Understanding the Mechanisms that Determine Feed Conversion Ratio in Chinook Salmon – Physiological and Behavioural Aspects

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Abstract

Finfish aquaculture production costs can be reduced by improving feed conversion ratio (FCR), the ratio of feed intake to weight gain. The aim of this thesis was to identify key factors that influence individual FCR variation in Chinook salmon (*Oncorhynchus tshawytscha)*. Feed efficient and inefficient Chinook salmon, in freshwater and saltwater, were identified using ballotini beads and X-radiography to determine individual feed intake. Comparisons of physiological traits and metabolism between the two FCR phenotypes found that freshwater and saltwater feed efficient fish consumed smaller meals, had higher growth rates, and retained a larger proportion of ingested protein and lipid. No detectable difference was found between FCR phenotypes with respect to maximum metabolic rate (MMR) or aerobic scope (AS). The minimal resting metabolic rate (RMR_{min}) varied between FCR phenotypes in Chinook salmon reared in saltwater but not in freshwater. Specific dynamic action was measured only in freshwater fish and did not differ between phenotypes when fed a set ration.

To assess feeding behaviour a novel application of the ballotini method was developed to determine the timing of feeding in fish. Two bead sizes (dual ballotini) were fed in different halves of the meal to assess when fish ate. No difference was found when the FCR phenotypes ate within the meal. Video imaging was used to analyse feeding behaviours and FCR more in-depth. Feed inefficient fish carried out more turns and swam further to obtain food and spat out more pellets, further increasing energy expenditure.

In conclusion, feed efficient Chinook salmon were faster growing, ate less and retained a higher proportion of ingested nutrients while exhibiting more efficient feeding behaviours. RMR_{min} was shown to be lower in feed efficient fish reared in saltwater, however, MMR and AS did not differ between FCR phenotypes regardless of salinity. The new knowledge that feed intake and behaviour are key drivers of FCR will enable industry to improve FCR. Feeding Chinook salmon to satiation increases overeating and spitting, reducing feed efficiency. Controlling feed intake, whether through selection for individuals that consume smaller meals or feeding a ration slightly below satiation, could improve farm FCR.

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Acronyms

AS – aerobic scope

CF – condition factor

DB – dual ballotini

DFI – daily feed intake

DWG – daily weight gain

ERE – energy retention efficiency

FCR – feed conversion ratio

FCR_E – feed efficient

FCR_I – feed inefficient

LRE – lipid retention efficiency

MMR – maximum metabolic rate

MO_2 – mass-specific oxygen consumption rate

PRE – protein retention efficiency

RMR – minimal routine metabolic rate

SDA – specific dynamic action

SFR – specific feed rate

SGR – specific growth rate

Chapter 1

General Introduction

1.1. Aquaculture

Worldwide fish consumption has increased faster than other forms of animal protein, resulting in aquaculture growing faster than other major food production sectors (FAO, 2018, 2022; Marine Harvest, 2012; MPI, 2013). Capture rates from wild fisheries have plateaued since the 1980s and are now even decreasing (Fig 1.1.; FAO, 2020; FAO, 2022). Aquaculture has expanded to include a wide range of fish, crustacean, shellfish, and algae species, enabling it to fill the gap between wild capture and overall human demand (FAO, 2022). The expansion and regular supply of aquaculture species, including salmon, have thus helped to meet consumer demands (FAO, 2022). Globally, finfish dominate aquaculture production.

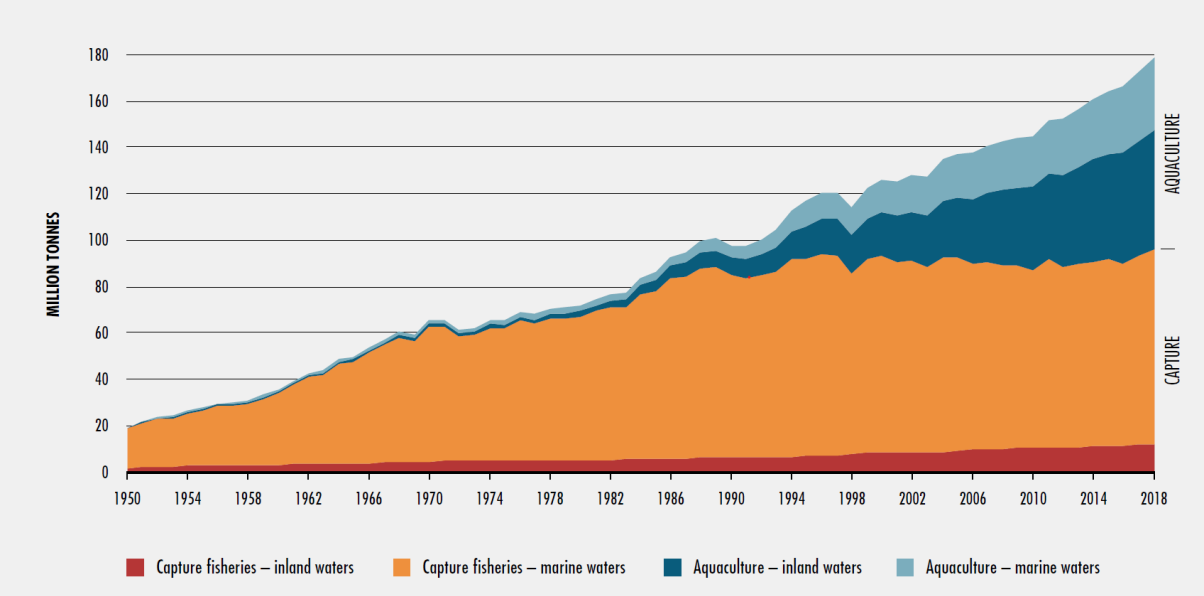

Figure 1.1. World capture fisheries (includes fish, crustaceans, shellfish) and aquaculture production. Figure sourced from FAO (2022). Note: excludes aquatic mammals, crocodiles, alligators and caimans, seaweeds, and other aquatic plants.

1.1.1. Salmon aquaculture

The salmonid fish family group has the largest commodity value, with 60% of salmon produced by aquaculture (FAO, 2018, 2022; Marine Harvest, 2012, 2014). Chinook salmon (*Oncorhynchus tshawytscha*) is noteworthy because it is an extremely high value salmonid species while only making up to 8% of farmed salmon worldwide (ISFA, 2015; Marine

Harvest, 2012; Ministry for the Environment, 2007). New Zealand produces farmed Chinook salmon, producing approximately 70% of global production (ISFA, 2015; Stenton-Dozey et al., 2020). Despite being a relatively small market, Chinook salmon reaches a premium price worldwide due to its flavour, texture, and nutritional profile (Araujo et al., 2021). However, there are several knowledge gaps for Chinook salmon, particularly an understanding of feed efficiency and its variability among individuals.

1.2. Feed conversion ratio

Feed conversion ratio (FCR) is a commonly used performance measure for feed efficiency and is defined as the amount of feed consumed divided by the weight gain of the animal in question across a set period of time (de Verdal et al., 2017a; de Verdal et al., 2017b; Knap and Kause, 2018). Feed conversion efficiency (FCE), also known as feed efficiency ratio, is also used as a feed efficiency measure but is defined as the weight gain of an individual divided by its feed consumption over a set period of time (Knap and Kause, 2018; Kolstad et al., 2004) and is, therefore, the reverse of FCR. The residual feed intake (RFI) is another feed efficiency trait based on the difference between an estimated feed intake, from a model accounting for maintenance and growth (de Verdal et al., 2017a; Pereira et al., 2022), and the actual feed intake of an individual. The difference is then divided by the weight gain to give a residual for each test animal (Silverstein et al., 2005). A negative or lower RFI residual is more favourable as it indicates the individual under investigation consumed less than estimated, suggesting a higher level of feed efficiency (de Verdal et al., 2017a; Martins et al., 2005). Other parameters can be included in the model, such as diet composition, temperature, photoperiod, feeding frequency (number of times fed per day), feeding rate, and time fed within a meal (de Verdal et al., 2017a; Pereira et al., 2022; Serpa et al., 2013; Sun et al., 2016). These models can range from simple to complex depending on the user's requirements but can change the accuracy of estimating feed efficiency. The New Zealand Chinook salmon industry uses FCR as a measure of feed efficiency, however, there is a substantial knowledge gap on FCR in Chinook salmon. Thus, this thesis used FCR due to its relevance to the industry.

Aquaculture production is generally considered more efficient than the farming of cattle, pigs, and poultry because fish generally require less feed per unit of weight gain (Jobling, 2011; Marine Harvest, 2014). This is because fish have significantly smaller maintenance

requirements than mammals of the same body size (Brett, 1972; Knap and Kause, 2018), therefore, the costs of converting feed into meat are lower in fish compared to warm blooded animals (Gjedrem et al., 2012; Jobling, 2011). However, even within fish, there is significant inter-species variation. For salmonids, Atlantic salmon (*Salmo salar*) have a relatively efficient FCR in the range of 1.1 – 1.2 (Cook et al., 2000; Mowi, 2019; Mundheim et al., 2004), meaning that it takes on average 1.1 – 1.2 kg of feed for Atlantic salmon to gain 1.0 kg of body mass. In contrast, farmed Chinook salmon have more variable FCR with an average value of 1.8 (Araujo et al., 2021; NZKS Company, 2019; Walker et al., 2012). It is important to note that FCR also varies with fish size, where smaller fish are usually more feed efficient than their larger conspecifics (de Verdal et al., 2017a; Jobling, 1993; Scholtens et al., 2022a).

Improving FCR is an important goal to improve the profitability and sustainability of fish farming (de Verdal et al., 2017a). One study suggests that improving the feed efficiency of Atlantic salmon worldwide by 2 – 5% would result in an annual global feed cost saving of US$42.9 – 107 million (de Verdal et al., 2017a). The Chinook salmon industry is small, but if all the 7,382 tonnes of Chinook salmon harvested by the New Zealand King Salmon Company (NZKS Company, 2022) in 2022 showed a 2 % FCR improvement, this one company could save 265,752 kg of feed each year, equating to an annual cost saving of approximately NZ$600,000. To improve feed efficiency the feed intake of a fish needs to be reduced, which means that either more fish can be produced with the same amount of resources and production costs (Besson et al., 2014; Gjedrem et al., 2012) or improving feed efficiency can improve the sustainability of fish farming (de Verdal et al., 2022). This is due to a reduction in the amount of nutrients lost as waste products, e.g. ammonia and phosphorus, which lessens nutrient loading into the environment (Eya et al., 2011). Despite a significant time under domestication and selective breeding since the mid-1990s (Symonds et al., 2019), Chinook salmon still remain far less feed efficient than other salmonid species (Scholtens et al., 2022a). Thus, a major goal of the Chinook salmon farming industry in New Zealand is to improve the FCR of this species (Araujo et al., 2021). Understanding why FCR varies between individuals could potentially help improve FCR through selective breeding, diet and/or husbandry changes.

Many traits influence FCR in an animal, including genetics (1.2.1), feed intake and weight gain (1.2.2.), metabolism (1.4.), behaviour (1.5.), protein turnover and body size (Daulé et al., 2014; Emmerson, 1997). External factors, such as nutrition, digestibility of the diet, as well as husbandry practices, also have a strong influence on FCR (de Verdal et al., 2017a). Feed intake, protein metabolism and feeding behaviours are also suggested to contribute to FCR variation in Chinook salmon and therefore warrant further investigation (Esmaeili et al., 2022a; Scholtens et al., 2022a).

1.2.1. Genetics and FCR

Understanding the extent of genetic variation in feed efficiency can determine whether it can be effectively selected for within breeding programs. Family has been shown to have a significant effect on feed efficiency as well as feed intake and growth (Eya et al., 2011). For example, FCR was seen to vary among rainbow trout (*Oncorhynchus mykiss*) families (Eya et al., 2011) with family accounting for 77 % of variation in Atlantic salmon (Kolstad et al., 2004). However, when selecting to improve FCR, Thodesen et al. (1999) only found a 4.6 % improvement per generation in Atlantic salmon and de Verdal et al. (2022) estimated a 4 % gain in FCR per generation in tilapia (*Oreochromis niloticus*) by selecting individuals with high and low FCR values. This was a lower improvement compared to growth which had an improvement rate of 12.5 % per generation (Gjedrem et al., 2012). Despite the lower heritability of FCR compared to growth, it is still possible to select for FCR in a breeding program, but gains are likely to be slower compared to selecting for growth.

It has been suggested that feed intake, growth and FCR can be improved by selection in Chinook salmon (Scholtens et al., 2022a). Although gains in Chinook salmon growth rates have been made in the last 20 – 30 years through selective breeding, the FCR of selected Chinook salmon has not improved greatly and is still higher than other salmonids. This suggests a low correlation between growth and FCR in this species (Scholtens et al., 2022a; Symonds et al., 2019). Therefore, it could be more beneficial to directly select for FCR or select for feed intake to get gains in FCR indirectly. However, due to the challenge of measuring individual feed intake, developing a selective breeding program for FCR improvement remains complex (de Verdal et al., 2022).

1.2.2. Feed intake and weight gain as interactive factors influencing FCR

Weight gain and feed intake measures collectively comprise FCR, so either one or both of these measures can be adjusted separately or simultaneously to influence FCR. Due to its role in FCR, improved growth is often used to improve FCR indirectly (de Verdal et al., 2017a). Coho salmon (*Oncorhynchus kisutch*) for example showed improvement in feed efficiency when selection for increased growth took place over 16 generations (Neely et al., 2008). However, the same principles cannot be applied to all species because the correlation of FCR, growth and feed intake is species-specific. FCR appears significantly correlated with the growth and feed intake of Atlantic salmon (Kolstad et al., 2004), whilst FCR was only related to growth, and not feed intake, of common carp (*Cyprinus carpio*; Zeng et al., 2018) and rainbow trout (Kause et al., 2006b; Silverstein, 2006; Silverstein et al., 2005). In tilapia, growth and feed intake were positively correlated, FCR and growth were negatively correlated, while FCR and feed intake were not correlated at all (de Verdal et al., 2022; de Verdal et al., 2017b). In brown trout (*Salmo trutta*), differences in feed efficiency were attributed to variation in feed intake but not to growth (Mambrini et al., 2004). Studies suggest that feed intake has a stronger correlation to FCR compared to growth in Chinook salmon and that by reducing feed intake the FCR of this species can also be improved (Esmaeili et al., 2022a; Scholtens et al., 2022a). Understanding the phenotypic correlations of growth, feed intake and FCR in Chinook salmon could provide insight into how to improve the FCR of this species.

FCR can be determined on a population or individual basis. Population-based FCR estimates use the collective feed intake and growth of a group of fish in a tank, pen, or cage across a set period of time. Population-based calculations are more commonly used on farms as measuring individual feed intake is difficult to achieve in this setting, and uneaten food cannot be recovered. An individual's FCR can be estimated from the population FCR by dividing the overall feed intake and growth by the number of individuals, but this tends to under or overestimate individual values as it does not allow for individual variation in either growth rate or feed intake. To select fish for a breeding program, it is important to know an individual's FCR (Kause et al., 2016). Therefore, tank-based trials are used to determine individual performance and have been shown to be representative of sea pen performance

(Scholtens et al., 2022b). While weight gain is relatively easy to measure in individually tagged fish, obtaining accurate measures of individual feed intake is more difficult.

The individual feed intake of fish is usually estimated using the following three methods: Individual rearing, video recording and the "ballotini method". Rearing fish in individual tanks allows individual feed intake to be determined directly (Besson et al., 2019; Martins et al., 2011a; Silverstein, 2006). This method involves feeding the tank a known amount of feed and recovering the uneaten feed. The uneaten feed is subtracted from the initial value, and as there is only one fish per tank, the feed intake can be associated with this individual. This feed intake method is beneficial as there are minimal disruptions to the fish and can, therefore, be measured across consecutive meals. It also provides feed intake information almost immediately. However, rearing fish individually requires many aquaria, which can confound environmental effects (de Verdal et al., 2017a). More crucially, rearing fish individually does not account for complex social interactions which can influence feeding behaviour under commercially relevant conditions (de Verdal et al., 2017a; Gilmour et al., 2005; MacLean and Metcalfe, 2001). Since the FCR of individually reared fish may not correlate with group-reared conspecifics (Rodde et al., 2021b), individual-based FCR measures may not provide information that is transferable to commercial settings.

