Morphometric relationships as computer vision parameters for growth development and sexual maturation status of Atlantic salmon (*Salmo salar*)

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Content

Abstract

The scope of computer vision (CV) assessment of free-swimming fish in aquaculture is large, and the development of CV and Artificial Intelligence (AI) driven fish parameters is still at an early stage of development. Little published documentation exist of on CV parameters that are relevant to measure for interpreting fish welfare and performance. The current study seeks to identify morphometric relationships in fish as CV parameters of biological relevance related to growth development and sexual maturation status. This is done by investigating how the morphometrical relationships evolve with growth rate, and whether the relationships can predict the trajectory of sexual maturation in individual Atlantic salmon. The data used was collected from large (4376 ± 1208 g, mean ± SD) Atlantic salmon (n=80) in flow-through tanks (Ø=7 m, depth 1.5 m) over a period of six months, where the fish were repeatedly netted out and individually measured for weight (g), length (mm), and photographed at both lateral sides.

The distance between key points of the fish was measured in ImageJ and used to form the ratios: head length/standard length (StdL), eye diameter/StdL, upper jaw/StdL, body height central/StdL, body height anal/StdL, and body height central/anal. The ratios were analysed relative to individual growth rate and sexual maturation status. The body height ratios were strongly correlated with condition factor, and the eye, jaw, and head length in ratio with standard length indicated potential of representing previous and current growth performance. There was a large difference in the ratios between mature and immature fish, where a head length/StdL ratio above 0.2 and a jaw/StdL above 0.1 is a strong indication that a fish is maturing (longer jaw and a hook formation). These two ratios, as well as the body height central/anal ratio, were significantly different between immature and mature fish, also at an early stage of maturation. This suggest that the three ratios could be used for distinguishing between mature and immature fish, as well as early detection of sexual maturation in individual fish.

1. Introduction

1.1 Automation of the aquaculture industry

The increasing global demand for high quality protein is pushing the development of novel technologies within the fish farming industry. The Norwegian salmon farming industry has since 1970 developed into becoming one of the largest producers of Atlantic salmon, where in the later years it has been a vanguard for innovative technologies with rapid development of sea lice detection, biomass calculation, and cage technology ("Verdiskaping Basert På Produktive Hav i 2050" 2012; Garlock *et al.*, 2019; FAO 2020). Another contributing factor to the resent interest in new technologies, is the restrictions put upon the industry in order to reduce its negative impact on the environment, such as the spread of salmon lice (*Lepeophtheirus salmonis),* pollution from excrements and undigested feed, and escapees (Glover *et al.*, 2012; Taranger *et al.*, 2015; Olaussen, 2018). These restrictions put an economic liability on the industry through the need of laborious monitoring of biomass and lice numbers, and through the need for expensive and invasive lice treatments (Liu and Bjelland, 2014; Olaussen, 2018). Most of the novel technologies being develop today are either aiming at reducing costs and time spent on the abovementioned problems through automation (Costa *et al.*, 2006; Føre *et al.*, 2018), or by reducing the problems all together through replacing the traditional in-shore sea cages (Bjørndal and Tusvik 2019; Bjørndal and Tusvik 2020). The economic incentive to solve these problems is obvious, and so is the negative impact these problems have on the welfare of farmed, wild, and cleaner fish.

One of the largest, and most limiting, problems in the Norwegian salmon farming industry is the ectoparasite, usually referred to as salmon lice, which causes increased mortality rates and poor fish welfare to both farmed and wild salmon (Olaussen *et al.* 2013; Taranger *et al.*, 2015; Overton *et al.*, 2019). The salmon lice problem also causes indirectly welfare problems and high mortality rates for cleaner fish used to combat the lice (Stien *et al.* 2020; Stien, 2022). The lice levels have become a problem with the increased production of salmon, which caused the Norwegian government to introduce new laws and regulations to limit the spread of lice (Jansen *et al.*, 2012). As a solution, the traffic light system was introduced, which divides the coastline into 13 different production areas and legislate a biomass limit depending on the status for each area. There is also a maximum limit to how many female lice per fish allowed depending on production area and season. To make sure that their

production is within the bounds of the law, farmers need to have control of the total biomass in production at all times, and do weekly counting and reporting of lice numbers (*Lov om akvakultur (akvakulturloven) - Lovdata*).

This need of always having control over important parameters during production, has developed into the idea of Precision fish farming (PFF), based on the 40 years of livestock farming (Føre *et al.*, 2018). This idea relies on innovative technologies to closely monitor, control, and improve different parameters during production. It seeks to create a data base of knowledge in order to transition from traditional experience-based farming to knowledge-based farming, and in doing so increase environmental sustainability, welfare, and biomass produced. An emerging technology that can play a key role in PFF, is the use of subsea cameras to collect, analyze, and store valuable information. The use of cameras and other sensory technologies have the potential to automate some of the manual labor, and we have already seen the first farmers getting approval for weekly lice reporting through the use of subsea cameras (*Historisk dag for automatisering og maskinlæring i havbruk - Aquabyte*, no date). It has also the potential to take PFF to new levels through enabling selective sorting, meaning that if key fish parameters (e.g. weight, health status, parasite load, sexual maturation) can be extracted, it could be used for selective sorting for different purposes, such as slaughter (e.g. superior fish) or selective treatment of certain groups instead of the total fish group (Misimi et al. 2007).

1.2 Computer vision as a solution

Computer vision (CV) attempts to mimic humanlike perception capabilities by recognising valuable information from an image or continuous image streams through the use of computers. It also seeks to understand, learn, and take actions based on the information it attains (Lecun *et al.*, 2015; Ji, 2020). The use of CV in the salmon farming industry is nothing new, and the technology has already been applied to, among other things, automatic estimation of individual body weight (Beddow and Ross, 1996), and assessment of biofouling on nets (Gansel *et al.*, 2017). Though some of these technologies have already been under development for a couple of decades, the technology has just recently started being adopted and deployed on a larger scale. Several companies are currently developing CV based tools to enhance the information flow in aquaculture (e.g. Aquabyte, Creatview, MSD, Stingray),

where key biological characteristics as fish growth rate, parasite infestation levels, and disease detection are main aims.

While analysis of monoscopic images can provide a wide range of valuable information (e.g. lice counting), the use of two lenses in a stereoscopic setup adds the extra layer of depth, which makes it possible to measure distances within the image. This is done by the lenses taking a picture simultaneously and by knowing the distance between the two lenses. Applied to subsea observation of free-swimming fish it allows for measuring the distance between key points of the fish in order to extract valuable information, such as weight and biomass (Beddow and Ross, 1996; Lines *et al.*, 2001).

1.3 The artificial brain - Big data, AI, ML, DL

As we move a process from human effort to be done autonomously by a machine or computer, we need to replace the human brain. This process of developing and theorizing computer systems that can be applied to tasks usually associated with human intelligence is called Artificial intelligence (AI) (Joiner, 2018). The artificial brain can be primitive, consisting only of a few lines of code, or it can be advanced, consisting of complex code which execute several functions at once. The code, or rather the software, can continuously be improved through updates, where new or improved lines of code are added either directly to the source code, or in external sources such as a database (Holton, 2007). There is also a method of improving software continuously without the need for software developers to add or improve the code directly. This method, called Machine learning (ML), aims at training the code, or rather the algorithm, to be able to make accurate predictions based on historical data. The accuracy of the predictions depends on the algorithms experience, which the algorithm attains from being "fed" with historical data (Jordan and Mitchell, 2015). ML is a subset of the broader category of AI, and within ML we find a subset called Deep learning (DL). While traditional ML require labelled and structured sets of data, which is done by human involvement in the data that is being "fed" to the algorithm, DL detects patterns by using a complex structure of algorithms modelled on the human brain. This allows for processing of unstructured dataset, thus requiring less human intervention (Lecun *et al.*, 2015; Voulodimos *et al*., 2018). To do predictions at a human level or better, the algorithm needs more data than any human can comprehend. Such large sets of data or often referred to

as Big data, which differ from other sets on data based on the three v's: volume, velocity, and variety (Mcafee and Brynjolfsson, 2012).

1.4 The artificial eye - The use of camera technology

As with the human brain, the artificial brain needs sensory input to execute certain functions. The better the hardware, the more accurate the input. As observation technology keep advancing, and the inputs becomes more accurate, the possibility of what can be measured keep increasing (Casey and Cornillon, 1999). The advance of technology comes with another benefit, that observation and communication devices keep getting smaller (MacK, 2011). This allows for camera devices to be of a manageable size, such that they can be deployed and used in commercial sea cages. Using subsea cameras (Fig. 1.1) for visual observation of fish allows for non-invasive assessment of a much large sample size than under the conventional practise of manual fish sampling by capture and observations in air. Strategic camera sampling may also be better towards attaining a better accuracy in terms of data that is representative for the whole population in e.g. a sea cage. The camera can be fitted with lights allowing for images to be taken continuously even during night-time, or at depths where the natural light is dim. Also, by having the opportunity to adjust the position of the camera, either manually or through a winch, the camera can be positioned according to predictable diurnal and environmental driven changes in swimming depth (Oppedal *et al.*, 2011), or horizontal space use as by changes in water current strength with tidal water (Johansson *et al.*, 2014).

Figure 1.1: Camera from Aquabyte used in the experiment.

1.5 Development of CV fish parameters in aquaculture production

As mentioned, the conventional fish observation and sampling methods in salmon aquaculture are visual observations from sub surface cameras of free-swimming fish, which is predominately used for appetite observation, or direct visual observation and grading of rather small samples captured, anaesthetized, and inspected in air. Use of CV in continuous image streams allows for extraction of key fish parameters that can describe the distribution within fish groups of individual size (growth rate over time), parasite load and its development, welfare indicators (Stien *et al.*, 2013; Noble *et al.*, 2018) including symptoms of disease or mechanical damage (e.g. wounds and scale loss), and sexual maturation status. Knowledge of such biological characteristics can be important for feedback to feeding control, sea lice management, preventive health measures, harvest planning, and estimation of the group status for pre-harvest sale of the fish (e.g. percent that is classified as superior fish) (Aunsmo *et al.,* 2013; Føre *et al.,* 2018).

While the scope for CV assessment of free-swimming fish in aquaculture is large, the data attained should be of biological relevance to make sense for the end users such as fish farmers, veterinarians, or researchers. Today, the development of CV and AI driven fish parameters is still at an early stage, and little published documentation exist of its precision in single fish measurement or accuracy of estimating the distributions within fish groups. While AI is commonly used to target key features descriptive of the abovementioned parameters, input from manual observations/annotations are required to guide the process by e.g. labelling the known conditions factor of individual fish, or sexually mature vs. not mature fish. Key parameters, e.g. by length relationships between key points in a fish can also be used in guiding AI processes, or work as stand-alone input for CV measurements. Such can be hight and length relationships in the fish to estimate their condition (Beddow *et al.*, 1996), distinguish between wild and farmed fish (Solem *et al.*, 2006; Solem *et al.*, 2011) etc. To achieve such data of high quality, experimental setups which allow for comparison with manual assessment are required. Preferably with tracking of individual fish over time to investigate the trajectories of characteristic to enable understanding of the biological impact, underlaying causes (e.g. environment or operational procedures) and early detection of both good and bad welfare and production performance. For example, while a mature salmon appears distinctively different from a non-mature fish with regards to skin colour and head

vs. full body length (Aksnes *et al.*, 1986; Leclercq *et al.*, 2010), it is not known how early maturation can be visually detected.

1.6 Key fish parameters

Fish parameters such as growth rate and condition factor are fundamental for interpreting both the production performance and health status of fish. These parameters are, however, not constant, even in fish that excel, and must be interpreted in context of the natural variations in the rearing environment, as well as manipulations (e.g. artificial light) and operational procedures (Oppedal *et al.*, 1999; Stien *et al.*, 2013). While image analysis can be used to assess the online and historical status of individual fish and the fish group, the greatest value is in enabling prediction of future welfare and performance. For this, is understanding of trajectories for morphometrical relationships (body ratios) a possible method to reveal future performance, as well as life-history traits such as sexual maturation.

1.6.1 Condition factor

Traditionally the condition of a fish is qualitative evaluated based on visual appearance, such as how the width is compared to the length, where a fat fish is considered to be of better condition compared to a thinner one. A quantitative approach to evaluate the condition of a fish is by measuring the condition factor. The condition factor is a widely accepted standard measurement used to assess the nutritional status of fish (Nash *et al.*, 2006). It is calculated by using the formula: $K = (Weight * Length^{-3}) * 100$, where weight is in grams (g), and length in centimetres (cm) measured from the tip of the snout to the end of the tail at the centre, usually referred to as the fork length (Endal *et al.*, 2000). The normal range of the condition factor for salmon is from 0.9 to 1.6, where a 0.9 or less are considered skinny, and a 1.6 and above are considered to be a fat fish. The condition factor can fluctuate throughout the production cycle, and can be influenced by seasons, temperature, feeding regime, sex, sexual maturation, gut size, and life stage (Oppedal *et al.*, 1999; Stien *et al.*, 2013; Noble *et al.*, 2018). As mentioned, stereoscopic images can measure the weight of a fish through lateral measurements, which allows for continuous evaluation of the condition factor. This information can be of great value for the harvest planning and pre-harvest sales.

1.6.2 Specific growth rate

The specific growth rate (SGR) is the daily increase or decrease of weight measured in percentage of the total weight for an individual or a group. Given a known period of time and the change of weight during that time frame, the SGR can be calculated using the formula: $SGR = 100 * (LN(End\ weight) - LN(Start\ weigth))/(days)$, where end weight is the weight at the end of the period and start weight is the weight at the beginning (Brett and Grover, 1979). The SGR is usually higher for smaller fish, and as the fish grows bigger the percentage daily increase gets smaller (Austreng *et al.*, 1987; Aunsmo *et al.*, 2014). A negative SGR for a given period indicates a reduction in weight, a positive indicates weight increase, and an SGR of 0 indicates no net change in weight. The SGR is influenced by seasonal changes such as temperature and day length, where higher temperatures and longer days are associated with increased SGR (Aunsmo *et al.*, 2014). It can also be affected by water quality, stress, nutrition, sexual maturation, and social interactions (Noble *et al.*, 2018). Tracking the daily growth rate with the use of stereoscopic cameras can provide valuable insights for the end user in and of itself, or in combination with other parameters of relevance (e.g. health status, feed use, temperature) to find potential causations or correlations.

1.7 Morphometrics as CV parameter

The quantitative characterisation and analysis of body proportions, shape and size in organisms is usually referred to as morphometrics (Fig. 1.2) (Arendt, 2015). Morphometrics can act as a key stand-alone parameter, or in combination with other parameters of relevance (e.g. visual cues) to guide the AI process. To gain insight and use morphometrics in any useful way, it is important to identifying which changes and differences in morphometrics that are of value. This could for example be analysing the disproportional growth of the eyes compared to the rest of the body in Atlantic salmon, which is showed to grow at a steady pace, resulting in an eye vs. body ratio that can be used to evaluate the difference in growth rate between groups and individuals (Solem *et al.*, 2006; Solem *et al.*, 2011). This was confirmed by Lange (2021), who also found that head measurements (upper jaw, snout to pectoral fin and operculum) in ratio to the standard length, were smaller for larger compared to smaller fish. This suggest that the head, similarly to the eye, grows at more a steady rate compared to the rest of the body, and that such ratios can be used as proxies for growth development. Pankhurst et al. (1994) got similar results when comparing slow growing wild

Rainbow Trout (*Oncorhynchus mykiss*) with fast growing farmed, which showed that farmed trout often had a more disproportional body. Devlin et al. (2012) also got similar results when comparing genetically modified salmonids with an accelerated growth to non-transgenic fish, which showed that that the transgenic fish had significantly smaller eyes compared to the non-transgenic fish of same size.

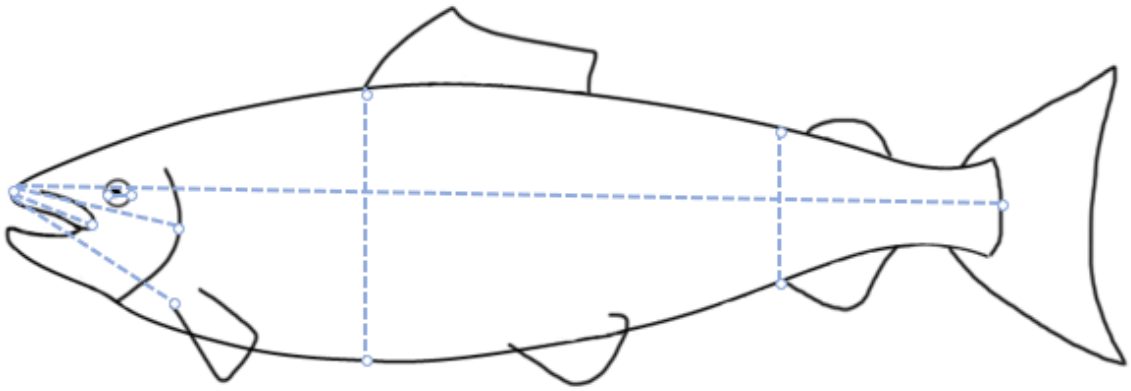


Figure 1.2: Illustration of measured lengths between key points.

1.8 Sexual maturation

Early sexual maturation in salmon farming is unwanted as it stunts growth, lead to poor filet quality, and is associated with welfare issues (Aksnes *et al.*, 1986; Skarstein *et al.*, 2001; Hvas *et al.*, 2021). Early detection of maturing individuals in a fish group, can therefore be of value for harvest planning to minimise potential costs associated with sexual maturation (Johnston *et al.*, 2006). Males usually reaches sexual maturity sooner than females, thus early maturation in salmon production is mainly a problem with males (Taranger *et al.*, 2010). Compared to females, sexually mature males develop distinct head features, such as longer jaws and the distinct hook on the lower jaw. Even though sexually matured females and males are both found to grow allometrically compared to immature salmon, which have an isometric weight-length relationship, the males tends to develop a more squared body figure (Leclercq *et al.*, 2010). Both sexes also develop a darker skin colour during maturation (Aksnes *et al.*, 1986).

The distinct head morphometrics along with the squared body shape and dark colour can function as key features in early detection through the use of stereo cameras. Also, due to the process of growing gonads being energy draining, salmon tend to have a period of rapid growth prior to showing changes in appearance and in morphometric signs (Taranger *et al.*,

2010). As growth can potentially be closely monitored using CV, this period of rapid growth can be used in combination with visual and morphometric cues to predict and track sexual maturation. The use of morphometric features to detect maturation in the early stages have not been researched to a large degree, although Kadri *et al.* (1997) did look at head height, body weight, and fork length, at the early stages of maturation to determine if it was possible to differentiate between sexual mature and immature salmon. This study showed that there was no simple way of differentiating using morphometric dynamics and concluded that it was not possible to detect maturation through a quantitative approach. The study did, however, only look at the dorsoventral axis, and not the anteroposterior axis, which Lange (2021) found to have key morphometric differences between sexually mature and immature salmon besides colour and shape.

