Morphometric Relationships as Indicators of Sexual Maturation in Atlantic Salmon (*Salmo salar*)

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Contents

Abstract

This study aimed to identify morphometric relationships in fish that could serve as indicators for status on sexual maturation in Atlantic salmon (*Salmo salar*). To achieve this, 250 salmon were netted out and individually weighed, measured for fork length, and photographed over six months. Further, the distance between morphometric key points were digitally measured for each individual using the photographs. Gonad weight was measured in order to calculate the gonadosomatic index (GSI) as a degree of sexual maturation status. The ratios of snout-eye length to head length, snout-eye length to fork length, head length to fork length, body-height-central to fork length, body-height-anal to fork length, anal-caudal-fin to fork length, anal-caudal-fin to body-height-central, body-height-anal to body-height-central, where analyzed. Our results reveal that the snout/head ratio and the snout/fork length ratio are statistically significant indicators of sexual maturation. Specifically, a Generalized Linear Model (GLM) test showed that the snout/head ratio, snout/fork length and head/fork length had a significant relationship with GSI in August and November. The study also unveils complex interactions between growth metrics such as length, weight, condition factor (K), and specific growth rate (SGR) with GSI, indicating that the relationship between growth and sexual maturation undergoes seasonal fluctuations. Mature fish were found to allocate energy differently from immature fish, particularly near the spawning season, confirming a shift from somatic growth to reproductive activities. Our study suggests a multi-metric approach is crucial for a nuanced understanding of salmon physiology. The findings point to specific morphometric ratios as reliable indicators for assessing sexual maturation in salmon, especially during August and November.

Abbreviation list (alphabetic order)

BPG	-	Brain-pituitary-gonad
GLM	-	Generalized Linear Model
GSI	-	Gonadosomatic index
K	-	Condition Factor
L:D	-	Light Conditions
PIT	-	Passive integrated transponder
SGR	-	Specific Growth Rate
Vgll3	-	Vestigial-like family member 3

1. Introduction

1.1 Norwegian aquaculture

Atlantic salmon (*Salmo salar*) farming in Norway started in the 1970s, and Norway has grown to become one of the largest producers of salmon in the world (Blix & Myhr, 2023; Iversen et al., 2020). In 2022, Norway exported seafood for a value of NOK 151.4, where salmon accounted for the largest share, with 70 percent of the total value (Skaug, 2023). The increasing global demand for high-quality protein to feed the world's population gives the aquaculture industry an optimistic prospect for the future. Leading researchers has an ambition that Norway shall increase production, with double output by 2030 and fivefold by 2050 (Hersoug, 2021). However, production is restricted due to environmental and biological factors, such as salmon lice, disease, escapes, emission, and feed sustainability (Fiskeridepartementet, 2021). Salmon lice(*Lepeophtheirus salmonis*) and escapes are considered to be the two of the most critical factors as this negatively affects the wild salmon stock (Hersoug, 2021). Another aspect to consider is fish welfare, where the mortality in production today is very high. Salmon mortality has not changed much in the last five years, with more than 50 million salmon dying during the sea phase. In 2021, the exact number was 54 million, which makes up to15,5 percent of the total production in Norway (Sommerset et al., 2022).

Early maturation in aquaculture may negatively affect animal welfare, economic profit and the environment. Sexually mature salmon allocates energy to gonadal growth and reproduction, rather than basal processes such as muscle growth and maintenance of other body processes. This results in a reduced immune system and susceptible to disease (Taranger et al., 2010). If mature salmon escape, there is a risk of reproduction, which could potentially damaging the wild stock, producing hybrids not well suited for life in the wild (Glover et al., 2017). According to the "Fish Health Report 2022," using data collected from the Norwegian Food Safety Authority, out of the 1,253,560-ton salmon slaughtered in 2022, nearly 15% were downgraded due to injuries, defects, and maturation. Of these, around 12.5% were downgraded due to maturation (Sommerset et al., 2023). A recent research report titled "Analysing mortality patterns in salmon farming using daily cage registrations" examined mortality rates of salmon from ten different hatcheries. The study collected data on the daily mortality records of 21 million salmon from stocking to harvest in 2017 and 2018. The results showed a total mortality of 1,797,467, with 1.8% of the deaths attributed to sexual maturation

(Persson et al., 2022). Identifying early maturation in salmon is an agreement that benefits fish welfare, the environment, and the economy.

1.2 Sexual maturation in farmed salmon

Sexual maturation in salmon is a complex and multi-dimensional process with significant implications for biological welfare and economic productivity. Natural maturation cycles in salmon generally occur during the summer months, leading to spawning activities in the fall. By winter, the majority of salmon revert to a non-mature state, although some may still show signs of sexual maturity (Taranger et al., 2010), the process follows temperature and day length (Mobley et al., 2021). Early puberty in farmed salmon presents many challenges, including adverse effects on growth, flesh composition, and overall welfare, not to mention its potential genetic impact on wild populations (Taranger et al., 2010).

The biological complexity of maturation in salmonids involve a mix of internal mechanisms and external conditions. Proximate environmental factors such as temperature and photoperiod affect anatomical and physiological processes, while ultimate factors, like competition and stress exposure, drive evolutionary adaptation and diversification (Rivera et al., 2021). Genetically, the vgll3 gene plays an essential role in governing sexual maturation and is also involved in regulating vertebrate adiposity, tying it to metabolic status and timing of maturity (Ayllon et al., 2019). The brain acts as the central processing unit, orchestrating these varied signals to initiate reproductive maturity (Mobley et al., 2021).

As salmon transition from juveniles to sexually mature adults, they develop a range of capabilities for gamete production and behavioral patterns conducive for mating, often influenced by factors such as size, growth rate, and fat deposition (Taranger et al., 2010; Thorpe et al., 1998). This transformation activates the brain-pituitary-gonad (BPG) axis, driving the salmon toward sexual maturity. The energy expenditure associated with the formation of gametes, the emergence of secondary sexual traits, and behaviors related to mating is significant in salmonids. In Atlantic salmon, it's estimated that nearly 59% of their energy reserves are consumed for reproductive purposes in both males and females (Fleming, 1998). However, farmed salmon face unique challenges compared to their wild counterparts. For example, they don't have the opportunity to migrate to freshwater environments for spawning, which can lead to harmful health consequences if not managed properly (Taranger et al., 2010). Physical changes like jaw hooking, especially prevalent in males, can lead to

injuries and deformities, highlighting the need for early detection and intervention strategies for maturation (Ashley, 2007; Iversen et al., 2016).

The economic implications of sexual maturation are critical for the sustainability of salmon farming. Norwegian Food Safety Authority has outlined classification criteria for salmon, categorizing them based on quality as superior, ordinary, production, or discarded. Among the leading causes for downgrade in these classifications is sexual maturation, often coupled with other factors like injuries or diseases that inherently affect salmon (Sommerset et al., 2023). These downgrades have economic consequences, reducing the market value of the fish and increasing the risk of diseases and mortality (Sommerset et al., 2022; Sommerset et al., 2023).

In summary, the ability to control sexual maturation in farmed salmon would not only alleviate biological and welfare concerns but also substantially improve production efficiency and profitability. Advanced maturation, especially when premature, elevates production costs due to diminished growth rates, reduced feed conversion efficiency, and the emergence of undesirable gender-specific traits (Rivera et al., 2021). Therefore, an integrated approach to understanding and managing sexual maturation in salmon is indispensable for both animal welfare and economic sustainability.

1.3 Growth factors

The relationship between sexual maturation and growth is tightly intertwined in the life cycle of salmon (Hansen et al., 1992). Sexual maturation is a natural biological process wherein fish develop reproductive organs and attain the ability to reproduce. Growth plays a central role in facilitating this process, as fish must reach a specific size and developmental stage before they can mature and successfully spawn (Leclercq et al., 2010; Mobley et al., 2021). Research has shown that salmon with rapid growth tend to mature earlier (Taranger et al., 2010). Adequate growth ensures that individuals attain the necessary physical condition, energy reserves, and size to support the reproductive demands associated with maturation (Agarwal, 2008). Optimal growth is vital for the transition to sexual maturity, as it directly influences the timing, quality, and reproductive potential of salmon. Understanding and managing the intricate relationship between growth and sexual maturation in salmon is imperative for the research and to one day be able to control it (Mobley et al., 2021).

1.3.1 Condition factor

Condition factor (K) of fish is determined by using their weight and fork length (measured in centimeters) to calculate a factor called K. This factor can be used as an indicator for health, categorized on a scale from excellent (above 1.6) to extremely poor (below 0.9). Generally, K falls within the range of 0.9 to 1.6, and is impacted by factors such as age, sex, season, maturation stage, gut fullness, and fat reserves (Stien et al., 2013).

1.3.2 Specific growth rate

Specific growth rate (SGR) is a measure of how quickly an organism grows. SGR reflects the weight increase of the organism during a specific period (Gjerde et al., 1994). The SGR is a measure of how much a fish has developed on average each day, expressed as a percentage. A positive number indicates weight gain, while a negative number indicates weight loss (Endal et al., 2000). For salmon farming, SGR plays a significant role in assessing the growth performance of fish. Several factors affect SGR, including feed quality, feeding rate, water temperature, and stocking density (Føre et al., 2016).

1.3.3 Gonadosomatic index

Gonadosomatic index (GSI) is a measurement that helps to determine the reproductive condition of an organism by comparing the weight of its gonads (ovaries or testes) to its total body weight. Essentially, GSI is a way to gauge the proportion of gonad weight to overall fish weight. This metric has been frequently utilized to assess the timing of reproduction (Flores et al., 2014).

1.4 Identifying sexual maturation

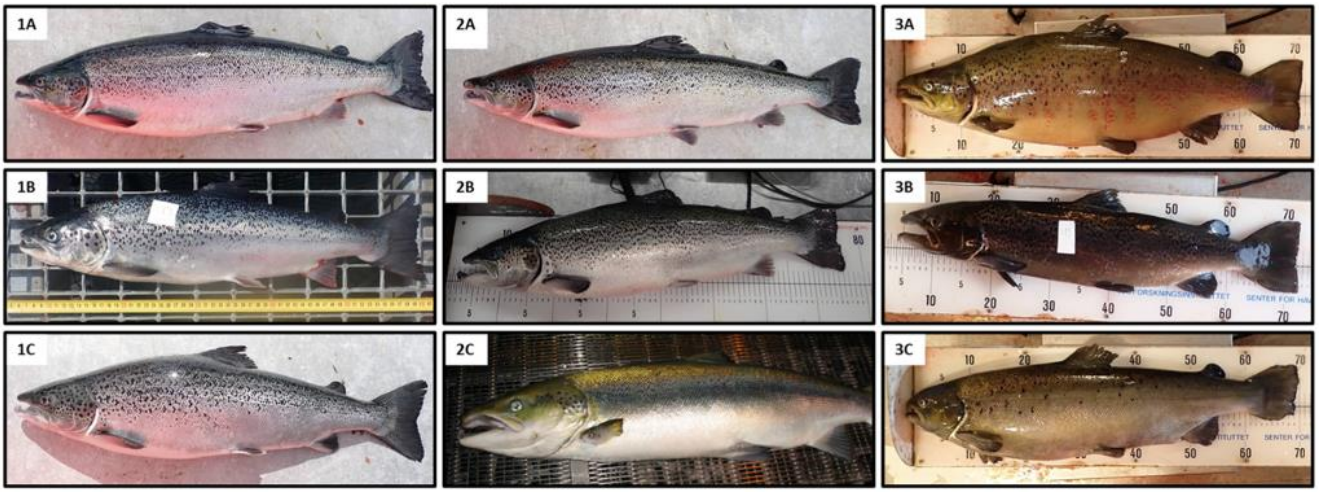


Figure 1.1: Overview over maturation score 1, 2 and 3 in Atlantic Salmon. Image from Nilsson et al, 2022.

In aquaculture settings, sexual maturity in fish is a process that usually occurs earlier in males than in females, although both sexes eventually reach maturity. External signs can serve as indicators of this transition. For instance, male fish develop noticeable morphological changes like an elongated jaw and a pronounced hook at the tip of their lower jaw (Figure 1.1, A-C, based on a maturity score of 1). These features are most commonly observed during late spring-ealy summer season. Juvenile salmon might also exhibit a subtle thickening at the front end of their lower jaw, although this is less noticeable than the mature hook formation. As the summer season advances, additional changes can be observed. The fish undergo color variations, particularly on the dorsal (upper) region of their bodies. Alongside this, the jaw continues its elongation and development (Figure 1.1, 2A-C, rated on a score of 2). By the time fall arrives, the fish exhibit their full spawning coloration, characterized by a brownish tint and anatomical modifications that include a more prominently arched back (illustrated in Figure 1.1, 3A-B). Female fish, although they don't manifest the pronounced jaw features seen in males, also go through discernible changes as they reach sexual maturity. These include alterations in body coloring and shape (Figure 1.1, 3C). The complete set of these characteristics, both in males and females, corresponds to a maturity level labeled as score 3 (Nilsson et al., 2022). Heightened sexual motivation in salmon is often influenced by these observable changes, such as more vibrant coloration and increased size, which are designed to make them more appealing to the opposite sex for the purpose of finding a mate (De Gaudemar et al., 2000).

1.4.1 Morphometrical measurements

Morphometrics is a method for quantitatively analyzing an animal's size and shape, as well as the relationship between the two (known as allometry) typically at specific points on the body (Dujardin, 2017). Variations in body measurements across salmonid populations from diverse regions have been observed, with particular attention to head size, body length, and eye diameter (Solem & Berg, 2011; Solem et al., 2006). An earlier morphometric analysis was conducted on Atlantic salmon (Figure. 1.2) by Kadri et al. in 1997. They explored the early differentiation between salmon on the verge of sexual maturation and those that were still immature. They assessed whether maturation could be anticipated based on various measurements such as body proportions, length from snout to forked tail, and height of both head and body. Their findings suggested that there isn't a straightforward mathematical method to consistently differentiate between the two stages of salmon. However, the body measurements could aid in visual categorization (Kadri et al., 1997). Their research focused on the dorsoventral (top-to-bottom axis) of the head to spot differences, neglecting the front-to-back head axis and the length of the jaw. It's essential to explore the anteroposterior (front-to-back axis) to definitively determine if head size varies between mature and immature salmon. In addition the anal caudal fin/fork length ratio has not been explored as a morphometric measurement before, the analysis aiming to detect if this measure could also serve as indicator for maturity status in salmon.