The video method records entire meals, the video footage can then be used to measure feed intake of individually identified fish, e.g. external tags, fin clips or dye (Adams et al., 1995; de Verdal et al., 2017a; Øverli et al., 1998). This method involves little or no disruption to the fish during feeding and can be easily measured across multiple meals (de Verdal et al., 2017a), similar to the individual rearing method above. Moreover, the video method accounts for social interactions that are excluded during individual rearing. However, this technique only works for small numbers of fish, as a higher number of individuals results in fish obscuring one another, thus preventing visual observations of feed intake. Therefore, observations of feeding and behaviours at lower densities may not represent higher densities seen on-farm (de Verdal et al., 2017a; Rodde et al., 2020). This method is also very time-consuming and requires the implantation of external tags or similar to track individuals.

The final method, and the one selected for this thesis, is known as the "ballotini method" and uses X-radiography to estimate individual feed intake through the addition of X-ray

opaque ballotini (glass beads) to the diet (Bégout et al., 2012; Difford et al., 2023; Esmaeili et al., 2021; McCarthy et al., 1992; Walker et al., 2012). The known inclusion rate of the ballotini is determined by X-raying known weights of feed and counting the number of beads present from the X-rays to create a standard curve (Handeland et al., 2008). The ballotini are visible in the X-ray of the gastrointestinal tract (Fig. 1.2.), allowing the total amount of food consumed by individual fish to be estimated after counting the beads in the X-ray and interpolating feed intake from the standard curve. The diet containing the ballotini is only fed to fish on the days that measures of feed intake are required; a commercial diet without the beads is fed to the fish at all other times (Moutou et al., 1998). After feeding a meal containing the beads, the fish are anaesthetised and X-rayed before gut evacuation occurs (Difford et al., 2023; McCarthy et al., 1992). Gut evacuation rates can be determined by repeatedly X-raying the same fish to determine when feed begins to leave the stomach (Walker et al., 2012). Gut evacuation varies between species but also with fish size, environmental conditions (e.g. water temperature) and feed, so it is important to consider this during experiments.

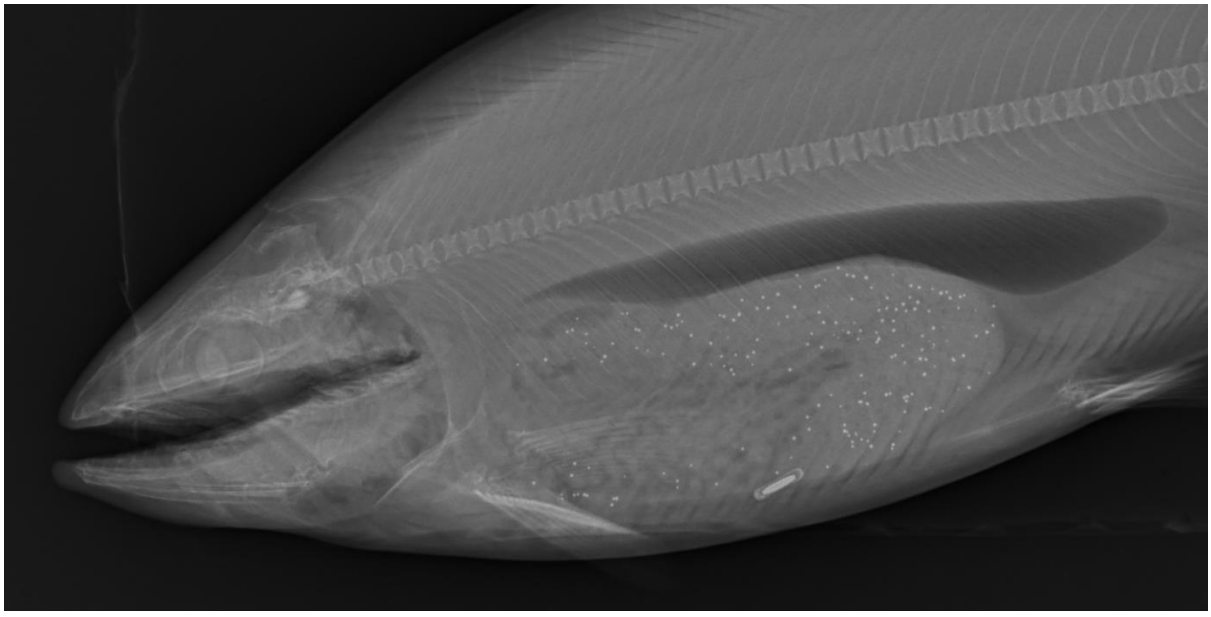

Figure 1.2. X-ray of Chinook salmon after being fed a diet containing ballotini at the Cawthron Aquaculture Park.

The ballotini method has the advantage that feed intake can be estimated in individual fish reared in large, commercially relevant groups (Bégout et al., 2012; Jørgensen and Jobling, 1992; McCarthy et al., 1992; McCarthy et al., 1993). The ballotini method provides a highly accurate estimate of feed intake from a single meal being measured, but it should not

be assumed that feed intake in other meals is always similar (de Verdal et al., 2017a; Walker et al., 2012). Salmonids are known to be variable in their daily feed intake, with individuals feeding heavily, lightly, or not at all (Kause et al., 2006b; McCarthy et al., 1992). It is therefore important to make repeated measures of feed intake in each individual to improve the reliability of the ballotini method (Grima et al., 2008). However, the repeated use of anaesthetic and handling of fish, and evacuation of ballotini, limit the number of repeated measures (de Verdal et al., 2017b; Grima et al., 2008). Therefore, unlike the previous methods, feed intake from consecutive meals cannot be measured on the same day as several days or weeks are required for fish to recover before the next measurement is attempted. This is the one major drawback of using the ballotini method. However, the ballotini method was selected over the other methods for this thesis due to its ability to estimate feed intake accurately at commercially relevant densities. This thesis used the ballotini method to estimate the FCR of farmed Chinook salmon as well as assign individuals to one of three main FCR phenotype categories: feed efficient (FCR_E), intermediate feed efficiency or feed inefficient (FCR_I), to compare how certain traits or variables varied between phenotypes and which had the most influence.

1.3. Body Composition

As feed intake is difficult to measure in fish, a practical proxy for estimating FCR could be beneficial, as this could eliminate the need to measure feed intake which is difficult on-farm. Nutrient composition has been hypothesised to be a useful tool in estimating FCR in mammals and poultry as well as fish (Knap and Kause, 2018). However, protein composition has low levels of variation in fish (Kause et al., 2009; Knap and Kause, 2018), which was also seen in Chinook salmon (Araújo et al., 2023; Glencross et al., 2022). On the other hand, lipid composition has been shown to be highly variable in fish (Kause et al., 2009) and has been shown to be a useful proxy for estimating FCR in rainbow trout (Kamalam et al., 2012; Kause et al., 2016; Kinghorn, 1983). These studies found that leaner fish have better feed efficiency which is thought to be due to a lower metabolic cost of growth, as protein deposition has a lower energetic cost compared to lipid deposition (de Verdal et al., 2017a). Near infrared spectroscopy can be used as a non-invasive technique to measure lipid content of whole live fish and has been shown to estimate values very similar to traditional chemical analysis (Folkestad et al., 2008). This thesis will examine whether there is any correlation between

FCR and lipid composition in Chinook salmon to assess the potential of using lipid composition as a proxy for FCR.

1.3.1. Nutrient retention

It has been argued that feed efficiency alone is a limited measure and only accounts for feed input instead of the more important nutritional content of feed and how it is incorporated into the animal's body (Azevedo et al., 2004a; Fry et al., 2018). Nutrient retention efficiencies consider the feed composition, the amount of feed eaten and animal production (growth; Jobling, 2011), providing a more in-depth understanding of feed efficiency. Retention efficiency values are determined by dividing the total amount of the nutrient gained (e.g. protein) by the total amount of the nutrient consumed. An initial (C_i) and final whole-body composition (C_f) is required to determine how nutrients are gained. However, as composition sampling is terminal, this method is currently limited as C_i and C_f cannot be measured on the same individual. Therefore, C_i has to be determined from multiple untracked conspecifics at the initial time point. Using the total estimated feed intake of fish across the whole study period and the concentration of the specific nutrient in the feed, the difference in body composition is then compared to the total consumption of the nutrient according to the following equation:

$$RE\ (\%)=100 \times \left((C_f w_f - C_i w_i)(C_{Fe} \times TFI)^{-1}\right)$$

Where retention efficiency (RE) can be protein retention efficiency (PRE), lipid retention efficiency (LRE) or energy retention efficiency (ERE), C_f is the final nutrient concentration of the fish (protein, lipid, or energy), C_i is the initial nutrient concentration of the fish, C_{Fe} is the selected nutrient concentration of the feed, and TFI is the total feed intake during the set period (g; Bendiksen et al., 2003; Biswas et al., 2005).

Nutrient retention efficiency allows a direct assessment of feed efficiency by looking at how much of each nutrient is deposited as growth from the feed consumed (Esmaeili et al., 2021). Protein retention efficiency has been correlated to feed efficiency in rainbow trout (Eya et al., 2013; Silverstein et al., 2005) and Atlantic salmon (Kolstad et al., 2004), indicating that nutrient retention is potentially an essential component of efficient growth in salmonids. Kolstad et al. (2004) also measured lipid and energy retention efficiency (LRE and ERE, respectively) in Atlantic salmon but detected no link to FCR in this species. However,

European sea bass (*Dicentrarchus labrax*) with poor feed efficiency had lower LRE (Peres and Oliva-Teles, 1999). Energy retention efficiency (ERE) also appears to be species-specific and varies with body size in salmonids, which may be due to differences in protein and lipid deposition, and may also be related to feed efficiency (Azevedo et al., 2004a, 2004b). However, ERE was not found to differ greatly in rainbow trout (Overturf et al., 2013). Nutrient retention efficiencies are able to provide additional details for feed efficiency, specifically in terms of which nutrients an individual is retaining from the feed. Therefore, nutrient retention efficiencies have been included in this thesis to further understand feed efficiency in Chinook salmon.

1.4. Metabolism

McCarthy et al. (1994) and Tuzan et al. (2019) suggest that differences in feed efficiency are due to metabolic differences. An animal's metabolism includes a wide variety of processes involved in homeostasis (including ion exchange and acid-base regulation), tissue maintenance and growth (including protein turnover), digestion, activity, and reproduction (Jobling, 2011; Kornberg, 2020; McKenzie, 2011). The energy required for these processes is produced via cellular respiration, whereby ingested food and/or stored energy such as lipid, amino acids, or glycogen are broken down into various substrates (e.g. pyruvate, succinate, malate, or glutamate). These substrates feed into aerobic or anaerobic glycolysis to produce adenosine triphosphate (ATP; Nelson and Chabot, 2011), depending on whether sufficient oxygen is available.

Aerobic respiration produces far more ATP molecules (~ 38) than anaerobic respiration (2 ATP; Hoar, 1983; McKenzie, 2011). However, anaerobic respiration is used when energy demand exceeds oxygen availability, or aerobic capacity, for example, during burst or high intensity swimming activity (McKenzie, 2011; Thorarensen, 2011; Wedemeyer, 1996). Anaerobic respiration is even less energy efficient, as it produces lactic acid as a waste product that subsequently needs to be oxidised for removal. This results in an oxygen debt for which the payback can last more than 24 hours in salmonids (Thorarensen, 2011; Wedemeyer, 1996).

1.4.1. Measuring metabolic rates in fish

Rates of whole-animal metabolism are influenced by various factors, including activity, physiological state, body mass, temperature, food intake, and oxygen availability (Chabot et al., 2016b). These can be measured by using various techniques that aim to either directly measure energy production or proxies of energy production (e.g., oxygen consumption). Direct calorimetry measures the heat production, produced during catabolic reactions, of an animal to determine energy use and is the most accurate method as it includes both aerobic and anaerobic respiration (Lighton, 2008b; Nelson and Chabot, 2011; Regan et al., 2013; van Ginneken and van den Thillart, 2009). Though it can be used to determine metabolic rate in fish, direct calorimetry is not a commonly used method in aquatic physiology due to direct calorimetry being difficult to measure in aquatic animals as the temperature production required for this method is much lower in fish (van Ginneken and van den Thillart, 2009). Due to this, indirect methods are used preferentially.

Indirect calorimetry measures the oxygen consumption of an organism, and mass-specific oxygen consumption ($\dot{M}O_2$) is used as the main proxy for measuring metabolic rate in fish (Lighton, 2008b; Nelson, 2016). Oxygen consumption can be used as an alternative to direct calorimetry due to its role in aerobic metabolism. However, $\dot{M}O_2$ does not account for anaerobic metabolism so it may differ from an organism's true metabolic rate (Cech Jr and Brauner, 2011; Nelson and Chabot, 2011). Consequently, it is considered a measurement in its own right (Nelson, 2016; Nelson and Chabot, 2011). Despite this limitation, it is the most practical estimation of metabolic rate in fish.

Oxygen consumption is measured using the method of respirometry, which requires the use of a respirometer or swim flume. A swim flume, or swimming respirometer, allows water flow within the respirometry chamber so fish swim against a current (Cech Jr and Brauner, 2011). This setup is ideal for active fish, such as Chinook salmon, that cannot be held immobile. Three types of respirometers are used in fish physiology: closed, flow through and intermittent. Closed, static, or constant volume respirometry involves fish being in an air-tight container with a fixed volume of water long enough to produce a measurable decrease in oxygen concentration (Lighton, 2008a). This allows $\dot{M}O_2$ to be measured but results in other environmental changes, such as increasing carbon dioxide and varying pH, which limits the accuracy of $\dot{M}O_2$ measures due to their influence on

metabolism. The length of the experiment is also restricted by the available oxygen as it will eventually be depleted by the fish (Cech Jr and Brauner, 2011). In flow through systems, the respirometer is constantly supplied with fresh oxygenated water and oxygen is measured at both the inflow and outflow of the tank (Álvarez and Nicieza, 2005; Lighton, 2008a). This method allows the constant replenishment of oxygen, but water flow needs monitoring to ensure a certain oxygen differential across the probes (Svendsen et al., 2016). Errors can be introduced into this method when water mixing within the flume is insufficient, flow rates vary, or small drifts in the oxygen probes.

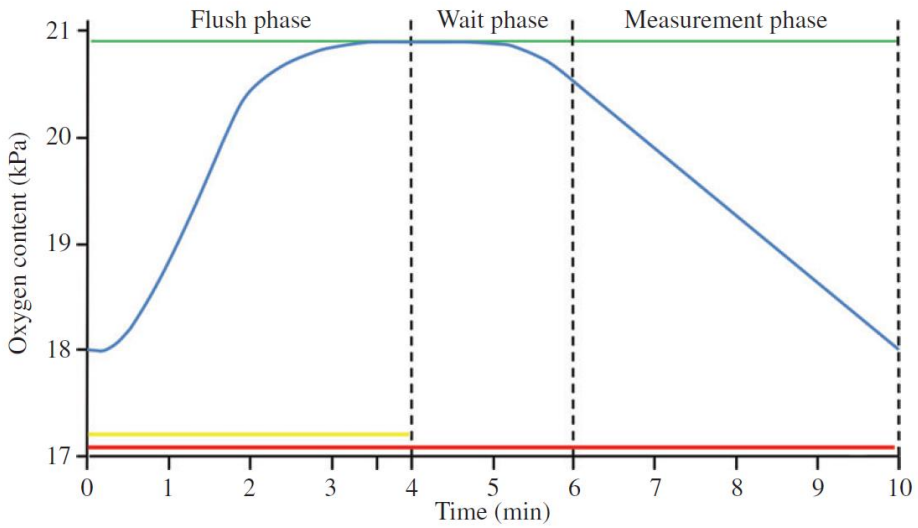

Figure 1.3. The three phases of intermittent respirometry, one complete respirometry measurement cycle is represented. The figure shows the oxygen levels inside the respirometer chamber (blue line) and in the reservoir tank (green line). The flush pump (yellow line) is active during the flush phase, whereas the recirculation pump or impeller (red line) is active constantly. The flush phase is repeated after the measurement phase. Durations of the three phases can be changed to match the experimental conditions. Image sourced from Rosewarne et al. (2016).