1.9 Objectives and aims

This thesis seeks to identify CV parameters with regards to growth dynamics and sexual maturation by comparing the development of morphometric indicators. This is done by tracking the growth and maturation status of 80 individuals over a period of six months, and measuring potential key head and body relationships. This thesis will investigate and try to answer two main questions:

- **Question 1:** How does morphometric relationships evolve with growth rate?
- **Question 2:** Can morphometric relationships predict the trajectory of sexual maturation in individual Atlantic salmon?
    - **Secondary question:** How will morphometric relationships indicative of sexual maturation develop post maturation?

2. Material and method

2.1 Location and experimental design

The experiment was conducted at the Matre Research Station at the Institute of Marine Research, in western Norway (60°N). Atlantic salmon (n=80) were collected from one sea cage at the IMR Solheim research site (Masfjorden) on the 3rd of June 2021 and transferred to an outdoor tank at the Matre Research station. The fish were not measured at transfer, but a sample (n=164 fish) from the same cage and same day showed an average weight of 4376 ± 1208 g (mean ± SD) and length of 70.4 ± 5.6 cm. Before transfer, the fish had undergone a standard 14-month production cycle in an open sea cage (12 × 12 m and 14 m deep) under a natural photo regime. The fish were left to acclimatize to the tank environment until the first recording of size and photos of all individual fish was carried out on the 5th of August 2021, followed by the same recordings at 28th of September, 9th of December and 17th of January 2022.

2.2 Tank environment and feeding

Two identical flow-through tanks (Ø=7 m, depth 1.5 m) were used, which the fish alternated between during the periodic samplings. The inflowing water was spread from a vertical pipe with perforation from surface to the tank bottom and directed along the tank wall, which created a circular current with decreasing current speed from the wall towards the tank centre where the effluent water left the tank. The water was a mix between sea and freshwater to keep a stable salinity at 25ppt to prevent bacterial infections in the fish, and the temperature was maintained at ~9° and oxygen levels were always above 90% saturation in the inflowing water. The flow was set to 500 l min^{-1} which secured normoxic conditions (>80% oxygen sat.) in the effluent water at all times. Water quality was logged by IMR custom made computer software (SD Matre, Normatic, Nordfjoreid, Norway). The tank had a roof, with a 1.5 × 3.5 m opening from the tank centre to the tank wall which opened for daylight. A stereo camera (Aquabytes camera v2.1, Fig. 1.1) was positioned at mid-depth 0.5 from the tank centre and directed towards the tank wall. This camera was used for continuous observation of the fish from 5th of August, and had two LED lamps (10k lumen each) which was always on throughout the experimental period. The fish were daily fed an ad libitum ration of 9 mm

pellets (Spirit, Skretting, Stavanger, Norway) between 0900 and 1100 and 14 and 1600, using an automatic feeder (TA1, Betten Maskinstasjon, Vågland, Norway).

2.3 Collection of ground truth data

Collection of ground truth data were done a total four times. Upon recording of individual data, the water level in the tank was lowered to 30 cm depth, before the fish were first sedated using AquiS (5 mg/L). Fish were then netted out and anesthetized with Finquel (0.1g/ L) in a holding tank. Then fish were individually measured for weight (g) and length (mm) and photographed in air at both lateral sides of the body using the same optics and image storage as for the stereo-camera deployed in the tank (Fig. 2.1).

Figure 2.1: The Aquabyte enclosure setup.

2.4 Manual pairing of individuals

The fish were paired using manual recognition mainly through visually matching the melanophore spot patterns on the head, following the method described by Stien *et al.* (2017). For each sampling the images were sorted in two folders, one for the images of the left side

of the fish and one for the right side. Each sampling was pared with the previous one for easier matching between sampling images per individual fish. To match a new sample with the previous, either the left- or the right-side image were used to compare to the images in the corresponding folder of the previous sample. Which of the two sides to use were determined by which of them that contained the most unique spot patterns, or by having a spot that contained one or more distinctive features such as larger in size, fade in the middle or a non-circular shape. For each individual fish that was matched, the matching image in the folder was taken out of the folder, decreasing the number of images to compare with for the next individual.

2.5 Morphometric measurements

The length measurements were done using monoscopic images (from the left lens) analysed in the image processing package Fiji, which is a distribution of the program ImageJ bundling useful and non-programmer friendly plugins facilitating scientific image analysis (Schindelin *et al.*, 2012).

ImageJ (Fiji distribution), URL: https://imagej.net/software/fiji/downloads

The camera position did vary slightly between each sampling due to the use of different scales, tables, or other smaller variables. Therefore, a calibration in ImageJ had to be done for the first image in each sampling using the fish itself as a size reference, since the fork length had been measured manually. Calibrating using measuring tape as size reference was done, but proved to be less accurate than using the fish itself, probably because the width of the fish made the distance from the camera to the side of the fish smaller compared to the distance between the camera and the measuring tape on the table. To test if the calibration in ImageJ was accurate for a sample, random images from the same sample were selected and measured for fork length using ImageJ. The ImageJ fork length were then compared to the manually measured fork length. As an example, for the first sampling a random selected group (n = 19) had a manually measured fork length of 754 ± 33 mm (Mean ± SD) and the measured fork length in ImageJ was 757 ± 30 mm, which is not significantly different ($p = 0.24$, paired t-test). The distances measured in ImageJ (Fig. 2.2) were snout to pectoral fin base, snout to the middle of the operculum, eye diameter measured horizontally, snout to the end of the upper jaw, body height central, and body height above the anal tract. These lengths were divided by

standard length to form the morphometric ratios used. The body heigh central in ratio with anal height was also studied. The standard length was measured using ImageJ, from the snout to the last tail bone, not including the fin. The standard length was used to form the morphometric relationships, as opposed to the fork length, to eliminate potential errors associated with tail damage.

Figure 2.2: Lateral measurements: Standard length (yellow), body height central (red), body heigh anal (orange), snout to operculum (green), snout to pectoral fin base (blue), upper jaw (purple).

2.6 Potential errors

Out of the 80 fish put in the tank in June 74 survived the six months, were one died between the August and September sampling, and the five others between the December and January sampling. The data from the fish that died during the experiment were not used in this thesis. Data from three other individuals were also excluded, were one of them was a sexually matured female and the other two had errors in the measured ground truth data. That leaves data from a total of 71 individuals used in this thesis. Out of the 71 individuals, 31 had an abbreviated snout, presumed to be caused by snout wounds. Out of the 31, 21 had had an abbreviated snout from the first sampling and 10 developed a shorter snout during the six months (Fig. 2.3).

The distance between the tip of the snout and to the end of the operculum is often used as a distance to represent total head length (Kadri *et al.* 1995; Solem *et al.* 2011). This distance may not be the best to represent head length due to opercular shortening and other irregularities often being observed in farmed salmon, caused by either malformations in early stages (Leliūna et al. 2012), or due to wounds and mechanical erosion (e.g. nipping) (Blaker et al. 2022). In free-swimming fish there is also some opercular movement that could potentially make the measurement less reliable as head length compared to, for example, the

snout to pectoral fin base distance. Out of the 71 fish, ~90% had a shortened operculum on at least one of the sides. This was minor for most individuals, but enough to get a glimpse of the gills, as seen in figure 2.3 on the right image. The snout to pectoral fin distance is also used to represent head length (Solem et al. 2006; Beacham et al. 2011; Solem et al. 2011), and a group of 16 individuals (the fish with the least opercular irregularities) selected from the immature fish, showed that the snout to operculum and the snout to pectoral fin length are strongly correlated (cor. (R) = >0.95, $p < 0.00001$) for all samplings. The snout to pectoral fin distance was on average 9.2 ± 2.9% (Mean ± SD) ($p < 0.00001$, paired t-test) longer than snout to operculum for all samplings combined. The percentage difference did, however, vary between samplings where the smallest difference was recorded on the first sampling (dif. = 7.6 ± 1.8%, $p < 0.00001$), and the largest on the third sampling (dif. = 11.4 ± 3.7%, $p < 0.00001$). Because of the potential errors associated with using the operculum as an end point, and the association between the two head lengths, the snout to pectoral fin was selected and used to represent head length.

Figure 2.3: Comparison of snout length for two individuals from the January sampling. The one on the left has a shorter snout than normal. The one on the right has a normal snout. The right one also has a shorter operculum than normal, where the gills can be seen.

2.7 Development of wounds

Due to many fish developing wounds all 71 individuals were scored for wounds for all four samplings according to the Laksvel guidelines (Fig. 2.4)(Nilsson *et al.,* 2022). This was done to account for potential correlations with growth and sexual maturation. Each side of the fish were scored, where the total score for each individual was based on the side with the most

wounds (Table 2.1). The scoring system uses a scale from 0 to 3, where each individual is manually evaluated through visual inspection. A score 0 is given to individuals with no wounds on either side. A score 1 is given to fish with small or healed wounds (Fig 2.4 1A-1C). Score 2 is given to fish with multiple score 1 wounds or one smaller open wound (2A-2C), and score 3 is given to fish with severe open wounds (3A-3C).

Table 2.1: Wound scores (according to Laksvel guidelines) for all 71 individuals for the four samplings. The score is given based on the one side of the fish that has the highest score.

(dd.mm.yyyy)	Score 0	Score 1	Score 2	Score 3
05.08.2021	34	29	7	1
28.09.2021	33	22	16	0
09.12.2021	12	21	38	0
17.01.2022	18	16	37	0

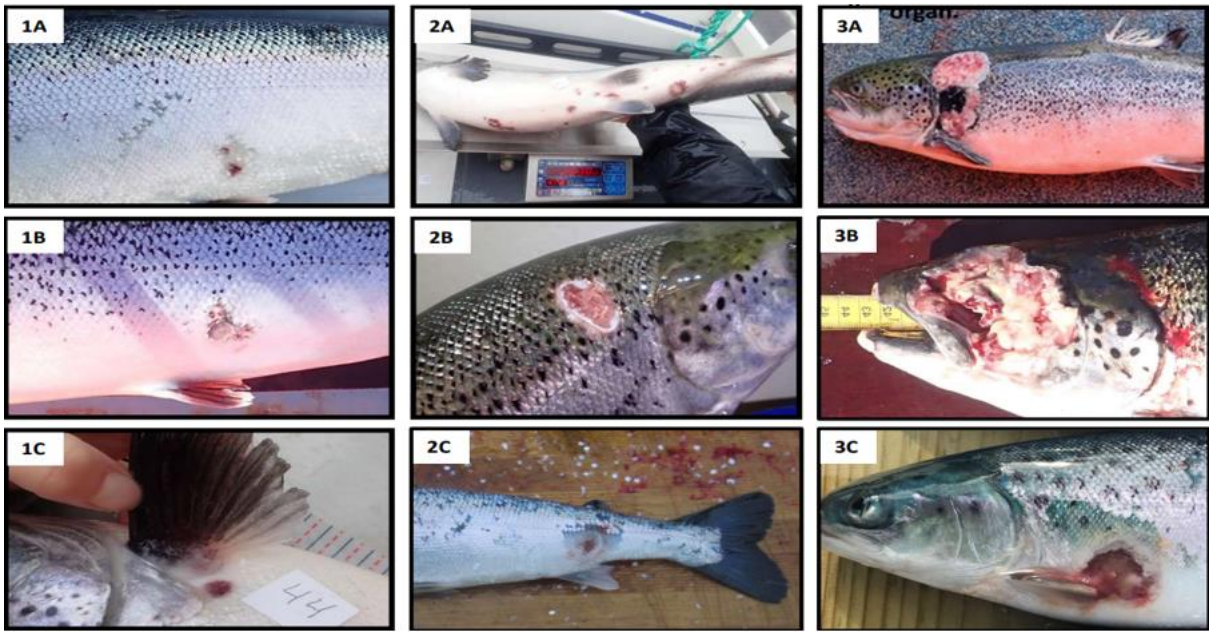


Figure 2.4: Visual scoring of wounds based on IMR's guidelines. 1A-1C = score 1, 2A-2C = score 2, 3A-3C = score 3. Image from Nilsson et al. (2022).

2.8 Evaluating signs of sexual maturation

To identify and track the development of sexually maturing individuals, a scoring system according to the Laksvel guidelines was used (Fig 2.5) (Nilsson *et al.*, 2022). This system uses a scale of 0 to 3, where a score 0 implies no signs of maturation and a score 3 implies full maturation. As most mature fish were males (n = 19), and only one female, only males were studied in this thesis. The following explanation of the scoring system is with regards to visual characteristics of sexually mature males. With the first signs of sexual maturation a

score 1 is given, where these individuals are starting to develop longer jaws and are showing signs of a hook formation on the lower jaw. At this stage the fish still maintain its natural body shape and colour (Fig. 2.5 1A-1C). A score 2 is given to individuals that are starting to develop a darker skin colour, and the masculine head features are further developed (2A-2C). A score 3 is given to fully matured individuals, where the masculine head features are fully developed, the body has a squared shape with a thicker tail end, and the colour is dark (3A-3C).

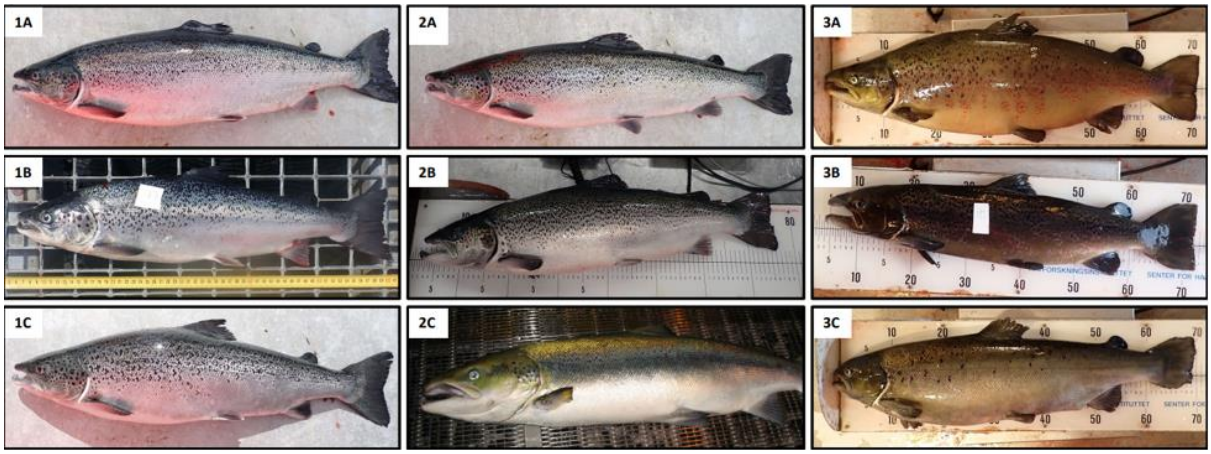


Figure 2.5: Visual scoring of sexual maturation according to MRI's guidelines. 1A-1C = score 1 (all males), 2A-2C = score 2 (all males), 3A-3C = score 3 (all males except 3C). Image from Nilsson et al. (2022).

2.9 Grouping

To identify potential differences in morphometrics between fish of different sizes, three groups (Large, Medium, and Small) were made from the 51 immature fish. Each group consisted of seven fish selected based on weight for the last sampling, where the Large group contained the seven largest, Small the seven smallest, and the Medium group seven fish in the middle. Seven individuals were selected for each group to maintain statistical power and at the same time represent a significant difference between the groups. To compare sexually mature fish with immature two groups were made, where the Mature group consisted of the 19 males that matured during the experiment, and the Immature group consisted of the 51 immature fish.

2.10 Statistical analysis

The statistical analyses of the data were done in R – 4.2.0, which is a software for statistical computing and graphics. The software is downloaded through the CRAN network, which is

R's main repository with servers storing up-to-date versions of R and its packages. The software was run though the integrated development environment RStudio 2022.02.2+485 (Prairie Trillium), a free open-source application that makes using R easier and more efficient. Multiple R packages were used: dplyr for data manipulation, readxl for importing data from excel, ggpubr and ggplot2 for data visualisation, and tidyverse to create a better environment for data science.

R base downloaded from CRAN, URL: https://cran.r-project.org/

RStudio, URL: https://www.rstudio.com/products/rstudio/download/#download

R packages:

- dplyr, URL: https://cloud.r-project.org/web/packages/dplyr/index.html
- readxl, URL: https://readxl.tidyverse.org/
- ggpubr, URL: https://cran.r-project.org/web/packages/ggpubr/index.html
- ggplot2, URL: https://cran.r-project.org/web/packages/ggplot2/index.html
- tidyverse, URL: https://cran.r-project.org/web/packages/tidyverse/index.html

All data used in R was stored and sorted in Microsoft 365 Excel (2022 version), where also all figures were made.

2.10.1 Statistical analysis of distribution

To analyse the distribution of data points to determine which statistical test to use, the data was analysed visually with a density plot (ggdensity) and a qq plot (ggqqplot), and statistically with a Shapiro wilk test. To test if the variance between two samples were significantly different or not an F-test was used. A confidence level of 5% was used for all tests.

2.10.2 Statistical analysis of growth development for the three selected groups

To compare the growth indicators (weight, standard length, condition factor, and specific growth rate) between the three growth groups, a one-way analysis of variance (ANOVA) was used to determine if there were any significant difference of means. An ANOVA was carried out for each of the four samplings to test if the difference between the groups were present at all sampling points. As the ANOVA only tests if at least one of the groups differ from the rest, the results from the ANOVA was used in a post hoc test to see if there was a significant

difference between each pair of the tested groups. The post hoc test used was the Tukey's honestly significant difference (HSD). If the change in a growth indicator appeared to be minimal for a group between two samplings, a paired t-test was used to test if the change was significant.

2.10.3 Statistical analysis of morphometric development for the three selected groups

To compare the five morphometric relationships (eye diameter/StdL, body height central/StdL, body height anal/StdL, upper jaw/StdL, and head length/StdL) between the three growth groups, an ANOVA was used for the four samplings separately, and a Tukey's HSD to compare each pair. A paired t-test was used to test if the change between two samplings were significant for a group. To test for correlation between the growth indicators (weight, standard length, and condition factor) and the five morphometric relationships a Pearson's product-moment correlation was used between each indicator and relationship, which measures both the direction and strength of the association between the two.

2.10.4 Statistical analysis of growth development for the Mature and Immature group

To compare the growth indicators (weight, standard length, condition factor, and specific growth rate) between the Immature and Mature group, a Welch two sample t-test was used for each sampling to determine if there were any differences between the means of the two groups. Doing a t-test for each sampling allows for tracking whether the difference between the groups stays the same or varies over time. A paired t-test was used to see if the change in growth indictor from one sampling to another were significant for a group.

2.10.5 Statistical analysis of morphometric development for the Mature and Immature

To compare the five morphometric relationships between the Immature and the Mature group, a Welch two sample t-test was used. A paired t-test was used to test if the change in a relationship from one sampling to another were significant for a group. To test for correlation between the growth indicators (weight, standard length, and condition factor) and the five morphometric relationships, a Pearson's product-moment correlation was used between each indicator and relationship.