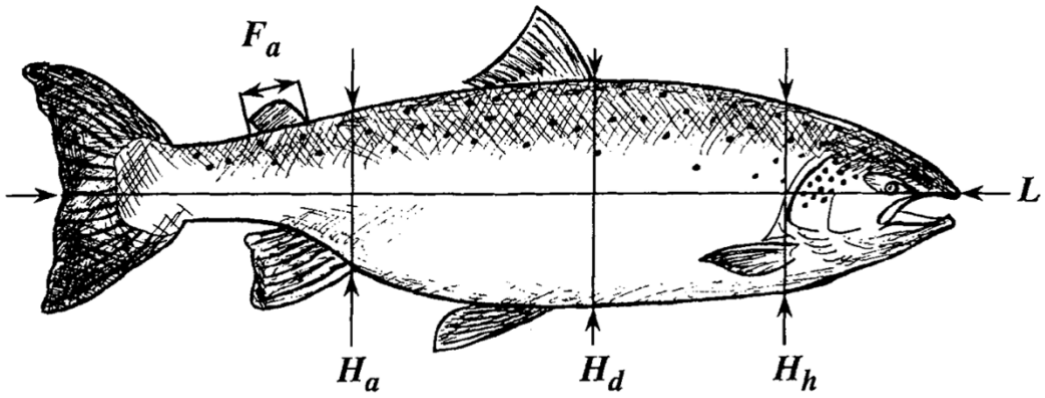


Figure 1.2: Overview of various morphometric measurements for Atlantic salmon. L: fork length, H_h = head height (just behind operculum), H_d = body height (prior to dorsal fin), H_a = body height (prior to anal fin) and F_a= Adipose fin. Image from Kadri et al. (1997).

1.5 Objectivities and aims

This study aims to find morphometric measurements and image analysis techniques that can be used to detect early maturation in farmed salmon. The analysis has used data collected during the maturation period of seven months where 36 fish each month. The study analyzed different body ratios of the fish whole fish, and head.

Main question: Can morphometric measurements and image analysis techniques be used to detect early maturation in farmed salmon?

Secondary questions:

- Can growth factors, length, weight, K, and SGR provide sufficient information to identify and determine early maturation?
- How is growth related to the GSI?
- Which morphometric measurements are most effective in detecting early maturation in farmed salmon?
- Can the length between the anal and caudal fin serve as an indicator for detecting early maturation?
- Can head analysis alone be the indicator for detecting early maturation?

2 Material and method

2.1 Experiment setup

Salmon of the Aquagen strained were cloned as described in (Hansen et al., 2020), with three different clonal lines incubated in separate cabinets. The eggs hatched between January 20 and January 29, 2021. The fish start fed between February 28 and March 11, 2021, in 1m (400 L) tanks at 13°C. On June 11, 2021, they were transferred to 1.5 m (1000 L) tanks at ambient temperature (mean temperature 12°C, range 9-15°C). The fish was under continuous light (24:0 L:D) from hatching to October 5, 2021.

The individuals used in the current experiment were transferred to separate tanks with a volume of 1.5 m^3 on October 5, 2021. Each tank accommodated one line of fish, with approximately 200 fish per tank. The average weight of the fish at the time of placement was between 109 g and 171 g. Starting from the placement date, the fish were subjected to a 12:12 light-dark (L:D) photoperiod, simulating winter conditions. This light regime was maintained until November 17, 2021. Subsequently, the fish were exposed to continuous light (24:0 L:D) to initiate smoltification, representing a spring-like signal. Between January 5, 2022, and January 12, 2022, the fish were exposed to a salinity of 20 parts per thousand (ppt). Following this period, they were transferred to full seawater (34 ppt) until May 5, 2022. The fish were vaccinated with Alpha Ject micro six and Alpha Ject 1PD on March 2, 2022. On March 29, 2022, they were individually tagged with 12 mm Passive Integrated Transponder (PIT) tags and transferred to a five m^3 tank in a common garden setup, where all groups were mixed. Originally, there were meant to be 800 fish, but due to injuries, some had to be euthanized, leaving a total of 735 fish in the setup. The fish were transferred to sea cages with dimensions of 5 m × 5 m with an approximate depth of 6 m on May 12, 2022.

The experiment took place at the Matre Research Station at the Institute of Marine Research. 600 male salmon were chosen from 3 different clonal lineage (Hansen et al., 2020), in addition to 200 "normal" salmon. Only data from the clonal salmon were used in the current study. All fish were PIT tagged, measured, and photographed before being transferred to a seawater cage on May 12, 2022. We conducted follow-up recordings on June 7th, July 5th, August 11th, September 14th, October 17th, and November 9th of the same year.

2.2 Manual sampling

The manual sampling for data collection was conducted seven times in total. 36 fish were netted out of their tank in May and sea cage from June to November and anesthetized with Finquel (0.1g/L) in a holding tank. Each fish was scanned for PIT, weight (g), and length (mm) measured individually. Additionally, both lateral sides of the fish's body were photographed in the air. The fish was photographed next to a ruler, giving us a key point for length, for measurements of the fish (Figure 2.1). The fish was first euthanized with a Finquel (0.2g/L) in a separate bucket (leaving them in longer then for anastatic), then blood samples were taken before dissecting to remove and weigh the gonads (Figure 2.1). In July, we observed a distinct variation in the GSI between immature and early maturation stages in certain fish samples. Consequently, a GSI threshold of 0.1 was established to classify fish as mature. It is worth mentioning that in November, there were two fish with GSI values slightly above the set threshold for maturation (>0.1), specifically 0.11 and 0.14. However, based on coloration and shape, these individuals appeared to be immature and were therefore classified as such.

Due to a substantial proportion of the fish population being sexually mature, a non-random sampling approach was adopted. The decision to exclude sexually immature individuals in October and September was made to preserve the immature individuals for November, with the intention of obtaining better data for that specific month when maturation was expected to be complete. Clearly stating this rationale will provide transparency and context for the data collection process.

In our data analysis, we opted not to differentiate among the three distinct clonal lines. This decision was taken primarily because segmenting the data further would result in sample sizes too small for robust statistical analysis. However, it's crucial to acknowledge that incorporating clonal line differentiation might have offered more nuanced insights. In the May sample group, there were four fish production fish, that were not part of the clonal lines. The specific group affiliation could not be determined due to a technical failure during the sampling process. However, this is unlikely to impact the conclusions of the study, as May is not a critical month for the data foundation of this research project.

Figure 2.1: Aerial photograph of fish, utilized for data extraction and subsequent analysis.

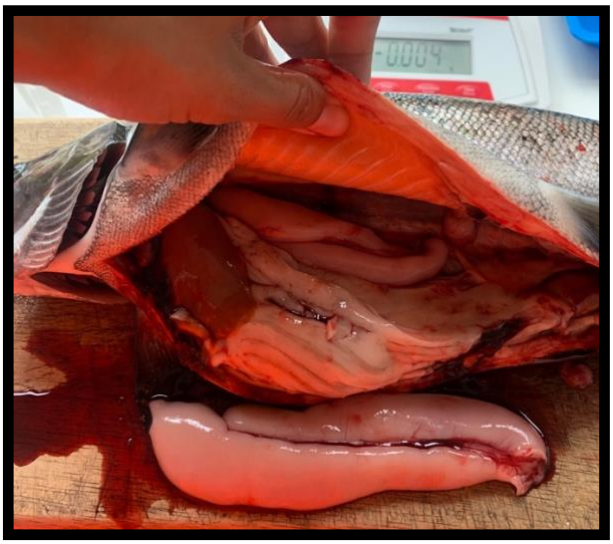


Figure 2.2: Image of the gonad, captured during sampling.

2.3 Growth factors

SGR was calculated with weight measurements from May(old weight) to the sampling month(new weight). The following equations have been used:

- K = 100*weight/fork length^3.
- SGR (%/day) = 100 * (LN (new weight) – LN (old weight)) / (new date-old date).
- GSI = 100*gonad weight /total body weight.

2.4 Measuring morphometrics in ImageJ

The length measurements were conducted using monoscopic images and analyzed with the ImageJ Java program. To access ImageJ, please refer to the following URL: https://imagej.nih.gov/ij/download.html

A calibration process was implemented within ImageJ for the initial image of each sampling. This calibration involved using the ruler you can see in figure 2.2, setting the scale for the picture in the program.

The measured distances in ImageJ included snout-to-pectoral fin base, snout to middle of eye (Figure 2.3), fork length, central body height, and body height above the anal tract, anal tract to caudal fin (Figure 2.4). These measurements were then divided by the fork length to yield the morphometric ratios utilized. Additionally, the relationship between central body height and anal height was explored, and central body height and anal tract to caudal fin.

Figure 2.3: Lateral head measurements: snout-eye(red line) and snout-to-pectoral fin base (purple line).

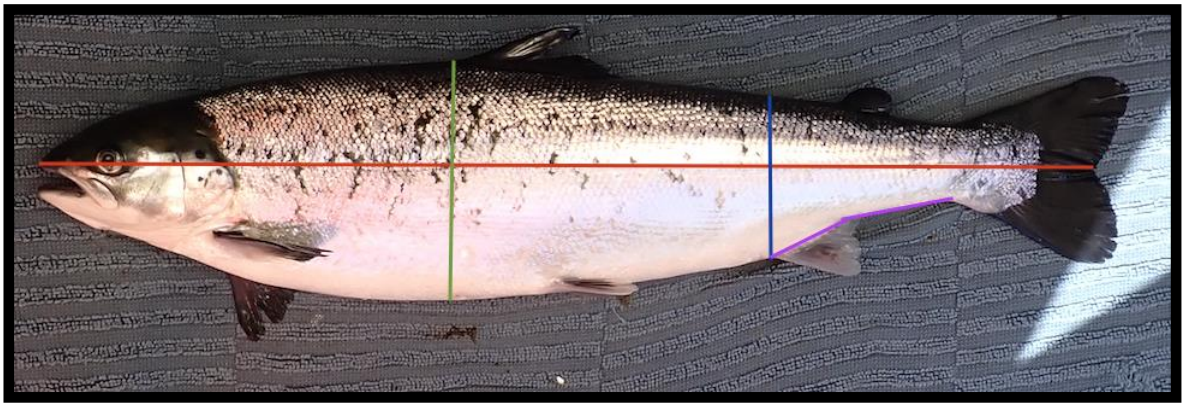


Figure 2.4: Lateral body measurements: Fork length (red line), body-height-central(green line), body-height-anal(blue line), anal-caudal-fin (purple line).

2.5 Data analysis

The data was analyzed and plots were made in the programming tool R and R Studio. Including Microsoft Excel for the data setup and production of tables. To access R and R Studio, please refer to the following URL: https://www.r-project.org/

Average and standard deviation were calculated to assess the spread in the growth of the population. In August, and November, there were two visible groups were observed (immature and mature fish), extra analyzed where done separately for average and standard deviation for each group. Furthermore, various plots were generated to visually explore the dataset and identify patterns and trends in the growth factors.

The GSI and different growth relationship was explored with a Generalized Linear Models (GLMs) and a Pearson's product-moment correlation analysis for each month on length, weight, K and SGR.

A GLM test was applied to all the morphometric measurements relative to the GSI for each month. This step aimed to pinpoint specific morphometric measurements warranting further investigation. Further analysis was conducted on snout/head, snout/fork length, head/fork length and anal caudal fin/fork analyzing correlations with indicators: fork length, weight, K, and GSI. The analysis employed a multi-step analytical approach to investigate the relationship between the morphometric measurement ratios and the indicators; fork length, weight, K and GSI. On each indicator, GLM test, Welch two-sample t-test and Pearson’s product-moment correlation analysis was applied to all the ratios for each month. To give an overview of the transformation the ratios were plotted against each indicator for every month.

3 Results

3.1 Growth in population

From May to November 2022, the fish population displayed a consistent growth trajectory in both weight and length. Average weight surged from 826g in May to 2678g (Figure 3.1 B) in November, while average length increased from 39.3cm to 57.8cm (Figure 3.1 A). Throughout this period, the K hovered consistently between 1.3 and 1.4 (Figure 3.1 C). The SGR peaked in July at 0.97%/day and then gradually diminished by November at 0.70%/day(Figure 3.1 D). The GSI exhibited a notable surge in August, reaching a peak in September before declining in the subsequent months (Figure 3.1 E).

In May and June and all GSI values were <0.1. In July GSI values were more spread, with some fish having clearly higher GSI than observed in May and June, and from August GSI values were split in two distinct groups of either >1 or below 0.1 (Figure 3.1E). Based on this, fish with GSI above 0.1 was considered maturing. When stratifying the population into mature and immature subsets for the months of July, August, and November, more nuanced patterns began to appear. In July, mature fish had an average weight of 1493g and an average length of 47.1cm(Figure 3.1 A and B). Their K was 1.42, and SGR was 1.08%/day (Figure 3.1 C and D). In contrast, immature fish in July averaged 1300g in weight and 45.8cm in length(Figure 3.1 A and B)., with a K of 1.34 and an SGR of 0.901%/day (Figure 3.1 C and D). By August, mature fish weighed on average 1889g and measured 51.2cm in length The K for this group was 1.39, and the SGR was 0.97%/day (Figure 3.1, Table A.2, appendix). Immature fish in August had an average weight of 1551g and an average length of 47.9cm. Their K was 1.38, and the SGR was 0.78%/day (Figure 3.1, Table A.3, appendix). By November, mature fish exhibited an average weight of 2373g and an average length of 56.8cm. The K value had dropped to 1.28, and the SGR was 0.61%/day. Meanwhile, immature fish in November weighed on average 3157g and measured 59.3cm in length, displaying a K of 1.49 and an SGR of 0.84%/day. It's crucial to note that for the months of September and October, data was only available for mature fish as a significant portion of the population had matured, as the few immature fish remaining were deliberately not sampled until the November sampling.