Intermittent respirometry is a combination of the first two methods, where short periods of closed chamber measurements are followed by flush periods to reoxygenate the water from the reservoir tank (Fig. 1.3.). This method allows more control over oxygen levels while also ensuring removal of CO_2 and other waste products. A wait period is used prior to the measurement period to ensure the water is thoroughly mixed, preventing variation of oxygen throughout the swim flume. Using intermittent respirometry uses the best features of the other two methods while limiting their problems, which allows accurate oxygen consumption measurements (Svendsen et al., 2016). Due to this reasoning, intermittent respirometry was used in this thesis.

1.4.2. Standard metabolic rate, growth and FCR

The standard metabolic rate (SMR) of fish is the equivalent of basal metabolic rate (BMR) in mammals and represents the minimum amount of energy required for core body function (i.e. basic protein turnover, heart rate, ventilation, and blood flow etc.) in an unfed immobile, post-absorptive animal (Armstrong et al., 2011; Clark et al., 2013; Nelson, 2011). It can be difficult to measure SMR in active species, such as Chinook salmon, as they cannot be held immobile. Instead, an alternative measure often used is routine metabolic rate (RMR); RMR measures fasting oxygen consumption but allows for some normal swimming activity (Brett, 1972; Chabot and Ouellet, 2005). This metabolic rate is also known as the maintenance energy requirement, and the processes that make this up include cell transport, tissue repair and renewal, protein turnover, and protein, lipid, and carbohydrate synthesis (Jobling, 2011). The transport of substrates and metabolites in and out of cells is thought to contribute to a sizeable proportion of maintenance metabolic processes. In mammals, it has been estimated that sodium pump activity alone makes up 20 – 40% of BMR (Jobling, 1993, 2011), which may also hold true for fish. In addition to transport, protein synthesis is an energetically expensive process and is estimated to make up 12 – 25 % of SMR (Houlihan et al., 1995; Jobling, 1985, 1993; Lyndon et al., 1992; Metcalfe et al., 2016). The importance of protein deposition to SMR/RMR is seen in cod (*Gadus morhua*), where rates of protein deposition remained increased following metabolism returning to baseline following digestion (Lyndon et al., 1992). This appeared especially important in actively growing animals.

Growth requires high levels of protein synthesis, so it is unsurprising that growth and metabolic rate proxies (i.e., oxygen consumption rates) often show a positive relationship in fish (Jobling, 1985; Metcalfe et al., 2016). Understanding the relationship between metabolic rates and growth could therefore be used to select for fish with fast growing genotypes (Álvarez and Nicieza, 2005). It has been shown that SMR and growth rate have a positive correlation in artificial rearing conditions for brown trout (Álvarez and Nicieza, 2005) and coho salmon (Van Leeuwen et al., 2012) where high metabolic rates are linked with high growth rates (Bochdansky et al., 2005; Zeng et al., 2017). In Atlantic salmon, fish with higher SMR values were also shown to have a faster metabolic recovery time following feeding, so they can increase their food processing potential (Millidine et al., 2009).

However, this relationship has been seen to occur at high feed rates but appears to become negatively related at low feed rates (Armstrong et al., 2011). This is because a high SMR becomes a hindrance as maintenance requirements need to be met before energy can be partitioned to growth, while those with lower SMR values can meet those requirements more easily, thus leaving more energy for growth from the same sized meal. However, most fish farms feed to satiation, so fish do not tend to be feed limited. The relationship between growth and metabolic rates are relatively unknown in Chinook salmon, but it is hypothesised that individuals with high growth rates would also have increased SMR.

In agricultural species, such as cattle and sheep, feed efficiency and metabolic rates also appear correlated, with more efficient individuals having lower metabolic rates (Arndt et al., 2015; Chaves et al., 2015; Paganoni et al., 2017). This is assumed to also occur in fish species where a high FCR indicates that a significant proportion of ingested energy is being lost in the form of heat and metabolic products, especially in active species (Nisbet et al., 2012). However, the relationship between feed efficiency and metabolic rates is not well understood in fish species. It is theorised that improved feed efficiency occurs in fish with low SMR/RMR values due to their lower maintenance costs (Zeng et al., 2017). Evidence for this appears to hold true because individual fish with lower maintenance values are shown to be more feed efficient in hapuku (*Polyprion oxygeneios*; Fig. 1.4.; Khan et al., 2014), Chinese crucian carp (Cyprinus carpio; Zeng et al., 2017) and European seabass (Rodde et al., 2021a). However, in European sea bass, this relationship is seen at a population level, but not within individuals and in Chinese crucian carp, this relationship was only seen when feed was restricted. Therefore, RMR cannot always be used as a predictor of efficiency at an individual level (Rodde et al., 2021a). Determining how RMR varies among individuals and how it relates to FCR is poorly understood in Chinook salmon, so it would be useful to determine if any correlations exist.

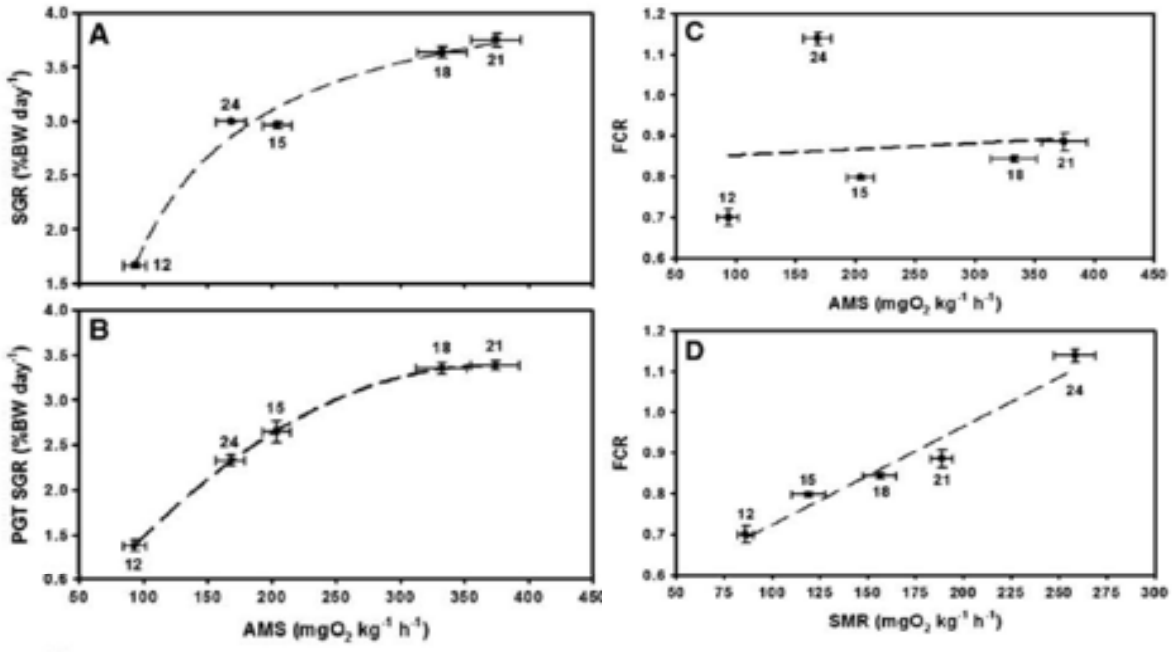

Figure 1.4. Specific growth rate (SGR; A), SGR post-trial (B) and feed conversion ratio (FCR; C) compared to aerobic metabolic scope (AMS) and FCR compared to SMR (D) in hapuku*. Figure sourced from Khan et al. (2014).*

1.4.3. Aerobic scope, growth and FCR

The capacity for growth is closely linked to an individual's ability to maximise their aerobic scope (AS; Claireaux and Lefrancois, 2007). The AS, also known as aerobic metabolic scope (AMS), of an individual is the difference between SMR/RMR and maximum metabolic rate (MMR; Claireaux and Lefrancois, 2007). MMR is the highest rate of oxygen consumption possible by an individual fish (Auer et al., 2017; Metcalfe et al., 2016; Norin and Clark, 2016). AS represents the physiological capacity for non-maintenance processes, such as growth, swimming, and digestion (Claireaux and Lefrancois, 2007; Clark et al., 2013; Fry, 1957). The way AS will be utilised will vary depending on growth potential, meal sizes, feeding behaviours, swimming style, environmental factors and more (Claireaux and Lefrancois, 2007; Clark et al., 2013; Khan et al., 2014; Leal et al., 2021; Marras et al., 2013). How growth and digestion occupy the available AS likely varies between individuals; therefore, differences in AS may be associated with variation in FCR.

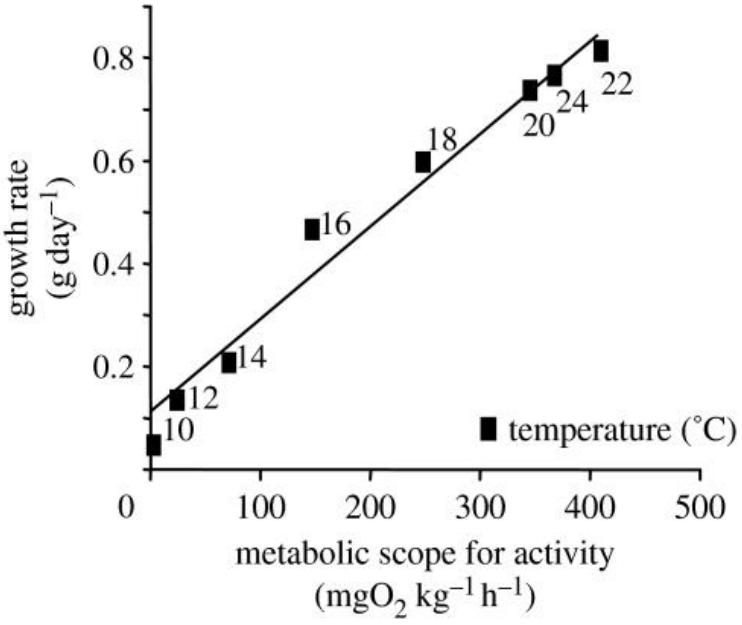

Figure 1.5. The influence of temperature on AS and growth, sourced from Claireaux and Lefrancois (2007).

There is confounding evidence on the relationship between AS and growth depending on species. In some species, such as hapuku and brown trout, AS is positively correlated with growth (Fig 1.4.; Fig 1.5.; Auer et al., 2015; Claireaux and Lefrancois, 2007; Khan et al., 2014), so as AS increases so do growth rates. This is thought to be due to an increased energy potential above maintenance for growth to occur (Leal et al., 2021). However, some species show a trade-off between growth traits (SMR) and active performance associated traits (MMR and AS), as seen by a negative relationship between growth and AS in rainbow trout and coho salmon (Allen et al., 2016; Rosenfeld et al., 2020). It is hypothesised that feed efficient Chinook salmon will have larger AS potential as they have been shown to have higher growth rates (Esmaeili et al., 2022a). Furthermore, protein synthesis, a required component for body growth, is an energetically expensive process (Jobling, 1985; Metcalfe et al., 2016) which has been shown to be upregulated in feed efficient Chinook salmon. Esmaeili et al. (2022a) examined proteomic differences between feed efficient and inefficient phenotypes and found that there were increased levels of proteins involved in protein synthesis in feed efficient Chinook salmon compared to feed inefficient individuals. Therefore, looking at the metabolism of different FCR phenotypes could provide an understanding of FCR variability but also act as an indirect selection criteria (Rodde et al., 2021a).

1.4.4. Specific dynamic action

Another influence on the correlation between AS and growth is thought to be due to the amount of energy required to digest a meal. This is because an individual's capability to digest a meal is restricted by the size of their AS (Claireaux and Lefrancois, 2007). Therefore, how feed efficient and feed inefficient Chinook salmon process meals metabolically could provide insight into how the available AS energy is used and, in turn, feed efficiency, especially since it has been shown that feed inefficient individuals consume significantly larger meals (Esmaeili et al., 2021; Esmaeili et al., 2022a). Specific dynamic action (SDA), also known as heat increment of feeding or feeding metabolism, is the postprandial metabolic cost of digesting, absorbing, and assimilating a meal (Bureau et al., 2003; Chabot et al., 2016a; Fu et al., 2005; Nelson, 2011; Norin and Clark, 2017). It is commonly viewed and quantified as a temporary rise in oxygen consumption after a meal that forms a peak and then reduces back to baseline over time (Fig. 1.6.). Quantifying SDA, therefore, provides an understanding of how individuals digest and utilise feed at a metabolic level. Understanding how SDA varies among individuals may help understand variation in feed efficiency.

There are several contributors to the increased energy requirements of SDA: 1) an increase in swimming activity at feeding time which can be either aerobic metabolism which creates an immediate oxygen demand, or anaerobic burst swimming, which creates an oxygen debt that must be repaid (Thorarensen, 2011); 2) digestion and absorption, which is made up of production and secretion of digestive enzymes, mechanical digestion of food in the mouth and stomach, gastrointestinal tract contractions for gut motility, and the active transport of nutrients into cells; 3) the formation and interconversion of substrates and their retention in tissues, e.g. storage of excess nutrients in the form of lipids, glycogen etc.; 4) post-absorptive protein synthesis; and 5) the formation and excretion of metabolic waste products, in which the deamination of amino acids plays a major role (Bureau et al., 2003; Chabot et al., 2016a; Cho et al., 1982; Jobling, 1993; Nelson, 2011; Soofiani and Hawkins, 1985).

Fish with higher growth rates are expected to have an increased SDA which is largely due to tissue synthesis (Jobling, 1981), particularly protein deposition (Carter and Brafield, 1992). This was seen by Brown and Cameron (1991), who showed that inhibiting protein synthesis correlated with the absence of a SDA response. So growth rates are thought to be

associated with an individual's capacity to process feed (Dupont-Prinet et al., 2010). Therefore, the magnitude of SDA may positively correlate with feed efficiency of fish. There has been little research on this topic; therefore, examining how SDA varies in fish with differing feed efficiencies is a valuable area of study

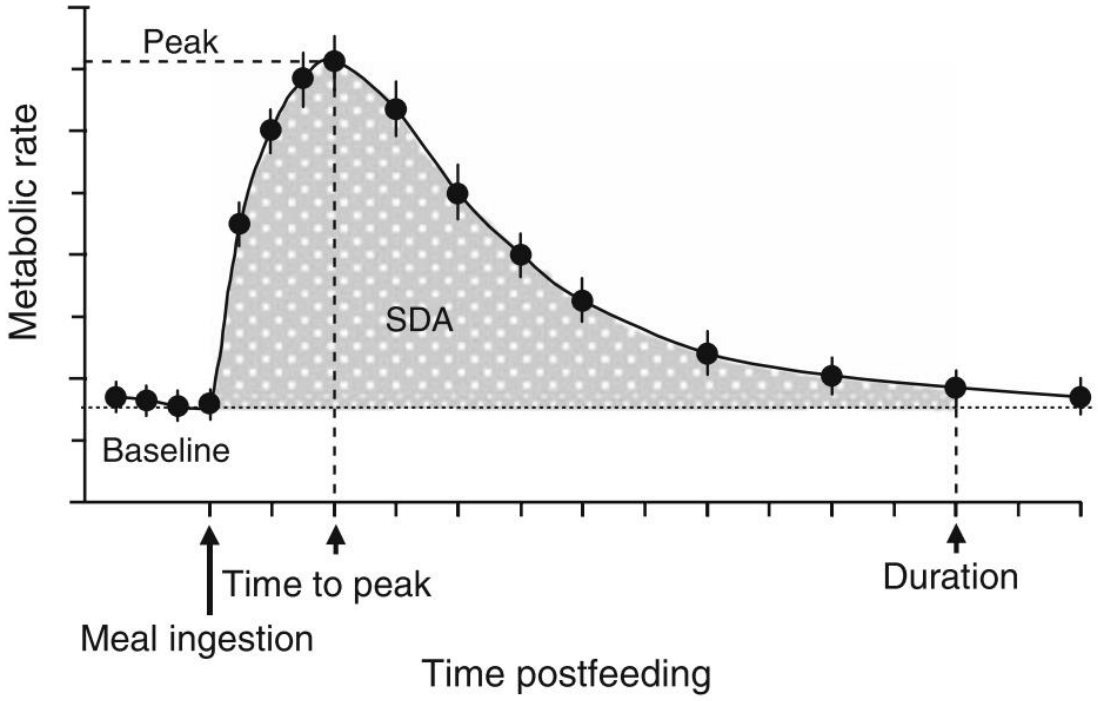

Figure 1.6. Hypothetical postprandial metabolic rate plotted against time from the point of consuming a meal to show a "SDA curve". Annotated with select SDA variables, sourced from Secor (2009).