3. Results

3.1 Analysis of the influence of wounds

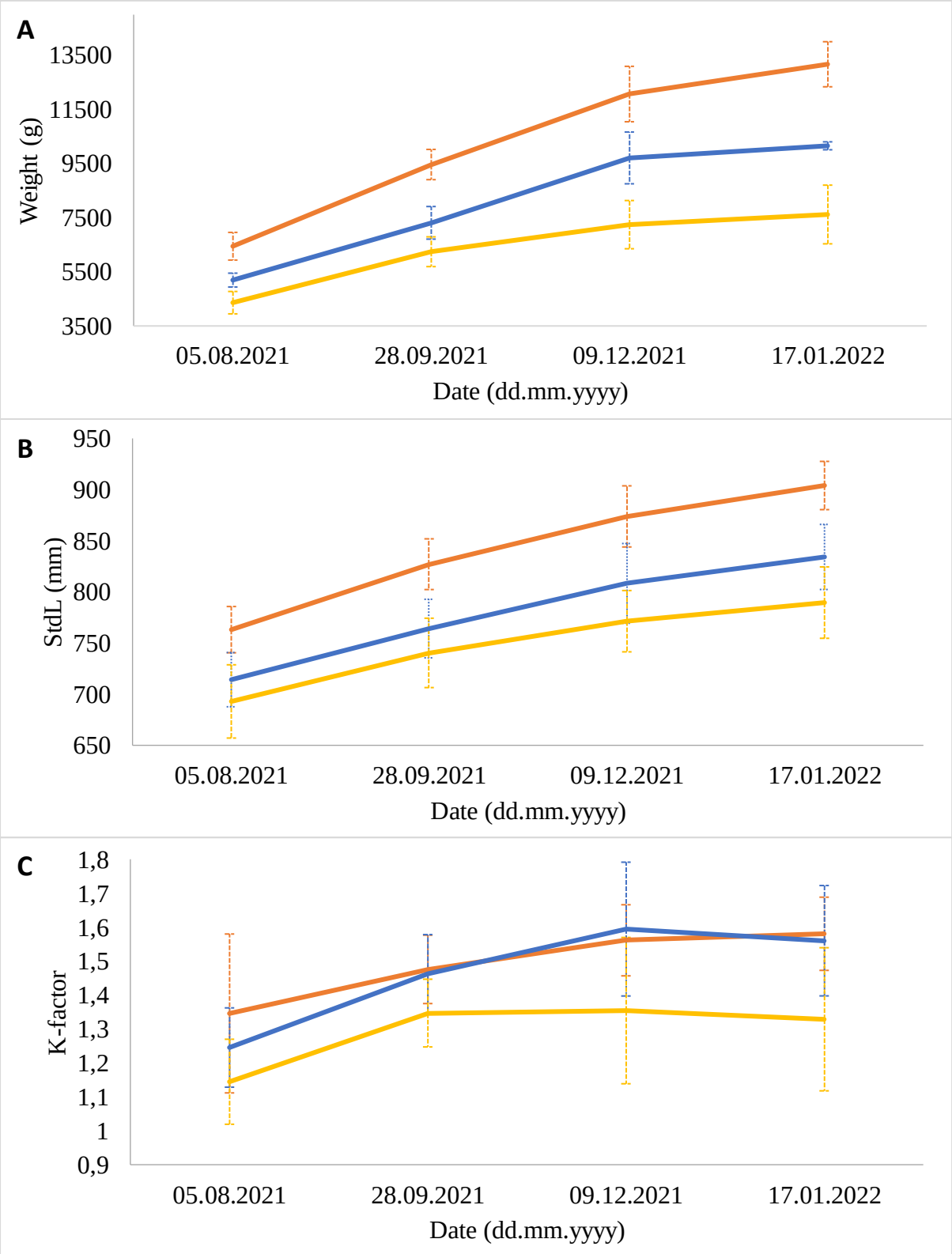
As mentioned earlier, the fish were grouped by wound score and analysed for significant differences in the means of weight and length. No significant difference was recorded between the groups ($p > 0.15$), signifying that the development of wounds did not have an effect on growth performance. To test for a correlation between maturation and wound development a Chi-square test of independence was used for wound score and maturation score. This test showed that there was no significant relationship between maturation and wound status ($p > 0.11$).

3.2 Analysis of the three selected growth-groups

3.2.1 Growth over time for the three selected groups

All three groups had a weight increase between each sampling (Fig. 3.1A), where the Large group grew the most from start to end with a mean increase of 6723 ± 607g (mean ± SD), the Medium grew 4961 ± 250g, and the Small group 3257 ± 1226g. The weight difference for the three groups was significant during the entire period, where large vs. small had the largest difference for all samplings ($p < 0.00001$). Large vs. medium had the second largest difference ($p < 0.01$) for all samplings and medium-small had the smallest difference ($p < 0.001$) for all samplings, with a few individuals overlapping on the second sampling. The difference in mean weight was largest for the last sampling when the large group was 13168 ± 833g, medium 10156 ± 146g, and the small group 7618 ± 1084g. Compared with weight, the groups had a more similar increase in standard length (Fig. 3.1B). Length was for all samplings significant higher for the Large vs. Medium group ($p < 0.02$) and Large vs. Small ($p < 0.001$). The Medium vs. Small was not significantly different in length for the first three samplings ($p > 0.12$), but the difference increased for each sampling and a significant difference was recorded for the last sampling ($p = 0.034$), where the Small had a standard length of 790 ± 35mm, Medium group 834 ± 32mm, and Large 904 ± 24mm. The condition factor was not significantly different between the groups until the last sampling (Fig. 3.1C), where Small had a lower condition factor than Medium ($p = 0.044$) and Large ($p = 0.028$).

The specific growth rate for the three periods decreased with each period for all groups (Fig. 3.1D) and was not significant different between the groups ($p > 0.05$) for any period.



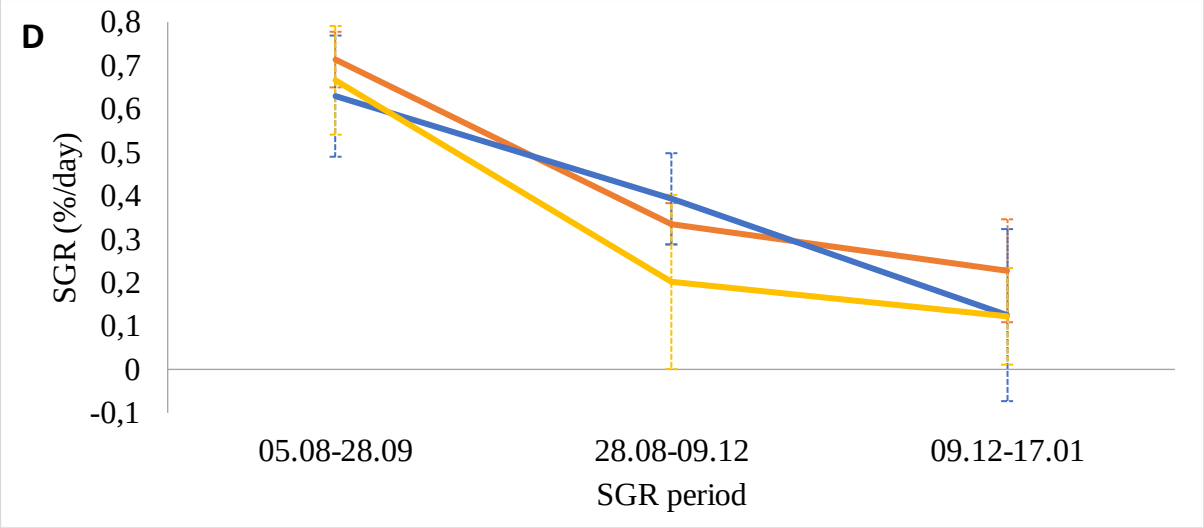


Figure 3.1: The average growth development over time for Large (orange), Medium (blue) and Small (yellow) groups. A = Weight, B =Standard length, C = Condition factor, D = Specific growth rate

3.3 Analysis of head morphometrics for the three selected groups

As the head measurements (upper jaw and head length) are in ratio with standard length, a decrease in the head ratios implies that the three head measurements became smaller relative to the full body length of the fish. There was no significant difference between the groups for the upper jaw ratio for any samplings ($p > 0.05$) except for the third, where Medium had a lower ratio than Large ($p = 0.0014$) and Small ($p = 0.0094$) (Fig. 3.2). The head length ratio was not significantly different between the groups for any sampling ($p > 0.05$), except for the third sampling where Medium had a lower ratio than the Small ($p = 0.016$).

The two head ratios were negative correlated with the three growth indicators where head length/StdL (weight: $p < 0.0001$, cor.(R) =-0.45, standard length: $p < 0.001$, cor. -0.40, condition factor: $p < 0.001$, cor. -0.38) (Fig. 3.3) had an overall stronger correlation than the upper jaw/StdL ratio (weight: $p < 0.001$, cor. =-0.36, standard length: $p < 0.01$, cor. -0.34, condition factor: $p < 0.01$, cor. -0.29) (Fig. 3.4 and appendix Fig. A.1).

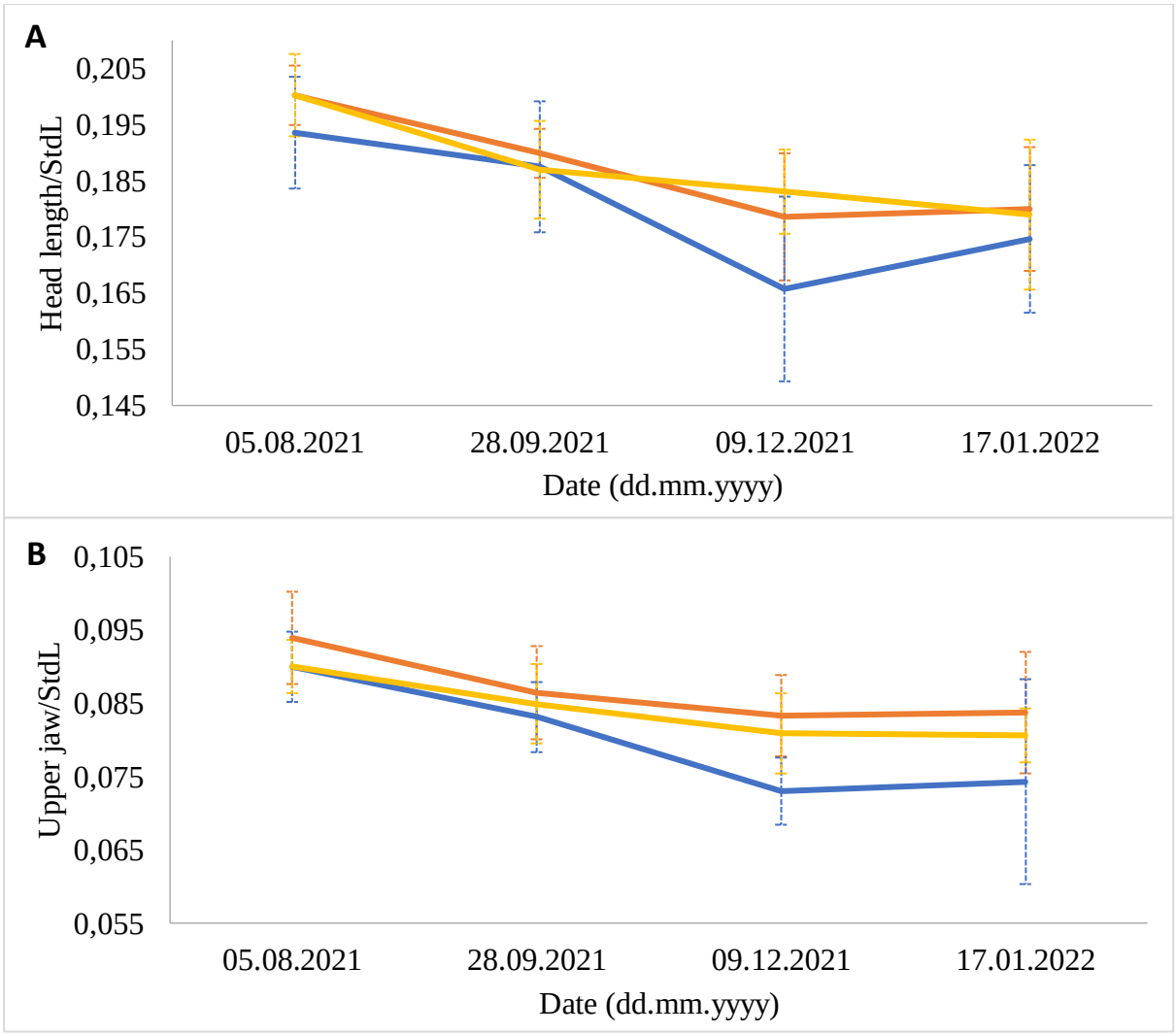
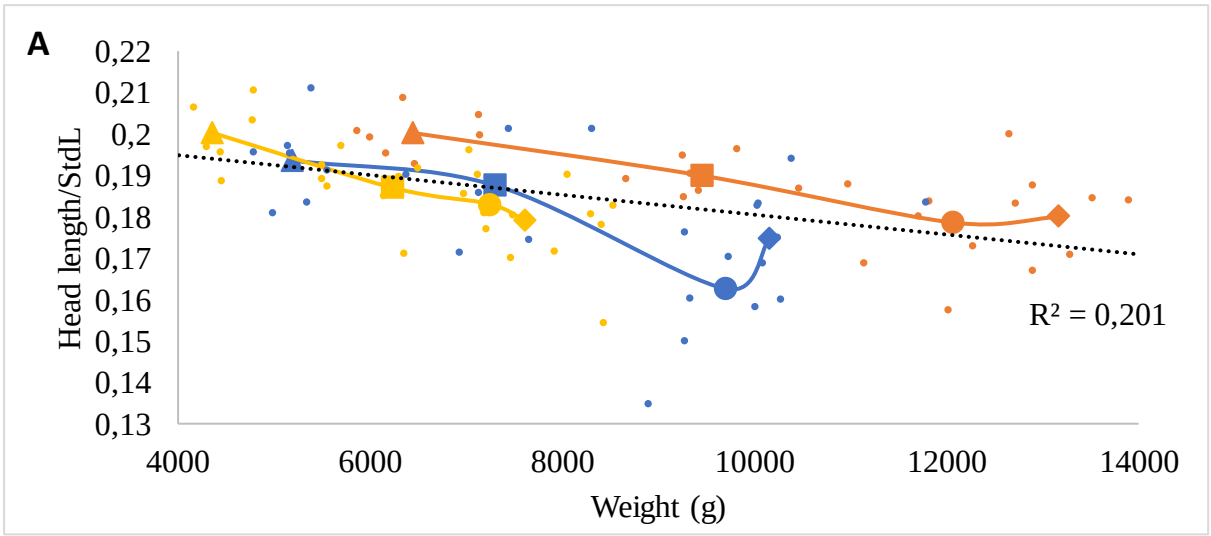


Figure 3.2: The mean development for morphometric relationships with standard deviations over time for the Large (orange), Medium (blue) and Small (yellow) group. A = head length/standard length ratio, B = Upper jaw/standard length ratio.



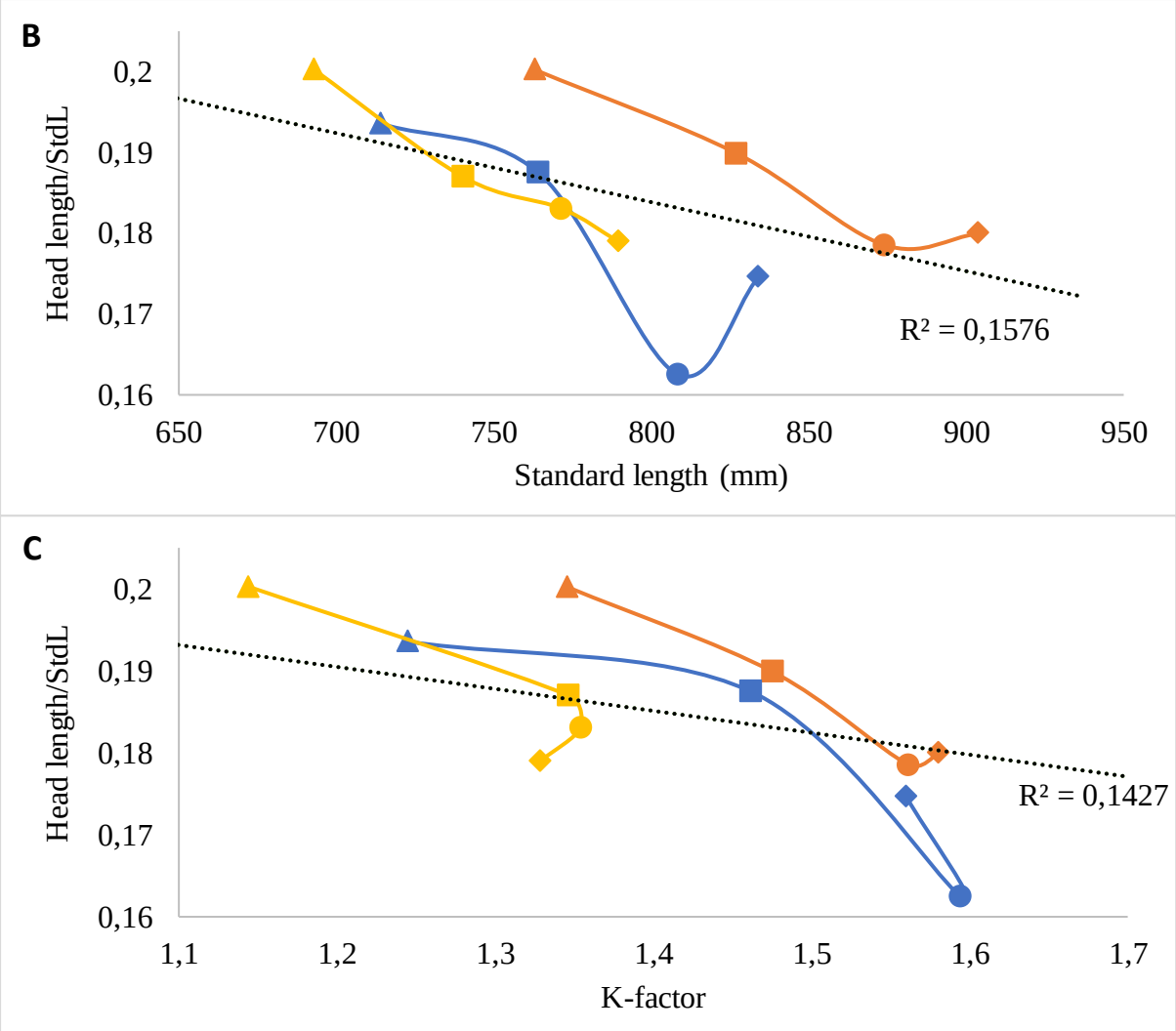


Figure 3.3: Head length/standard length ratio compared to A = weight, B = standard length and C = Condition factor over time for the Large (orange), Medium (blue) and Small (yellow) group. The dotted black line is a fitted linear model between the morphometric relationship and the growth indicator, and it is accompanied by its coefficient of determination (R^2). The symbols on each line represents sampling number: Triangle = 1, square = 2, circle = 3, and tilted square = 4.