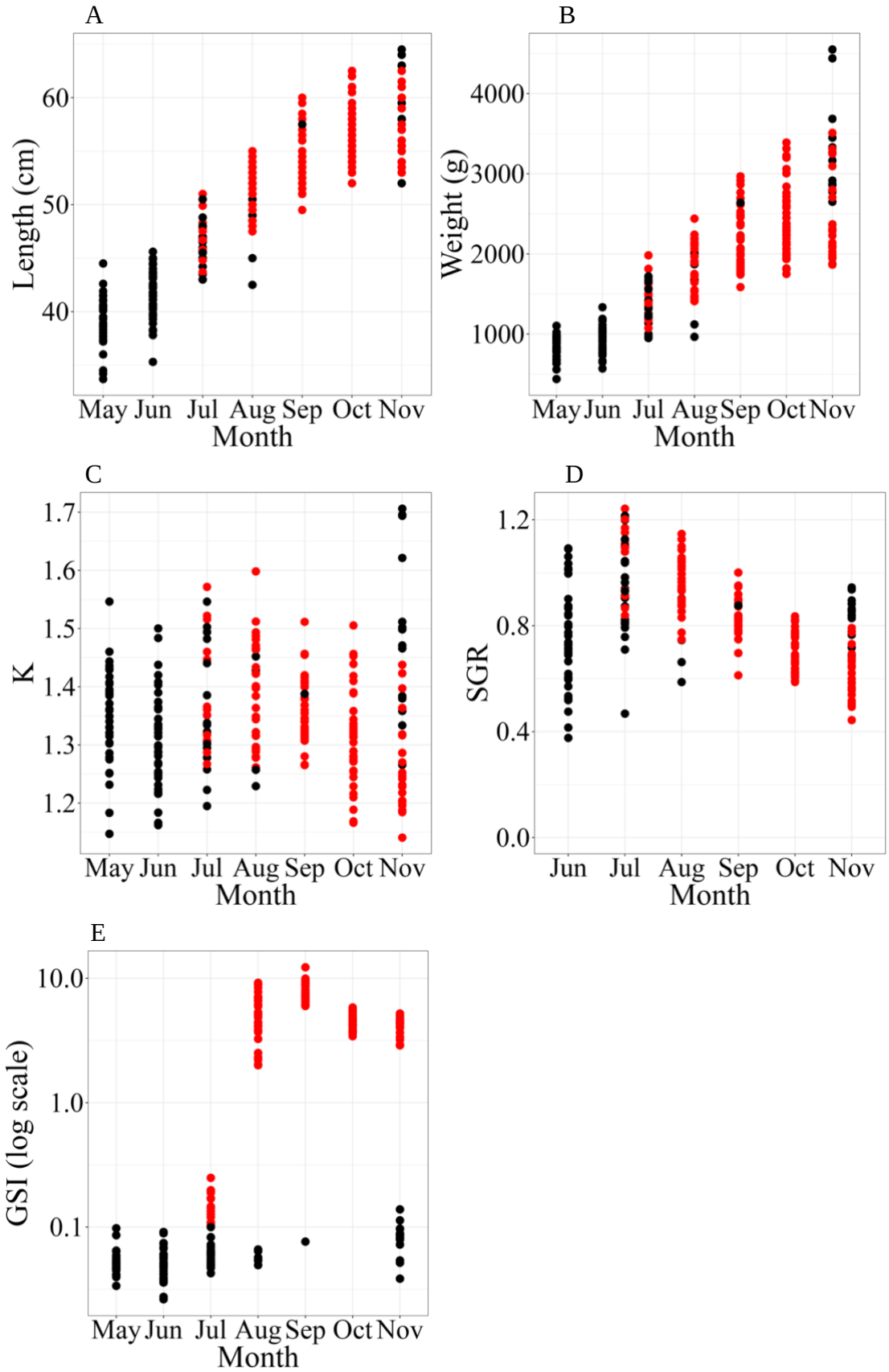


Figure 3.1, A-E: This figure presents the growth trends of the population across various metrics, each plotted against the month for mature(red) and immature(black) salmon. The x-axis represents the month, while the y-axes correspond to different growth metrics: length (cm), weight (g), K, SGR, and GSI.

3.2 Gonadosomatic index analysis

3.2.1 Length

A GLM analysis conducted for each month showed distinct findings regarding the relationship between fish length and GSI. May displayed a significant negative relationship ($\beta = -0.0024$, $p = 0.0057$, Figure 3.2 A), while June and July had no significant relationship ($\beta = -0.0011$, $p = 0.53$, Figure 3.2 C, and $\beta = 0.0063$, $p = 0.16$, Figure 3.2 E, respectively). August revealed no significant relationship ($\beta = 0.074$, $p = 0.68$, Figure 3.2 G), while September ($\beta = -0.40$, $p < 0.001$, Figure 3.2 I), October ($\beta = -0.26$, $p = 0.001$, Figure 3.2 K) and November ($\beta = -0.28$, $p = 0.0093$, Figure 3.2 M), showed a significant negative relationship

3.2.2 Weight

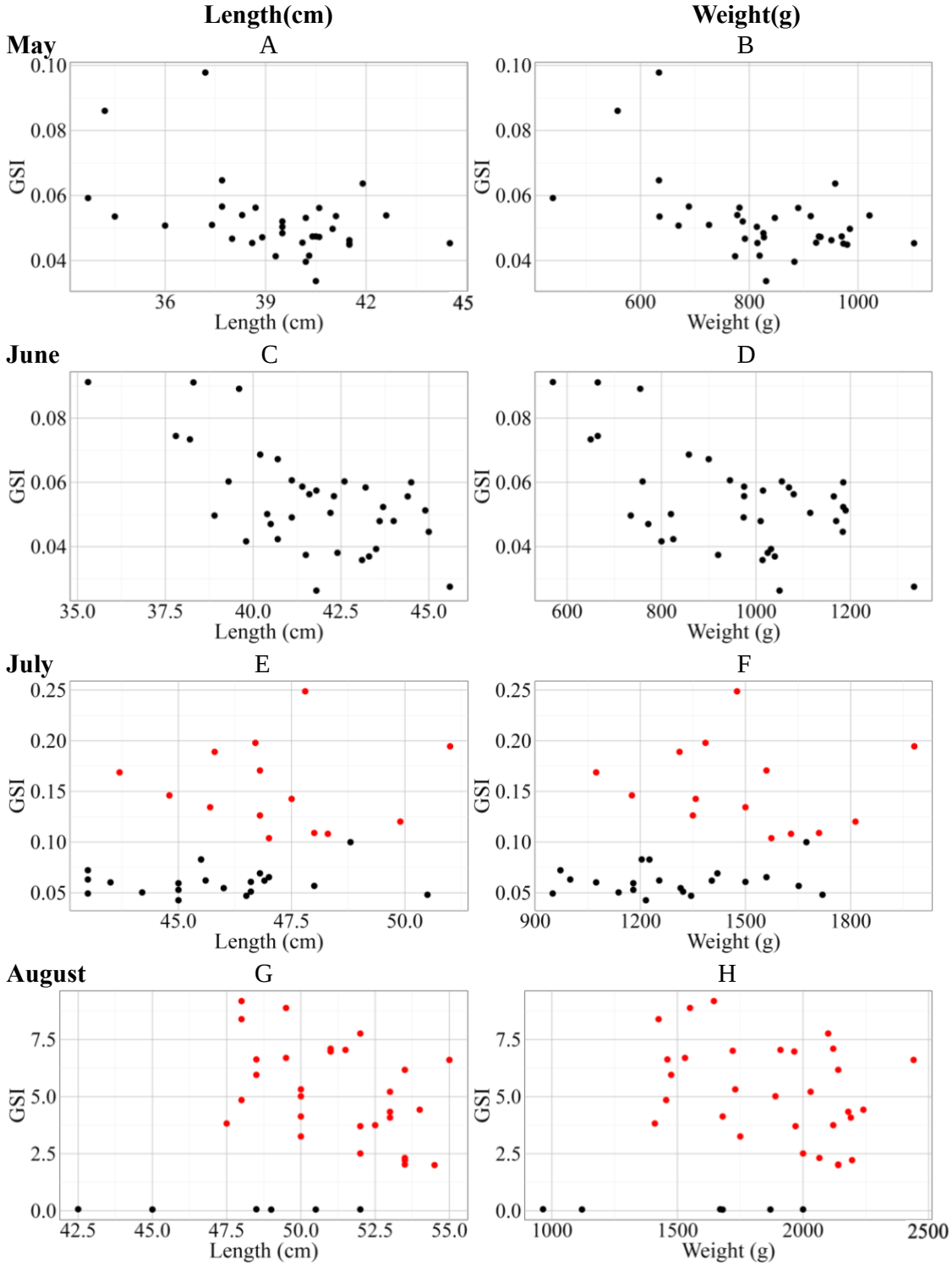
The GLM analysis revealed a significantly negative relationship in May ($\beta = -4.19e-05$, $p = 0.0026$, Figure 3.2 B). June showed no significant positive relationship ($\beta = 3.90e-06$, $p = 0.85$, Figure 3.2 D), and July also showed no significant positive relationship ($\beta = 5.10e-05$, $p = 0.16$, Figure 3.2 F). In August, the relationship was no significant but positive ($\beta = 0.0002972$, $p = 0.834$, Figure 3.2 H). Interestingly, September revealed a strong and significant negative relationship ($\beta = -0.002813$, $p = 0.00014$, Figure 3.2 J). October followed a similar trend, with a significant negative relationship ($\beta = -0.0015$, $p < 0.001$, Figure 3.2 L). November continued this trend, also showing a significant negative relationship between weight and GSI ($\beta = -0.0020$, $p < 0.001$, Figure 3.2 N).

3.2.3 Condition factor

The GLM revealed no significant relationship in May ($\beta = -0.023$, $p = 0.33$, Figure B.1 A, appendix). June displayed a no significant relationship ($\beta = 0.035$, $p = 0.48$, Figure B.1 B, appendix). July showed no significant relationship ($\beta = 0.097$, $p = 0.32$, Figure B.1 D, appendix). August also presented a no significant positive relationship ($\beta = 1.32$, $p = 0.81$, Figure B.1 F, appendix). September exhibited a significant negative relationship ($\beta = -12.47$, $p = 0.03$, Figure B.1 H, appendix). Notably, October and November yielded significant negative relationships ($\beta = -7.41$, $p < 0.001$, Figure B.1 J and $\beta = -11.081$, $p < 0.001$, Figure B.1 L, appendix).

3.2.4 Specific growth rate

The GLM revealed in June, no significant positive realationship (β = 0.0053, p = 0.80, Figure B.1 C, appendix). July showed a significant positive relationship (β = 0.11, p = 0.03, Figure B.1 E, appendix), and August(β = 6.36, p = 0.089) and September(β = -2.25, p = 0.61). October (β = -6.047, p = 0.0013, Figure B.1 K, appendix) and November (β = -12.7684, p < 0.001, Figure B.1 M, appendix), both showed significant negative relationships.



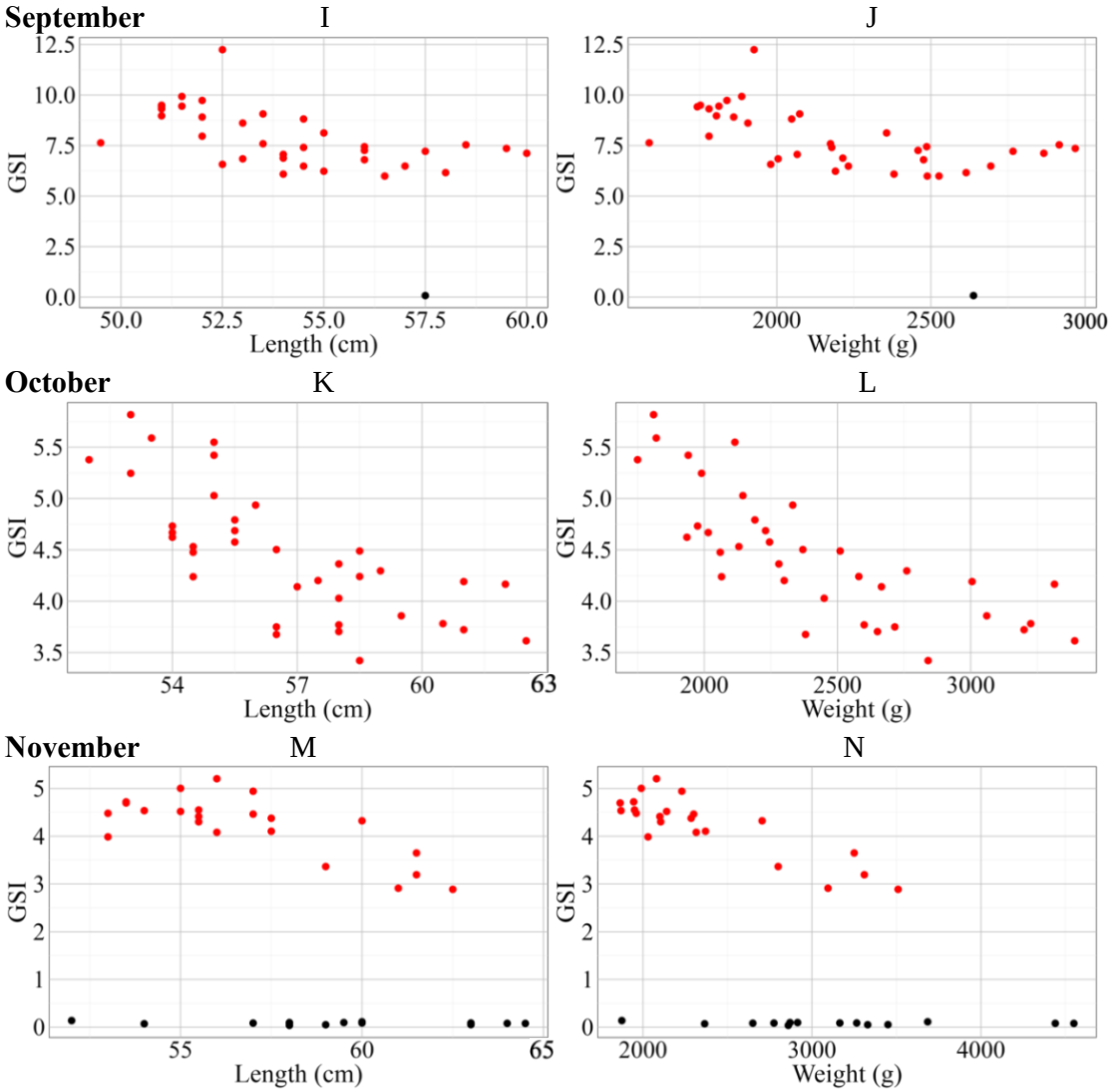


Figure 3.2, A-N: Relationship between GSI and fish length (cm), weight (g) for each month.

3.3 Morphometrical measurements

The application of a GLM has illuminated the relationship between the GSI and the morphometric measurement ratios. This analytical method has pinpointed specific ratios that warrant a more in-depth exploration. The ratios with significant p-values during the months of August and November are prioritized (Table 3.2).

Table 3.2: Overview of statistical analysis for morphometrical measurement ratios for all months. The ratios examined include Snout/Head, Snout/Fork Length, Head/Fork Length, Body Height Central/Fork Length, Body Height Anal/Fork Length, Anal Caudal Fin/Fork Length, Anal Caudal/Body Central, and Body Height Anal/Body Central. The symbol "x" indicates ratios that are statistically significant for a given month.

Morphometrical measurement ratios	Month	P-value	Significant
Snout/head	May	0,607	
Snout/head	June	0,53	
Snout/head	July	0,348	
Snout/head	August	p<0,001	x
Snute/fork length	June	0,29	
Snute/fork length	July	0,518	
Snute/fork length	August	p<0,001	x
Snute/fork length	September	0,006302	x
Snute/fork length	October	0,0283	x
Snute/fork length	November	p<0,001	x
Hode/fork length	May	0,858	
Hode/fork length	June	0,699	
Hode/fork length	July	0,792	
Hode/fork length	August	p<0,001	x
Hode/fork length	September	0,001	x
Hode/fork length	October	0,167	
Hode/fork length	November	p<0,001	x
Body height central/fork length	May	0,2805	
Body height central/fork length	June	0,936	
Body height central/fork length	July	0,232	
Body height central/fork length	August	0,829	
Body height central/fork length	September	0,001932	x
Body height central/fork length	October	0,007169	x
Body height central/fork length	November	0,00683	x
Body height anal/fork length	May	0,2805	
Body height anal/fork length	June	0,936	
Body height anal/fork length	July	0,232	
Body height anal/fork length	August	0,829	
Body height anal/fork length	September	0,001932	x
Body height anal/fork length	October	0,007169	x
Body height anal/fork length	November	0,00683	x
Anal caudal fin/fork length	May	0,756	
Anal caudal fin/fork length	June	0,313	
Anal caudal fin/fork length	July	0,0275	x
Anal caudal fin/fork length	August	0,923	
Anal caudal fin/fork length	September	0,465	
Anal caudal fin/fork length	October	0,958	
Anal caudal fin/fork length	November	0,372	
Anal caudal/body central	May	0,259	
Anal caudal/body central	June	0,544	
Anal caudal/body central	July	0,886	
Anal caudal/body central	August	0,774	
Anal caudal/body central	September	0,75623	
Anal caudal/body central	October	p<0,001	x
Anal caudal/body central	November	0,0018	x
Body height anal/body central	May	0,0083	x
Body height anal/body central	June	0,4925	
Body height anal/body central	July	0,250677	
Body height anal/body central	August	0,54602	
Body height anal/body central	September	0,0311	x
Body height anal/body central	October	0,827	
Body height anal/body central	November	0,97102	

3.3.1 Snout/head ratio

One of the most scrutinized aspects of salmonid morphology in this study is the snout/head ratio.