SDA profiles (Fig. 1.6.) can be analysed in several ways as there are various ways in which the SDA of feed efficient and feed inefficient fish might vary. For example, any number or combination of the following variables could vary: 1) the duration of SDA; 2) the peak level of SDA; 3) the total energy required to process the meal; 4) the proportion of AS that the SDA peak takes up, known as the percentage reduction of AS; and 5) the proportion of the energy consumed via the meal that is then expended on SDA, known as the SDA coefficient (Dupont-Prinet et al., 2010; Fu et al., 2005; Jordan and Steffensen, 2007; Khan et al., 2015; Pirozzi and Booth, 2009). It is possible that one or a combination of the variables above could differ among individuals resulting in FCR variation as the more an individual's AS is taken up by SDA through total time or total energy requirements, the less energy available for other functions (Jutfelt et al., 2021). For example, Southern catfish (*Silurus meridionalis*)

with higher maintenance requirements had a shorter SDA duration (Fu et al., 2018), indicating that the time to clear SDA and return to baseline level was shorter, which in turn meant that SDA was processed quicker and may have lead to improved feed efficiency. The SDA coefficient estimates energy efficiency by comparing the amount of energy consumed in the meal to the total SDA energy required to digest that meal (Secor and Faulkner, 2002). Therefore, giving a percentage of the meal's energy that is used to digest the meal itself, which ranges from approximately 5 – 20 % in fish (Dupont-Prinet et al., 2010; Priede, 1985). Based on the above, SDA coefficient, SDA peak and the percentage reduction of AS are theorised to be important variables in determining the feed efficiency of Chinook salmon.

While feeding fish in a swim flume to determine SDA is possible, not all species will willingly feed in a respirometer. A way around this involves gavage feeding, where feed that is first soaked in water to soften the pellets is placed in the stomach using forceps or via a tube (Altimiras et al., 2008; Frisk et al., 2013; Thorarensen and Farrell, 2006). Prior to this process, a sham-feeding protocol is required to account for the increased oxygen consumption due to the stress of gavage feeding and handling. The sham-feeding protocol follows the same process as gavage feeding but without adding feed. Altimiras et al. (2008) found that $\dot{M}O_2$ returned to base level and plateaued 2 hours after the sham feeding in sea bass. However, Dupont-Prinet et al. (2010) found that the effect of handling lasted 3 hours. The effect of sham feeding on oxygen consumption is then subtracted from the gavage fed fish to remove the stress effect of handling, and the resulting $\dot{M}O_2$ is assumed to be due to SDA (Dupont-Prinet et al., 2010).

1.5. Behaviour

As previously discussed, feed is required to meet the energy requirements of an individual's biological process, including maintenance, growth, activity, and reproduction. However, to maximise energy accumulation, a fish needs to optimise feed intake through not just the amount of feed consumed but also the amount of energy spent foraging, i.e. the behaviours performed by animals to identify, capture, and consume feed (Killen, 2011). It is important to understand how fish eat to optimise feed intake of all individuals and minimise feed loss. However, it is hard to predict when a fish will eat and how much (Jobling, 2011). Therefore, ignoring natural feeding behaviours of fish can impact production and fish welfare and lead to problems in an aquaculture context (Damsgård and Huntingford, 2012;

Jobling et al., 2012a). Not considering how fish naturally want to feed and what stimulates them to feed can lead to inadequate feed intake, which can impact production through reduced growth, increased feed wastage and increased mortality while affecting fish welfare through ill health (Jobling et al., 2012a). It is suggested that some variation seen in growth rates among a population of farmed fish is due to behavioural differences (Huntingford et al., 2006). Therefore, understanding these inter-species and intra-individual differences is important.

1.5.1. Feeding behaviours

Individual differences in feeding activity may play a major role in explaining variation in FCR and feeding behaviours, and therefore, have the potential to act as predictors of FCR, as seen in African catfish (*Clarias gariepinus;* Martins et al., 2005). As feed intake is one of the main components of FCR, it stands to reason that any behaviour involved in the acquisition of food (e.g., monopolisation of space and/or resources, the timing of feeding within the meal, aggressive manoeuvres) would potentially exert a strong influence over feed efficiency, especially if individual feeding activity varies the energy expended (Martins et al., 2011b). As fish compete for available feed, activity levels and social interactions increase (Zhao et al., 2017), increasing overall energy expenditure during feeding (Jobling, 2011). Increasing energetic expenditure also detracts energy from growth (e.g., aggressive interactions, changes in swimming style and/or speed) and may contribute to feed efficiency variation. Examining the behaviours of feed efficient and feed inefficient fish is an under-researched area that warrants investigation.

Feeding involves a complex series of behaviours that starts with the fish identifying a food item and leads up to the point where the food is ingested or rejected. Feeding in a captive setting provides a different feeding environment than the wild. A regular feeding schedule means fish do not need to search for feed outside of these times actively. Therefore, during most of the day, fish tend to swim more uniformly throughout the tank, as feeding stimulus is absent. This is assumed to be energetically optimal for fish as they naturally use flow and schooling as a behavioural strategy to reduce drag and lower their energetic costs of locomotion (Fish, 2010; Killen et al., 2012). The addition of feed to the tank changes the activities expressed by fish. Firstly, a fish needs to search for food and detect it to orient towards the feed item (Jobling et al., 2012a; Stradmeyer, 1989).

Locomotion behaviours are required to orientate and get to the feed, including turning, acceleration, and manoeuvring (Rice and Hale, 2010). However, activity is energetically expensive, and moving body mass across a distance or changing direction comes at a cost (Adams et al., 1995; Careau et al., 2008; Fish, 2010; Hughes and Kelly, 1996; Rice and Hale, 2010). The rate of sharp-angled turns and swimming speeds increases with the addition of feed (Andrew et al., 2004b; Webb, 2011; Zhao et al., 2017). The energetic cost is further increased when fish begin performing locomotion other than in straight or linear swimming motions (Webb, 2011). Manoeuvrers can be hard to study as such a wide range of different behaviours can be performed in a range of combinations (Webb, 2011). Therefore, it is important to understand the behaviours that Chinook salmon choose to execute during feeding, which could also provide insight into the energy expenditure of this species and examine if different FCR phenotypes choose different feeding behaviours or perform them at different frequencies.

To successfully complete the feeding manoeuvre after the individual has reached the food item, it must then grasp and ingest the item (Jobling et al., 2012a; Stradmeyer, 1989; Stradmeyer et al., 1988). There are three main mechanisms for prey capture: suction, where the mouth expands to draw water into the mouth; ram, where a fish swims with its mouth open to obtain the prey item; and manipulation, which is essentially biting the item to bring it into its mouth (Higham, 2011). However, food items are not necessarily eaten immediately as they can be rejected at multiple times throughout the process. For example, once food items are approached, they can be ignored or, if ingested, be rejected and spat out (Jobling et al., 2012a; Stradmeyer, 1989). Therefore, the number of successful attempts can give an indication of energy efficiency. If an individual performs a large number of feeding attempts but rejects a large proportion of food items, then energy expenditure is occurring without compensation from the feed. While Chinook salmon feeding behaviours have been studied in the wild (Neuswanger et al., 2014), their behaviours in captivity are much less understood. Further research is required to explore how Chinook salmon feed in captivity which could provide important insight for improving feeding husbandry practices.

Feeding behaviours that can be analysed include feed intake, where the total number of pellets ingested by individual fish during a meal can be counted (Adams et al., 1995; de Verdal et al., 2017a; Øverli et al., 1998). It is also possible to measure chewing rates,

spitting, the total time spent feeding, and how long into the meal the fish begins and finishes feeding (Andrew et al., 2004a; Martins et al., 2011b; Martins et al., 2005). Understanding how the time spent feeding is used could give an indication of energy expenditure. If more time is spent capturing, e.g. such as time to get to the feed, or handling feed, e.g. time to decide whether to eat an item or spitting, this distracts from further feeding behaviours as well as taking energy away from performance traits such as growth or reproduction (Killen, 2011). For example, some studies have found that feeding behaviours correlated significantly with residual feed intake (for definition, see Section 1.2.). In particular, it was found that more efficient fish took longer to begin eating, ate for less time, and were less active (Martins et al., 2011b; Martins et al., 2005). Fish that spend less time feeding presumably use less energy, sparing more energy for metabolism and growth (Hart, 1986), which could improve feed efficiency. Thus, differences in feeding activity result in variation in energy expenditure, contributing to differences in feed efficiency (Martins et al., 2011b), as efficient fish are likely using less energy during feeding, leaving more AS available for growth. However, it is important to note that these studies were performed on individually housed fish and therefore did not include social interactions which would occur in a farming environment, though they suggested that this did not impact results. These behaviours could all provide an insight into energy requirements of individuals during feeding, which may vary with FCR phenotypes.

In African catfish, it has also been shown that feeding behaviours have a high repeatability. For example, fast eaters remain fast eaters in the same environment (Martins et al., 2005), suggesting that individual differences in feeding activity may, in part, explain variation in feed efficiency. Based on the evidence above, especially the studies of Martins et al. (2005) and Martins et al. (2011b), feeding behaviour could feasibly be used to predict and select for FCR, leading to fish welfare and financial benefits for aquaculture. However, in the absence of evidence, especially on commercially farmed salmonid species, more work is required to understand which behaviours are performed by individuals with differing FCR phenotypes, whether they remain consistent over the long term and if it is possible and practical to use feed behaviour as a way of determining feed efficiency.

1.5.2. Aggression and social hierarchies

Aggression and the maintenance of social hierarchies can be very energetically expensive, resulting in wasted energy that an individual cannot use for growth (Damsgård and Huntingford, 2012; Gilmour et al., 2005) and potentially feed conversion. However, fish often use aggressive behaviours as a tactical way of maintaining preferential access to feed and other resources (Killen, 2011). Aggression is classified as a behaviour that has the potential to or actually does cause harm to another individual and is carried out to gain or maintain resources (e.g. food, feeding sites, territory, mates, and spawning sites) or dominance (Damsgård and Huntingford, 2012). Intraspecific aggression is an issue in aquaculture (Adams et al., 2000) as it leads to uneven feed distribution amongst fish (Cutts et al., 1998), injury to other fish, such as damage to fins (MacLean et al., 2000; Moutou et al., 1998) or skin and scale loss. Aggression also results in increased stress amongst subordinates, as evidenced by increased cortisol levels (Pottinger and Carrick, 2001). Subordinate fish are forced to feed irregularly by dominant fish and, therefore, often have a lower feed intake (Bégout et al., 2012; MacLean et al., 2000). The maintenance of social hierarchies is energetically expensive for all involved; dominant fish use more energy on activity (Killen, 2011), while subordinates have less access to feed and can be immune suppressed (Damsgård and Huntingford, 2012; Noble et al., 2007). Regardless of an individual's role in an aggressive encounter, there is an increased energy cost and risk of injury (Damsgård and Huntingford, 2012).

Aggression can be quantitatively analysed in a variety of ways. Examples include the time taken to begin fighting, the frequency of attacks, and the length of a fight (Pinho-Neto et al., 2014). This can be further quantified by the type of agonistic activity such as approach, where a fish moves towards another to displace it; chasing; a nip, which is a bite to a neighbour or an attack which has the addition of a charge prior to biting (Adams et al., 1995; Øverli et al., 1999; Pottinger and Carrick, 2001). Other dominant behaviours can include maintaining territory observed by their position in the tank (bottom, midwater) for ideal conditions to limit locomotion (Pavlov et al., 2010) or to favour feed acquisition (Pottinger and Carrick, 2001; Reinhardt, 2001; Unrein et al., 2018). Also, fish that are the first to begin feeding are likely more dominant (Pottinger and Carrick, 2001). In addition, it is also possible to analyse subordinate behaviours such as escape (Øverli et al., 1999) and not eating or

eating later than other fish (Pottinger and Carrick, 2001). It is then possible to identify whether a fish is dominant or subordinate based on the frequency that these behaviours are occurring, the more aggressive moves executed or the more fights won, the more dominant an individual is (Adams et al., 1998; Damsgård and Huntingford, 2012).

Aggressive behaviours result in a social hierarchy amongst fish where dominant fish tend to have preferential access to feed and typically feed voraciously if feed is unlimited. Because of this, aggression has been observed to be higher during feeding (Damsgård and Huntingford, 2012; Noble et al., 2007). This is related to the ability to monopolise limited food resources, i.e. the more aggressive fish eat more pellets than subordinate fish (Adams and Huntingford, 1996; MacLean and Metcalfe, 2001; Øverli et al., 1998). It also results in subordinates having increased amounts of stress and cortisol levels (Gilmour et al., 2017; Gilmour et al., 2005; Montero et al., 2009; Pottinger and Carrick, 2001), causing a suppression of growth performance (Abbott and Dill, 1989; Christiansen and Jobling, 1990; de Verdal et al., 2019; Montero et al., 2009), feed intake (Montero et al., 2009), metabolic rate (Sloman et al., 2000), and feed efficiency (de Verdal et al., 2019; Martins et al., 2011b; Martins et al., 2008; Martins et al., 2006). Therefore, subordinates are more likely to have lower growth rates and be less feed efficient. Studies on Chinook salmon suggest that feed inefficient individuals eat a larger share of the meal compared to efficient Chinook salmon (Esmaeili et al., 2022a), and individuals that eat more are more likely to be dominant (Alanärä, 1996; Esmaeili et al., 2022b). Therefore, it is possible that in Chinook salmon, inefficient fish are more likely to be dominant. However, this observation was based on proteomics and feed intake of the individuals and did not include any behavioural observations, so further research incorporating direct observations of behaviours of Chinook salmon and their potential impact on feed efficiency is still required.

1.5.3. Methods to analyse fish behaviour

Behaviour can be measured in various ways in fish depending on the behaviour of interest. Indirect methods can be used to infer encounters with other fish or feed intake; this can include analysing scars, fin wear and stomach contents analysis (Bégout et al., 2012). However, more direct observational methods can be used to understand fish behaviour in culture systems. These methods include video monitoring, electronic tags, echo integration and demand feeders (Alanärä and Brännäs, 1993; Bégout et al., 2012; de

Verdal et al., 2017a; Oppedal et al., 2011). Video recording is a common method used to analyse behaviours, allowing in-depth analysis with little disruption to the fish. However, as mentioned previously, a limitation of this method is the number of fish that can be analysed at one time, potentially limiting the influence of social interactions.

In terms of feed intake, the ballotini method is a good method for assessing groups of fish at similar densities of farming conditions. This method can also be modified to provide additional information on fish feeding behaviours. For example, previous studies have shown that the incorporation of different sized beads into different feeds is a useful tool for determining feed selection preference (Amundsen et al., 1995; Christiansen and George, 1995; Thorpe et al., 1990; Toften et al., 2003; Toften et al., 1995) as feed containing two bead sizes can be fed within the same meal and are able to be differentiated in X-rays.

Other feed intake studies have indicated that the timing of feeding within a meal can be related to feed efficiency (Martins et al., 2011b; Martins et al., 2005). In a novel approach, this thesis will assess whether the same feed made using two different bead sizes can be fed at different times during the meal to determine when individuals with differing FCR phenotypes are feeding. This will be tested by using feed containing one bead size for the first half of the meal followed by feed containing a second sized bead in the second half. If successful, this 'dual ballotini' method will then be incorporated into the feed intake assessments to investigate the timing of feeding, requiring less time than analysing video footage. This method could then be used for direct comparison between performance traits, such as growth or FCR, and feeding behaviours in fish.