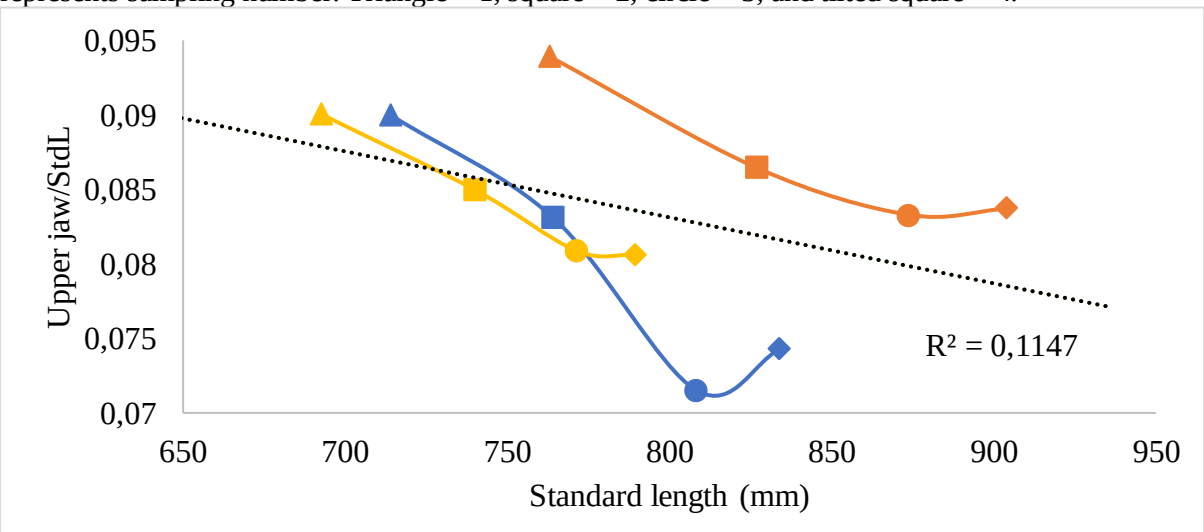


Figure 3.4: Upper jaw/standard length compared to standard length over time for the Large (orange), Medium (blue) and Small (yellow) group.

3.4 Analysis of eye morphometrics over time for the three selected groups

The eye ratio was not significantly different between the groups for any samplings ($p > 0.05$) (Fig. 3.5), except for the third sampling where the Large (0.0141 ± 0.0011, mean ± SD) and the Medium (0.0146 ± 0.0016) groups had a larger decrease than the Small (0.0168 ± 0.0009) compared to the second sampling. This resulted in the Small having a larger ratio than Large ($p = 0.0037$, dif. = 19.2%) and the Medium group ($p = 0.017$, dif. = 15.1%). The eye diameter/standard length ratio was negatively correlated with all three growth indicators (Fig. 3.6 and appendix Fig. A.2) having the strongest correlation with weight ($p < 0.00001$, cor. -0.65) and standard length ($p < 0.00001$, cor. -0.65), and less so with condition factor ($p = 0.0014$, cor. -0.34).

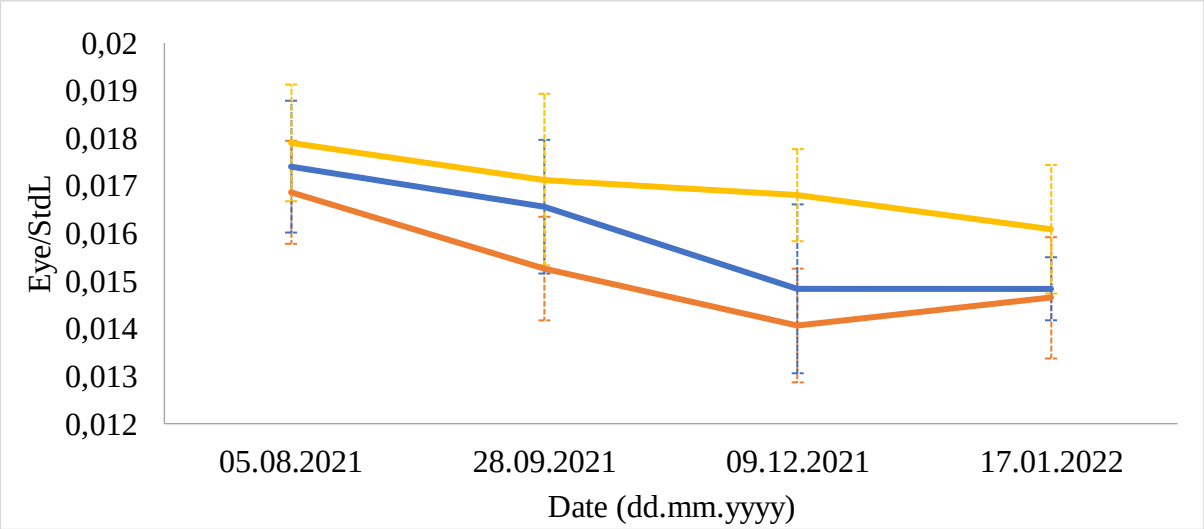


Figure 3.5: The mean eye diameter/standard length ratio with standard deviations over time for the Large (orange), Medium(blue) and Small (yellow) groups.

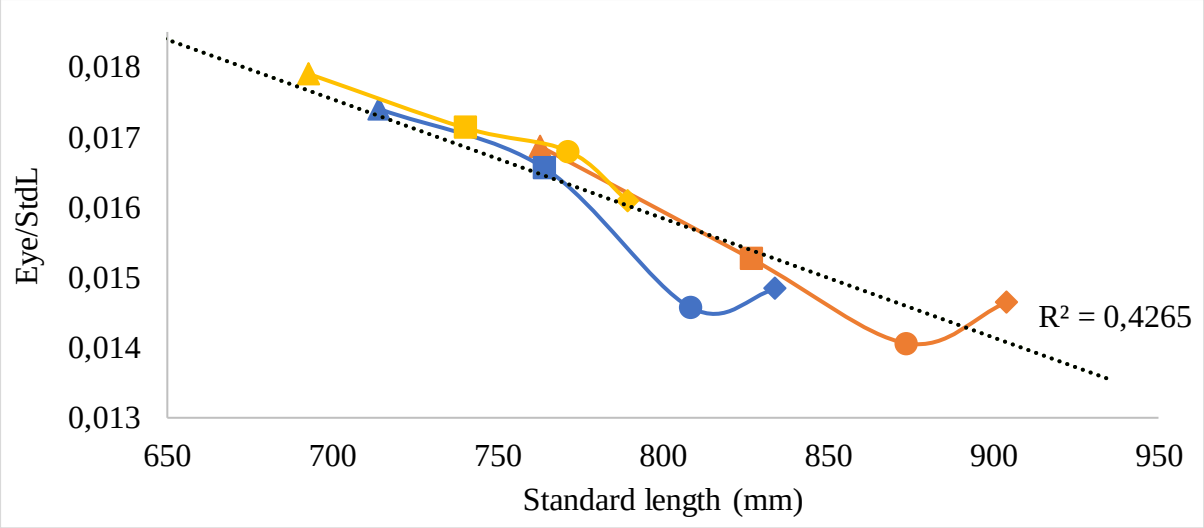


Figure 3.6: Mean eye diameter/standard length ratio compared to standard length over time for Large (orange), Medium (blue) and Small (yellow) groups.

3.5 Body height central and anal over time

The body height central and anal ratios both increased until the third sampling ($p < 0.01$), where the two ratios for all groups levelled out between the third and last sampling with no significant change ($p > 0.7$) (Fig. 3.7). The body height central ratio was larger for the Large group compared to the Small ($p = 0.0017$, dif. = 9.2%), and Medium compared to Small ($p = 0.0019$, dif. = 6.7%) during the first sampling, where the Large group had a ratio of 0.26 ± 0.008 (mean ± SD), Medium 0.25 ± 0.008 and Small 0.24 ± 0.012 (Fig 3.7A). There was also a significant difference for Large vs. Small ($p = 0.027$, dif. = 10.1%) during the last sampling where the Large group still had the largest ratio (0.29 ± 0.009). The body height anal ratio was only significantly different for Large vs. Small on the first ($p = 0.043$, diff. = 7.1%), second ($p = 0.022$, diff. = 7.1%), and last ($p = 0.032$, diff. = 9.3%) sampling where the Large group had the largest ratio for all dates (Fig 3.7B).

The body heights were both positively correlated to all three growth indicators, where BHC/StdL (weight: $p < 0.00001$, cor. = 0.77, standard length: $p < 0.00001$, cor. = 0.49, condition factor: $p < 0.00001$, cor. = 0.90) (Fig. 3.8A, Fig. 3.9A and appendix Fig. A.3) and BHA/StdL (weight: $p < 0.00001$, cor. = 0.73, standard length: $p < 0.0001$, cor. = 0.45, condition factor: $p < 0.00001$, cor. = 0.89) (Fig. 3.8B, Fig. 3.9B and appendix Fig. A.4) both showed strong correlation with the indicators.

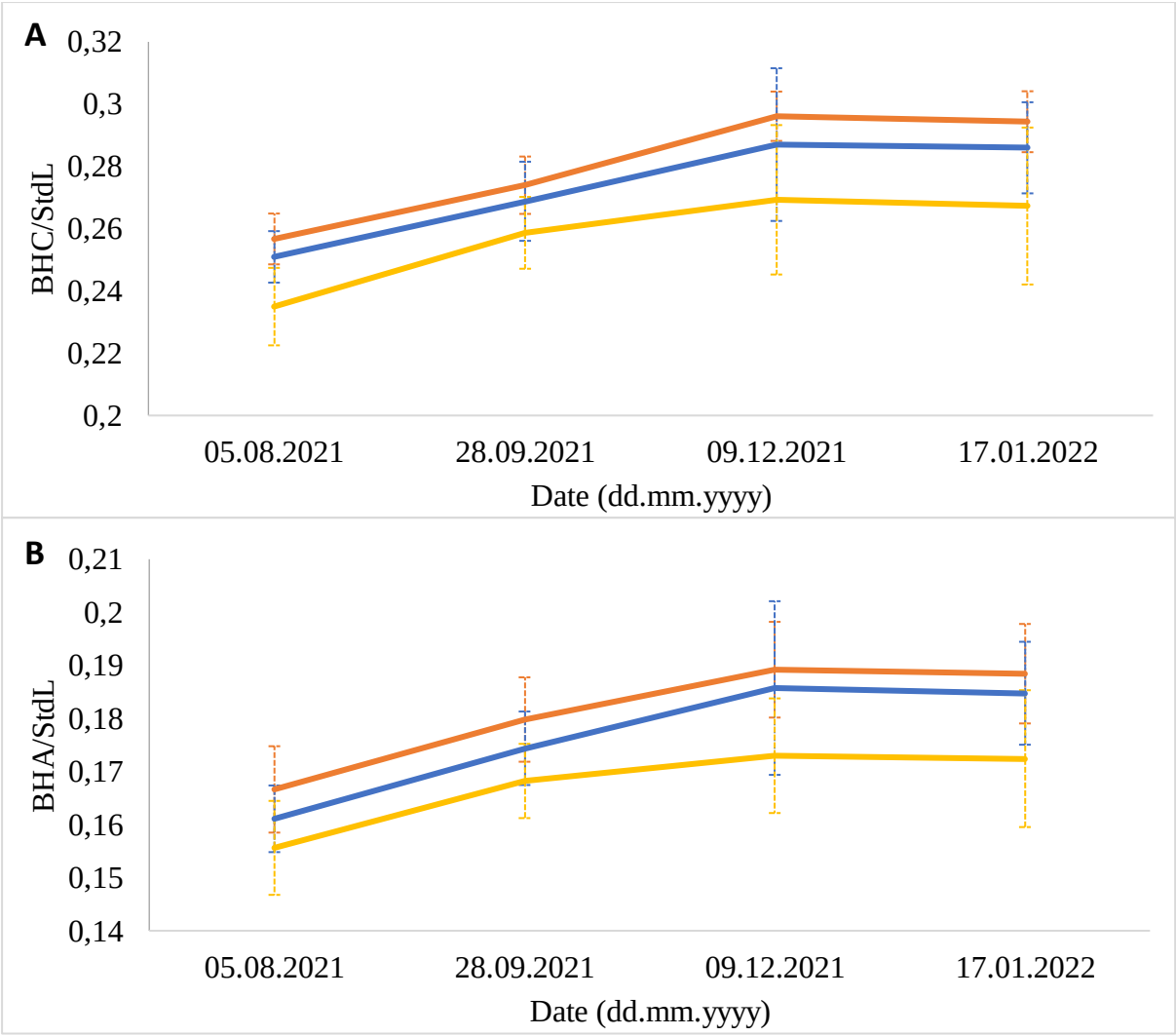
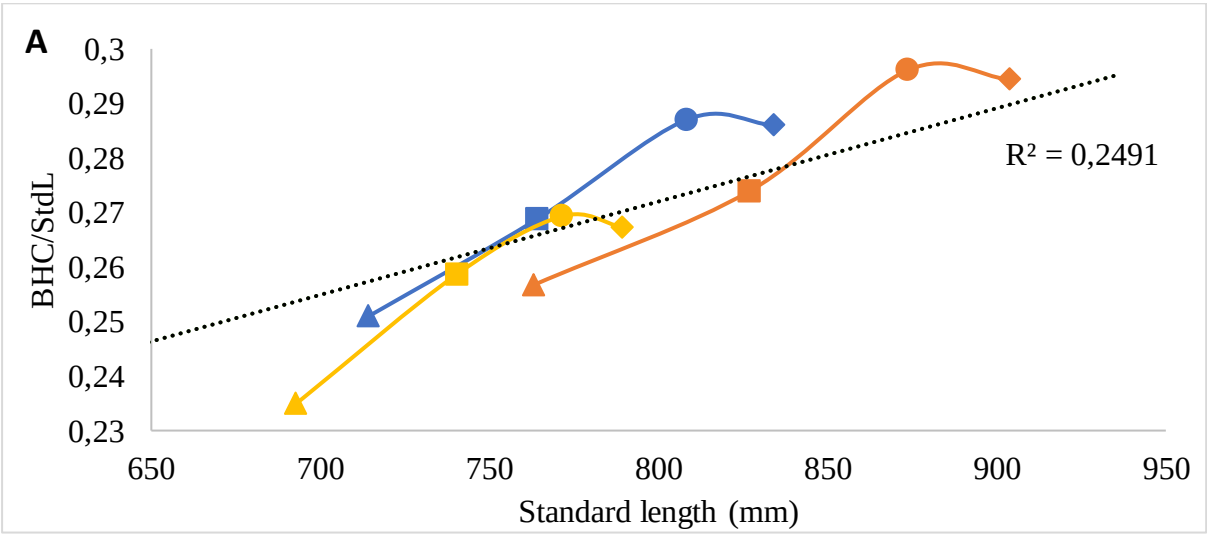


Figure 3.7: Mean body height/standard length ratios over time for Large (orange), Medium (blue) and Small (yellow) groups. A = body height central, B = body height anal.



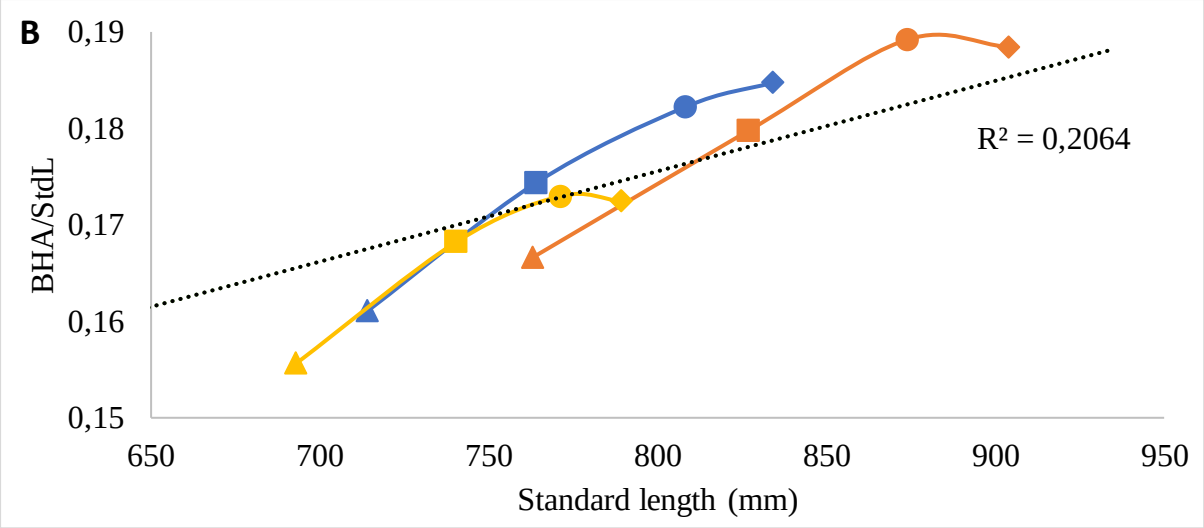


Figure 3.8: Mean body height/standard length ratios compared to standard length over time for Large (orange), Medium (blue) and Small (yellow) groups. A = body height central, B = body height anal.