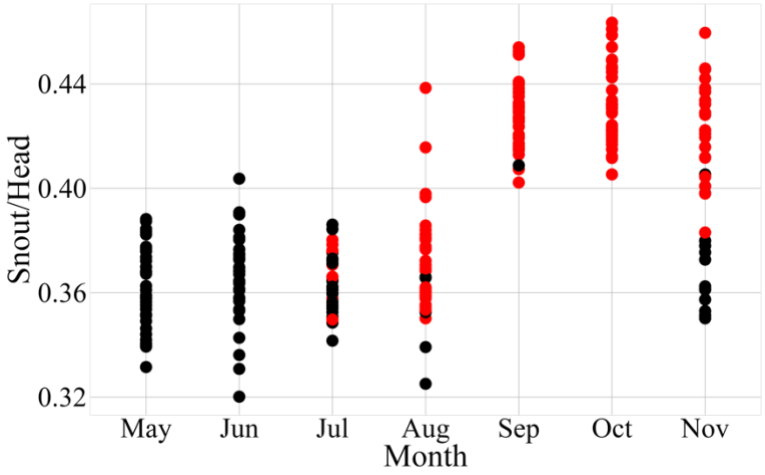


Figure 3.3: Monthly distribution of snout/head ratio, the data reveals two distinct groups, representing immature (black) and mature(red) individuals.

The GLM test showed that GSI was significant in August ($\beta = 0.0037$, $p = 0.0028$, Figure 3.4 L) and November ($\beta = 0.015$, $p < 0.001$, Figure 3.4 U). Pearson's correlation test revealed a significant positive correlation with K (cor = 0.42, p = 0.012, Figure 3.4 K) and with GSI (cor = 0.38, p = 0.019, Figure 3.4 L) in August, while in November, we observed a strong positive correlation between snout/head ratio and K (cor = 0.55, p = 0.004 Figure 3.4 T) and GSI (cor = 0.78, $p < 0.001$, Figure 3.4 L). Furthermore, Welch t-tests showed significant differences between mature and immature fish, with mature fish having a higher mean snout/head ratio in August with a percentage of 8.57% (mature: 0.38, immature: 0.35), p=0.012) and November a percentage of 16.22% (mature: 0.43, immature: 0.37, p<0.001)(Figure 3.3 and 3.4).

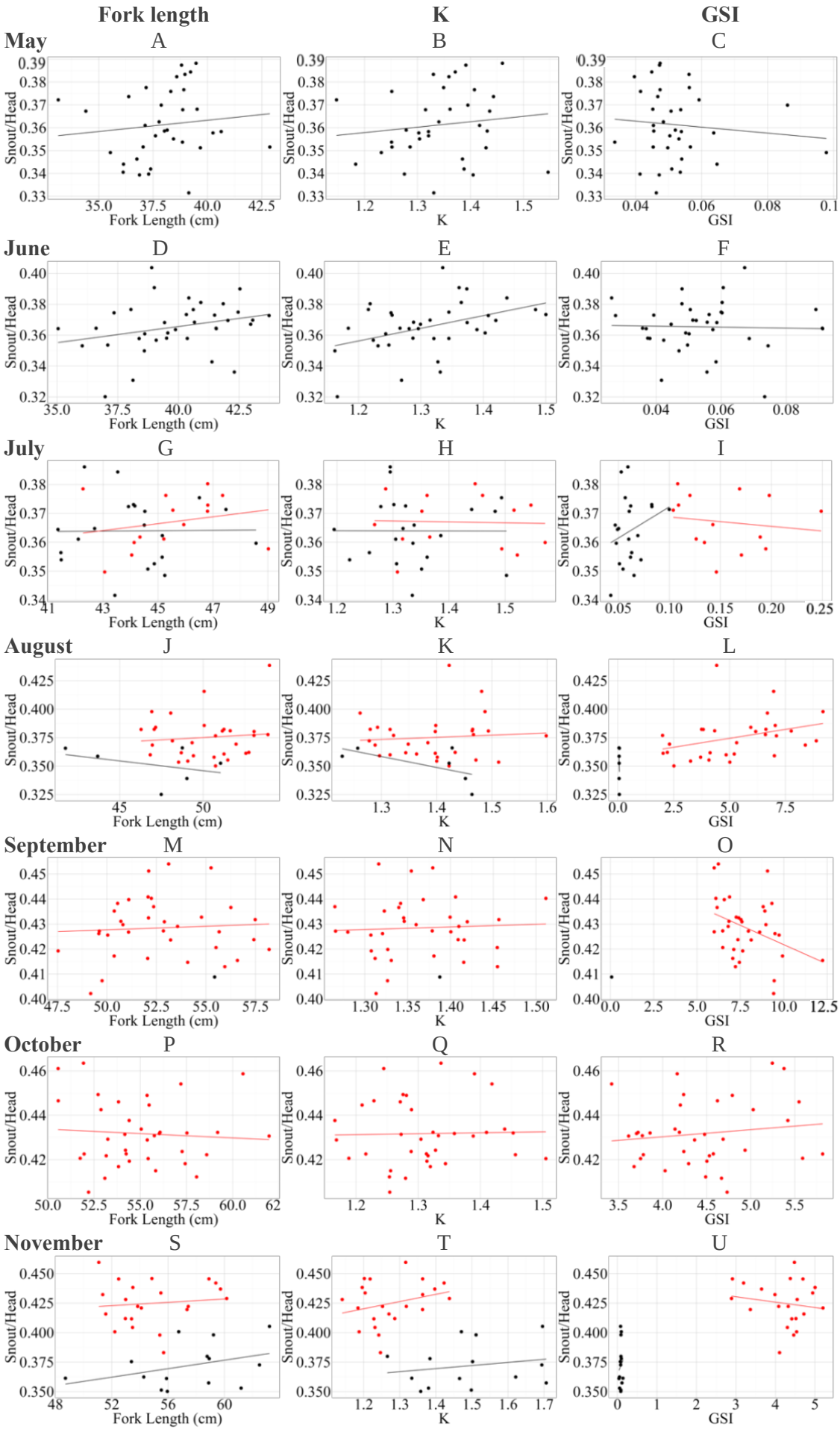


Figure 3.4, A-U: Overview of snout/head ratio, fork length, K, and GSI for salmon in the months of May-November. The data reveals two distinct groups, representing immature(black) and mature(red) individuals.

3.3.2 Snout/fork length ratio

This part presents snout size in relation to body length.

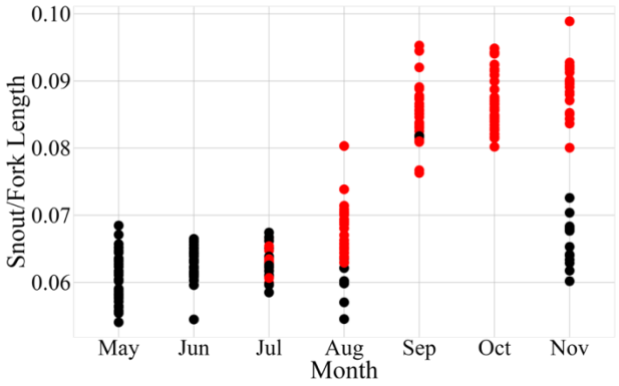


Figure 3.5: Monthly distribution of snout/fork length, the data reveals two distinct groups, representing immature (black) and mature(red) individuals.

The GLM test revealed that GSI had significant p values in August ($\beta = 0.00096$, $p < 0.001$, Figure 3.6 L) and November ($\beta = 0.0060$, $p < 0.001$, Figure 3.5 and 3.6 U). Pearson's correlation showed a significant positive correlation between snout/fork length ratio and GSI (cor = 0.58, $p < 0.001$, Figure 3.6 L) and with fork length (cor = 0.33, $p = 0.048$, Figure 3.6 J) in August. Conversely, in November, there was a strong positive correlation between snout/fork length ratio and GSI (cor = 0.93, $p< 0.001$, Figure 3.6 U), as well as a strong negative correlation with K (cor = -0.65, $p < 0.001$, Figure 3.6 T) and a moderate one with weight (cor = -0.44, $p = 0.0069$, Figure B.2 N, appendix). Welch's t-tests indicated significant differences between mature and immature individuals, with mature fish having higher mean snout/fork length ratios in both August (mature: 0.067, immature: 0.060, $p < 0.001$) and November (mature: 0.089, immature: 0.066, $p < 0.001$) (Figure 3.5 and 3.6)

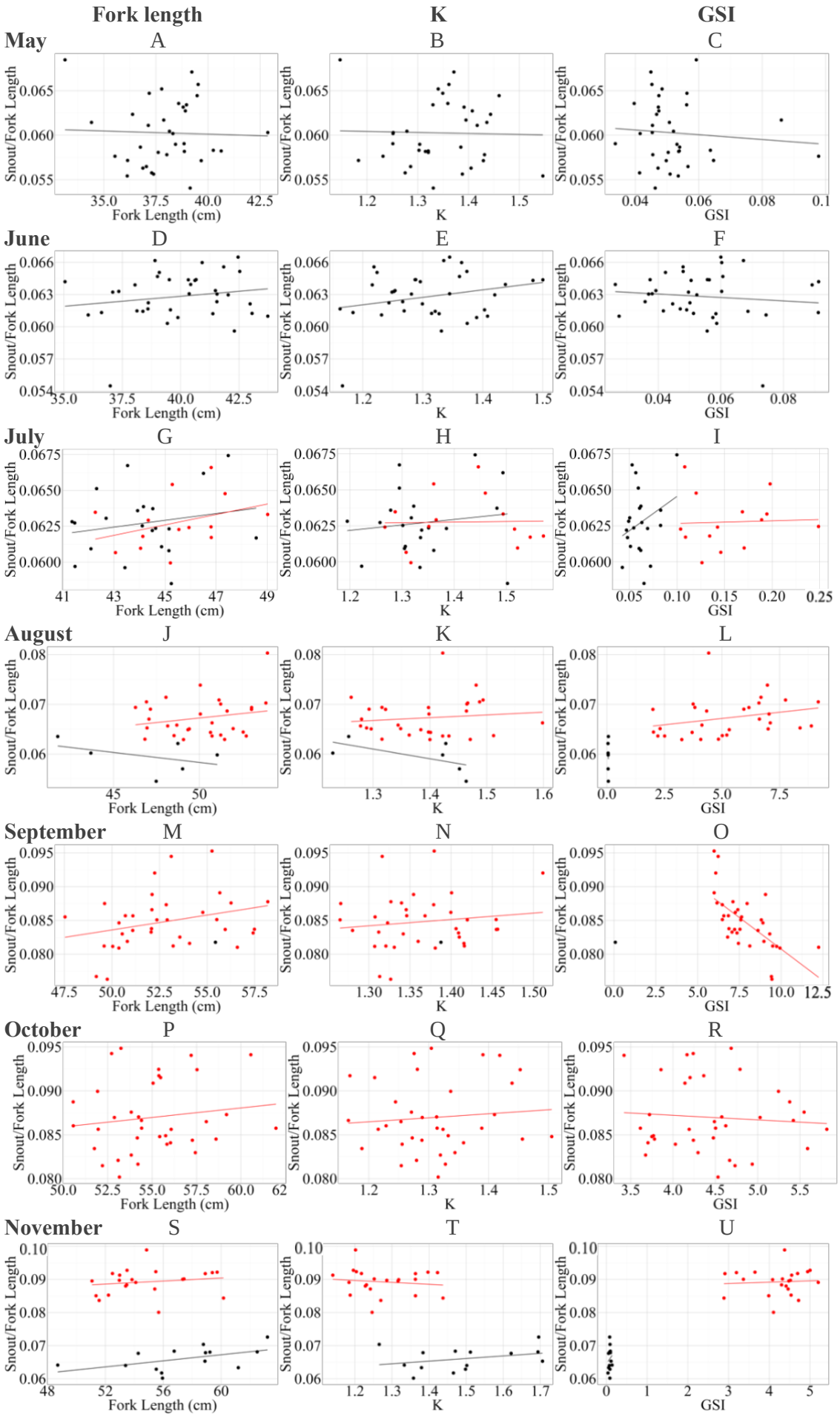


Figure 3.6, A-U: Overview of snout/fork length ratio, Fork Length, K, and GSI for Salmon in the Months of May-November. The data reveals two distinct groups, representing immature (black) and mature(red) individuals.

3.3.3 Head/fork length ratio

The head/fork length ratio is an important morphometric parameter that represents the balance between head and body length.

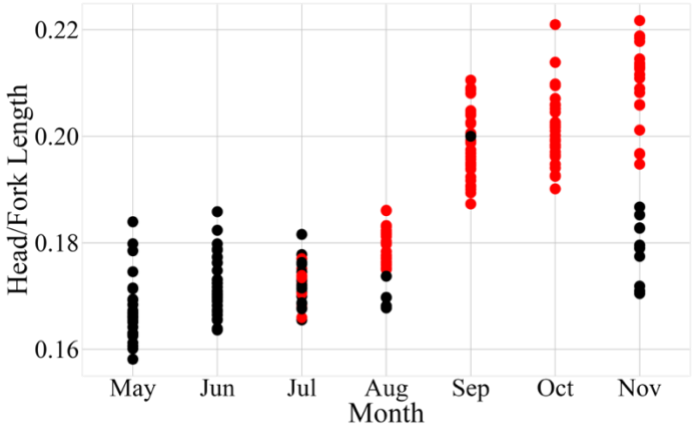


Figure 3.7: Monthly distribution of head/fork length, the data reveals two distinct groups, representing immature (black) and mature(red) individuals.