1.6. Aims and hypotheses

Chinook salmon are currently less feed efficient and have high intraindividual variation in FCR values compared to other salmonid species. Knowledge gaps need to be filled for this species to develop effective best practice and improve FCR. This thesis aimed to investigate physiological and behavioural factors that may underlie variation in FCR in farmed Chinook salmon with a particular focus on feed intake, nutrient retention, metabolism, and feeding behaviours.

Chapter 2

This chapter aimed to better understand some of the physiological factors that may influence FCR in Chinook salmon, including traits such as growth, feed intake, nutrient retention efficiency and various aspects of metabolism in saltwater reared Chinook salmon. The following parameters were examined for correlations with FCR and differences between FCR phenotypes:

- RMR_{min}, MMR, and AS.
- Protein, lipid, and energy retention efficiencies.
- Protein and lipid body composition.
- Feed intake and growth.

It was hypothesised that feed efficient fish would have a lower RMR_{min} requiring less energy to meet maintenance requirements but would also have a larger AS leaving more energy available for growth. It was also hypothesised that efficient fish would have higher growth rates and retain a larger proportion of protein, lipid and energy compared to their feed inefficient conspecifics. The relationship of lipid composition to FCR was also investigated to see if it could be used as a proxy for FCR in Chinook salmon.

Chapter 3

This chapter aimed to determine the values of different physiological traits (growth and nutrient retention efficiencies) and metabolic rates (RMR, MMR, AS) as well as SDA in Chinook salmon and compare performance across FCR phenotypes. The following traits were quantified in freshwater Chinook salmon:

- RMR_{min}, MMR, and AS values.
- SDA variables.
- Protein, lipid, and energy retention values.
- Feed intake and growth.
- The interaction of the above with FCR and between FCR phenotypes.

In addition to hypotheses in Chapter 2, it was also hypothesised that feed inefficient fish would use more energy during SDA (whether it be total energy or a larger SDA peak). This

would therefore take up a larger proportion of their AS (higher AS reduction percentage) thus, leaving less energy available for growth.

Chapter 4

This chapter aimed to determine if the ballotini X-radiography method could be adapted into a dual ballotini method by using different sized ballotini to determine the timing of feeding behaviour, and therefore establish potential links in feed timing with FCR efficiency between different FCR phenotypes. In doing so, this chapter aimed to resolve the following specific questions in freshwater Chinook salmon:

- Can two bead sizes be fed at the same time and differentiated in the X-rays?
- Can the dual ballotini method be used to determine timing of feeding in individuals?
- Are there differences in feed timing between the different FCR phenotypes?

It was hypothesised that feed efficient fish would begin feeding later in the meal to avoid the highly competitive environment seen when feed first enters the tank, therefore, lowering energy usage during feeding.

Chapter 5

This chapter aimed to define and quantify behaviours expressed by freshwater Chinook salmon during feeding, by answering the following questions:

- What behaviours do Chinook salmon perform in a tank during feeding?
- Do these behaviours occur consistently across multiple meals?
- Do the behaviours carried out differ between FCR phenotypes?

It was hypothesised that inefficient Chinook salmon would carry out more energy expensive behaviours as well as more behaviours overall thus contributing to their poor FCR values.

Chapter 2

The relationship of feed intake, growth, nutrient retention, and oxygen consumption to feed conversion ratio of farmed saltwater Chinook salmon (*Oncorhynchus tshawytscha*)

2.1. Introduction

Aquaculture is growing faster than other primary food production sectors and contributed 46 % of all fish production in 2018 (FAO, 2020). With wild capture fisheries remaining stable since the late 1980s, aquaculture has helped bridge the gap between customer demand and product supply (FAO, 2018). However, for aquaculture to be sustainable, there is a need to farm fish as efficiently as possible which can be achieved by maximising the overall level of production combined with minimum feed input (Besson et al., 2014; de Verdal et al., 2017a; Gjedrem et al., 2012). Farmed salmon is the most lucrative intensively farmed fish group and it is estimated that improving the feed efficiency of Atlantic salmon (*Salmo salar*) by just 2 – 5 % would result in annual feed cost savings of US$42.9 – 107 million worldwide (de Verdal et al., 2017a). Optimisation of feed use would improve protein retention efficiency and reduce the output of waste nutrients (e.g. ammonia and phosphorus) to the environment (Eya et al., 2011; Kause et al., 2016). Chinook salmon (*Oncorhynchus tshawytscha*) is regarded as a premium salmon species that presents a niche high value-low volume product compared to the Atlantic salmon market (Stenton-Dozey et al., 2020). A major goal of the Chinook salmon farming industry in New Zealand is to improve feed conversion ratio (FCR; Araujo et al., 2021). To achieve this a better understanding of FCR and the factors that drive it is needed.

Feed conversion ratio (FCR) can be estimated by dividing the total weight of feed consumed by the total weight gain of a group (or individual) over a set period, with lower values indicating better feed efficiency (de Verdal et al., 2017a; Knap and Kause, 2018). Atlantic salmon have a relatively efficient FCR in the range of 1.1 – 1.2 (Cook et al., 2000; Mowi, 2019; Mundheim et al., 2004), whilst farmed Chinook salmon are reported to have a more variable range of FCR with an average value of 1.8 (Araujo et al., 2021; NZKS Company, 2019; Walker et al., 2012). When estimating FCR, weight gain is straightforward to measure in individually tagged fish. However, measuring the feed intake for those individuals is far

more challenging. There are three main methods used to measure individual feed intake directly. The first involves rearing fish individually and measuring feed intake directly (Besson et al., 2019; Martins et al., 2011a; Silverstein, 2006). However, this does not account for complex social structures, such as feeding hierarchies, that influence feeding behaviours (de Verdal et al., 2017a; Gilmour et al., 2005; MacLean and Metcalfe, 2001). The second method involves recording the feeding behaviour of externally tagged fish and determining feed intake from the video footage (Øverli et al., 1998; Smith et al., 1995). This method works for small numbers of fish if the whole tank can be viewed. Unfortunately, increasing the number of individuals to commercial densities results in fish obscuring one another and prevents accurate visual-based estimates of feed intake, and it is very time-consuming (de Verdal et al., 2017a; Rodde et al., 2020). The final method involves taking X-radiographs of fish that have consumed feed with known levels of indigestible X-ray opaque beads ("ballotini"). This method has the advantage that feed intake can be estimated in individual fish reared in large groups (Bégout et al., 2012; Jørgensen and Jobling, 1992; McCarthy et al., 1992; McCarthy et al., 1993) and has already been used to estimate the FCR of individually tagged, group-reared Chinook salmon (Walker et al., 2012). Thus, the X-radiography method was selected to determine feed intake in the current study.

Weight gain and feed intake measures collectively comprise FCR, so either one or both of these measures can be adjusted separately or simultaneously to influence FCR. FCR is significantly correlated with both growth and feed intake in Atlantic salmon (Kolstad et al., 2004), while in common carp (*Cyprinus carpio*; Zeng et al., 2018) and rainbow trout (*Oncorhynchus mykiss*; Silverstein, 2006; Silverstein et al., 2005) FCR was only related to growth and not feed intake. In tilapia (*Oreochromis niloticus*) growth and feed intake were positively correlated, FCR and growth were negatively correlated while FCR and feed intake were not correlated at all (de Verdal et al., 2017b). This complex relationship between FCR, growth and feed intake is species-specific and currently unknown for Chinook salmon.

It has been argued that feed efficiency alone is a limited measure and only accounts for feed input instead of the more important nutritional content of feed and how it is incorporated into the animal's body composition (Azevedo et al., 2004a; Fry et al., 2018). It is therefore important to also examine how individual nutrients are retained. Retention efficiency values are determined by dividing the total amount of the nutrient gained (e.g.

protein) by the total nutrient consumed. Protein retention efficiency is correlated to feed efficiency in rainbow trout, indicating that nutrient retention is potentially an essential component of efficient growth in salmonids (Eya et al., 2013; Silverstein et al., 2005). In European sea bass and Atlantic cod, fish that had poor feed efficiency also tended to have low lipid retention (Du et al., 2005; Peres and Oliva-Teles, 1999). However, in Atlantic salmon, lipid retention did not correlate with feed efficiency (Kolstad et al., 2004). Energy retention efficiency (ERE) is species-specific and varies with body size in salmonids, which may be due to differences in the proportion of protein and lipid deposition and may also be related to feed efficiency (Azevedo et al., 2004a, 2004b).

Metabolism comprises a series of chemical reactions within the cells of an individual and provides the energy for essential life support (body maintenance) and non-essential life processes, such as growth, movement, and reproduction etc. (Kornberg, 2020). Growth requires high levels of protein synthesis, which is an energetically expensive process, so it is not surprising that growth and metabolic rate proxies (i.e. rates of oxygen consumption) often show a positive relationship in fish (Jobling, 1985; Metcalfe et al., 2016). The minimum rate of oxygen consumption to support core body maintenance (i.e. protein turnover and repair, heart rate, ventilation, and blood flow) in an unfed immobile, post-absorptive ectotherm is usually termed the standard metabolic rate (SMR; Armstrong et al., 2011; Clark et al., 2013; Nelson, 2011). Protein synthesis comprises a relatively large proportion (12 – 25 %) of SMR (Houlihan et al., 1995; Jobling, 1993; Lyndon et al., 1992), so it could be theorised that SMR would be positively linked to growth and potentially FCR. However, protein synthesis within SMR does not seem to lead to growth, as protein synthesis is equal to protein degradation at this level of body maintenance (Houlihan et al., 1995). SMR could be used to determine which of these apply for Chinook salmon; however, some species, including Chinook salmon, are highly stress-sensitive, so it is difficult to hold these species immobile to measure SMR. Instead, these species are held in a swim flume respirometer at the lowest possible swimming speed (e.g. 0.5 body lengths [BL]/s). However, this estimate of minimal oxygen consumption is not truly SMR and is instead defined as minimal routine metabolic rate (RMR_{min}), where RMR is fasting oxygen consumption with slightly less rigor and allows for some normal activity (Brett, 1972; Chabot and Ouellet, 2005) and serves as the closest approximation to SMR in a stress-sensitive species, such as Chinook salmon.

Metabolic rates above SMR typically support growth and other non-maintenance processes, such as swimming etc., but are constrained by the upper metabolic rate, defined as maximum metabolic rate (MMR; Auer et al., 2017; Metcalfe et al., 2016; Norin and Clark, 2016). The difference between SMR and MMR defines aerobic scope (AS; Claireaux and Lefrancois, 2007; Clark et al., 2013; Fry, 1957), and this can correlate positively with growth across individuals (Claireaux and Lefrancois, 2007). This is presumably because a larger AS can accommodate a higher level of protein synthesis, hence growth. However, differences in MMR and AS between different FCR phenotypes have not been measured in fish and warrants further investigation given the high cost of protein synthesis (Houlihan et al., 1995).

This study examined the FCR of farmed saltwater Chinook salmon under semi-commercial tank conditions. The ballotini X-radiography method was used to determine the feed intake of individual fish and assign individuals into groups fish based on their FCR phenotype. How growth, feed intake, nutrient retention levels, and various oxygen consumption rates (RMR_{min}, MMR, AS) correlate with FCR phenotypes was examined to assess which factors may be identified as key drivers of FCR in Chinook salmon. This can then be used to better understand the factors potentially driving FCR in Chinook salmon and develop a more informed selective breeding goals.

2.2. Materials and methods

2.2.1. Fish and trial set up

All-female Chinook salmon were sourced from a commercial salmon hatchery (Sanford's Kaitangata) and reared in freshwater by a commercial company, Salmon Smolt New Zealand, Kaiapoi, before transfer to the Finfish Research Centre at the Cawthron Aquaculture Park, Nelson, New Zealand, on 18 December 2018. A total of 2,186 juveniles were anaesthetised in tricaine methanesulfonate (TMS; 65 mg/L) and individually tagged with passive integrated transponder (PIT) tags (HIDGlobal, EM4305, 12 mm long and 2 mm diameter glass tags) by inserting the tag into the abdominal cavity via a small incision (< 5 mm) between the pectoral and pelvic fin using a disinfected scalpel blade. The fish were transferred into four 8,000 L tanks (528 – 558 fish per tank) containing water with a salinity of 18 – 20 ppt at 15.2 ± 0.1 °C on arrival. The temperature was increased by 0.5 °C per day up to 17 °C starting on 19 December 2018. Fish were acclimatised to full saltwater (35 ppt)

over 16 days, and 44 – 45 days after transfer all fish were assessed for weight and length and re-distributed evenly into five 8,000 L tanks (420 – 435 fish per tank) for the start of the trial.

A recirculation system provided clean oxygenated saltwater at 17.1 ± 0.2 °C to all tanks. The photoperiod was set to 24-hours artificial light throughout the trial to align with common practice on Chinook salmon farms, as this inhibits sexual maturation in the fish. The fish were hand fed once daily to satiation in the morning on a commercial diet (Skretting Orient A 2000, Table 2.1.). Pellet size was increased as the fish grew (4, 6, and 9 mm) following the manufacturer's guidelines. The feed bucket was weighed before and after the meal to determine the weight of feed delivered to each tank. Uneaten pellets sank to the tank bottom and were collected with a swirl separator. Approximately 15 minutes after each meal, the uneaten feed was recovered from each tank because the pellets are stable and do not breakdown during this time. Pellets were then dried before being counted using an automated counter (Contardor2, PFEUFFER GMBH, Kitzingen, Germany). The number of uneaten pellets was multiplied by an average pellet weight and subtracted from the total feed delivered to the tank to calculate the total feed consumed by the tank for each meal.

Table 2.1. Diet compositional data

	4 mm	**6 mm**	**9 mm**
Protein (%)	44	42	36
Lipid (%)	24	29	26
Moisture (%)	8.9	8.3	8
Ash (%)	8.2	9	5
Energy (kJ/100 g)	1636	1804	1574

2.2.2. Fish growth assessments

Fish were weighed five times throughout the trial to assess growth (Fig. 2.1.); individual feed intake was measured during three of these assessments. Prior to any handling event, fish were crowded in their rearing tank. Groups of fish were then netted and anaesthetised in 200 L bins containing TMS (65 mg/L) until they lost equilibrium and became unresponsive

to touch. Oxygen levels within the bins were monitored during handling. Fish were scanned into a computer using a microchip tag reader (Avid-Power Tracker VI, Avid Identification Systems, Inc. CA, USA), weighed using a digital balance (to 1 g), and their fork length measured (to 1 mm). The external appearance of fish was assessed, and only presumed healthy fish that had gained weight since the last assessment were kept for the following assessment. At each of these assessments, fish were culled as required to keep densities below 26 kg/m^3. During assessment 2, fish were redistributed into 11 tanks to allow for further growth and to maintain target densities.

2.2.3. Ballotini method and estimation of daily feed intake

The X-radiography method used to measure the daily feed intake was based on the method of Talbot and Higgins (1983) and Walker et al. (2012). X-ray images were obtained using an Atomscope HFX90V EX9025V portable X-ray unit (DLC Australia Pty, Ltd, Melbourne, Australia) and Canon CXDI-410C Wireless Cesium Amorphous Silicon digital radiographic receptor (DLC Australia Pty, Ltd, Melbourne, Australia).