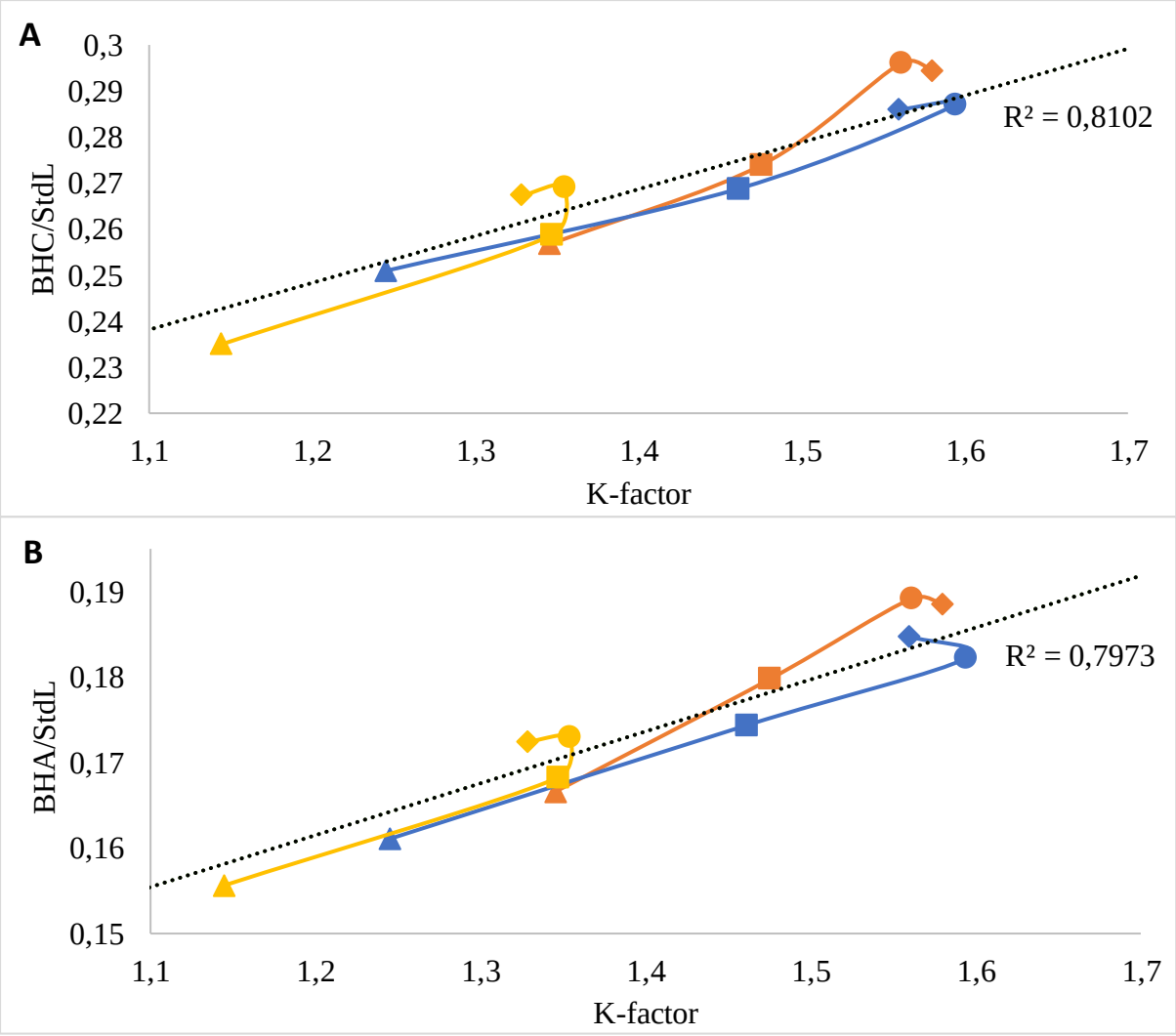


Figure 3.9: Mean body height/standard length ratios compared to K-factor over time for Large (orange), Medium (blue) and Small (yellow) groups. A = body height central, B = body height anal.

3.6 Analysis of the sexually mature group

3.6.1 Analysis of growth trajectory for the sexually matured group compared to the Immature group

Out of the 19 sexually matured individuals 17 started showing visual signs of maturation, as described by Nilsson et al. (2022), already from the first sampling (Fig. 3.10A), and the last two showed the first signs on the second sampling. All 19 appeared as fully mature with brown skin coloration on the third sampling on the 12th of December (Fig. 3.10C), and on the last sampling 9 of them had turned back to silvery coloration (Fig. 3.10D), but still maintained the characteristic squared body shape and masculine head features.

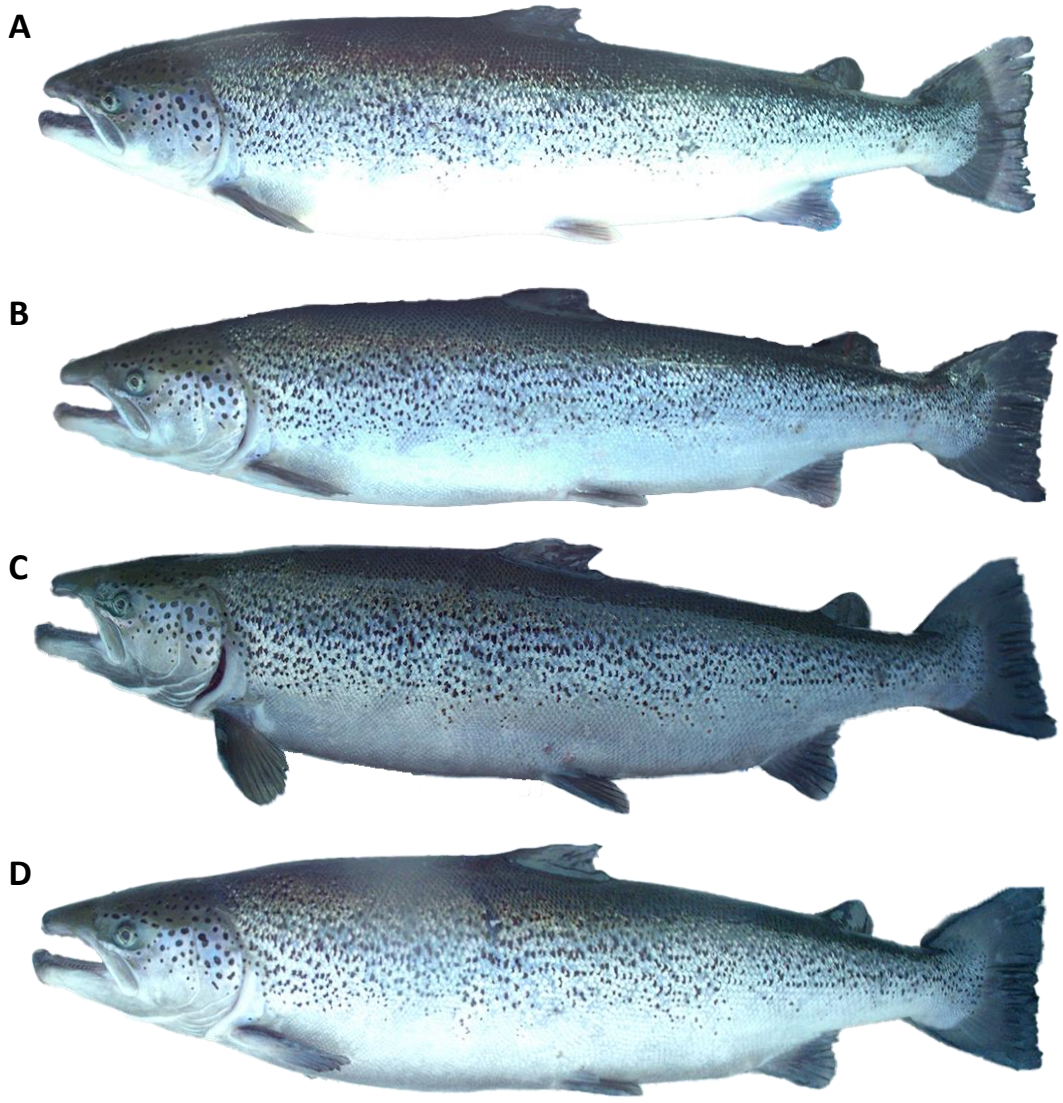


Figure 3.10: Maturing process of an individual over the four samplings.

The Mature and Immature group started off in August with no significant difference in weight ($p = 0.82$, ~5600g), standard length ($p = 0.53$, ~735mm), or condition factor ($p = 0.41$ ~1.23,) (Fig. 3.12). Between the first and second sampling the two groups started to differentiate, which is reflected in the specific growth rate between the two groups for that period ($p < 0.00001$, dif. = 0.633), where the Mature group had a mean daily weight increase of 0 ± 0.22% (mean ± SD), and the Immature group 0.64 ± 0.12% (Fig 3.12D). This difference in daily weight increase during the first 54 days resulted in a significant difference in weight on the second date ($p < 0.00001$, dif. = 2167g), where the Immature group had grown to 7853 ± 1153g (mean ± SD) which is a weight increase of 41%, while the Mature group did not have a significant weight change ($p = 0.7$, paired t-test) (Fig. 3.12A).

Although the Mature group did not have a significant change of weight from the first to the second date, a small but significant increase of standard length was recorded ($p = 0.00023$, dif. = 15.6mm) (Fig. 3.12B). The Immature group grew 7.1% in standard length during the same time frame, resulting in a significant difference between the groups of 31mm ($p < 0.01$). Compared to weight the standard length had more over lapping data points, where some of the individuals in the Mature group had a longer standard length than the mean length for the Immature group. Between August and September, the condition factor for the Mature group slightly decreased ($p < 0.01$, dif. = 0.066), while the Immature group had an increase of 14.5%, resulting in a significant difference between the groups of 24% ($p < 0.00001$) (Fig. 3.12C).

By the third sampling the weight, standard length and condition factor had increased for the Immature group (Fig. 3.11A), where the 72 days period led to an 21.9% increase ($p < 0.00001$) in mean weight to 9579 ± 1604g, 5.4% increase ($P < 0.00001$) in standard length to 819 ± 42mm, and an increase in condition factor of 4.3% ($P = 0.015$) to 1.49 ± 0.15 (Fig. 3.12). The specific growth rate did, however, decrease for the period compared to earlier, signifying that the weight increase rate was slowing down (Fig. 3.12D). The 72 days period led to no significant change for all growth indicators ($p > 0.1$ for all indicators) for the Mature group (Fig 3.11B).

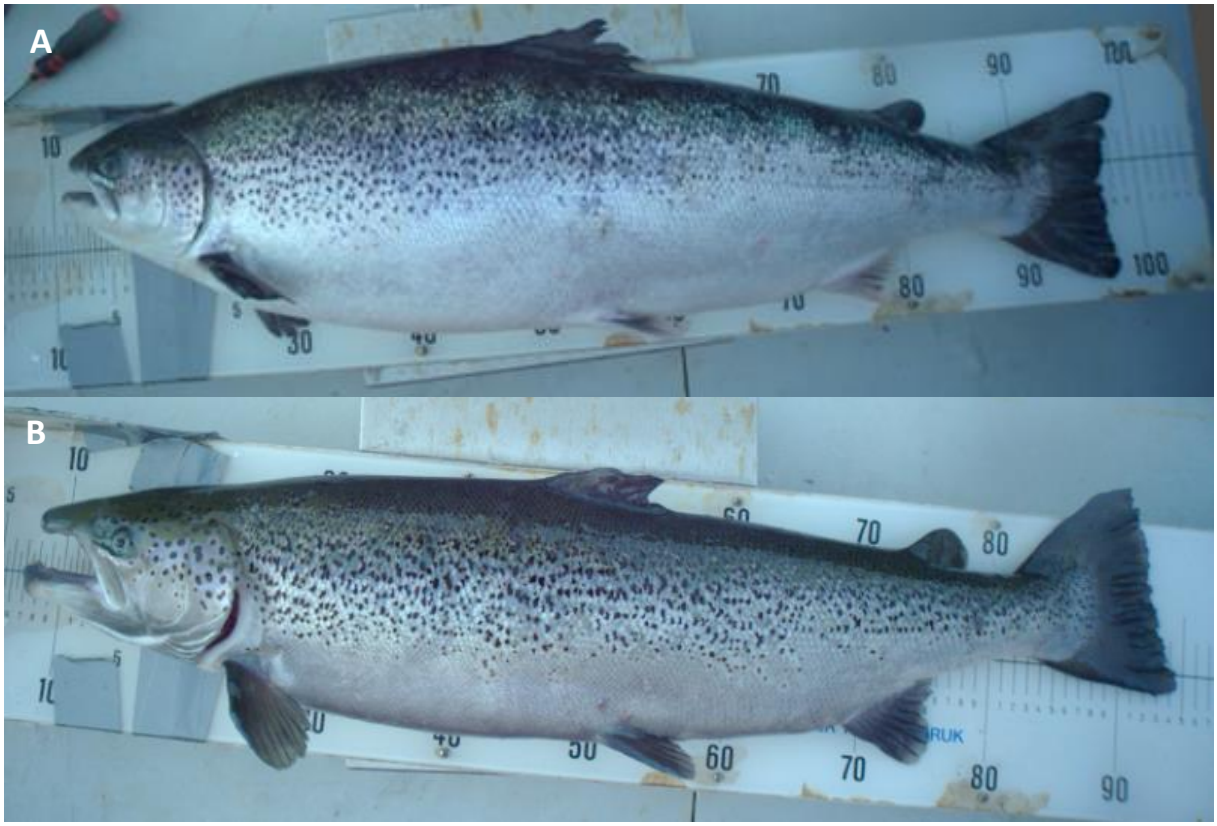
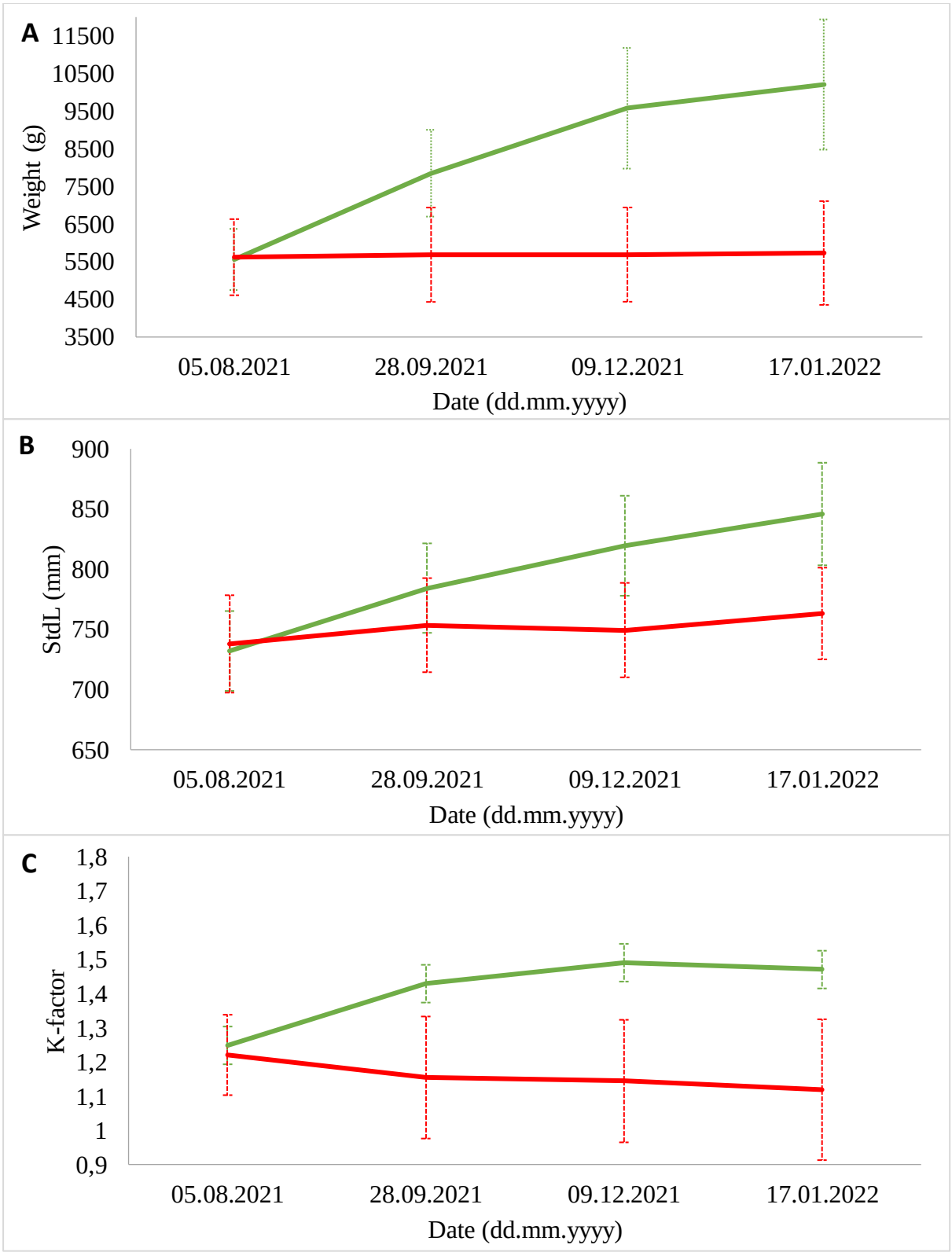


Figure 3.11: Image A: immature and B: mature fish from the December sampling.

The 39 days long period from the third to the last sampling, the Immature group had continued its growth, but with a diminishing rate. This is reflected by the specific growth rate for the last period which decreased to 0.16 ± 0.13% compared to 0.27 ± 0.12% during the previous period (Fig. 3.12D). This resulted in a 6.6% weight increase during the last period, ending with the Immature group having a mean weight of 10209 ± 1730g, which is a total increase of 84% from the start (Fig. 3.12A). The Mature group did not change weight between the third and the last sampling ($p = 0.63$). This means that during the 165 days the experiment lasted, the Mature group did not change weight from start to finish ($p = 0.71$), resulting in a large difference between the two groups ($p < 0.00001$, dif. = 4476g). For the Mature group, a small but significant increase of standard length was recorded between the third and the last sampling ($p < 0.0001$, dif. = 13.8mm). This resulted in a total increase for the whole experiment of 25mm ($p = 0.0001$), which is an increase of 3.4%. The total increase of standard length for the Immature group during the 165 days was 15.6% from 732 ± 33mm to 846 ± 43mm, resulting in a significant difference between the two groups ($p < 0.00001$, dif. = 82.7mm).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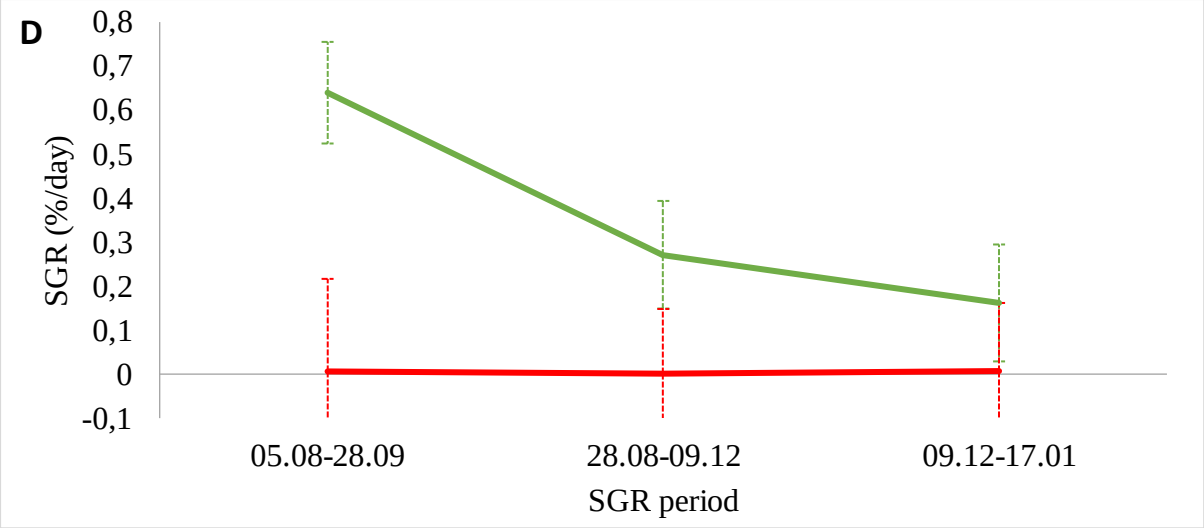


Figure 3.12: The mean growth development with standard deviations over time for the Immature (green) and Mature (red) group. A = Weight, B=Standard length, C = Condition factor, D = Specific growth rate.