GLM in August showed both the K (β = -0.033, p=0.049, Figure 3.8 K) and GSI (β = 0.00089, p< 0.001, Figure 3.8 L) had a significant influence on the head/fork length ratio. Moving to November, GSI (β = 0.0079, p< 0.001, Figure 3.8 U) remained highly significant, whereas weight (β = 0.000017, p=0.0504, Figure B.2 Z, appendix), and K(β = -0.042, p=0.0501, Figure 3.8 T) were almost significantly correlated. The Pearson correlation test showed a significant positive correlation between head/fork length ratio and fork length (cor=0.47, p=0.0034, Figure 3.8 J), weight (cor=0.44, p=0.0071, Figure B.2 U, appendix), and GSI (cor=0.52, p< 0.001, Figure 3.8 L) in August. In September, there was positive correlations with fork length (cor=0.38, p=0.022, Figure 3.8 M) and weight (cor=0.40, p=0.013 Figure B.2 W, appendix), while GSI had a negative correlation (cor=-0.51, p< 0.001, Figure 3.8 U). In November, a negative correlation was found with fork length (cor=-0.28, p=0.094, Figure 3.8 S), weight (cor=-0.47, p=0.0036, Figure B.2 Z, appendix), and K (cor=-0.68, p< 0.001, Figure 3.8 T), and a positive correlation with GSI (cor=0.92, p< 0.001, Figure 3.8 U). Mature fish had significantly higher mean head/fork length ratio values than immature fish in August (mature: 0.18, immature: 0.17, p< 0.001). In November, this mean difference was even more pronounced (mature: 0.21, immature: 0.18, p< 0.001) (Figure 3.7 and 3.8).

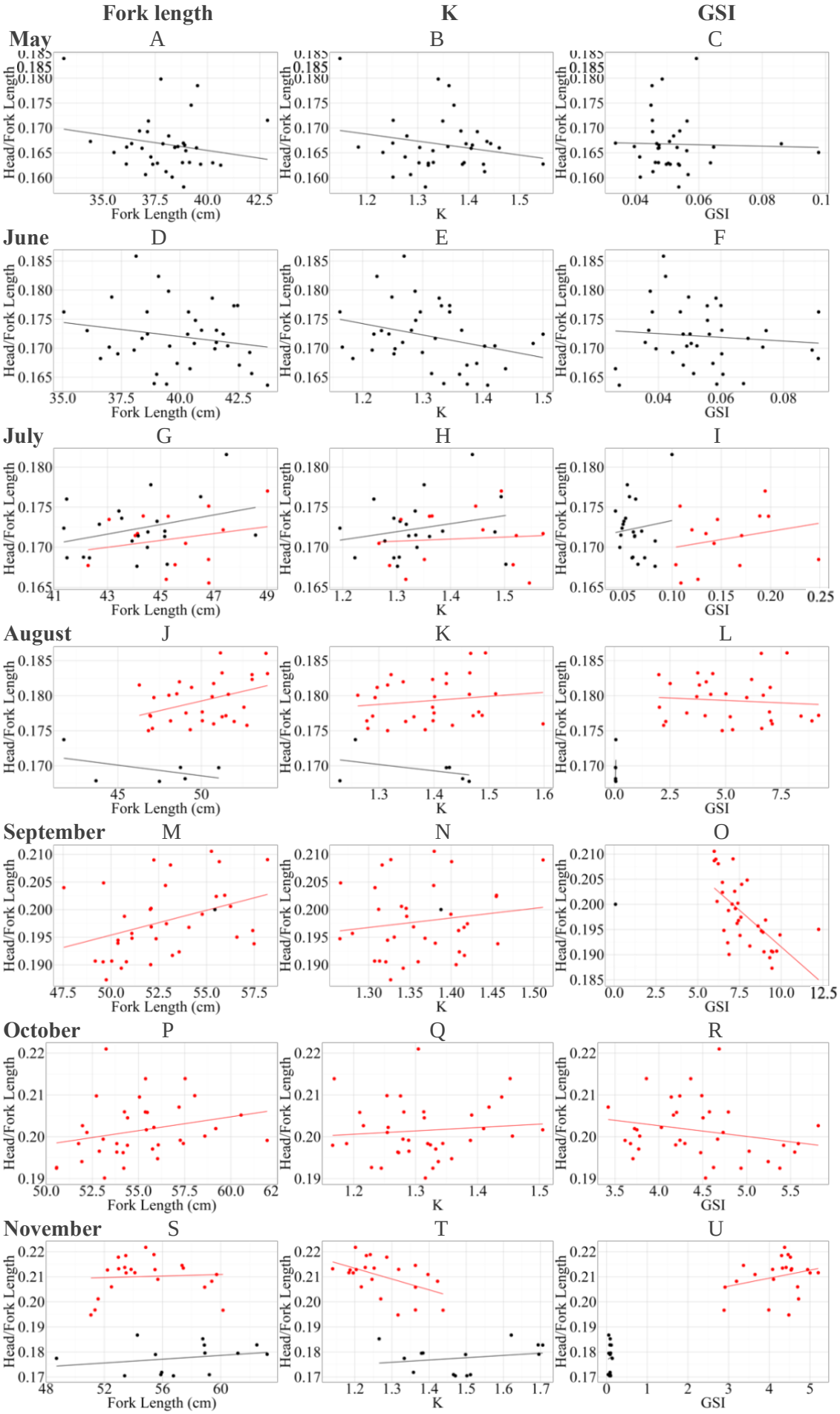


Figure 3.8 A-U: Overview of head/fork length ratio, Fork Length, K, and GSI for Salmon in the months of May-November. The data reveals two distinct groups, representing immature (black) and mature(red) individuals.

3.3.4 Anal caudal fin/fork length ratio

Data analysis for anal to caudal fin divided by fork length ratio, to explore the ratio as an indicator for detecting early maturation.

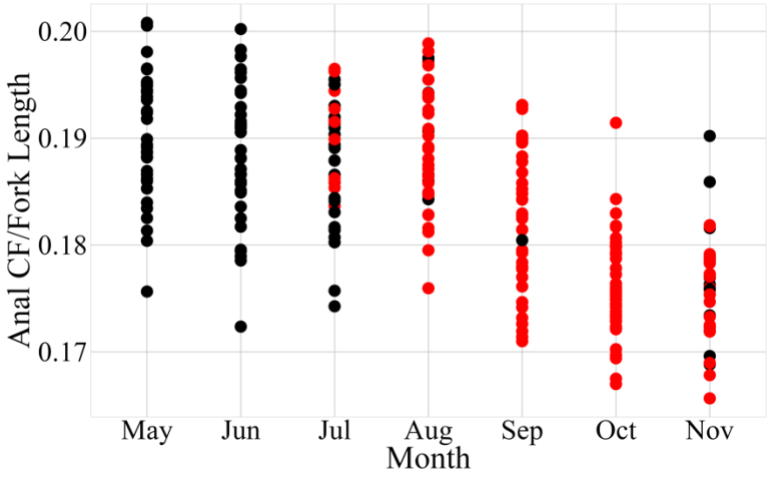


Figure 3.9: Monthly distribution of anal caudal fin/fork length, the data reveals two distinct groups, representing immature (black) and mature(red) individuals.

The GLM test revealed that GSI was found to significantly influence this ratio in July (β =0.051, p=0.0029, Figure 3.10 I) and November (β = -0,0014, p=0.024, Figure 3.10 U). Pearson's correlation test observed a positive correlation for K in May (corr=0.34, p=0.049, Figure 3.10 I), for GSI in July(corr=0.41, p=0.014, Figure 3.10 I) and a negative correlation for fork length in September (corr=-0.33, p=0.048, Figure 3.10 O). Additionally, Welch's t-tests showed in July, mature fish had a slightly higher mean anal caudal fin/fork length ratio (0.19) compared to immature fish (0.18), with the difference being statistically significant (p=0.021). However, this difference was not consistent across other months, as evidenced by the p-values in August (p=0.63) and November (p=0.25) (Figure 3.9 and 3.10).

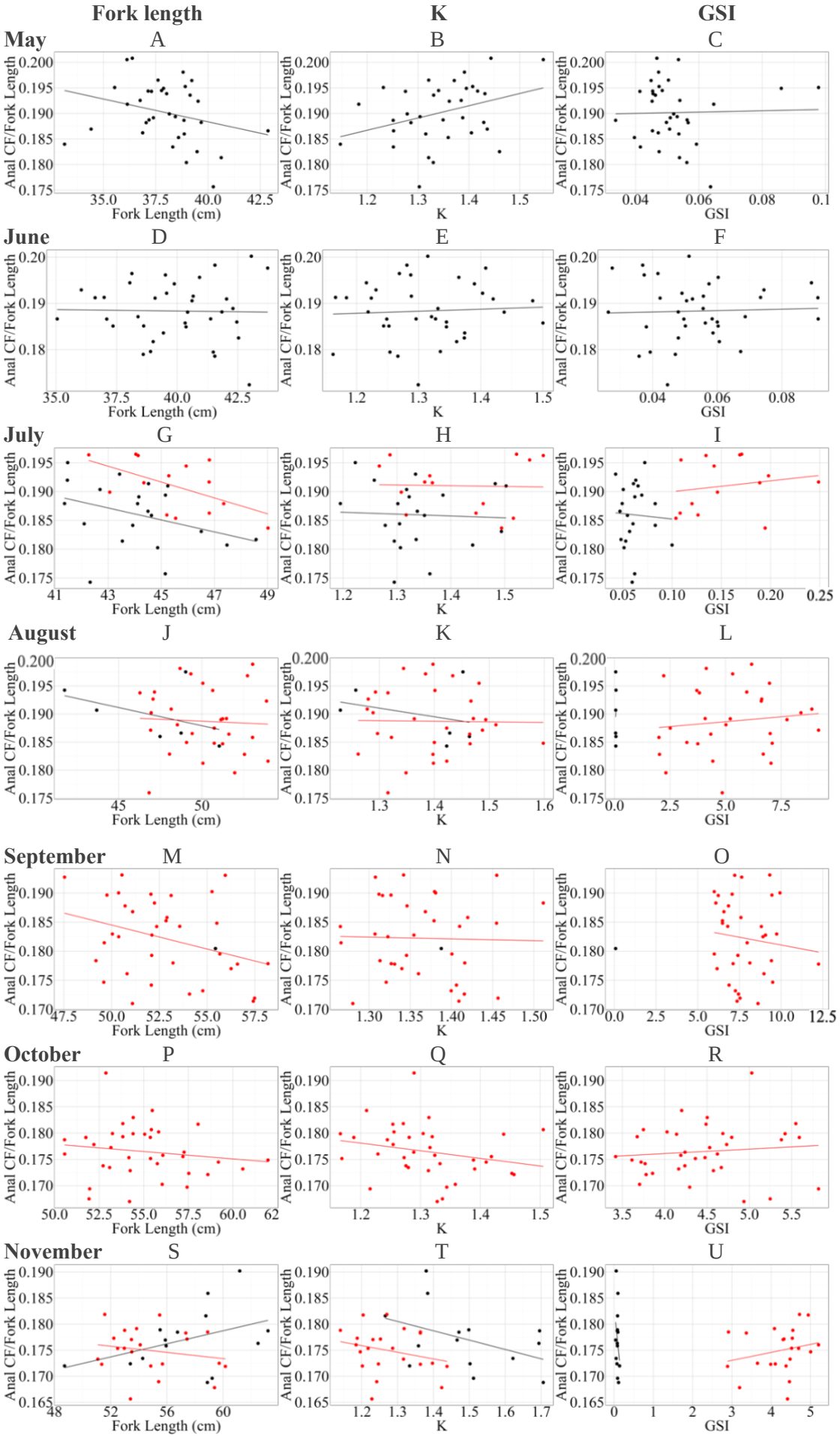


Figure 3.10, A-U: Overview of anal caudal fin/fork length ratio, Fork Length, K, and GSI for salmon in the months of May-November. The data reveals two distinct groups, representing immature (black) and mature(red) individuals.

4 Discussion

4.1 Discussion of methods

4.1.1 Experiment Setup

The experiment conducted at Matre Research Station represents a controlled environment in simulating salmon's natural life cycle, for observing fish growth, behavior, and morphometric data. The choice of PIT tagging, often employed in fisheries research, allowed for individual identification and efficient data collection for each salmon throughout the experiment, including individual growth rates. Additionally, the timeline and the frequency of recordings give a detailed longitudinal insight into the salmon's growth and development over the maturation phases of their life cycle. The switch from a 12:12 L:D photoperiod to continuous light simulates seasonal changes in natural habitats, triggering smoltification—a critical physiological process in salmonids (Björnsson et al., 2011). Exposing the fish to gradual salinity levels helps to acclimatize them to seawater conditions while minimizing potential stress.

4.1.2 Manual Sampling

Manual sampling serves as a robust and reliable method for data collection in fisheries research. The use of Finquel ensures the humane handling of the salmon, further enhancing the ethical integrity of the data collection process. Moreover, the standardized photographic documentation, taken from a fixed distance and angle, provides a reproducible method for analyzing morphometric data. This choice of methodology is particularly advantageous for the reliable extraction of size and shape characteristics from fish photographs. While other methods like underwater stereo camera offer the benefit of less handling stress for the fish, they can introduce variations in size and shape measurements due to fluctuating distances and angles. Therefore, manual sampling combined with standardized photography was preferred for its reliability and precision in assessing the biological principles under study.

Additionally, it's worth discussing the use of ImageJ for measuring morphometric data as an alternative to direct measurements on the fish during sampling. Utilizing ImageJ, a widely recognized and validated image processing tool, ensures accurate and standardized measurements. This method allows for shorter handling time of the fish and enhances the

precision of the measurements, particularly when a ruler is included in every image for calibration purposes. This minimizes potential errors due to variations in the camera setup or in the manual measurements, making it an appealing alternative for ensuring more standardized and reliable data.

In July, we noticed that some fish started to show early signs of becoming mature. The GSI, a measure we use to understand fish maturity, was different for these fish compared to those that were still immature. To make our study clearer, we set a GSI level of 0.1 as the point where we consider a fish to be mature (visible in figure 3.1 E). This helped us focus our analysis on spotting early maturation.

The decision to include mature fish in specific months, while methodologically sound, could introduce biases. It's commendable that this was clearly acknowledged. The decision not to differentiate between clonal lines might overlook potential genetic effects (Hansen et al., 2020), but it's understandable given the need for larger sample sizes in statistical analyses.

One fish from the July sampling was excluded from the dataset due to inconsistencies in its measured length, which was shorter in July than in May when it was transferred to the sea. Such discrepancies led to a negative SGR, possibly due to a data entry error during measurement. Given that the remaining sample size included 35 fish from this sampling, the exclusion of this one fish is unlikely to substantially impact the overall findings.

4.1.3 Data Analysis

Utilizing tools such as R and R Studio, in conjunction with Microsoft Excel, offers robust analytical capabilities. The range of the analysis, which encompasses everything from average growth calculations to GLM modeling, provides a thorough understanding of the dataset. By adopting a multi-step approach, especially concerning morphometric measurement ratios, it's ensured that the subtle nuances within the data are captured. The GLM is employed to clarify the nature of the linear relationship between the morphometric measurement ratios and their potential predictors. By using the Welch two-sample t-test on all ratios for each predictor and month, potential statistical differences between mature and immature fish are identified. Furthermore, Pearson’s product-moment correlation analysis is undertaken to reveal monthly associations between the morphometric measurement ratios and indicators.