Skretting supplied feed mash, with ballotini beads incorporated, made to the same specifications as the commercial feed used in the trial. Two different pellet sizes (6 and 9 mm) containing ballotini were manufactured using this mash by CSIRO Australia's feed

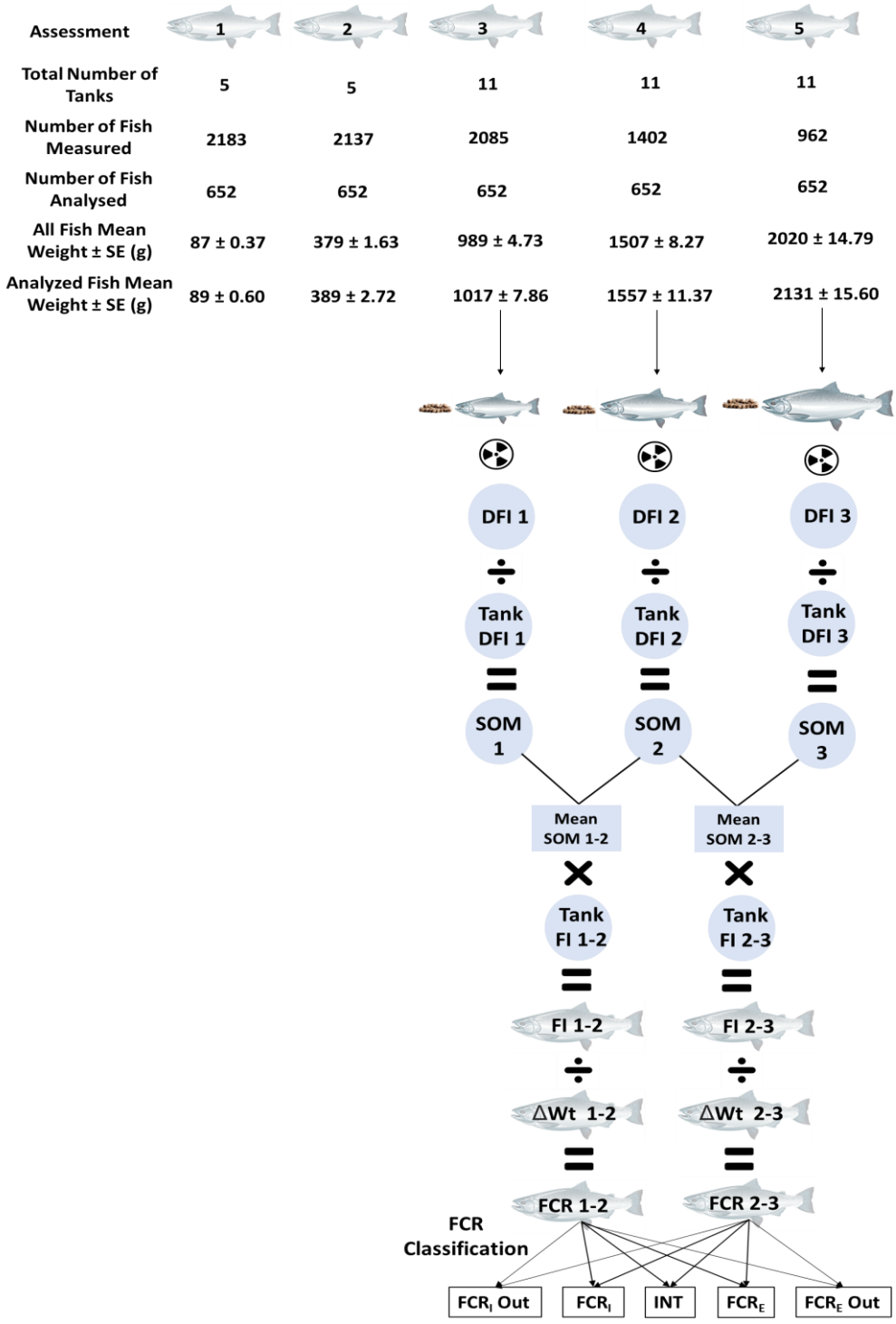

Figure 2.1. Schematic diagram of weight assessments (1 – 5) and daily feed intake assessments (DFI 1 – 3). The share of the meal (SOM) is determined from DFI assessments for each fish by dividing the individuals' DFI by the overall tank feed intake based on the sum of X-radiography images. The mean SOM between the periods (e.g. SOM 1 – 2) was used to multiply the total feed consumed by the tank between the two assessments (e.g. Tank FI 1 – 2) to estimate the total feed eaten by an individual (e.g. FI 1 – 2). The estimated feed eaten by the individual is then divided by the weight gained (ΔWt) by the individual between assessments (e.g. ΔWt 1 – 2) to estimate its FCR (e.g. FCR 1 – 2). The two FCR estimates are then used to determine if an individual is efficient (FCR_E), intermediate (INT), inefficient (FCR_I), inefficient outlier (FCR_I Out), or efficient outlier (FCR_E Out).

extrusion laboratory using a twin-screw extruder. The ballotini were 0.5 mm diameter ceramic zirconium silicate ("ZS type") SiLibeads® supplied by Sigmund Lindner GmbH, Germany. The beads were added to the feed during manufacture at an inclusion rate of 1 % in 6 mm feed and 1.25 % in 9 mm feed. For each diet, a series of samples of known weight, ranging from one pellet to an amount higher than an expected meal size, were X-rayed. Beads in each sample were counted using a semi-automated bead counting software, "Bead Counter", developed by AgResearch Ltd, New Zealand (P Smale, Personal communication), where beads are marked with a coloured dot. X-rays were manually checked to account for potentially missed beads which were manually added to the count. Final bead counts were then plotted against the weight of the sample to create a calibration curve. Curve intercepts were always forced to zero, and pellet-size-specific calibration curves were made.

For each feed intake assessment, fish in their tanks were fed the ballotini feed to satiation by hand. Fish were then crowded, anaesthetised, measured for weight and fork length, and X-rayed. After X-raying, the fish were immediately returned to the same tank to recover. The final ballotini counts were used to determine the daily feed intake (DFI) of individual fish. As feed intake can vary from day to day (Kause et al., 2006b), repeated feed intake measurements are needed to improve the reliability of this method (Grima et al., 2008). Therefore, DFI measurements were done three times at approximately 6-week intervals. An interval of 6-weeks allowed the fish to recover from handling which only impacted feed intake for 6 – 8 days before returning to normal levels.

2.2.4. Individual FCR calculation

Fig. 2.1. outlines the process of identifying an individual's estimated FCR based on an individual's weight and DFI estimates at the three-timepoints using X-radiography. The sum of all the individual DFI estimates of the fish in a tank was used to calculate a tank DFI for each tank at each assessment. The individual DFI values were then divided by the tank DFI to estimate a percentage share of the meal (SOM) for each fish at each assessment, as per the following:

SOM = (individual DFI (g) / tank DFI (g)) x 100

To estimate the amount eaten by an individual on the days between DFI assessments (e.g. the period between DFI 1 and DFI 2), the mean SOM from DFI 1 and DFI 2 was

multiplied by the total feed consumed by the tank over the entire period between those two timepoints.

The estimated total feed intake of each individual was then used to calculate FCR for each period between DFI measurements according to:

FCR = total feed eaten (g) / weight gain (g)

Thus, two measures of FCR were estimated: between DFI 1 and 2 (FCR 1 – 2) and between DFI 2 and 3 (FCR 2 – 3). A low FCR value indicates a more efficient fish.

2.2.4.1. Specific growth rate

Specific growth rate (SGR) was calculated according to:

$$SGR = \frac{\ln(w_f) - \ln(w_i)}{days} \times 100$$

Where SGR is the specific growth rate (%/day), w_f is the final weight (g), w_i is the initial weight (g), and days is the number of days between measurements (Biswas et al., 2005).

2.2.4.2. Daily weight gain

Daily weight gain (DWG; g/day) was calculated according to:

$$DWG = \frac{w_f - w_i}{days}$$

2.2.4.3. Identification of feed efficient and inefficient phenotypes

Fish were categorised as having efficient (FCR_E), intermediate, or inefficient (FCR_I) phenotypes using a novel method modified from Esmaeili et al. (2021), based on three DFI measurements and two FCR estimates (Fig. 2.1.). Firstly, any fish with outlying SGR values (including values beyond 1.5 times the inter-quartile range of the data distribution or negative values) was removed from the analysis. Fish with DFI values that were less than the weight of one pellet were also excluded as the feed weight could not be accurately estimated. Only fish with complete data for all 3 DFI measurements were used in the analyses. Fish that showed outlying FCR values (values beyond 1.5 times the inter-quartile range of the data distribution) were also excluded.

For each period (FCR 1 – 2 and FCR 2 – 3), FCR was plotted as a box and whisker graph to determine the median and the first and third quartile of the distribution of FCR values (Fig. 2.2.). Values smaller than the first quartile were classified as FCR_E, values between the first and third quartiles were considered intermediate, and values greater than the third quartile were considered FCR_I. Fish that fell within the 1.5 interquartile range above and below the first and third quartile were classified as inefficient outliers or efficient outliers, respectively.

FCR varies with fish size; smaller fish are more efficient than their larger conspecifics (de Verdal et al., 2017a; Jobling, 1993; Kause et al., 2016). Therefore, fish were categorised as efficient or inefficient, rather than using absolute values, similar to Esmaeili et al. (2021). The categories were compared at both periods to select fish that had a consistent phenotype throughout the trial. Fish that remained within the same category for both FCR 1 – 2 and FCR 2 – 3 were considered consistent. Fish that were intermediate at one period and either efficient or inefficient at the other were categorized as efficient or inefficient, respectively. Fish that moved between any other categories between periods were classified as inconsistent and excluded from the analyses.

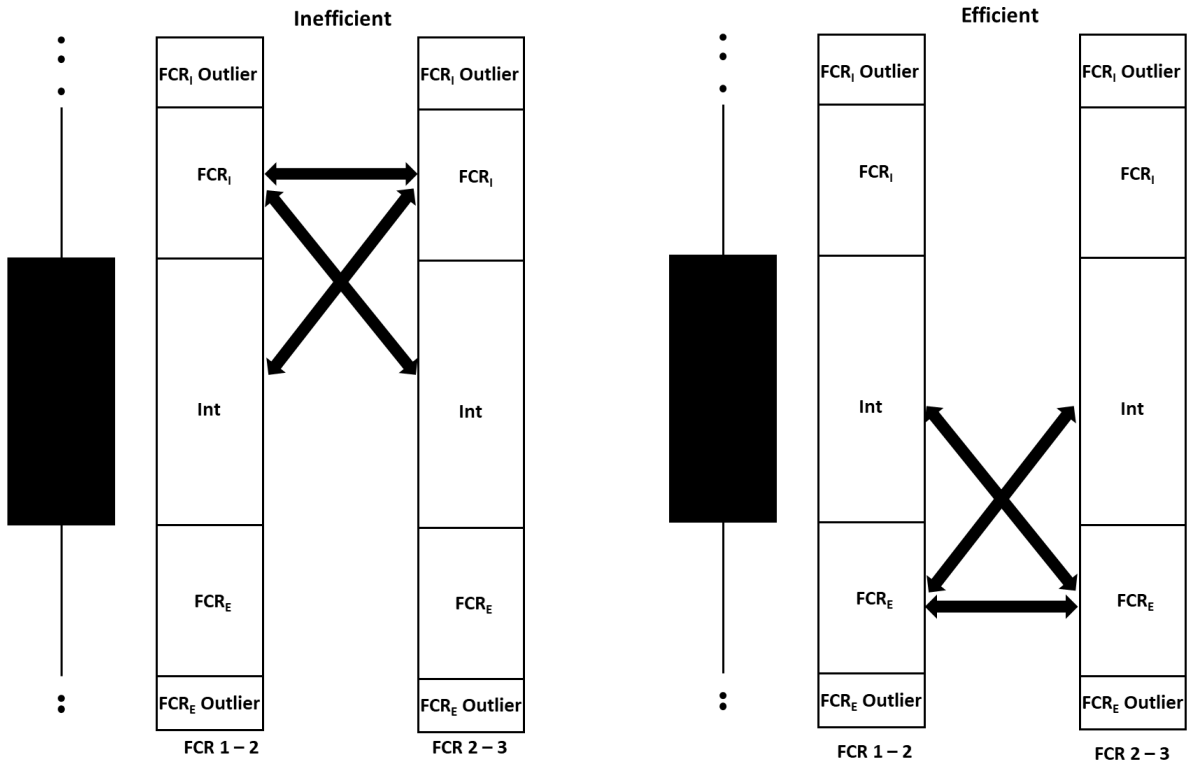

Figure 2.2. Box and whisker plots of estimated FCR across FCR 1 – 2 and FCR 2 – 3 were used to assign FCR phenotypes. The upper and lower limits of the box are, respectively, 75th percentile and the 25th percentile, values that fall within the box are classified as intermediate. The whiskers extend to the minimum and maximum values within 1.5 of the interquartile range, the values within the upper whisker are considered inefficient (FCR$_I$) while values within the lower whisker are classified as efficient (FCR$_E$). The dots represent the outliers and fish that fall above or below the whisker are classified as FCR$_I$ and FCR$_E$ outliers, respectively. Fish that were FCR$_I$ at both time points or intermediate at one period and FCR$_I$ at the other were considered FCR$_I$, the same is true for FCR$_E$.

2.2.5. Fish trait calculations

2.2.5.1. Condition Factor

Condition factor was calculated with the following equation:

$$CF = \frac{w}{L^3} \times 100000$$

Where CF is the condition factor, w is the weight (g), and L is the length (mm).

2.2.6. Nutrient composition and retention efficiency

The proximate composition of both whole fish and feed was assessed in a commercial testing laboratory (Food Testing Laboratory of Cawthron Analytical Services; Nelson, NZ). Association of Official Analytical Chemists (AOAC) methods for crude protein (AOAC 981.10),

total lipid (AOAC 948.15), moisture at 105 °C (AOAC 950.46) and ash (AOAC 920.153) were used. Energy was estimated by multiplying the total whole-body protein (g/100g) by 17 and adding this to the total whole-body lipid (g/100g) multiplied by 37 (Food Standards Australia and New Zealand, 2020). Whole fish (without skin) were blended using a food processor to form a homogenous mixture for sampling. A sample of 1 g of feed was required for protein, 2 g for lipid, 3 g for moisture and ash.

Forty fish were taken for composition analysis at DFI assessment 1. These fish were used to create calibration curves for lipid and energy by plotting nutrient against body weight. As composition sampling is terminal, these equations were used to determine what the initial nutrient concentration (C_i) of the fish used for final nutrient concentration (C_f) would have been at DFI 1 based on their body weight at this time. As there is little variation in protein composition between individuals on a percentage of body-weight basis (Knap and Kause, 2018), the mean protein composition of the initial fish was used as an initial level.

Forty-seven fish were sampled at DFI assessment 3 for the final composition analysis. Only fish that showed consistent FCR phenotypes at FCR 1 – 2 and FCR 2 – 3 were selected to calculate retention efficiency, using the equation below. Nutrient retention was then compared to FCR, however, a single overall FCR value was calculated using the mean of the three SOM values (as per section 2.4). We estimated the total feed eaten by the tank's population between DFI 1 and DFI 3 and multiplied it by the mean SOM for those selected fish for this overall value. This value was divided by the total weight gain for each fish between DFI 1 and DFI 3.

$$RE\ (\%)=100 \times \left((C_f w_f - C_i w_i)(C_{Fe} \times TFI)^{-1}\right)$$

Where retention efficiency (RE) can be protein retention efficiency (PRE), lipid retention efficiency (LRE) or energy retention efficiency (ERE), C_f is the final nutrient concentration of the fish (protein, lipid, or energy), C_i is the initial nutrient concentration of the fish, C_{Fe} is the selected nutrient concentration of the feed, and TFI is the total feed intake during the set period (g; Bendiksen et al., 2003; Biswas et al., 2005).

2.2.7. Oxygen consumption rates

Only fish that had gained weight since the last assessment and had consistently efficient or inefficient FCR phenotypes were selected for respirometry. They were removed from the

main tanks and reared in a separate but identical 8,000 L tank in the same room, which received the same saltwater supply and feed until respirometry commenced. These fish were held at a lower density (12 kg/m^3) than the main trial fish. Three days prior to respirometry measurement, individual fish were removed from this tank to a 3,000 L holding tank on a separate water system, with the same temperature and oxygen regime, to be starved for 66 – 68 h prior to being transferred to a swim flume.