3.7 Analysis of head morphometrics for the Mature and Immature group

The two head measurements (head length and upper jaw) in ratios with the standard length tells a similar story when plotted over time (Fig. 3.13 and appendix Fig. A.5). On the first sampling both ratios were the most similar between the groups, but still significantly different ($p < 0.0001$ for both ratios) with the Mature group having larger ratios (head length/StdL: dif. = 6.9%, upper jaw: dif. = 8.8%) compared to the Immature. At this point there was some over lapping of individual fish.

From the first to the second date the ratios for Mature group increased ($p < 0.001$ for both ratios), while they decreased for the Immature group ($p < 0.00001$ for both ratios). This led to the Mature group having larger ratios for both the head length (dif. = 18.1%) and upper jaw (dif. = 25.2%) compared to the Immature group.

From the second to the third sampling the two ratios for the Mature group were not significantly different ($p > 0.2$), while the ratios for the Immature group decreased during the 72 days period, resulting in an even larger difference between the groups for both the head length ratio ($p < 0.00001$, dif. = 26.7%) and the upper jaw ratio ($p < 0.00001$, dif. = 34.8%).

Between the third and the last date, both ratios increased for the Mature group ($p < 0.04$ for both ratios), while only the head length ratio increased for the Immature ($p = 0.035$). This resulted in a peak for the ratios for the Mature group (head length/StdL = 0.225 ± 0.009,

upper jaw/StdL = 0.110 ± 0.006), and a significant difference between the two groups (head length: $p < 0.00001$, dif.: 25.9%, upper jaw/StdL: $p < 0.00001$, dif. = 35.6%). Compared with the percentage difference between the two groups on the previous sampling, suggests that the difference in the ratios between the groups reached a peak at the third sampling when the 19 sexually matured individuals appeared as fully matured.

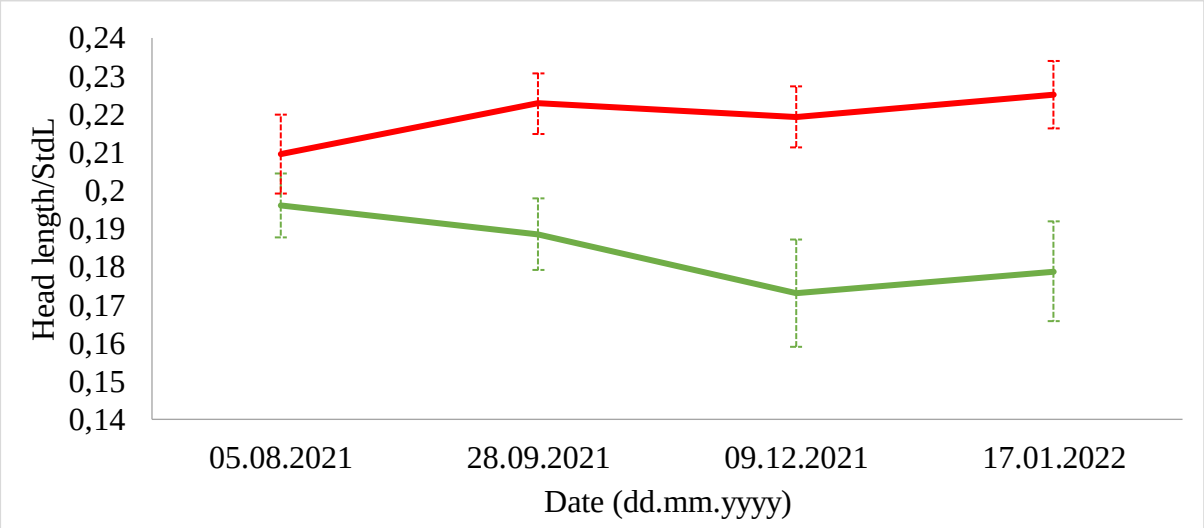


Figure 3.13: Mean snout-pectoral/standard length ratio with standard deviations over time for Mature (red) and Immature (green) group.

3.8 Analysis of eye morphometrics for the Mature and Immature group

The eye diameter/standard length ratio started off with no significant difference between the groups in August ($p = 0.45$, ratio = ~0.172), with a lot of overlap of individuals (fig. 3.14). Similarly, to the two head ratios, the eye ratio increased for the Mature and decreased for the Immature during the first 54 days. At this point there was a significant difference between the groups ($p < 0.00001$, dif. = 11.9%), where the Mature had a ratio of 0.0184 ± 0.0011, and the Immature 0.0164 ± 0.0014. From the second to the third sampling, the ratio for the Mature group did not change ($p = 0.83$), while the ratio for the Immature decreased to 0.0152 ± 0.0016, resulting in a 22% difference ($p < 0.00001$) between the groups. The eye ratio did not change in the Mature ($p = 0.38$) or Immature group ($p = 0.62$) after the December sampling, meaning that the eye ratio, similarly to upper jaw, reached a peak difference in December. The eye diameter did not change for the Immature group ($p = 0.47$) during the six months, while an 7% increase was recorded for the Mature group ($p = 0.025$) (Table. 3.2).

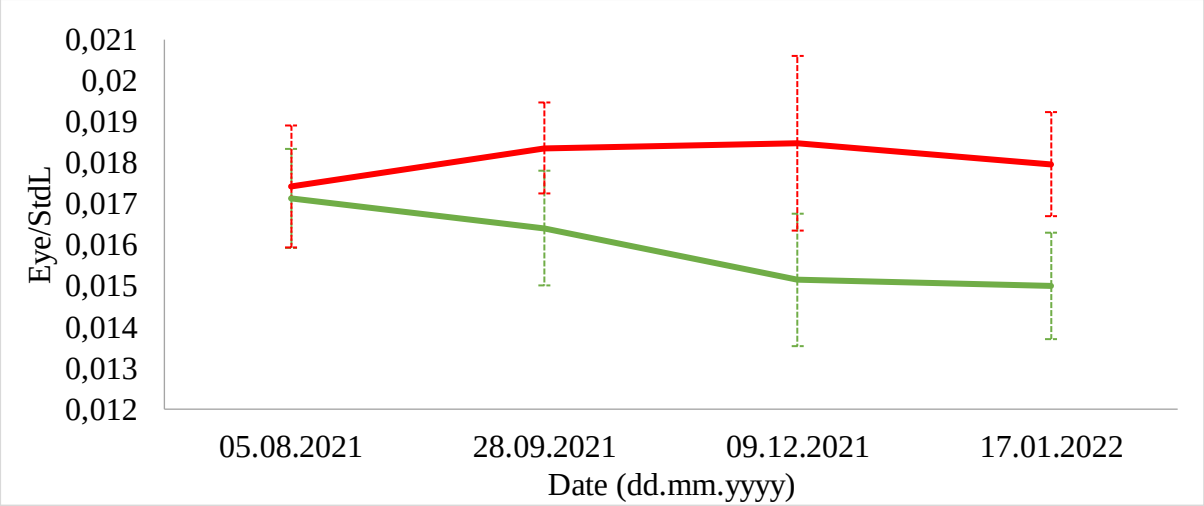


Figure 3.14: Mean eye diameter/standard length ratio with standard deviations over time for Mature (red) and Immature (green) group.

Table 3.2: Mean eye diameter (mm) with standard deviation for the Immature and the Mature group for all four samplings.

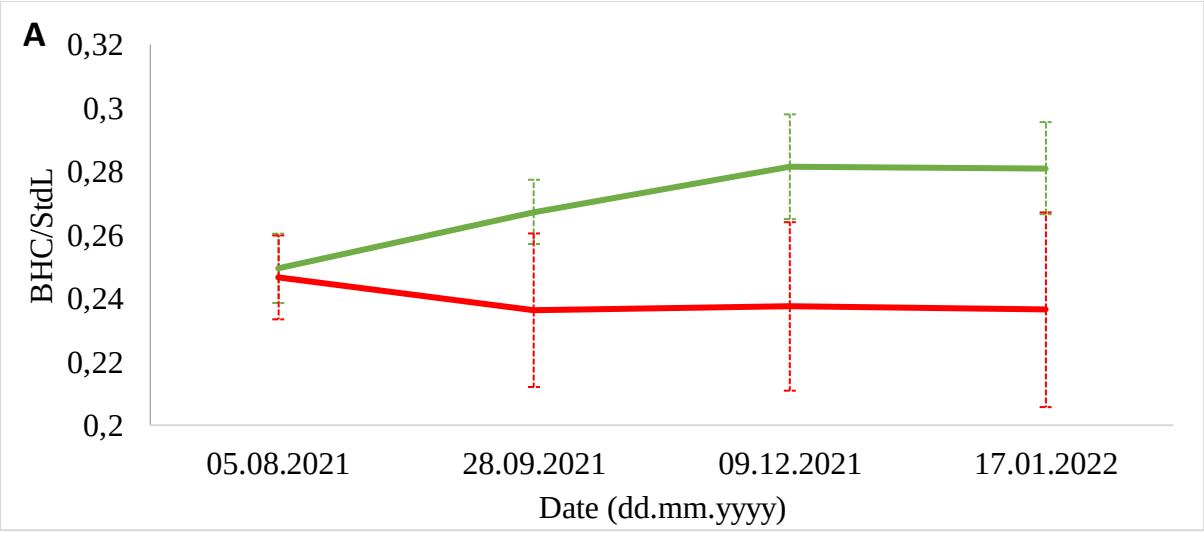
Groups	**Eye.Dia1**	**Eye.Dia2**	**Eye.Dia3**	**Eye.Dia4**
Immature	12,5 ± 0,9	12,9 ± 1,2	12,4 ± 1,2	12,7 ± 1
Mature	12,8 ± 1,2	13,8 ± 0,8	13,8 ± 1,3	13,7 ± 1

3.9 Analysis of body height morphometrics for the Mature and Immature group

Both central and anal body heigh in ratio with the standard length were not significantly different between the two groups on the first sampling in August ($p = 0.41$ and $p = 0.098$, respectively) (fig. 3.15). The two ratios increased for the Immature group from August to September (7.1% for BHC/StdL, 7.8% for BHA/StdL), while for the Mature the central height ratio decreased by 4.4% and the anal height did not have any change ($p = 0.42$). At this point the groups had differentiated significantly with regards to both BHC/StdL ($p < 0.0001$, dif. = 13.1%), and BHA/StdL ($p = 0.098$, dif. = 4.9%), where the Immature group had the largest ratios. On the third sampling, the two ratios for the Immature group had continued to increase (5.3% for BHC/StdL, 3.6% for BHA/StdL), while for the Mature the BHA/StdL had a 3.5% increase and the BHC/StdL did not change ($p = 0.074$). This resulted in the Immature group having a 18.6% ($p < 0.00001$) larger BHC/StdL ratio, and a 5.1% ($p = 0.012$) larger BHA/StdL ratio compared to the Mature group. Both body height ratios did not change in the

Mature ($p > 0.4$ for both ratios) or Immature group ($p > 0.8$ for both ratios) after the December sampling.

The BHC length for the Mature group was 182 ±14mm on the first sampling, and did not change significantly throughout the six months ($p = 0.8$), while it had a steady increase for the Immature group from 183 ± 12 on the first sampling to 238 ± 18 on the last (Table 3.3). For the mean BHA length there was a small but significant increase of 6.5% ($p = 0.026$) for the Mature group from the first to the last sampling, while for the Immature the BHA ratio increased 30%. The BHC and BHA length were not significantly different between the groups on the first sampling ($p > 0.13$), but since the body hights for the Mature group had a slow growth compared to the Immature, a significant difference between the groups was recorded on the three last samplings ($p < 0.0004$). Even though the BHC and BHA length were not significantly different between the groups on the first sampling, a significant difference in BHC/BHA ratio was recorded on the first sampling ($p = 0.00049$, dif. = 3.1%), where the immature had a larger ratio (Fig. 3.16). The difference in BHC/BHA ratio between the groups increased to 8% ($P < 0.00001$) from the first to the second sampling, and to 13% ($P < 0.00001$) from the second to the third sampling, where the difference between the groups was the largest for all samplings. From the third to the last sampling the ratio increased for the Mature group ($p = 0.023$), while it did not change for the Immature group ($p = 0.61$), resulting in a smaller difference between the two groups compared to the third sampling ($p < 0.00001$, dif. = 11.1%).



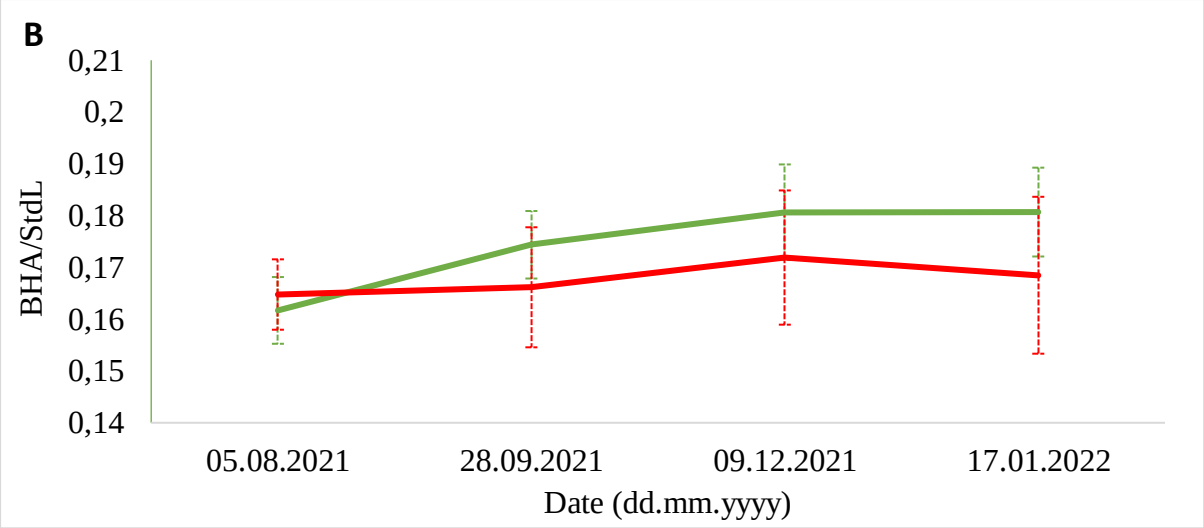


Figure 3.15: Mean body height/standard length ratios with standard deviation over time for Mature (red) and Immature (green) group. A = Body height central/standard length, B = Body height anal/standard length.

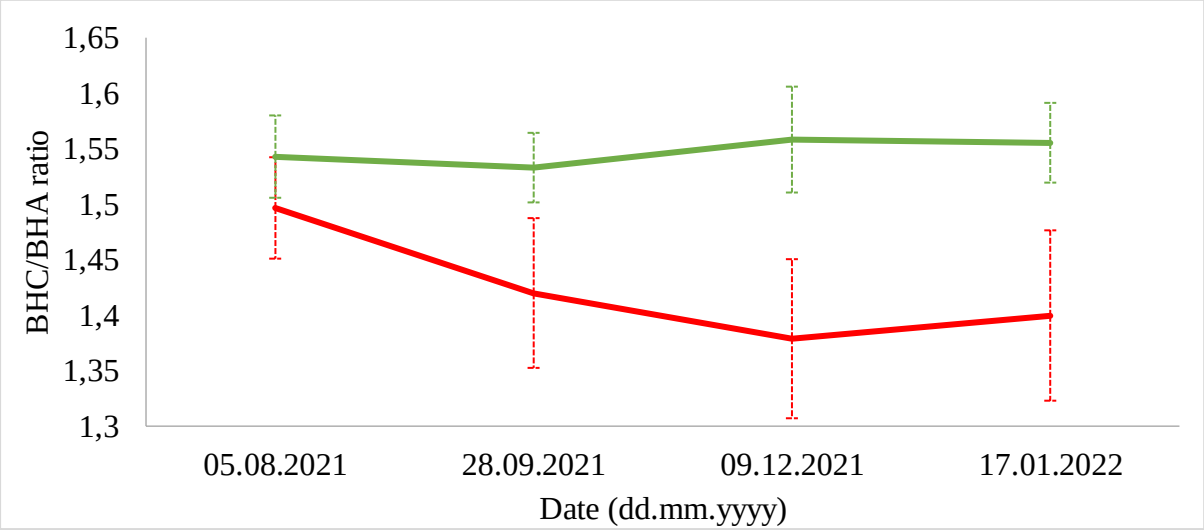


Figure 3.16: Mean body height central/anal ratio with standard deviation over time for the Mature (red) and Immature (green) group.

Table 3.3: Mean body height central and anal with standard deviation for the Immature and Mature group for all four samplings.

Groups	**BHC1 (mm)**	**BHC2 (mm)**	**BHC3 (mm)**	**BHC4 (mm)**	**BHA1 (mm)**	**BHA2 (mm)**	**BHA3 (mm)**	**BHA4 (mm)**
Immature	183±12	210±13	231±19	238±18	118±7	137±8	148±11	153±11
Mature	182±14	178±22	178±23	181±26	122±8	125±11	129±12	129±13

4. Discussion

4.1 Discussion of methods

4.1.1 Manual pairing of individuals

The pairing of individuals was done by comparing the spot patterns on the head, as described by Stien et al. (2017). This process was easier the more distinct spots the fish had on the head, and usually required just one spot that stood out for the individual to be easily recognised. Most fish (>90%) had one or more distinct spots on the head that were used for individual matching between samplings. If the spots on the head were fairly uniform and without any distinct features, the fish could be matched by having spots on certain areas, such as on or more spots on the upper jaw, or even by having spots right above the pectoral fin. Using the spot pattern on the head proved to be an accurate, though time consuming, way of recognizing individuals from a sample. The spot patterns did not, to any large degree, change throughout the experiment. The small changes that did occur were some fading of either one or multiple spots, mechanical damage, or the development of new spots. The largest and most noticeable spots on the first sampling were also the largest and most noticeable spots on the last sampling, which is also what Stien et al. (2017) found.

4.1.2 Measuring morphometrics accurately

Measuring the morphometrics as done in this study is relatively accurate due to the fish laying still under the enclosure on a flat table, however there is a few potential errors. The fish were not always placed in the middle of the image frame, since the fish were slippery and often moved due to the table not being perfectly levelled. This might be a source of potential error due to the angle not being consistent for every sampling. This could cause inaccuracies, especially with regards to head lengths as subtle changes in the angle can cause substantial errors in the lengths measured. The angle of the head may also be affected by the condition of the fish, as fish with high condition have a steeper angle towards the surface than thinner fish. Many fish had a shortening of the snout and/or the operculum, which might cause errors in the measured lengths. An example of this, is the development of head ratios for the Medium group (Fig. 3.2), where an unexpected dip on the third sampling caused a significant

difference between Medium vs. Small and Medium vs. Large. Close examining of the images showed that several of the fish in the Medium group had snout damage on the third sampling that later healed. Since each group only consisted of seven fish, having individuals with irregularities like snout damage or operculum shortening can cause a substantial error in the mean lengths measured. The potential error associated with operculum shortening was eliminated by using the snout to pectoral fin base as a measurement for head length. Applied to measuring free-swimming fish, the current ratios may be challenging if the fish angle relative to the camera is too large for precise observation of e.g. the tip of the snout, or if the fish body is curved by tail beat which will affect measurement of the standard length. Filtering of fish observations should, however, be possible to only analyse fish that will provide solid data.

