	3.043213905
Within Groups	771.8840639	191	4.041277822			
Total	1280.371804	193				

III.I.III – Condition factor (K-Factor) Levene's test

Table 32: Anova: Single factor results from the 1st k-factor sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is not less than 0.05, we fail to reject the null hypothesis. In other words, we don't have sufficient evidence to say that the variance between the three groups is different.

K1

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - K1	75	95.19522518	1.269269669	0.005692687
80r - K1	75	95.885428	1.278472373	0.003856682
50r - K1	75	97.00655121	1.293420683	0.00348891

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.02228533	2	0.011142665	2.563834841	0.079287579	3.036523693
Within Groups	0.964832657	222	0.004346093			
Total	0.987117987	224				

Table 33: Anova: Single factor results from the 2nd k-factor sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is less than 0.05, we reject the null hypothesis. In other words, we do have sufficient evidence to say that the variance between the three groups is different.

K2

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - K2	75	93.8787119	1.251716159	0.009089609
80r - K2	73	88.02797542	1.205862677	0.005948941
50r - K2	75	89.18787849	1.189171713	0.005079613

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.157132758	2	0.078566379	11.70372619	1.47832E-05	3.036897906
Within Groups	1.476846189	220	0.006712937			
Total	1.633978948	222				

Table 32: Anova: Single factor results from the 3rd k-factor sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is less than 0.05, we reject the null hypothesis. In other words, we do have sufficient evidence to say that the variance between the three groups is different.

K3

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - K3	75	91.32912999	1.217721733	0.008695979
80r - K3	72	84.73899064	1.176930426	0.004610224
50 - K3	74	88.2267895	1.192253912	0.006786707

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.062606674	2	0.031303337	4.654110924	0.010488347	3.037279048
Within Groups	1.466258008	218	0.006725954			
Total	1.528864681	220				

Table 35: Anova: Single factor results from the last k-factor sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is less than 0.05, we reject the null hypothesis. In other words, we do have sufficient evidence to say that the variance between the three groups is different.

K4

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - K4	73	88.88964334	1.217666347	0.006058248
80r - K4	73	86.52631425	1.185291976	0.007159752
50r - K4	48	56.72712059	1.181815012	0.005776581

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.052223919	2	0.026111959	4.077340976	0.018449808	3.043213905
Within Groups	1.223195278	191	0.006404164			
Total	1.275419197	193				

III.I.VI – Specific Growth Rate (SGR) Levene's test

Table 36: Anova: Single factor results from the 1st SGR sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is less than 0.05, we reject the null hypothesis. In other words, we have sufficient evidence to say that the variance between the three groups is different.

SGR1

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - SGR1	75	70.26788562	0.936905142	0.037940371
80r - SGR1	73	47.88017537	0.655892813	0.021709385
50r - SGR1	75	33.34961916	0.444661589	0.019144779

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9.146165058	2	4.573082529	173.8401002	5.19E-46	3.036897906
Within Groups	5.787376763	220	0.026306258			
Total	14.93354182	222				

Table 37: Single factor results from the 2nd SGR sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is not less than 0.05, we fail to reject the null hypothesis. In other words, we don't have sufficient evidence to say that the variance between the three groups is different.

SGR2

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - SGR2	75	59.85279658	0.798037288	0.543038326
80r - SGR2	71	47.2193613	0.665061427	0.041882933
50r - SGR2	74	48.92233748	0.661112669	0.04724805

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.901340955	2	0.450670477	2.100159357	0.124918691	3.037472278
Within Groups	46.56574905	217	0.214588705			
Total	47.46709	219				

Table 38: Single factor results from the 3rd SGR sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is less than 0.05, we reject the null hypothesis. In other words, we do have sufficient evidence to say that the variance between the three groups is different.

SGR3

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - SGR3	73	63.01051935	0.863157799	0.036672094
80r - SGR3	72	51.27829551	0.712198549	0.028549019
50r - SGR3	48	27.42211971	0.571294161	0.012718193

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.522689368	2	1.261344684	45.51752012	7.07E-17	3.043466449
Within Groups	5.265126249	190	0.027711191			
Total	7.787815618	192				

Table 39: Single factor results from the last SGR sample, Levene's test done on the Absolute Residuals down to r. The p-value of the one-way ANOVA is less than 0.05, we reject the null hypothesis. In other words, we do have sufficient evidence to say that the variance between the three groups is different.

SGR Overall

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100r - SGROVERALL	75	67.71446669	0.902859556	0.023107164
80r - SGROVERALL	74	49.3838596	0.667349454	0.017696793
50r - SGROVERALL	75	36.93411244	0.492454833	0.018016048

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.36171927	2	3.180859635	162.1620857	4.52E-44	3.036709945
Within Groups	4.334983583	221	0.01961531			
Total	10.69670285	223				

III.II – Kolmogorov-Smirnov test for normal distribution

Table 40: Results of Kolmogorov-Smirnov test for normal distribution for the 100r group.

Criteria	n	Maximum value	Kolmogorov-Smirnov critical values (.05 = 1.3581 /$\sqrt{n}$)
W1	75	0.88	0.1568
W2	75	0.97	0.1568
W3	75	0.96	0.1568
W4	73	0.96	0.1590
L1	75	0.91	0.1568
L2	75	0.93	0.1568
L3	75	0.91	0.1568
L4	73	0.93	0.1590
K1	75	0.93	0.1568
K2	75	0.85	0.1568
K3	75	0.87	0.1568
K4	73	0.87	0.1590
SGR1	75	0.94	0.1568
SGR2	75	0.70	0.1568
SGR3	75	0.91	0.1568
SGROVERALL	73	0.94	0.1590

Table 41: Results of Kolmogorov-Smirnov test for normal distribution for the 50r group.

Criteria	n	Maximum value	Kolmogorov-Smirnov critical values (.05 = 1.3581 /$\sqrt{n}$)
W1	75	0.84	0.1568
W2	73	0.83	0.1590
W3	72	0.85	0.1601
W4	73	0.83	0.1590
L1	75	0.80	0.1568
L2	73	0.82	0.1590
L3	72	0.85	0.1601
L4	73	0.84	0.1590
K1	75	0.97	0.1568
K2	73	0.95	0.1590
K3	72	0.93	0.1601
K4	73	0.96	0.1590
SGR1	75	0.94	0.1568
SGR2	73	0.86	0.1590
SGR3	72	0.95	0.1601
SGROVERALL	73	0.92	0.1590

Table 42: Results of Kolmogorov-Smirnov test for normal distribution for the 33r group.

Criteria	n	Maximum value	Kolmogorov-Smirnov critical values (.05 = 1.3581 / $\sqrt{n}$)
W1	75	0.96	0.1568
W2	75	0.93	0.1568
W3	74	0.93	0.1579
W4	47	0.79	0.1984
L1	75	0.88	0.1568
L2	75	0.91	0.1568
L3	74	0.94	0.1579
L4	47	0.78	0.1984
K1	75	1.00	0.1568
K2	75	0.96	0.1568
K3	74	0.97	0.1579
K4	47	1.00	0.1984
SGR1	75	0.91	0.1568
SGR2	75	0.71	0.1568
SGR3	74	0.80	0.1579
SGROVERALL	47	0.78	0.1984