Metabolic O_2 consumption rates, including resting (RMR_{min}) and maximum (MMR) rates, were determined using intermittent flow respirometry as described by Steffensen (1989). Two separate but identical swim-flume respirometers were used. Each consisted of a 182 L internal Perspex™ chamber that housed an individual fish, surrounded by a reservoir tank constantly supplied with filtered and oxygenated saltwater from the recirculation system that also supplied the holding tank from which the fish was transferred (Fig. 2.3.). A small Eheim™ pump connected the chamber and the reservoir tank (the 'flush pump') for water exchange. The internal chamber housed a temperature probe and a robust fibre-optic oxygen dipping probe (OXROB10-CL4, Pyroscience), connected to an oxygen meter (Pyroscience FirestingO_2, GmbH, Aachen, Germany). The oxygen probe was calibrated to 100 % and 0 % oxygen saturation using fully aerated saltwater and a sodium sulphite saturated solution, respectively. Calibration was carried out prior to adding a fish to the swim flume. A water current in the internal chamber was created using an impeller connected to a variable speed motor, the speed of which was manually controlled using a variable speed drive (WEG CFW500 Frequency Inverter, Georgia, USA).

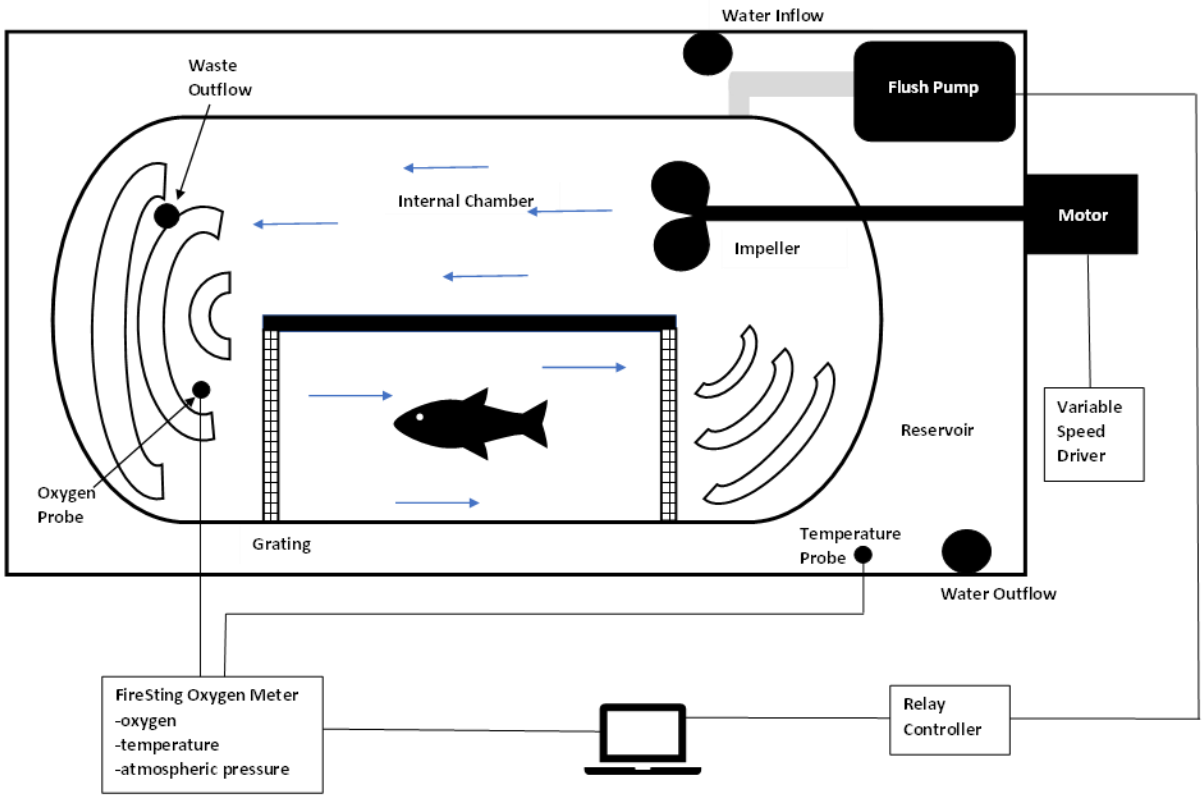

Figure 2.3. Schematic diagram of the swim flume setup. See Section 2.5.3. for written details.

The mass-specific oxygen consumption rate ($\dot{M}O_2$) was repeatedly measured by sealing a fish in the respirometer and carrying out repeated measurement cycles. Each cycle consisted of a "measurement period" where the respiratory decline in O_2 was measured, a re-oxygenation "flush period" of internal chamber water, and a one-minute "wait period". The wait period ensured thorough mixing of the water within the internal chamber. The duration of the measurement period was set to ensure that O_2 saturation declined by at least 5 % but never below 80 %. Customised software (Leigh Resp), developed by The University of Auckland, was used to control the flush pump after manual setting of the required length of "flush", "wait", and "measurement" periods. The software then recorded oxygen saturation and temperature measurements automatically into an MS Excel csv file. The decrease in chamber O_2 over time (α, $\Delta O_{2sat}/\Delta t$) was calculated automatically for each measurement period by Leigh Resp. $\dot{M}O_2$ in mg O_2/kg/hr was calculated using the following formula:

$$\dot{M}O_2 = \left(\frac{\left(\frac{\alpha}{100} \text{x } PO_2 \text{ x } V_{resp} \text{ x } \beta \text{ x } 60 \right) \text{x-1}}{M} \right) \text{x } 60$$

Where PO_2 is the measured partial pressure of oxygen at 100% saturation (kPa), V_{resp} is the volume of the respirometer minus the volume of the fish (L), β is the oxygen solubility constant (mgO_2/L/kPa) in water at a specific salinity (35ppt in this case), 60 converts seconds to minutes, -1 converts oxygen reduction to consumption, and M is the fish mass (kg). The overall equation is multiplied by 60 to convert min to hours.

Following the starvation period, the fish were anaesthetised using AQUI-S®, and weight, length, depth, and width measured for the $\dot{M}O_2$ and solid blocking calculations. Solid blocking is the effect of the fish itself 'blocking' the water, reducing the water velocity in the flume. The solid blocking calculation below corrects for this and calculates the corrected velocity (v_{cor}; Bell and Terhune, 1970) as follows:

$$v_{cor} = v \times \left(1 + \left(\left(\frac{L}{w+d}\right) \times 0.8\right) \times \left(\frac{0.25\pi wd}{S}\right)^{\frac{3}{2}}\right)$$

Where v is the velocity in the empty chamber (m/s) determined with a current meter in the empty swimming section of the flume, L is the fork length (m), w is the width of the fish (m), d is the depth of the body (m), and S is the cross-section of swim flume (m^2).

Fish were then placed in the flume, with a set (corrected) speed of 0.5 body lengths (BL)/s, and oxygen consumption measurement cycles initiated. The fish were left for 24 h to recover from handling stress and for respiration rates to decline to a steady low rate. Measurement cycle times varied between fish but the number of cycles within this period ranged from 78 to 167 cycles. The minimal routine metabolic rate (RMR_{min}) was then determined as the mean of the lowest 15^{th} percentile of the 24 h of measurements. Data was manually visually checked to ensure that RMR_{min} represented a minimum level.

The maximum metabolic rate (MMR) was measured by gradually increasing the speed in the chamber in increments of 0.5 BL/s up to 3.5 BL/s and then in increments of 0.25 BL/s until the fish could no longer maintain its swimming speed. Due to the potential stress sensitivity of Chinook salmon in the swim flume, it was decided that each speed would be maintained for one measurement cycle only. It was found that increased time spent at higher speeds resulted in fish moving rapidly around the chamber, losing their ability to

remain swimming in an upright position. MMR was defined as the highest $\dot{M}O_2$ value recorded during the entire swimming challenge.

$\dot{M}O_2$ was weight corrected to a standard 2.5kg fish using the calculation in Schurmann and Steffensen (1997):

$$\dot{M}O_{2(cor)} = \dot{M}O_{2(meas)} \times \left(\frac{w}{w_{(cor)}} \right)^{1-b}$$

Where $\dot{M}O_{2(cor)}$ is weight corrected $\dot{M}O_2$, $\dot{M}O_{2(meas)}$ is the measured metabolic rate ($mgO_2/kg/hr$), w is the weight (kg), $w_{(cor)}$ is the standard weight to correct all $\dot{M}O_2$ values to, and *b* is the metabolic scaling exponent. RMR_{min} was corrected using a scaling exponent (*b*) of 0.82 (Schurmann and Steffensen, 1997), while MMR was corrected using *b* = 0.918 (Glazier, 2009). The increase in *b* accounts for the increase in energetically expensive tissues used during swimming (Killen et al., 2010) and volume-related muscular power production (Glazier, 2009).

2.2.8. Statistical analysis

Mixed effect linear models (Zuur et al., 2009) were used to analyse the pairwise relationships between FCR, DWG and DFI. We used mixed-effect models with "tank" ID as a random factor to account for the non-independence of values coming from fish in the same tank. ANOVA and Tukey's HSD test were used to compare phenotypes across various traits (Table 2.2.). Mixed effect linear models were also used to compare RMR, MMR and AS between efficient and inefficient fish.

We also performed linear mixed effect models to analyse the relationship between FCR, DWG and DFI and nutrient retention efficiency (energy [ERE], protein [PRE] and lipid [LRE]). For this analysis, FCR and DFI values were natural log-transformed. We tested the log-transformed values for normality with an Anderson-Darling normality test (Thode Jr, 2002). We checked for any tank effect in all the models by comparing the mixed effect linear models with and without a random component, using ANOVA.

All statistical analyses were performed using 'R' (R Core Team, 2020). We used the package lmerTest (Kuznetsova et al., 2017) for performing the mixed effect linear models. Significance was accepted at $P < 0.05$.

2.3. Results

2.3.1. Daily weight gain, FCR, and daily feed intake

Fish that did not grow or demonstrated negative growth during the current study were excluded from the analyses. Specific growth rate (SGR) decreased over time from 0.94 % per day between approx. 1 kg – 1.6 kg, to 0.50 % per day when fish grew from approx. 1.6 to 2.1 kg (Table 2.2.).

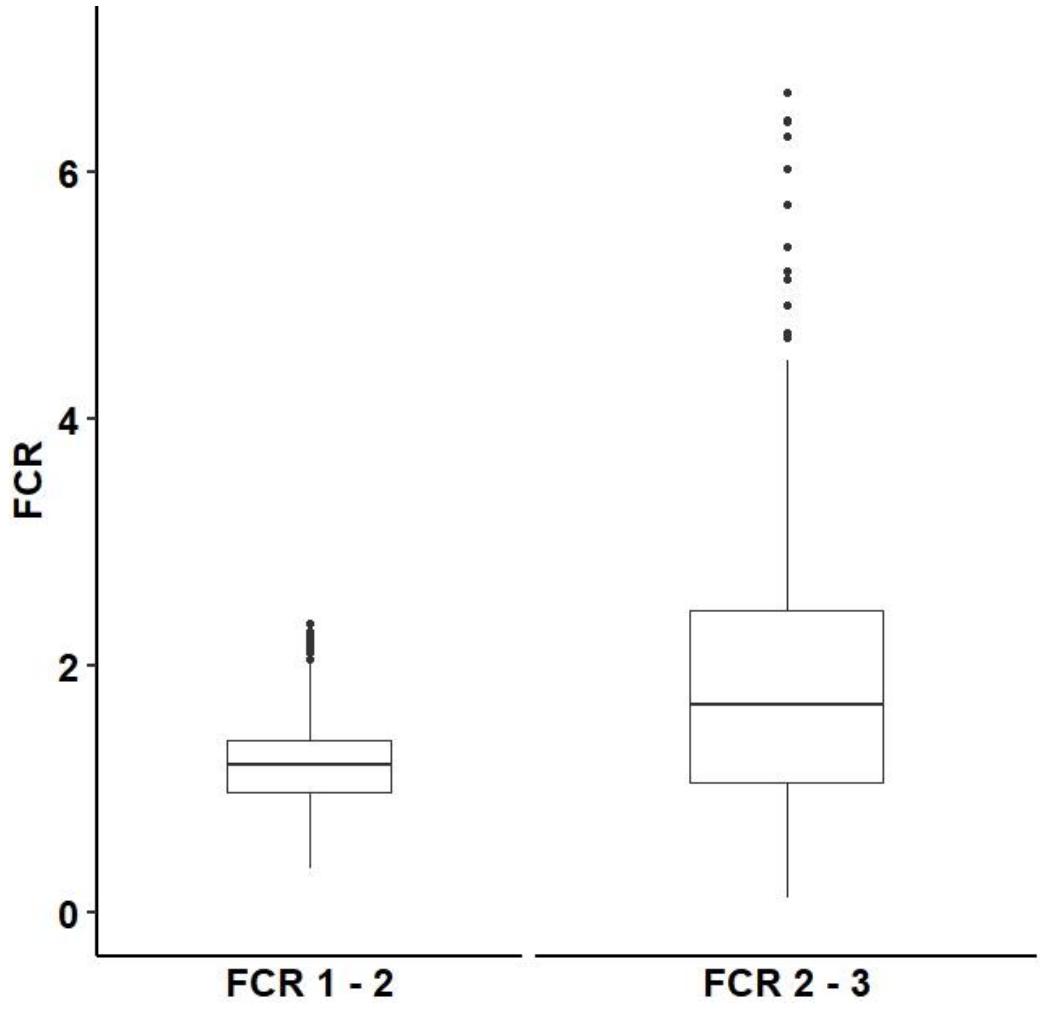

Figure 2.4. Box and whisker plot of estimated FCR across the FCR 1 – 2 and FCR 2 – 3 measurement periods. The upper and lower limits of the box are, respectively, 75th percentile and the 25th percentile. The bold line is the median of the values, the whiskers extend to the minimum and maximum values within 1.5 of the interquartile range, and the dots represent the outliers.

Overall, FCR worsened as fish increased in size; a 1 kg fish had an average FCR of 1.21, which increased to 1.89 at 2.1 kg (Fig. 2.4.). Examining the FCR of individual fish across the two measurement periods showed that FCR from the FCR 1 – 2 period was significantly correlated with FCR from the FCR 2 – 3 period for all fish (R^2 = 0.13, P < 0.0001; Fig. 2.5A.)

and between fish with consistent phenotypes (R^2 = 0.29, P < 0.0001). Of the 652 fish measured in total, 32 % were classified as feed-inefficient (FCR_I), 34 % were feed-efficient (FCR_E), 26 % were intermediate, and just 8 % of the fish were efficient outliers, inefficient outliers or inconsistent (Table 2.2.).

Table 2.2. Growth performance of the phenotypes at the daily feed intake (DFI) assessments. All data are mean values except the number of fish which is a total value. Values with different superscripts within a row are significantly different at P < 0.05 ("all fish" column excluded). Rows without superscripts have no significant differences between any groups. FCR: feed conversion ratio; SGR: specific growth rate; DWG: daily weight gain; DFI: daily feed intake; CF: condition factor; 1 – 2: measured between DFI 1 and DFI 2; 2 – 3: measured between DFI 2 and DFI 3; 1, 2 and 3 – measured at DFI 1, DFI 2 and DFI 3 respectively.