4.1.3 Ethical considerations

The fish was taken out of the tank a total of four times during this study, where it was manually handled, and spent some time on the table being measured and photographed. Some of them were also tagged in the adipose fin. Large salmon, as currently used, are more vulnerable during handling than smaller fish, as emphasized by industry mortality data connected with mechanical treatment for salmon lice (Stien *et al.,* 2018). The manual handling in combination with sexual maturation and wound development may have contributed to the rather high accumulated mortality rate of five percent over the six months of this study, which is similar to the mortality rate in commercial farming during the sea water production phase (Jensen *et al.,* 2020). It is reasonable to presume that the individuals that died had a period of bad welfare prior to death, and that the fish that developed wounds and/or sexually matured had a reduction of welfare (Aksnes *et al.,* 1986).

At the core of all the restrictions and regulations imposed on the Norwegian salmon farming industry, we find the interest of maintaining good fish welfare for farmed and wild salmon. Currently, there is no periodic reporting of welfare status from farmers other than the mortality rate, which is an indirect measurement of fish welfare. As this study seek to identify important CV parameters, this could potentially be used for evaluation of fish welfare in the future. It could also help reduce to invasive manual handling of the fish, which could be beneficial for many individuals in the future. This may justify the rather high mortality rate of five percent and the reduced welfare linked with wounds and sexual maturation.

4.2 Discussion of results

4.2.1 How does morphometric relationships evolve with growth rate?

Growth patterns

The three groups selected based on weight measured at the last sampling (end weight), were also significantly different at the first sampling with no overlapping between in weight the groups. If three groups were made based on start weight and not end weight, the means between the two sets of groups would be similar on the first sampling but not identical. This indicates that the individuals to a large degree maintained their rank in the size distribution, and that size within the current size distribution and span has a predictive value. For smaller salmon, size may have little predictive value, where the correlation between individual smolt size and harvest size is found to be relatively low (Gjerde *et al.,* 2006). The drivers for size and growth distribution within fish groups may be affected by numerous and largely unknown factors, where genetical dependent growth curves is one (Thorland *et al.*, 2020).

The three growth groups had similar growth dynamics throughout the six months, where the specific growth rate was not significantly different between the groups and decreased for each period. Since the daily percentage weight increase were equal for the groups, it is expected that the mean weight of three groups would differentiate more as time passes, since an equal SGR does not mean an equal weight increase. As the three groups had a similar SGR throughout the experiment and a significant difference in weight on the first sampling, the groups did differentiate more over time in weight. The mean condition factor was above 1.1 for all groups on the first sampling (group Small had three individuals with a K-factor close to 1), which is within the normal range in farmed groups of Atlantic salmon (Stien *et al.,* 2013). This means that even though the Large group had a ~50% larger mean weight than the small group on the first sampling, the Small group is still considered to be within the normal range of condition, and not loser fish. The condition factor does fluctuate during the production cycle (Oppedal *et al.,* 1999) which makes it inadequate as a stand-alone parameter to determine if a fish is considered a loser fish or not. It is also not always evident that a fish with low condition factor is an emaciated and moribund loser fish, or if it is emaciated but still has the potential for normal growth (Noble *et al.*, 2018). The Small group had an

increase of mean condition factor to above 1.3 from the first to the second sampling and remained at this level for the rest of the experiment. This confirms that the Small group is to be considered a group of normal growth, though at the lower end of the size distribution.

Head ratios

The two head ratios (head length/StdL and upper jaw/StdL) had a negative correlation with weight, standard length, and condition factor. The two head distances did increase as the fish grew larger, but in ratio with standard length they decreased (Fig. 3.3 and 3.4). This suggest that there is a negative allometric correlation, where the relative head size decreases with growth, which was also shown by Lange (2021). This means that the relative head size might be used as a measurement of previous growth. In order for the relative head size to be used as an accurate measurement for previous growth rate, one would expect that fish of different sizes would have different head ratios, were the larger the fish, the smaller the relative head size. But the findings in the current study contradicts this expectation, as there was no significant difference in head ratios between the groups (Fig. 3.2), even though the weight were significantly different between the groups for all samplings (Fig. 3.1). Although the difference in weight was large, the difference in standard length was less so, and as the difference in standard length increased the difference in the two head ratios increased as well. This suggests that the ratios can be used to measure previous growth, but more at the extreme (e.g. accelerated growth for superior fish, or stunted growth for loser fish). As the distribution of females and males were not accounted for in the current study, the small differences between the groups could be caused by an uneven distribution in the groups, as males tends have a longer head and therefore could e.g. be overrepresented in the Large group making the relative head size similar to the Medium and Small group. A difference was recorded between the groups on the third sampling, but this might be caused by the potential errors in measured head lengths for the Medium group as mentioned earlier.

Eye ratio

Both Devlin et al. (2012) and Lange (2021) found that the eye diameter grew allometrically with the body length, where the relative eye size is smaller for larger fish. This is partially confirmed in the current study, as the relative eye size did decrease over the six months for all

groups, but there was no significant difference in eye ratio between the groups on each sampling, except for the third, where the Small had larger relative eye size than Large and Medium. Even though there was no significant difference between the groups for the other samplings, Figure 3.5 does show an order were group Small has the largest eye/StdL ratio, followed by the Medium, and then the Large group. This, and the fact that there was a significant difference on the third sampling, does suggest that relative eye size might be a more sensitive measurement of previous growth rate compared to the relative head measurements.

The head length, upper jaw, and eye diameter in ratio with standard length are interesting morphometric relationships that have the potential of measuring growth performance. Applied to CV, these ratios can be closely and continuously measured against other parameters to gain key knowledge of how growth is affected by e.g. lice treatments or other invasive forms of handling. This allows for a more optimized production, and the ratios could possibly serve as important parameters in the transition from experience based to knowledge based salmon farming.

Body height ratios

The condition factor is a calculated number based on the relationship between weight and length of the fish, were the relationship between the two is of exponential nature. If a fish has a weight and length increase that follows the exponential curve between the two, the condition factor stays the same. If the growth do not follow the curve, e.g. the fish puts on more weight than length, the condition factor will increase. Thus, this extra weight increase will expand across the dorsoventral axis, making the body height in ratio with length a potential measurement of condition. As seen in figure 3.9, there is a strong positive correlation between the condition factor and the two body height ratios (cor. (R): BHC/StdL = 0.90 and BHA/StdL = 0.89), confirming the ratios could be used as an accurate measurement of condition. Lange (2021) also found a similar correlation between the body height ratios and the condition factor (cor. (R): BHC/StdL = 0.86 and BHA/StdL = 0.84).

The height ratios increased with the length and weight until the third sampling for all groups, where they levelled out towards the last sampling, even though the weight and length continued to increase. This was caused by a slower growth between the third and last sampling, which allowed for the weight to be distributed more evenly over the fish body and

not mainly across the dorsoventral axis as often seen for accelerated growth (Oppedal *et al.,* 1999). This corresponded well with the condition factor, that also levelled out between the third and the last sampling, and supports the use of body heights in ratio to the body length as measurements for condition, which is consistent with what Lange (2021) found.

The body heights in ratio with standard length can act as an alternative and accurate method of measuring the condition of a fish. Using stereoscopic cameras and CV technology to measure and analyse these two ratios could potentially give the end users key insight in the current status of the total population (e.g. what percent that is classified as superior fish). This information would be valuable for harvest planning and pre-harvest sales.

4.3 Can morphometric relationships predict the trajectory of sexual maturation in individual Atlantic salmon?

As mentioned earlier, 17 out of the 19 sexually mature individuals started to show visual signs of sexual maturation, such as longer jaws and development of hook, on the first sampling. Since the first signs were detected already on the first sampling in August, it would be valuable if there were a sampling or two before the first signs to collect data leading up to the morphological changes. This data could potentially have shown the accelerated growth often associated sexual maturation (Taranger *et al.*, 2010), or other growth and morphometric dynamics of value. It is interesting that there was no significant difference between the Mature and Immature group in size on the first sampling, where from the first to the second sampling all three growth indicators (weight, standard length, condition factor) were significantly lower in the Mature than the Immature fish (Fig 3.12). At this point, 14 of the individuals were scored with 2 (clear signs) according to the Laksvel guidelines (Nilsson *et al.,* 2022) for scoring of sexual maturation, and the five others were scored as 1 (early signs). This difference in growth performance between the Mature and Immature group comes from the Immature continuing an expected growth trajectory while the Mature group stagnated (Hvas *et al.,* 2021). This reduced growth in combination with welfare issues and low product quality is why sexual maturation is unwanted in commercial salmon farming (Aksnes *et al.,* 1986; Skarstein *et al.,* 2001; Hvas *et al.,* 2021).

Although the mean weight for the Mature group did not increase during the six months (Fig. 3.12A), there was a small but significant increase in standard length (3.5%) (Fig. 3.12B), which resulted in a small but significant decrease of condition factor (Fig. 3.12C). For the weight there was some overlapping on the second sampling, but not on the third when many mature fish were peaking in the maturation process, as indicated by observation of several mature males releasing sperm when handled during the sampling session. This corresponds with the relatively large time window and individual variation in timing of spawning within wild Norwegian salmon populations (Heggberget, 1988). For the fourth sampling, 13 out of the 19 mature fish had changed back to a silvery body colour while the rest remained brown, which may reflect the natural variation within wild stocks of Atlantic salmon where some fish dies after spawning while other migrates back to the sea, which Jonsson et al. (1991) found to be 64.5% (breeding survival rate) for males and 85.3% for females. The fact that 4 out of the 5 fish that died between the third and fourth sampling was mature fish that remained brown, is in support of this.

As mentioned earlier a correlation between size and sexual maturation is recorded in previous research, where an accelerated growth is often seen for the period leading up to the first visual signs of maturation in farmed Atlantic salmon (Taranger *et al.*, 2010). Because of this, one would expect the Mature group to be larger on the first sampling, or at least have a significantly larger condition factor compared with Immature group. As seen in figure 3.12 there was no significant difference in the growth indicators between the two groups on the first sampling, and the spread within the Mature group is large, suggesting that fish undergoes sexual maturation regardless of size and condition. Or alternatively, that the initiation of maturation differed between the fish, and thus some had a lower weight and condition factor due to low feed intake over a longer period than others. For all fish, the growth was possibly hampered after transfer from the sea cage and during acclimation to the experimental tank.

Head ratios

As mentioned, on the first sampling there was no significant difference in growth indicators between the Immature and Mature group. What is interesting is that a significant difference in the three head rations (snout to pectoral/StdL, snout to operculum/StdL, upper jaw/StdL) between the groups were recorded on the first sampling. This difference between the groups comes from the characteristic head features associated with sexually matured males, where

the head and jaws grows allometrically with the body length, resulting in longer relative head and jaws compared to immature fish (Leclercq *et al.*, 2010).

Lange (2021), who measured morphometric ratios in mature and immature harvest sized fish in February, found that sexually mature salmon had a head length/StdL ratio (snout to pectoral fin base/StdL) ratio above 0.2, and that if the ratio for a fish started to move close to 0.2 there was a strong possibility that the fish is maturing. The findings in the current study confirms this, as the mean head length/StdL ratio for the Mature group was 0.21 ± 0.01 already at the first sampling where the first visual signs of maturation appeared (Fig. 3.13). This head length/StdL ratio of 0.2 seem to be a good predictor of sexual maturation as all individuals in the Mature group had a had a ratio above 0.2 on the first sampling, except three individuals with ratios between 0.19 and 0.2. The mean head length/StdL ratio for the Immature group on the first sampling was 0.196 ± 0.008, with some individuals having a ratio right above 0.21. This show that a ratio over 0.2 should not be used as a conclusive parameter, but rather as an indication of sexual maturation. Interestingly, the mean head length/StdL ratio for the Immature group decreased throughout the experiment until the third sampling, where it increased slightly. For the Mature group, the mean snout to pectoral fin/StdL increased to 0.222 ± 0.01 from the first to the second sampling, where 14 out of the 19 individuals in the Mature group had a maturation score of 2. From the second to the third sampling, the ratio decreased to 0.219 ± 0.008, where at this point all 19 individuals had a maturation score of 3. This suggest that a fish with a ratio between 0.21 and 0.22 is highly likely to mature.

Almost the exact same differences between the two groups are seen with regards to the upper jaw/StdL (appendix Fig. A.5), where the Mature group had a ratio of 0.099 ± 0.006 on the first sampling, and the Immature group 0.091 ± 0.006. This confirms what Lange (2021) found, that if the ratio starts to move toward 0.1 or above, the salmon is most likely maturing. What is interesting is that the three individuals in the Mature group that had a snout to pectoral fin/ratio under 0.2 (substantial deviation from the mean), did not have the same deviation from the mean of the upper jaw ratio, meaning that their upper jaw/StdL ratio was close to 0.1. This is also seen the other way around, where four fish had an upper jaw/StdL ratio of less than 0.096 but had a snout to pectoral fin/StdL ratio of well above 0.2. This suggest that the two ratios should be used together to detect the first changes in maturing salmon, where either a jaw/StdL ratio close to 0.1 or a snout to pectoral fin/StdL ratio close to

0.2 is a strong indication that the fish is sexually maturing. It also suggests that early detection might be possible by only using the morphometrics of the head.

Eye ratio

The eye ratio was not significantly different between the Mature and Immature group on the first sampling, but was on the second, suggesting that the ratio can be used to predict sexual maturation, but at a later stage than with the head ratios. The eye diameter did increase for the Mature group from 12.85 ± 1.21 on the first sampling to 13.7 ± 1.03 on the last sampling, while it did not undergo a significant change for the Immature group (Fig 3.14). Because the Immature group had an increase in standard length, and the eye diameter stayed the same, the eye/StdL ratio decreased until the third sampling, where it levelled out until the fourth. For the Mature group the ratio increased from the first to the second sampling, where it levelled out and did not have a significant change for the two last samplings. That the eye diameter increased for the Mature fish and not for the Immature (Table 3.3) is interesting, and is also consistent with what Lange (2021) recorded. This means that the eye diameter and eye/StdL could be used to predict and measure sexual maturation, and should be interpreted as an effect from the voluntary fasting and thus arrested growth in maturing salmon. Something that is worth noticing is that the head ratios did vary more from sampling to sampling compared to the eye/StdL, suggesting that the head ratios might be better at not only predicting sexual maturation early, but also at measuring maturation at different stages (e.g. if a fish is assessed with score 2 for sexual maturation).

Body height ratios

There was no significant difference in body height ratios recorded between the Immature and Mature group on the first sampling (Fig. 3.15). The BHC/StdL ratio did decrease for the Mature group from the first to the second sampling, where it stayed throughout the last sampling (Fig 3.15A). For the Immature group the BHC/StdL increased from the first through the third, where it levelled out to the last sampling. Because the ratio changed in opposite directions for the two groups, a significant difference in the BHC/StdL ratio was recorded on the second sampling, and the two groups stayed significantly different throughout the experiment. This suggest that the body heights in ratio with standard length might be good indicators of sexual maturation and/or stagnated growth. Lange (2021) found

that the BHC/StdL ratio was equal, and that the BHA/StdL ratio was different between mature and immature fish, and that the ratios increased with size for both groups. This was partially confirmed in the current study, as the BHC/StdL ratio did increase with size within both groups, but there is a clear difference after the first sampling in mean BHC/StdL between the two groups (with some individuals overlapping). The same is found for the BHA/StdL (Fig. 3.15B), but with a smaller difference between the two groups and a lot more overlapping between individuals, suggesting that the BHC/StdL ratio is a better indicator for sexual maturation and stunted growth.

As sexually mature males have a more squared body shape, and to a large degree stop growing, one could expect both height ratios to be larger for Mature fish compared to Immature. The opposite is recorded in the current study. This is because the mean BHC length had no significant change for the Mature group during the six months while there was a small but significant increase in length for the same period, resulting in a decrease in BHC/StdL. The mean BHC length had an 30% increase from the first to the last sampling, resulting in an increase in BHC/StdL ratio. The mean BHA length increased for the Mature group during the six months (5.6%), but not as much as for the Immature group (30%). This difference in development of BHC and BHA length between the groups is interesting, and Lange (2021) argued that the relationship between BHA and BHC could be used as an indicator for sexual maturation. As seen in figure 3.16, there is clear difference in the BHC/BHA ratio between the Mature and Immature group, where the difference also was significant on the first sampling. This is an interesting finding as it suggests that the relationship between BHC and BHA could not only be used as a measurement for sexual maturation status, but also for early prediction.

In summary, the stunted growth for the Mature group was identified through the abovementioned ratios, where there was a significant difference between the two groups on the first sampling for the head length/StdL, upper jaw/StdL, and the BHC/BHA ratios. The eye/StdL, BHC/StdL, and BHA/StdL ratios were significantly different first on the second sampling. As sexual maturation is unwanted in commercial salmon farming, early detection through the use of CV technology would be valuable for farmers. The current study have confirmed that the head length/StdL, upper jaw/StdL, and the BHC/BHA ratio have the potential to be used as proxies for early detection of sexual maturation. As seen with regards to the head length/StdL and the upper jaw/StdL ratio, both ratios can be used together to improve the accuracy for detection of the early stages of sexual maturation.

4.4 Conclusion

In the current study, different morphometric relationships have been studied to identify potential proxies for growth development and sexual maturation. The head length, upper jaw, and eye diameter in ratio with standard length are interesting morphometric relationships as they can potentially measure previous growth rate and the current growth performance. The body heights in ratio with standard length showed to be strongly correlated with condition factor, making the two ratios measurements for condition without the need of knowing the weight of the fish. Applied to CV with continuous image streams of fish from subsea stereo cameras, morphometry could give the farmers a far greater insight in growth performance and the current status of the population compared to traditional manually sampling. Combined with other data of biological relevance, the morphometric ratios can be important for feedback to feeding control, preventive health measures, sea lice management, and harvest planning.

The findings in the current study shows that visual signs of sexual maturation are detectable earlier than loss of weight and condition factor in maturing individuals. At this stage of the maturation process, the three ratios head length/StdL, upper jaw/StdL, and BHC/BHA were significantly different between the Mature and Immature group, suggesting that they can be used for early detection of maturing individuals. The fact that the head length/StdL and upper jaw/StdL could be used together to better detect maturation is also valuable information as it suggest that it might be possible to detect maturation by only using the morphometrics of the head.

4.5 Future experiments

As mentioned above, the findings in the current study suggests that sexual maturation can potentially be detected by only using the morphometrics of the head. More research is needed on the head morphometrics, which could not only provide valuable information about maturation status, but potentially also for growth performance and as assessment of welfare status (e.g. snout damage, shortened operculum). As discussed earlier, the use of the current ratios may be challenging as it requires the fish to be at a certain angle relative to the camera,

and that the body in images of free-swimming fish is often curved by tail beats which affect the measurement of the body length. The use of only the head morphometrics as opposed to the use of the head relative to length, might be better at attaining accurate data, as it to some degree eliminates the errors associated with body movement, as well as fish overlapping each other in images. This assumes, of course, that the head morphometrics can provide data of biological relevance, which should be tested over a longer time scale, e.g. from smolt to harvest size, and over the full size distribution within groups of farmed salmon.