A key decision made during the analysis was to examine the data on a month-by-month basis. This choice is grounded in the understanding that seasonal variations likely affect maturation rates, making it inappropriate to aggregate data across all months.

In conclusion, the methods adopted throughout the research a well-structured, comprehensive, and scientifically rigorous approach to understanding salmon growth, morphology, and reproductive readiness. The choices made in experimental design, data collection, and statistical analysis ensure the reliability and validity of the findings.

4.2 Discussion of results

4.2.1 Can growth factors; length, weight, K and SGR provide sufficient information to identify and determine early maturation?

To address the central research question, of whether growth factors like length, weight, K, and SGR can provide sufficient information to identify and determine early maturation in salmon, we employed a suite of well-established equations for K, SGR, and GSI. These metrics are industry standards in fisheries biology, offering a holistic view of fish health, growth trajectories, and readiness for reproduction.

Our study from May to November 2022 sheds light on the complex interplay between growth metrics and sexual maturation in farmed salmon, with distinct patterns emerging in July. During this early stage, July and August, mature salmon had higher average weight, length, SGR, and K values compared to immature fish. These higher metrics suggest an initial focus on somatic growth and potentially early reproductive activities, corroborating established literature on the physiological shifts commonly associated with fish maturation: Indicating that prior to maturation, they tend to consume large amounts of food in order to build up their reserves, as the maturation process is known to be quite energy-intensive (Fleming, 1998; Mobley et al., 2021; Taranger et al., 2010). However, by November, this pattern noticeably reverses. While mature fish still continue to grow in weight and length, there is a decline in their K and SGR values, indicative of a reallocation of energy from growth to reproductive activities. This aligns well with existing research on the trade-off between growth and reproduction in aquatic species (Kadri et al., 1997; Peterson & Harmon, 2005; Taranger et al., 2010). Immature fish in November showed higher growth metrics, focusing their energy

primarily on somatic growth. This divergence in energy allocation between mature and immature fish underscores the need for a multi-metric approach to fully understand the complexities of fish physiology.

To summarize, our research highlights the complex connection between growth and sexual development in salmon. By analyzing various factors such as weight, length, K, and SGR, we can make assumptions about whether a group of fish is maturing or not. However, predicting this on an individual level may be challenging.

4.2.2 How is growth related to the gonadosomatic index?

Previous research has shed light on the intricate relationship between growth and sexual maturation in salmon(Hansen et al., 1992; Kadri et al., 1997; Peterson & Harmon, 2005; Taranger et al., 2010), and the current study has further illuminated this topic. By examining the connection between growth and the GSI.

The association between length and GSI demonstrates varying month-specific data. During June, July, and August, surprisingly none of the indicators; length, weight and K showed a significant realationship. Differing from previous studies (Leclercq et al., 2010; Mobley et al., 2021). However, a significant negative association was observed in September, October, and November, where size and GSI appear inversely related, supproting the energy trade-off hypothesis (Kadri et al., 1997; Peterson & Harmon, 2005; Taranger et al., 2010).

In terms of SGR, its relationship with GSI was inconsistent, but still revealing. June and August displayed no significant correlation, while July exhibited a positive relationship, implying that fish with faster growth rates during July are more likely to be mature (Leclercq et al., 2010; Mobley et al., 2021). October and November revealed a negative correlation, supporting the energy trade-off hypothesis (Kadri et al., 1997; Peterson & Harmon, 2005; Taranger et al., 2010).

To sum up, the connection between growth metrics and GSI in salmon is complicated and impacted by seasonal changes. SGR shows that higher values in summer might indicate maturation and length, weight, K and SGR showing a significant negative relationship indicating energy trade-off in the fall. However, in this study, the data analysis may not be

entirely trustworthy, showing some abnormal results compared to previous studies. It's possible that the reason for this is the small sample size used in our study.

4.2.3 Which morphometric measurements are most effective in detecting early maturation in farmed salmon?

Our data support the notion that morphometric measurements and image analysis can be powerful tools for detecting early maturation. The measurements provide comprehensive insight into the physiological and morphological changes that salmon undergo across different stages of sexual maturation. Specifically, ratios like the snout/head, snout/fork length, and head/fork length showed significant differences between mature and immature salmon. This echoes the work of Kadri et al. (1997), although with a broader focus that includes both dorsoventral and anteroposterior axes of the head.

We found statistically significant variations in the snout/head ratio concerning the GSI and K, particularly during the months of August and November. This provides substantial evidence that influences morphometric changes in salmon. Our study aligns with Kadri et al. (1997) in that specific morphometric variables could potentially aid in visual categorization, as we also found mature fish having a higher mean snout/head ratio than immature fish in the months of August and November. However, we extend upon their work by illustrating that snout/head ratio can be closely linked to GSI and K, supported in the master thesis of (Lange, 2021; Vambeseth, 2022).

GSI also had a strong positive correlation with the snout/fork length ratio, with more pronounced effects observed in November. Interestingly, the K showed a strong negative correlation with snout/fork length ratio in November, suggesting a potential inverse relationship between general body condition and sexual maturation in that specific month. These correlations imply that snout/fork length ratio can be a crucial variable in studying sexual maturation in salmon. The head/fork length ratio was influenced by GSI and K, but the strength and direction of these relationships varied across months. Mature fish consistently exhibited higher mean ratios than immature fish, thereby affirming the role of this morphometric measurement in early maturation detection.

In our examination of various morphometric ratios as indicators of early maturation in salmon, two metrics emerged as particularly noteworthy: the snout/head ratio and the snout/fork length ratio. Both ratios exhibited strong statistical significance across a range of analytical methods, bolstering their potential reliability as robust indicators of maturation. Moreover, these ratios demonstrated consistent statistical significance across temporal spans, effectively distinguishing mature from immature individuals in both August and November. Among these, the snout/fork length ratio displayed an exceptionally strong correlation with GSI, particularly in the month of November (cor = 0.93). This suggests that the snout/fork length ratio may be highly responsive to changes in maturation status and could serve as a sensitive marker for such biological transitions. While statistical robustness is paramount, practical considerations should not be overlooked. The snout/head ratio, despite being slightly less correlated with maturation indices, offers the advantage of easier detection in the field. Consequently, for researchers and practitioners seeking a morphometric ratio that combines strong statistical validation, temporal consistency, and robust correlation with established maturation indicators like GSI and K, the snout/fork length ratio stands out as the most compelling candidate based on our data.

In summary, our study confirms the utility of morphometric measurements and image analysis in detecting early maturation in salmon. Two ratios, snout/head and snout/fork length stood out as particularly significant indicators of maturation. These ratios were statistically robust, temporally consistent, and aligned well with established maturation indices like GSI and K. The snout/fork length ratio was especially noteworthy, displaying a high correlation with GSI, particularly in November. This makes it a sensitive marker for detecting biological transitions in salmon. Our work not only supports previous findings but also extends them by illustrating the correlation between these ratios and other physiological variables. Therefore, for those interested in a reliable, practical measure of early maturation in salmon, the snout/fork length ratio emerges as the most compelling choice based on our data. The snout/head ratio also exhibits a strong correlation and is simpler to measure in the field.

4.2.4 Can the length between the anal and caudal fin serve as an indicator for detecting early maturation?

The analysis of the anal caudal fin to fork length ratio yielded intriguing insights into the potential morphological differences between mature and immature fish. In July the GLM test

and the Pearson correlation test found a significant positive relation between the ratio and GSI, in addition to the t-test showing higher ratio values for mature than immature fish. This could suggest that during the developmental stages, immature fish may allocate a relatively larger proportion of their body length to the anal caudal fin, potentially for improved maneuverability and swimming efficiency. However, the ratio did not show significant values for any of the other months the findings remain speculative due to the complex interplay of various factors influencing fish morphology. Further research with a larger dataset and more detailed morphometric analyses could shed more light on the significance of this ratio in terms of its functional and ecological implications. Therefore, while we do not have sufficient data to definitively prove these hypotheses, the observed difference in the anal to caudal fin /fork length ratio presents an intriguing avenue for future investigations in fish morphology.

In summary, while GSI significantly influenced the anal caudal fin/fork length ratio, the correlations were inconsistent across different months, making it a less reliable indicator of early maturation, but an intriguing ratio to explore for further experiments.

4.2.5 Can head analysis alone be the indicator for detecting early maturation?

As to whether head analysis can alone be an indicator of early maturation, the data suggests that it could be. The snout/head ratio showed strong correlations with sexual maturation and can be easily measured, thus making it a promising candidate for a standalone indicator. In August the mean of the mature fish was 8.57% larger than the head ratio of the immature fish. This is supported by previous studies of (Lange, 2021; Vambeseth, 2022).

It is worth emphasizing that the elongation of the jaws primarily contributes to the increase in head length relative to the body in maturing fish. This finding supports the notion that measuring the jaw in relation to head length is sufficient for identifying maturation. This has significant implications for the methodologies applied in assessing fish maturation, particularly in contexts where imaging technology is used.
In underwater camera setups, or when using cameras designed for deceased fish examination, capturing a focused image of the fish's head is often easier than obtaining a comprehensive image of the entire fish. This practicality offers a significant advantage for researchers, making the head-based morphometric measures not only scientifically robust but also

logistically efficient. It streamlines data collection and minimizes the handling stress on the fish, which is an important consideration from both an ethical and a data integrity standpoint.

To summarize, head analysis appears to be a promising indicator of early maturation in fish, particularly when measuring the snout/head ratio or the elongation of the jaws in relation to head length. These measures are not only scientifically robust but also logistically efficient, making them a practical choice for researchers, especially in contexts where imaging technology is used.

4.3 Conclusion

Our study provides a comprehensive analysis of early maturation indicators in farmed salmon, revealing that while growth metrics like length, weight, K, and SGR are important, their relationship with maturation is seasonally complex and should be considered in conjunction with other variables like the GSI. We establish morphometric measurements as reliable indicators of maturation, with the snout/fork length ratio being particularly sensitive to biological transitions and the snout/head ratio offering the advantage of easier field measurement. Our data suggests that head analysis alone, snout/head ratio, can serve as a standalone, logistically efficient indicator of early maturation, whereas anal caudal fin/fork length did not. These findings advocate for a multi-metric approach and offer both theoretical and practical insights for future research and sustainable salmon farming.

4.4 Further experiments

This study's findings are intriguing, particularly the observed differences in the anal caudal fin to fork length ratio between mature and immature fish, a result that opens up new avenues for understanding fish morphology and behavior. However, these insights warrant consideration of certain caveats and future research avenues. One aspect to note is the use of genetically uniform clonal lines, which, while reducing variability and potentially refining our results, were not differentiated in our analysis due to concerns over small sample sizes. Future research could benefit from incorporating this genetic uniformity as a variable, ideally with larger sample groups to enhance the robustness of the findings. Additionally, the unique farming conditions under which the salmon were raised and the limited sample size could both influence the generalizability of our conclusions. Therefore, subsequent studies should validate the morphometric measures across varied salmon populations and environmental

settings to ensure broader applicability. An exciting prospect lies in the application of this methodology to underwater imaging of free-swimming fish populations, which not only motivated this study but also offers distinct advantages. Utilizing stereo-camera technology in underwater conditions can provide more accurate dimensional data while minimizing optical distortions and mitigating stress associated with fish capture and handling. Additionally, cameras capable of covering multiple depths in the pen could offer more representative measurements than traditional manual sampling methods, which are often biased. This multi-faceted approach would allow for a more comprehensive understanding of salmon maturation indicators, thereby enhancing both the scientific and welfare implications of the research.

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6 Appendix

Appendix A: Growth tables

Table A.1: Monthly changes in physical and biological parameters of the studied fish. The parameters presented include mean length, weight, gonad weight, K, SGR (percentage per day) and GSI.

Date (dd.mm.yyyy)	Length. (cm ± SD)	Weight (g ± SD)	Gonad Weight (g± SD)	K-factor	SGR (%/ day)	GSI
05.05.2022	39,3 ± 2,36	826 ± 144	0,423 ± 0,0804	1,34		0,0522
07.06.2022	41,6 ± 2,30	958 ± 185	0,503 ± 0,116	1,31	0,754	0,0543
05.07.2022	46,3 ± 2,00	1377 ± 249	1,40 ± 0,884	1,37	0,973	0,0987
11.08.2022	50,7 ± 2,70	1832 ± 336	80,8 ± 49,6	1,39	0,935	4,38
14.09.2022	54,3 ± 2,66	2207 ± 380	164 ± 37,8	1,36	0,835	7,62
17.10.2022	56,7 ± 2,69	2418 ± 453	105 ± 11,7	1,31	0,699	4,45
09.11.2022	57,8 ± 3,39	2678 ± 708	60,5 ± 47,5	1,36	0,696	2,61

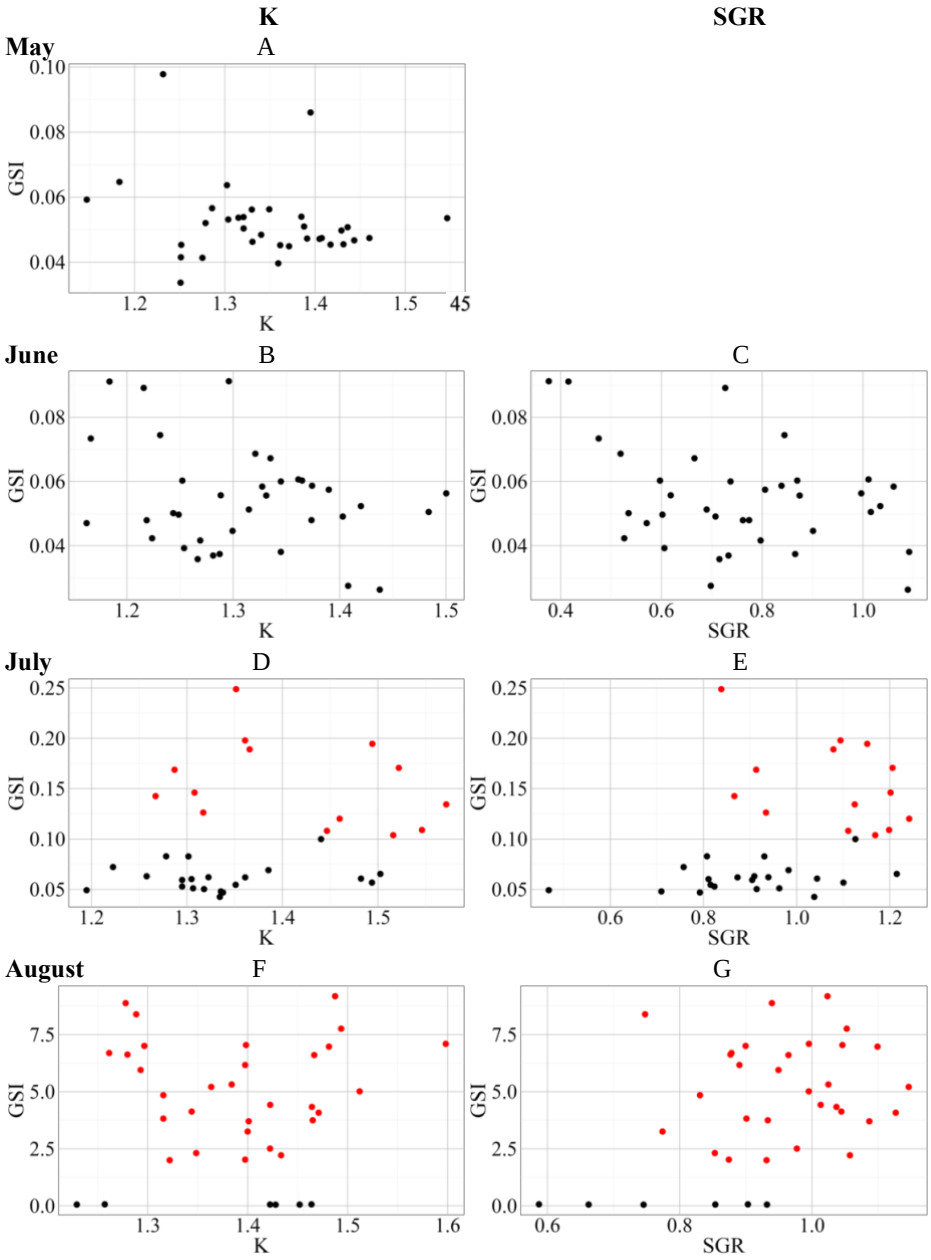
Table A.2: Mature fish: Monthly changes in physical and biological parameters of the mature studied fish. The parameters presented include mean length, weight, gonad weight, K, SGR(percentage per day) and GSI.