The scope of CV assessment of free-swimming in aquaculture is large, and morphometrics is only one out many potential parameters that can provide data of biological relevance. Other well-known characteristics, such as the brown coloration of matured fish, or wound development, should be further explored in context with other parameters of relevance. Welfare assessment through the use of CV might in the future provide a solid database of knowledge useful for fish farmers, veterinarians, or researchers, though it will require substantial research efforts as the scope of welfare assessment is large.

References

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Appendix

Appendix A: Additional figures

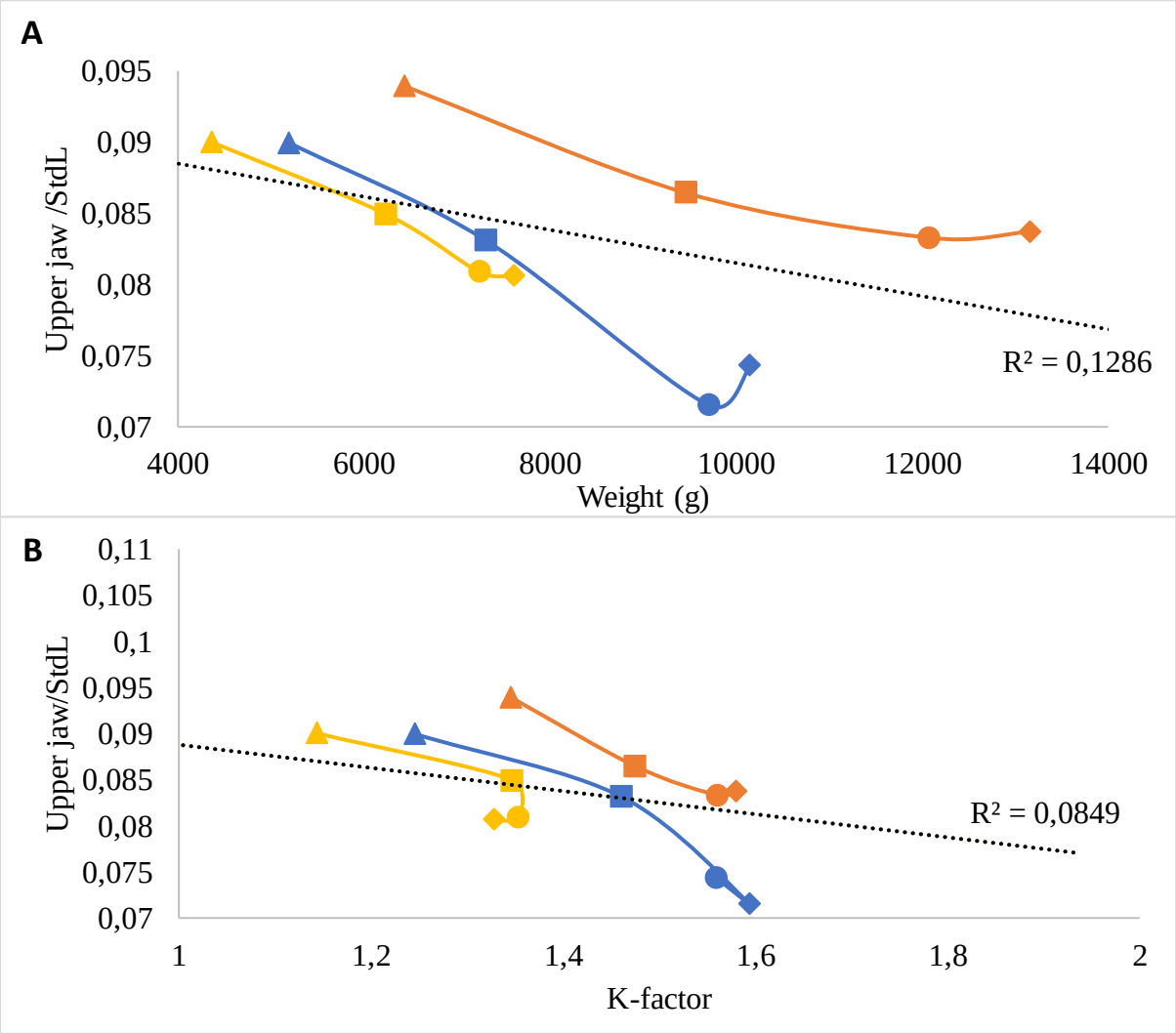

Figure A.1: Upper jaw/standard length ratio compared to A = weight, B = Condition factor over time for the Large (orange), Medium (blue) and Small (yellow) group. The dotted black line is a fitted linear model between the morphometric relationship and the growth indicator, and it is accompanied by its coefficient of determination (R^2).

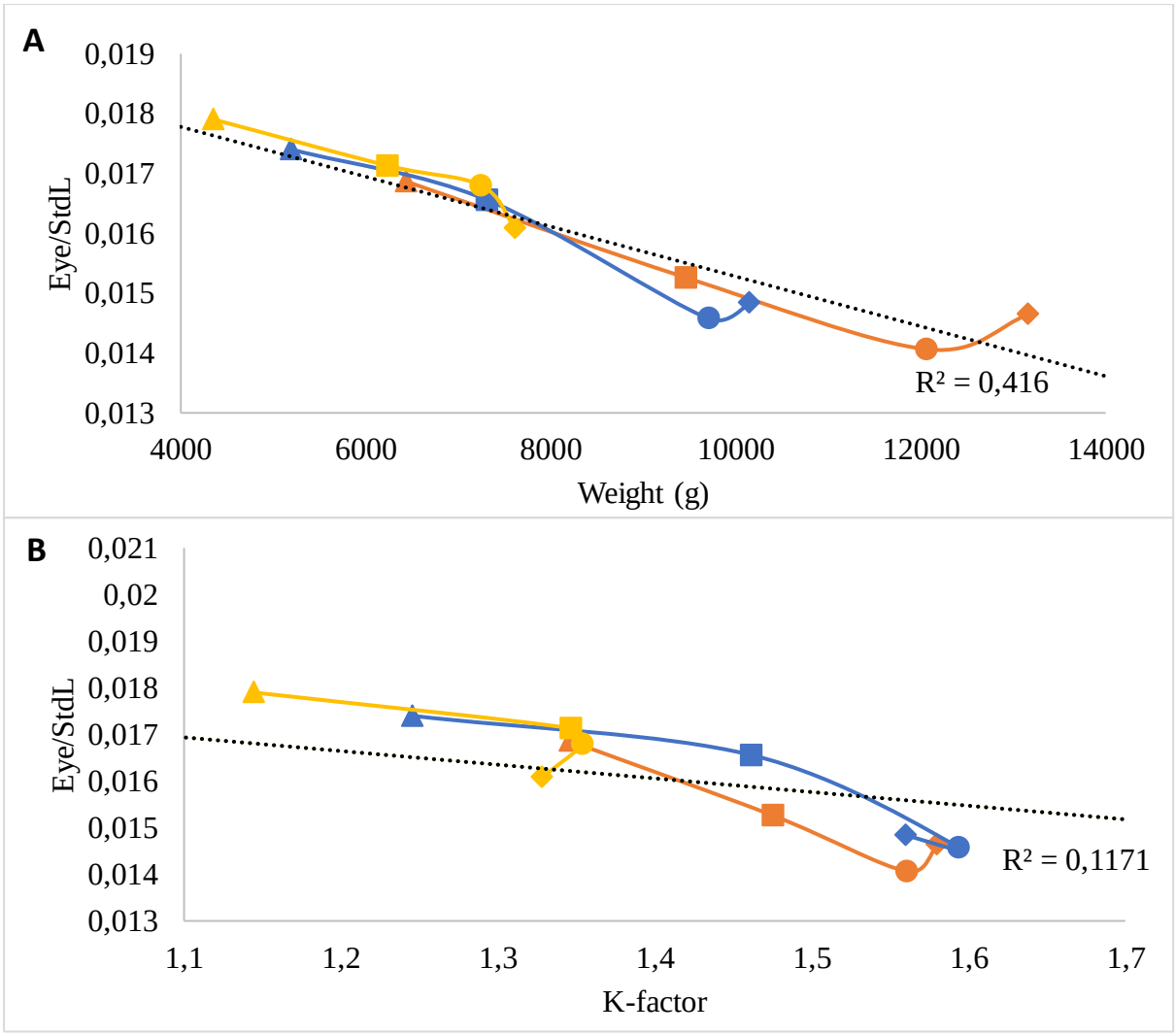

Figure A.2: Eye/standard length ratio compared to A = weight, B = Condition factor over time for the Large (orange), Medium (blue) and Small (yellow) group.

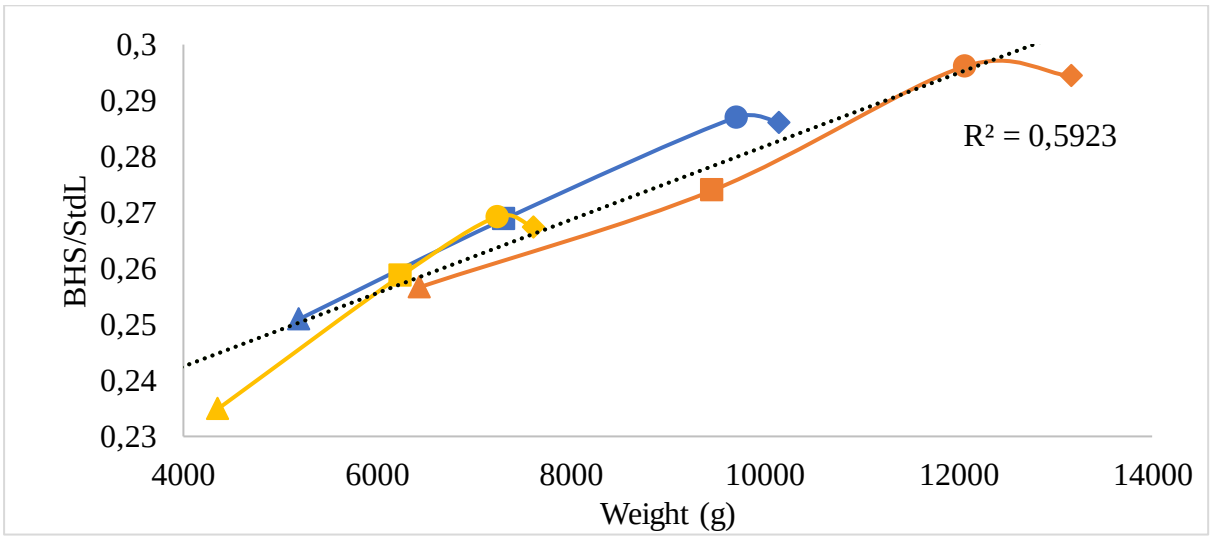

Figure A.3: BHC/standard length ratio compared to weight over time for the Large (orange), Medium (blue) and Small (yellow) group.

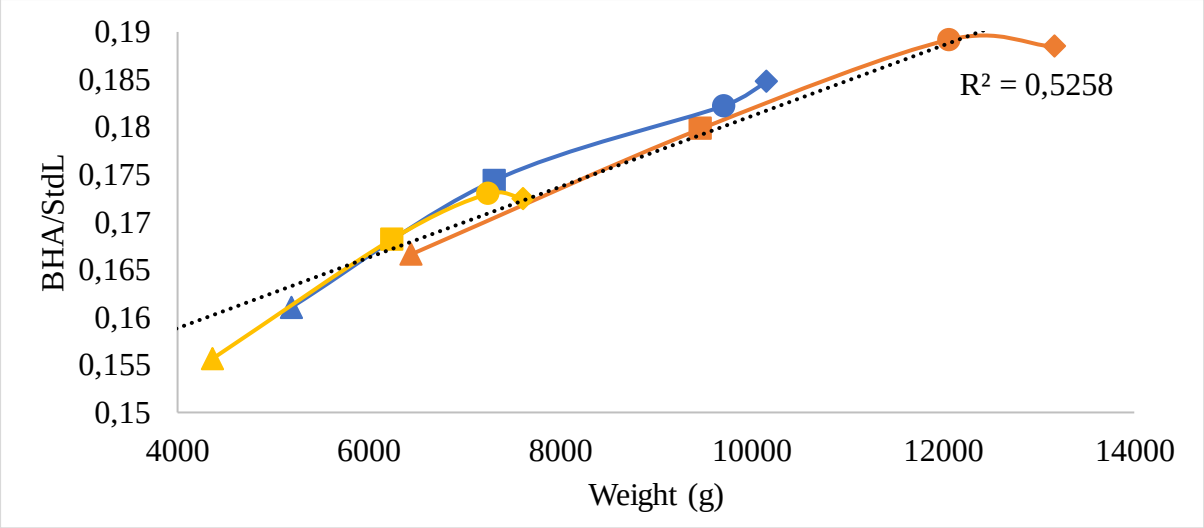

Figure A.4: BHA/standard length ratio compared to weight over time for the Large (orange), Medium (blue) and Small (yellow) group.

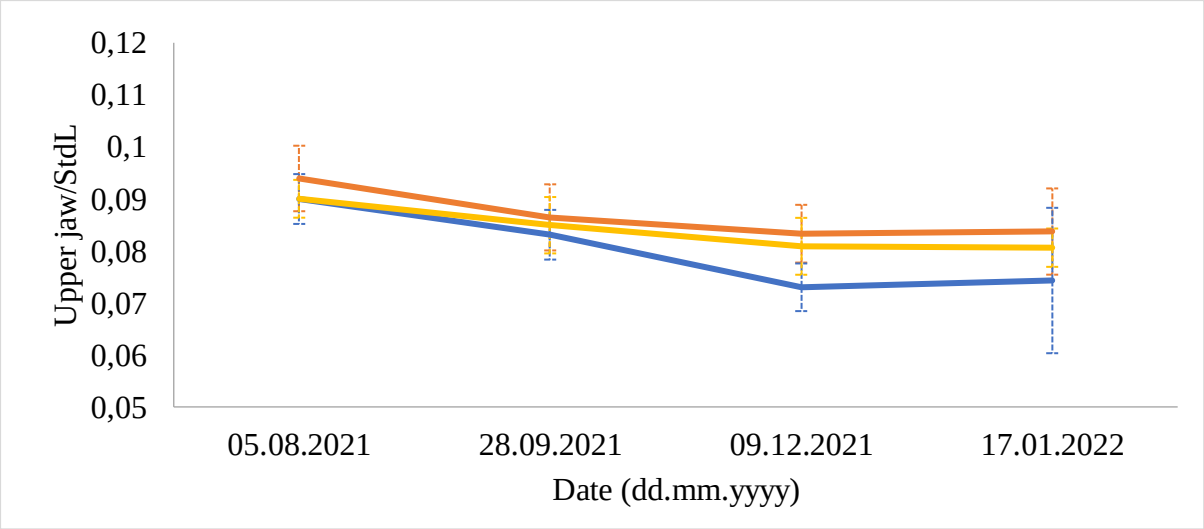

Figure A.5: Upper jaw/standard length ratio with standard deviations over time for the Large (orange), Medium(blue) and Small (yellow) groups.

Appendix B: Growth data

Weight (g)					
Date (dd.mm.yyyy)	Large	Medium	Small	Mature	Immature
05.08.2021	6445 ± 512	5194 ± 256	4360 ± 413	5621 ± 1011	5560 ± 813
28.09.2021	9464 ± 559	7310 ± 602	6242 ± 549	5686 ± 1253	7852 ± 1153
09.12.2021	12068 ± 1023	9708 ± 957	7243 ± 891	5691 ± 1252	9579 ± 1604
17.01.2022	13168 ± 833	10156 ± 146	7618 ± 1084	5733 ± 1378	10208 ± 1731

Table B.2: The average standard length for the five groups: Large, Medium, Small, Mature, and Immature during the four samplings.

Standard length (mm)					
Date (dd.mm.yyyy)	Large	Medium	Small	Mature	Immature
05.08.2021	763 ± 23	714 ± 27	693 ± 36	738 ± 41	732 ± 33
28.09.2021	827 ± 25	764 ± 29	740 ± 34	754 ± 39	784 ± 37
09.12.2021	874 ± 30	808 ± 39	771 ± 30	749 ± 39	819 ± 42
17.01.2022	904 ± 24	834 ± 32	790 ± 35	763 ± 38	846 ± 43

Table B.3: The average condition factor for the five groups: Large, Medium, Small, Mature, and Immature during the four samplings.

K-factor					
Date (dd.mm.yyyy)	Large	Medium	Small	Mature	Immature
05.08.2021	1,35 ± 0,23	1,25 ± 0,12	1,14 ± 0,13	1,22 ± 0,12	1,25 ± 0,14
28.09.2021	1,48 ± 0,10	1,46 ± 0,12	1,35 ± 0,22	1,15 ± 0,18	1,43 ± 0,10
09.12.2021	1,56 ± 0,11	1,59 ± 0,20	1,33 ± 0,21	1,14 ± 0,18	1,49 ± 0,15
17.01.2022	1,58 ± 0,11	1,56 ± 0,16	0,33 ± 0,12	1,12 ± 0,21	1,47 ± 0,14

Table B.4: The average specific growth rate for the five groups: Large, Medium, Small, Mature and Immature during the four samplings.

SGR					
Date (dd.mm.yyyy)	Large	Medium	Small	Mature	Immature
05.08.2021-28.09.2021	0,714 ± 0,069	0,629 ± 0,151	0,666 ± 0,135	0,006 ± 0,217	0,639 ± 0,117
28.09.2021-09.12.2021	0,335 ± 0,051	0,393 ± 0,114	0,201 ± 0,217	0,001 ± 0,151	0,271 ± 0,123
09.12.2021-17.01.2022	0,227 ± 0,128	0,125 ± 0,214	0,122 ± 0,120	0,007 ± 0,159	0,162 ± 0,134

Appendix C: R code

R packages used:

```
library(fs)

library(readxl)

library(ggpubr)

library(ggplot2)

library(tidyverse)
```

Testing for normality (continuous data):

```
ggqqplot(my_data$variable)

ggdensity(my_data$variable)

shapiro.test(my_data$variable)
```

Testing for equal variance (continuous data):

```
var.test(my_data$variable1, my_data$variable2)
```

Comparing the means of paired samples (continuous data):

```
t.test(my_data$variable1, my_data$variable2, PAIRED = TRUE, var.equal = FALSE, conf.level = 0.95)
```

Comparing the means of unpaired samples (continuous data):

```
t.test(my_data$variable1, my_data$variable2, PAIRED = FALSE, var.equal = FALSE, conf.level = 0.95)
```

Comparing the means of three samples (continuous data):

```
my_data %>% aov(variable ~ group, data = .) %>% TukeyHSD()
```

Testing for correlation (continuous data):

```
cor.test(my_data $variable1, my_data $variable2)
```

Testing for independence (categorical data):

```
my_data %>% select(variable1, variable2) %>% table() %>% chisq.test()
```