Date (dd.mm.yyyy)	Length (cm ± SD)	Weight (g ± SD)	Gonad Weight	K-factor	SGR (%/ day)	GSI
05.07.2022	47,1 ± 1,90	1493 ± 245	2,29 ± 0,720	1,42	1,08	0,154
11.08.2022	51,2 ± 2,18	1889 ± 295	96,8 ± 37,2	1,39	0,966	5,24
09.11.2022	56,8 ± 2,94	2373 ± 506	97,3 ± 10,0	1,28	0,607	4,21

Table A.3: Immature fish: Monthly changes in physical and biological parameters of the mature studied fish. The parameters presented include mean length, weight, gonad weight, K, SGR (percentage per day) and GSI.

Date (dd.mm.yyyy)	Length (cm ± SD)	Weight (g ± SD)	Gonad Weight	K-factor	SGR (%/ day)	GSI
05.07.2022	45,8 ± 1,94	1300 ± 226	0,805 ± 0,261	1,34	0,901	0,0616
11.08.2022	47,9 ± 3,54	1551 ± 416	0,883 ± 0,225	1,38	0,781	0,0575
09.11.2022	59,3 ± 3,61	3157 ± 728	2,59 ± 0,842	1,49	0,835	0,0835

Appendix B: Additional figures



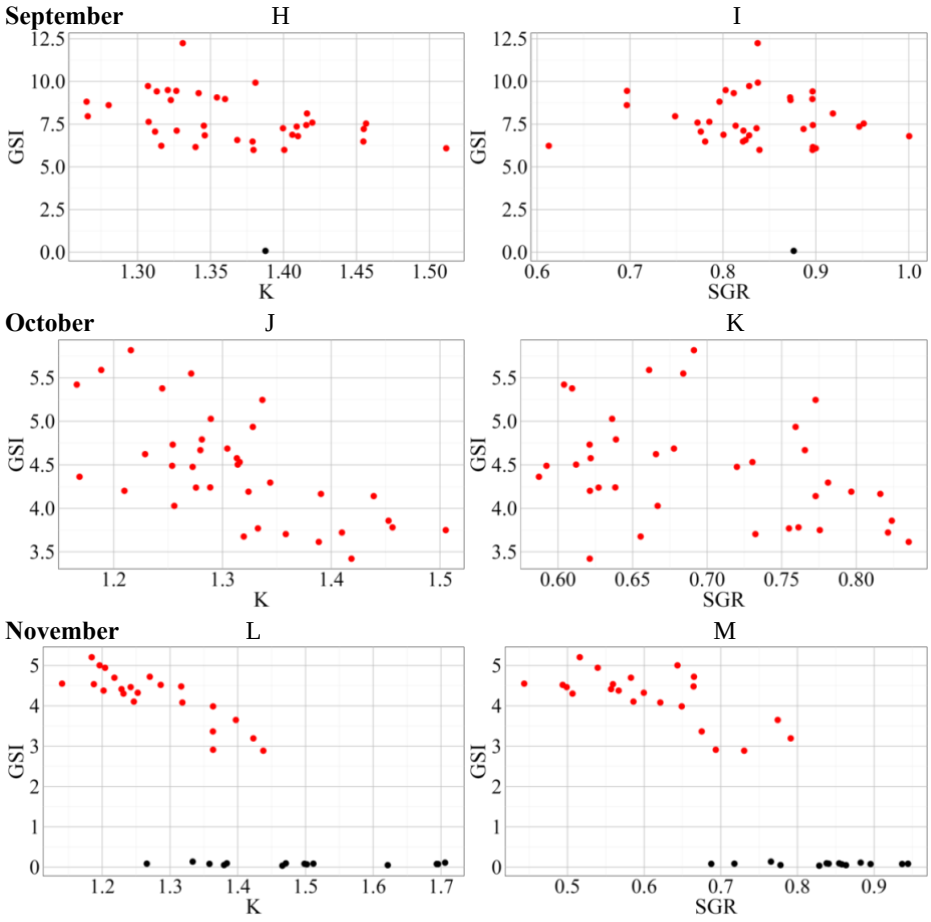
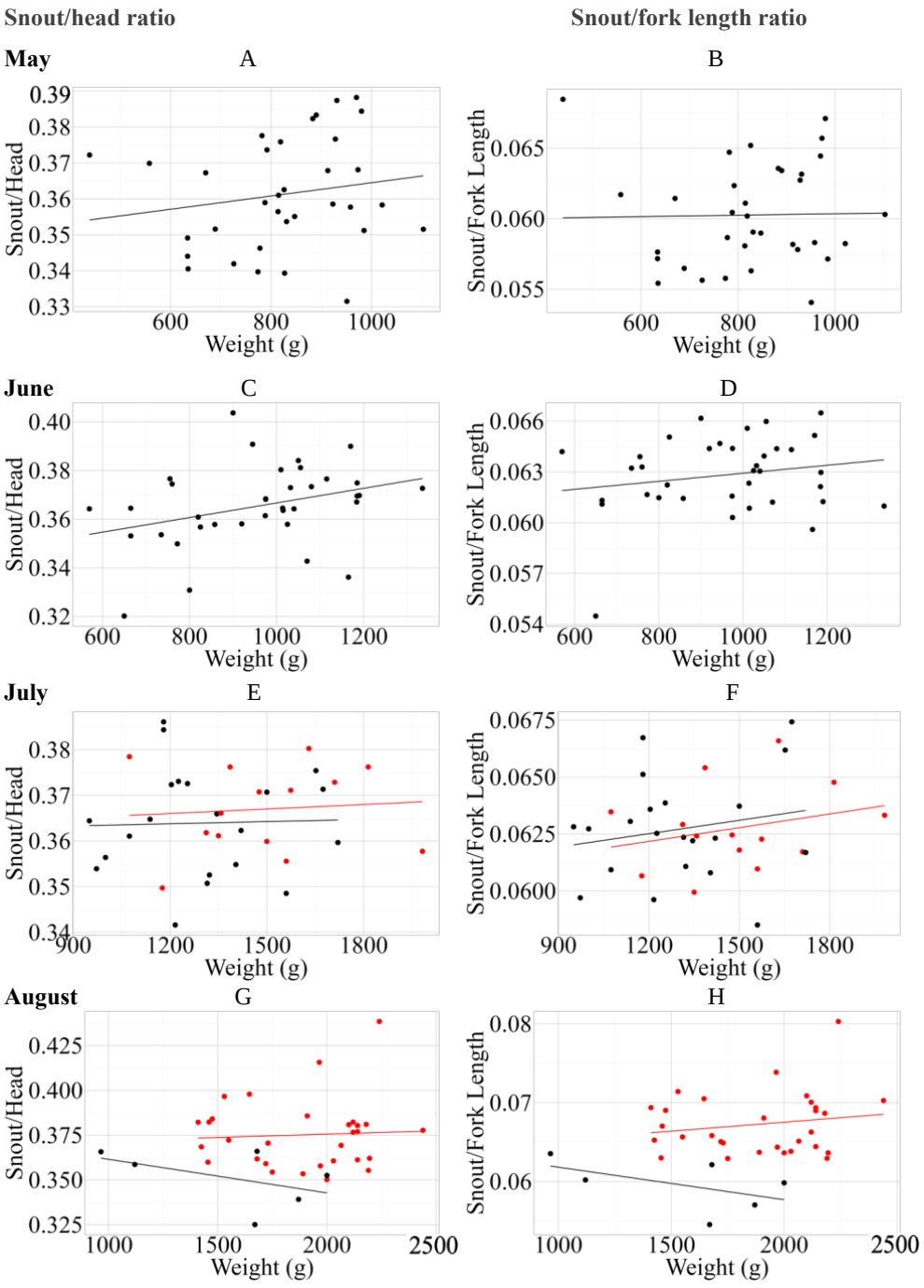
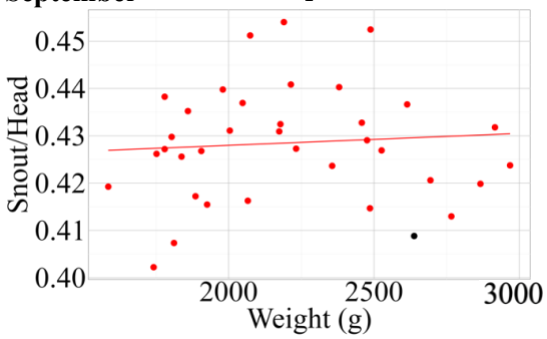


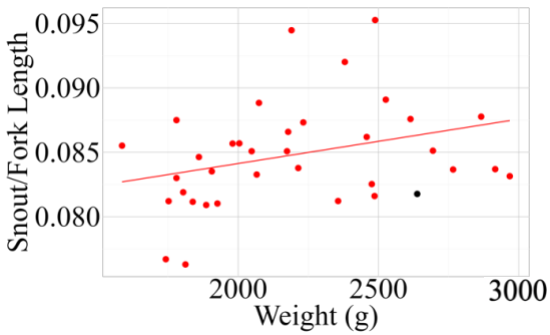
Figure B.1, A-M: Relationship between GSI and K and SGR for each month.


Snout/head ratio
Snout/fork length ratio
May
A
B
June
C
D
July
E
F
August
G
H
Snout/Head
Snout/Fork Length
Weight (g)

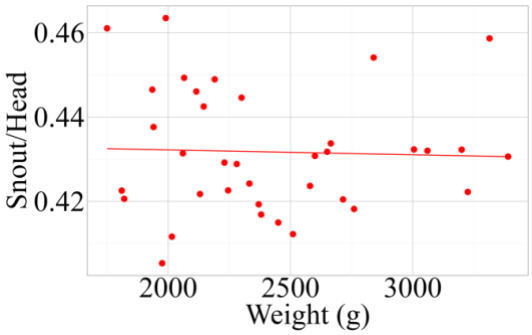
September I



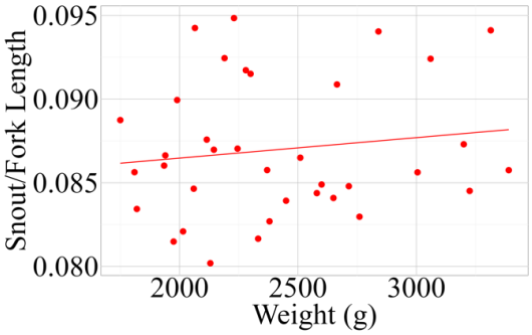
J



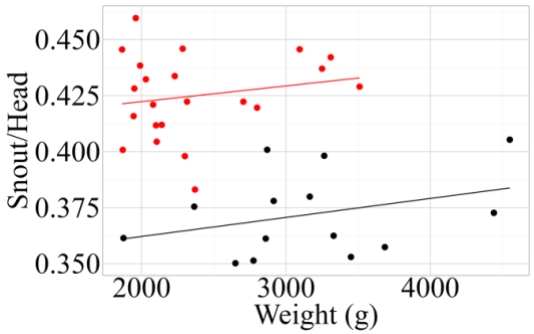
October K



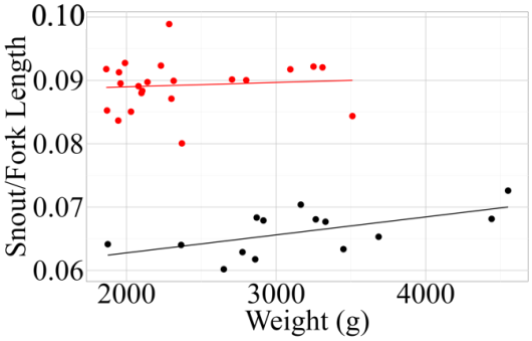
L



November M



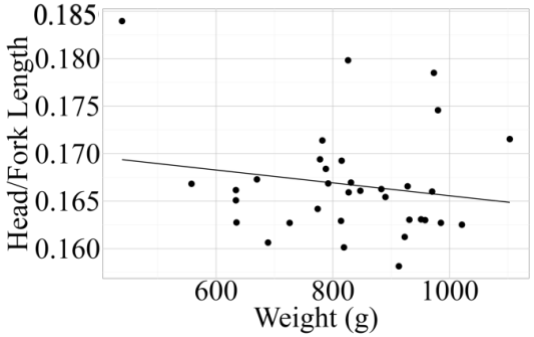
N



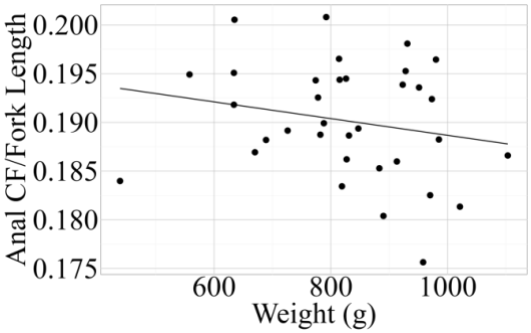
Head/fork length ratio

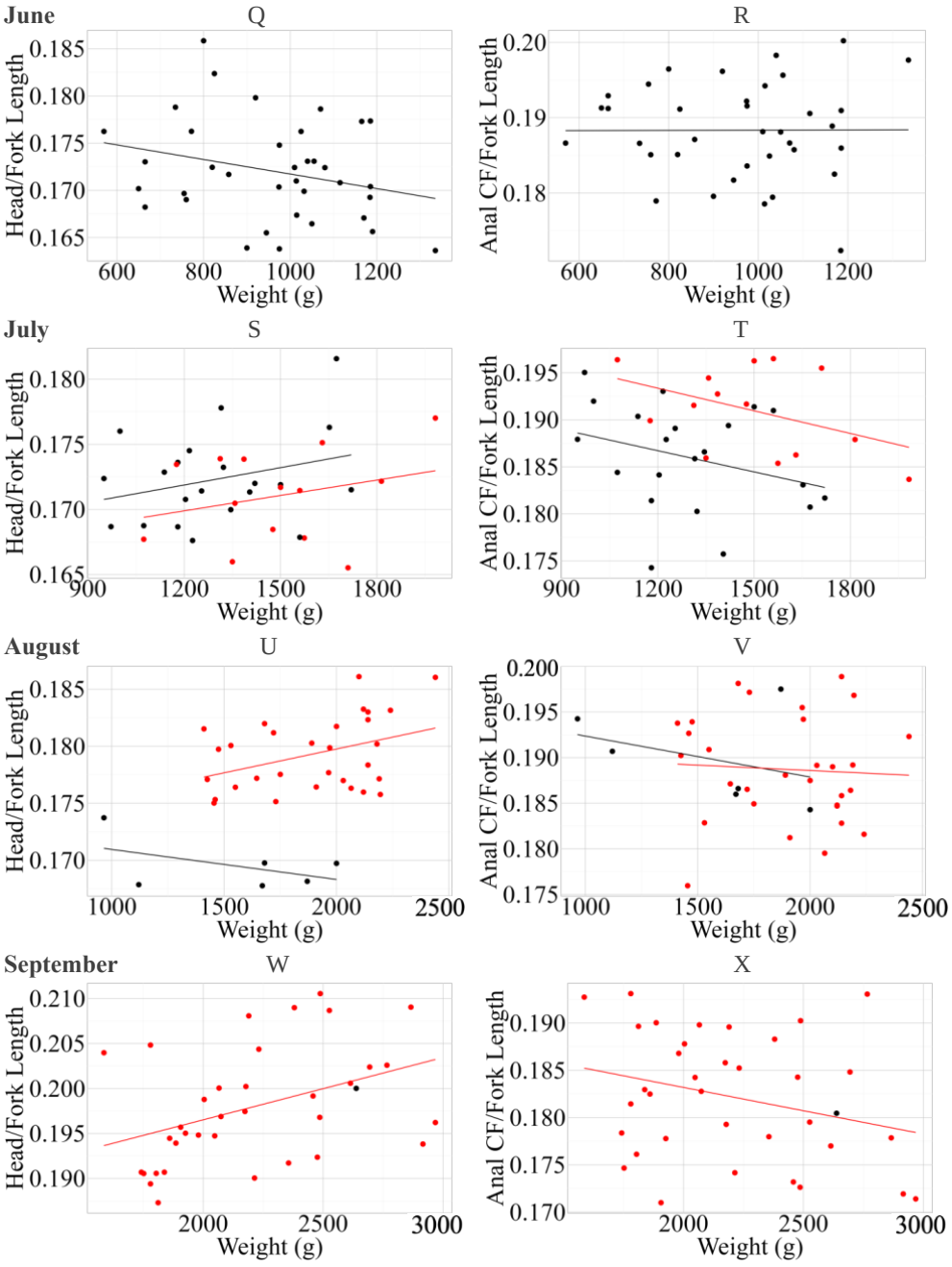
Anal caudal fin/fork length ratio

May O



P




June
Q
R
July
S
T
August
U
V
September
W
X
Head/Fork Length
Anal CF/Fork Length
Weight (g)

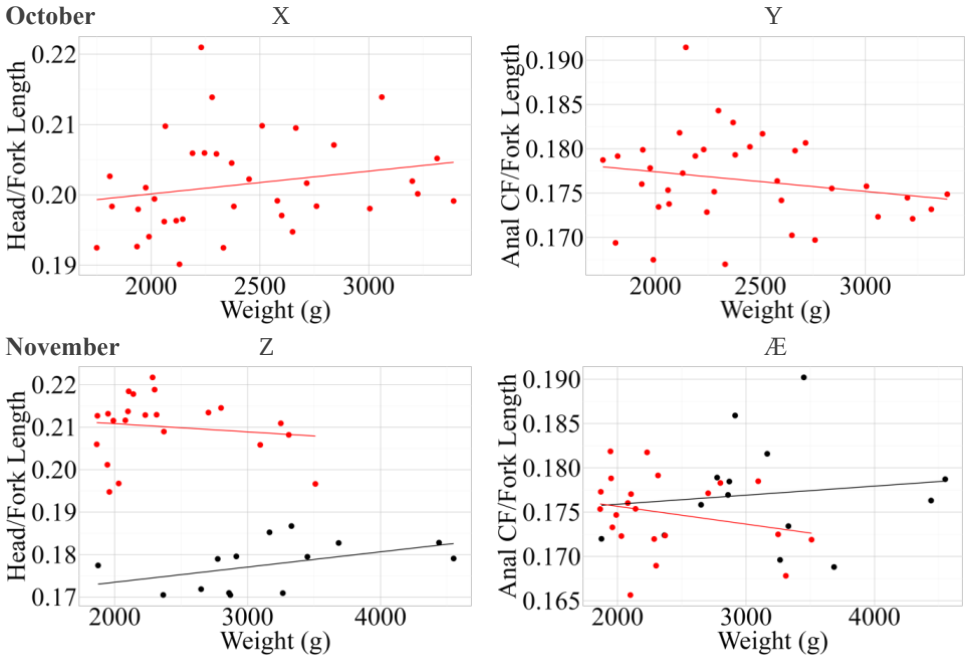


Figure B.2, A-Æ: Overview of weight distribution for ratios; snout/head, snout/fork length, head/fork length, and anal caudal fin/fork length for Salmon in the months of May-November. The data reveals two distinct groups, representing immature (black) and mature(red) individuals.

Appendix C: Coding from R

Initial settings

Installing packages:
install.packages(“lubridate”)
install.packages(“dplyr”)
install.packages(“ggplot2”)
install.packages(“readxl”)

Retrieving packages:
library(lubridate)
library(dplyr)
library(ggplot2)
library(readxl)

Loading data:
data.w <- read_excel("Alldata.xlsx")

Defining months:
months <- c("May", "June", "July", "August", "September", "October", "November")
start_dates <- as.Date(c("2022-05-01", "2022-06-01", "2022-07-01", "2022-08-01", "2022-09-01", "2022-10-01", "2022-11-01"))
end_dates <- as.Date(c("2022-05-31", "2022-06-30", "2022-07-31", "2022-08-31", "2022-09-30", "2022-10-31", "2022-11-30"))

Defining path(saving plots straight to computer folder):

```
output_folder <- "/Users/margretheoen/Dropbox/UiB/Master/data/grafer/"
```

Plots

Growth factors:

```
# Function to create and save a ggplot
create_and_save_plot <- function(data, measurement, y_label, log_scale = FALSE) {

# Create a ggplot with the specified data, measurement, and color by maturity
  plot <- ggplot(data, aes(x = factor(month), y = .data[[measurement]], color =
as.factor(mature))) +
    geom_point(na.rm = TRUE, size = 4) +  # Add points to the plot, removing NA values and
setting size to 4
    scale_color_manual(values = c("0" = "black", "1" = "red")) +  # Manually specify colors
for the factor levels
    geom_smooth(aes(group = interaction(month, year)), method = "lm", se = FALSE) +  #
Add a smoothed line
    labs(x = "Month", y = y_label) +  # Set axis labels
    theme_bw() +  # Use a black-and-white theme
    guides(color = FALSE)  # Remove the color legend

  # Apply log scale to y-axis if log_scale is TRUE
  if (log_scale) {
    plot <- plot + scale_y_log10()
  }

  # Save the plot as a PNG file in the specified folder
  ggsave(filename = paste0(output_folder, measurement, "_MONTH.png"), plot = plot)
}

# Use the create_and_save_plot function to create and save multiple plots
create_and_save_plot(data.w, "length.cm", "Length (cm)")  # Plot for Length (cm)
create_and_save_plot(data.w, "weight.g", "Weight (g)")  # Plot for Weight (g)
create_and_save_plot(data.w, "K", "K")  # Plot for K
create_and_save_plot(data.w, "SGR", "SGR")  # Plot for SGR
create_and_save_plot(data.w, "GSI", "GSI", log_scale = TRUE)  # Plot for GSI with a log
scale
```

GSI(showing only length, same was done for weight, K, SGR):

```
# Define a function to create a plot with ggplot2
create_plot <- function(data, x, y, color_column) {

# Create a ggplot using the specified data, x and y variables, and color column
  ggplot(data, aes(x = !!sym(x), y = !!sym(y), color = factor(!!sym(color_column)))) +
    geom_point(na.rm = TRUE, size = 4) +  # Add points to the plot, removing NA values and
setting size to 4
```

```
   scale_color_manual(values = c("0" = "black", "1" = "red")) +  # Manually specify colors
for the factor levels
   labs(x = x, y = y) +  # Set axis labels
   theme_minimal() +  # Use a minimal theme
   theme(legend.position = "none")  # Remove legend from the plot
}

# Specify the folder where the plot files will be saved
output_folder_length <- "/path/to/output/folder/"

# Loop over each month to create and save a plot
for (i in 1:length(months)) {
  # Filter data based on the start and end dates for the current month
  data_subset <- data.w %>% filter(date >= start_dates[i] & date <= end_dates[i])

  # Create the name for the output plot file
  plot_name <- paste0("GSI_vs_Length_", months[i], ".png")

  # Create the plot using the 'create_plot' function
  plot <- create_plot(data_subset, "length.cm", "GSI", "mature")

  # Save the plot to the specified folder, setting dimensions and resolution
  ggsave(filename = paste0(output_folder_length, plot_name), plot = plot, width = 10, height
= 6, units = "in", dpi = 300)
}
```

Morphometric measurement ratios (showing only snout/head ratio, same was done for: snout/fork length, head/fork length, anal caudal fin/fork length):

```
# Convert date to proper format and extract month, year
data.w$date <- as.Date(data.w$date)
data.w$month <- format(data.w$date, "%B")
data.w$year <- format(data.w$date, "%Y")

# Change the factor level from "x" to "1"
data.w$mature[data.w$mature == "x"] <- "1"

# Define month order and custom colors
month_order <- c("May", "Jun", "Jul", "Aug", "Sep", "Oct", "Nov")
custom_colors <- c("black", "red")

# Function to create and save the plot
create_plot <- function(data_subset, x_var, output_folder) {
  g_plot <- ggplot(data_subset, aes(x = .data[[x_var]], y = snout.head.ratio, color =
factor(mature))) +
    geom_point(na.rm = TRUE) +
    geom_smooth(method = "lm", se = FALSE) +
    labs(x = x_var, y = "Snout/Head") +
    scale_color_manual(values = custom_colors) +
    theme_minimal() +
```

```
    theme(legend.position = "none")

  # Save the plot
  ggsave(paste0(output_folder, unique(data_subset$month), "_", x_var, ".png"),
         plot = g_plot, width = 16, height = 10, units = "in", dpi = 300)
}
```

Analysis

Growth in population:

```
# Check if data exists and 'date' column is available
if (exists("data.w") & "date" %in% colnames(data.w)) {

  # Convert 'date' and extract 'month'
  data.w$date <- as.Date(data.w$date)
  data.w$month <- format(data.w$date, "%Y-%m")

  # Group by month and calculate stats
  average_data <- data.w %>%
    group_by(month) %>%
    summarise_at(vars(weight.g, length.cm, gonadeweight, K, SGR, GSI),
                 list(avg = mean, sd = sd), na.rm = TRUE)

  # Print stats
  print(average_data, width = Inf)

} else {
  print("Check if 'data.w' exists and has 'date' column.")
}
```

Growth mature fish (showing only mature, did same for immature):

```
# Check if data exists and if 'date' column is available
if (exists("data.w") && "date" %in% colnames(data.w)) {

  # Convert 'date' to Date format and extract 'month'
  data.w$date <- as.Date(data.w$date)
  data.w$month <- format(data.w$date, "%Y-%m")
  data.w$only_month <- format(data.w$date, "%m")

  # Group data and compute stats for mature fish in selected months
  average_data_mature_selected_months <- data.w %>%
    filter(mature == 1, only_month %in% c("07", "08", "11")) %>%
    group_by(month) %>%
    summarise_at(vars(weight.g, length.cm, gonadeweight, K, SGR, GSI),
                 list(avg = mean, sd = sd), na.rm = TRUE)

  # Print the computed stats
  print(average_data_mature_selected_months, width = Inf)
```

```
} else {
  print("Check if 'data.w' exists and has 'date' column.")
}
```

GSI on indicators (showing only length, same was done for: weight, K and SGR):

```
# Loop through each month and create GLM for length.cm
for (i in seq_along(months)) {
  subset <- data.w[start_indices[i]:end_indices[i], ]
  glm_formula <- formula(GSI ~ length.cm)
  glm_model <- glm(glm_formula, data = subset, family = gaussian)
  cat("Month:", months[i], "\n")
  summary(glm_model)
}
```

Morphometric measurement ratios (showing only snout/head ratio, same was done for: snute/fork length, body height central/fork length, body height anal/fork length, anal caudal fin/fork length, anal caudal/body central and body height anal/body central):

GLM

```
# Loop through each month and create GLM
for (i in seq_along(months)) {
  subset <- data.w[data.w$date >= start_dates[i] & data.w$date <= end_dates[i], ]
  glm_formula <- formula(GSI ~ snout.head.ratio)
  glm_model <- glm(glm_formula, data = subset, family = gaussian)
  summary(glm_model)
```

Morphometric measurement ratios for further research (showing only snout/head ratio, same was done for: snout/fork length, head/fork length, anal caudal fin/fork length)

```
# GLM Models
glm_list <- list()
for (month in months) {
  data_filtered <- filter(data.w, date >= start_dates[month] & date <= end_dates[month])
  glm_list[[month]] <- glm(snout.head.ratio ~ fork.length + weight.g + K + GSI, data =
data_filtered, family = gaussian)
}

# Pearson Correlations
correlation_results <- list()
for (var in c("fork.length", "weight.g", "K", "GSI")) {
  for (month in months) {
    data_filtered <- filter(data.w, date >= start_dates[month] & date <= end_dates[month])
    if (nrow(data_filtered) > 0) {
      correlation_results[[month]][[var]] <- cor.test(data_filtered$snout.head.ratio,
data_filtered[[var]], method = "pearson")
    }
  }
```

```
}

# Welch T-Tests
welch_ttest_list <- list()
for (month in selected_months) {
  data_filtered <- filter(data.w, date >= start_dates[month] & date <= end_dates[month])
  data_mature <- filter(data_filtered, mature == 1)
  data_immature <- filter(data_filtered, mature == 0)
  if (nrow(data_mature) > 0 && nrow(data_immature) > 0) {
    welch_ttest_list[[month]] <- t.test(data_mature$snout.head.ratio,
data_immature$snout.head.ratio, var.equal = FALSE)
  }
}
```