	Inefficient Outlier	Inefficient	Intermediate	Efficient	Efficient Outlier	Inconsistent	All Fish
Number of Fish	12	207	169	219	2	43	652
FCR 1 – 2	1.68^{a}	1.24^{b}	1.00^{c}	0.83^{d}	0.54^{e}	0.97^{c}	1.21
FCR 2 – 3	4.67^{a}	2.56^{c}	1.72^{d}	1.00^{e}	0.24^{e}	3.02^{b}	1.89
SGR 1 – 2 (%/day)	0.80^{d}	0.91^{bc}	0.94^{ab}	0.97^{a}	0.80^{abcd}	0.89^{cd}	0.94
SGR 2 – 3 (%/day)	0.38^{cd}	0.48^{b}	0.52^{ac}	0.53^{a}	0.57^{abcd}	0.45^{bd}	0.50
DWG 1 – 2 (g/day)	8.50^{a}	11.5^{bd}	12.11^{bc}	12.44^{c}	8.23^{abc}	10.82^{d}	11.84
DWG 2 – 3 (g/day)	5.42^{a}	8.67^{c}	9.65^{b}	9.91^{b}	8.69^{abc}	7.80^{c}	9.22
Weight 1 (g)	881	1015	1033	1023	858	984	1017
Weight 2 (g)	1269^{b}	1534^{a}	1585^{a}	1592^{a}	1238^{ab}	1481^{ab}	1557
Weight 3 (g)	1604^{d}	2083^{bc}	2171^{ab}	2210^{a}	1783^{bcd}	1978^{ac}	2131
Fork Length 1 (mm)	345	354	357	355	343	353	355
Fork Length 2 (mm)	384^{b}	400^{ab}	405^{a}	404^{a}	387^{ab}	399^{ab}	402
Fork Length 3 (mm)	421^{c}	446^{b}	452^{ab}	455^{a}	441^{abc}	445^{ab}	450
DFI 1 (g)	12.78^{ab}	13.27^{a}	11.70^{b}	12.03^{b}	8.61^{ab}	10.59^{b}	12.25
DFI 2 (g)	15.43^{ab}	14.82^{a}	12.32^{b}	8.62^{c}	0.91^{c}	9.67^{c}	11.72
DFI 3 (g)	16.29^{a}	13.42^{a}	10.41^{b}	5.35^{c}	1.28^{bc}	15.41^{a}	10.10
CF 1	2.14^{ab}	2.26^{ab}	2.25^{ab}	2.26^{a}	2.11^{ab}	2.20^{b}	2.25
CF 2	2.24	2.37	2.36	2.39	2.12	2.29	2.37
CF 3	2.15^{bc}	2.32^{ab}	2.33^{ab}	2.34^{a}	2.07^{abc}	2.22^{c}	2.32

At DFI 1 and DFI 2, the weight of FCR_E and FCR_I fish was not significantly different (P = 0.1, P = 0.28 for DFI 1 and DFI 2, respectively), but at DFI 3, the FCR_E fish were significantly heavier than FCR_I fish (P = 0.009). Condition factor did not differ significantly between FCR_E and FCR_I phenotypes at any of the three DFI assessments (Table 2.2.).

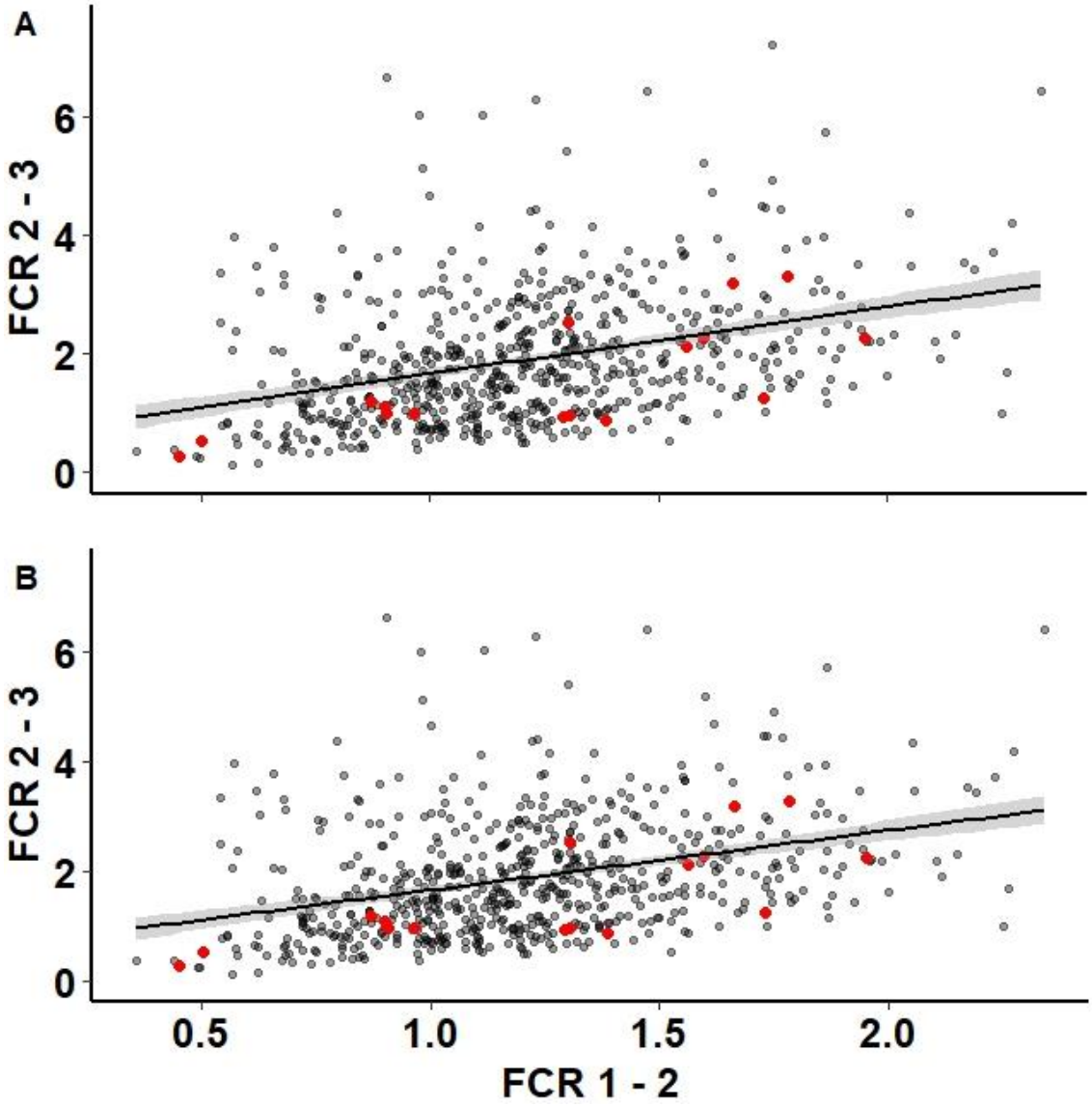

Figure 2.5. FCR of individual fish from the FCR 1 – 2 period against the FCR 2 – 3 period. A) All fish used in analyses (F = 97.67), B) Fish with consistent phenotypes only (F = 94.84). The red dots represent the fish used in respirometry work and show an even spread of FCR phenotypes.

DFI, from the end of the growth period, and DWG was correlated at FCR 1 – 2 (F = 118.1, R^2 = 0.15, P = 0.001; Fig. 2.6A.) and FCR 2 – 3 (F = 4.38, R^2 = 0.005, P= 0.04; Fig. 2.6D.). FCR and DWG had a weak but highly significant negative relationship in both FCR 1 – 2 (F = 27.61, R^2 = 0.04, P = 0.0001; Fig. 2.6B.) and FCR 2 – 3 (F = 146.5, R^2 = 0.18, P = 0.0001; Fig. 2.6E.), meaning that the feed efficient fish grew significantly faster than feed inefficient fish.

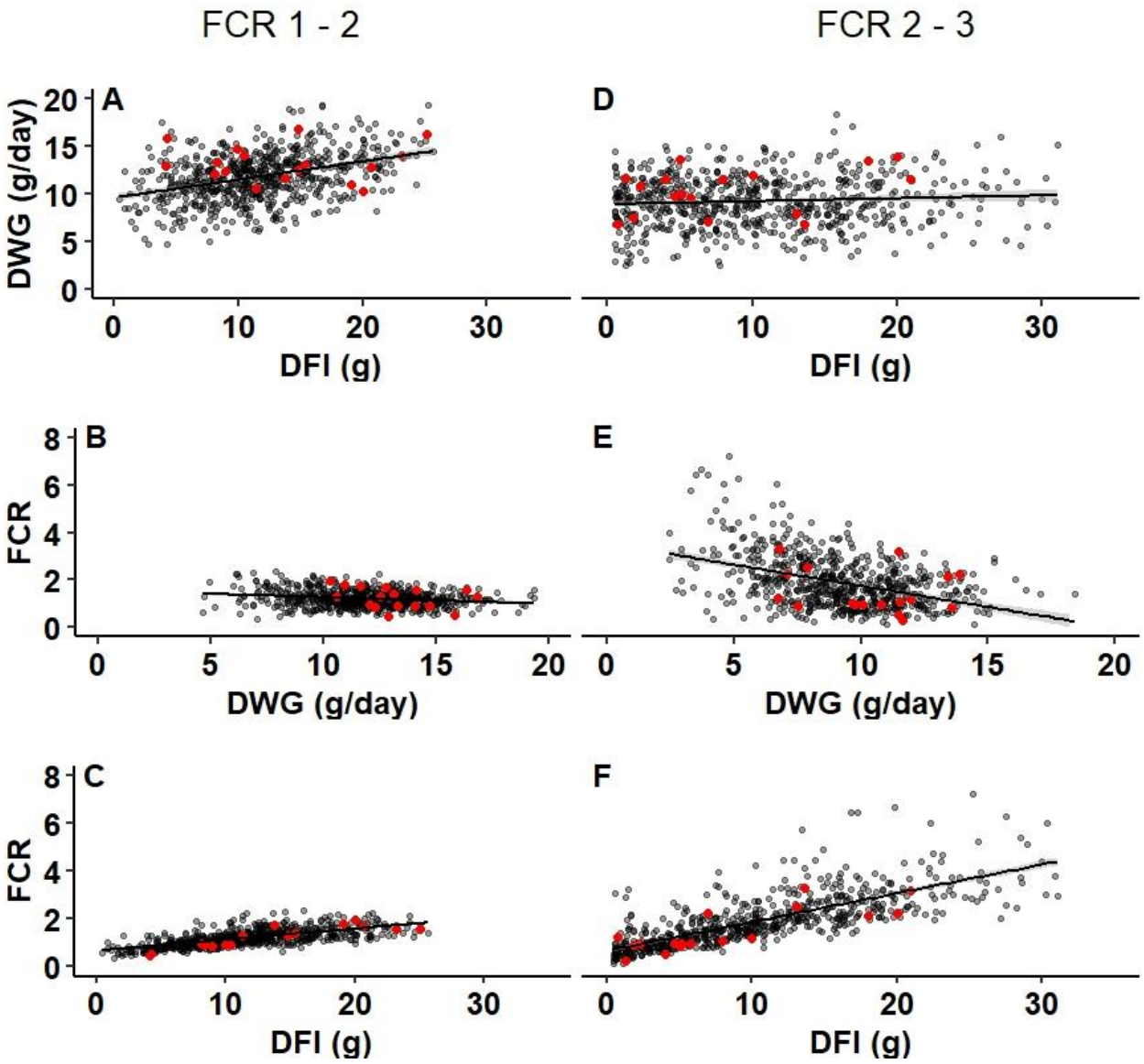

Figure 2.6. The relationship between daily weight gain (DWG), feed conversion ratio (FCR), and daily feed intake (DFI). A – C refer to timepoint "FCR 1 – 2"; D – F refer to timepoint "FCR 2 – 3". Red dots represent the fish used for respirometry and show an even spread of FCR phenotypes.

FCR had a strong positive correlation with DFI at FCR 1 – 2 (F = 548.8, R^2 = 0.46, P < 0.0001; Fig. 2.6C.) and FCR 2 – 3 (F = 910.8, R^2 = 0.58, P < 0.0001; Fig. 2.6F.) meaning feed efficient fish ate smaller meals at all time periods (Table 2.2.).

2.3.2. Nutrient retention efficiency, body composition and FCR

Protein, lipid, and total energy retention efficiencies were all significantly correlated with FCR (Fig. 2.7.): FCR vs PRE (F =223.5, R^2 = 0.81, P < 0.001), vs LRE (F = 145.8, R^2 = 0.73, P < 0.001), vs ERE (F = 181.7 R^2 = 0.77, P < 0.001). Log-transformed DFI was also significantly correlated with PRE (F = 57.26, R^2 = 0.55, P < 0.0001), LRE (F = 44.52, R^2 = 0.49, P < 0.0001), and ERE (F = 45.31, R^2 = 0.49, P < 0.0001). DWG did not show a significant correlation with PRE (F = 1.38, R^2 = 0.01, P = 0.25< 0.05), LRE (F = 1.59, R^2 = 0.01, P = 0.21< 0.05), and ERE (F = 1.63, R^2 = 0.14, P = 0.21). Protein, lipid, and energy retention efficiencies were all significantly higher in FCR_E fish compared to FCR_I and intermediate fish (Table 2.3.). No significant tank effects were detected for any of the variables. Whole body protein and lipid composition did not correlate with FCR (Fig. 2.8.; F = 0.49, R^2 = -0.01, P = 0.486> 0.05 and

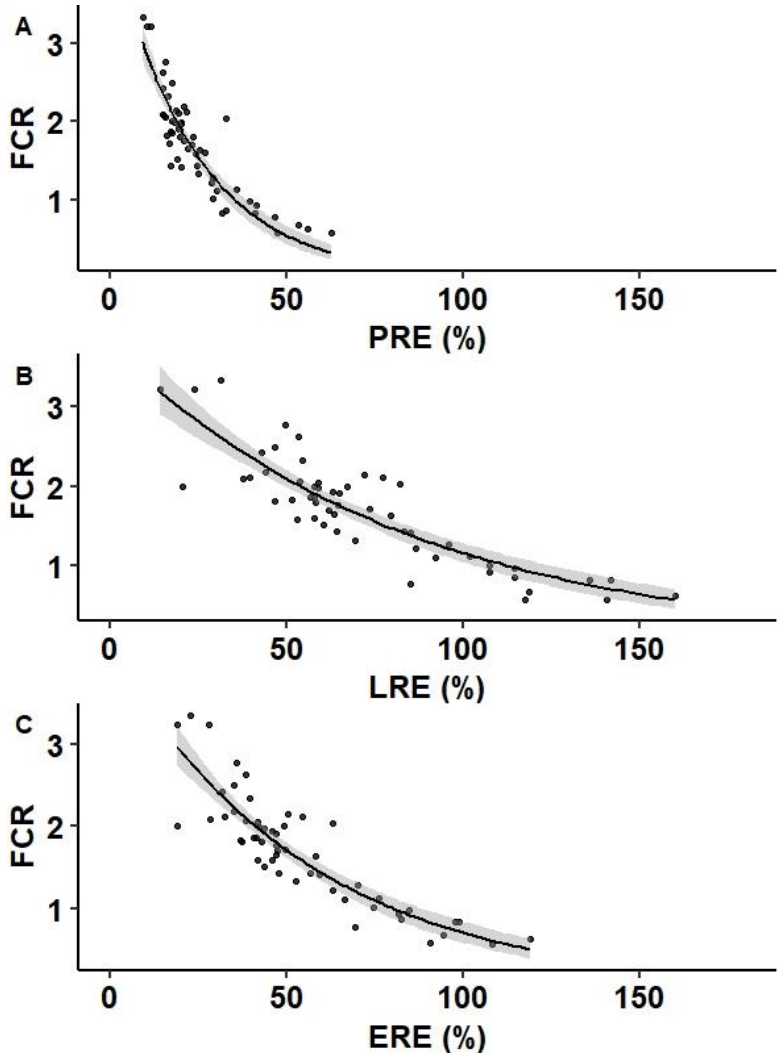

Figure 2.7. The relationship between nutrient retention values and FCR. A) Protein retention efficiency (PRE) vs feed conversion ratio (FCR). B) Lipid retention efficiency (LRE) vs FCR. C) Energy retention efficiency (ERE) vs FCR.

F = 0.422, R^2 = -0.013, P = 0.52 respectively). The body composition of FCR_E were not significantly different to FCR_I for protein (Table 2.3.; F = 0.001, P = 0.82) or lipid (F = 1.13, P = 0.174).

Table 2.3. Mean protein (PRE), lipid (LRE) and energy (ERE) retention efficiencies, whole body protein and lipid composition in each feed conversion ratio (FCR) phenotype. Values with different superscripts within a row are significantly different at P < 0.05. Rows without superscripts have no significant differences between groups.

	Efficient	**Intermediate**	**Inefficient**
Number of Fish	15	15	17
PRE (%)	39.22[a]	22.48[b]	17.79[b]
LRE (%)	108.56[a]	67.85[b]	52.49[b]
ERE (%)	81.18[a]	49.77[b]	39.69[b]
Protein Composition (%)	15.73	15.69	15.67
Lipid Composition (%)	27.87	29.06	29.18

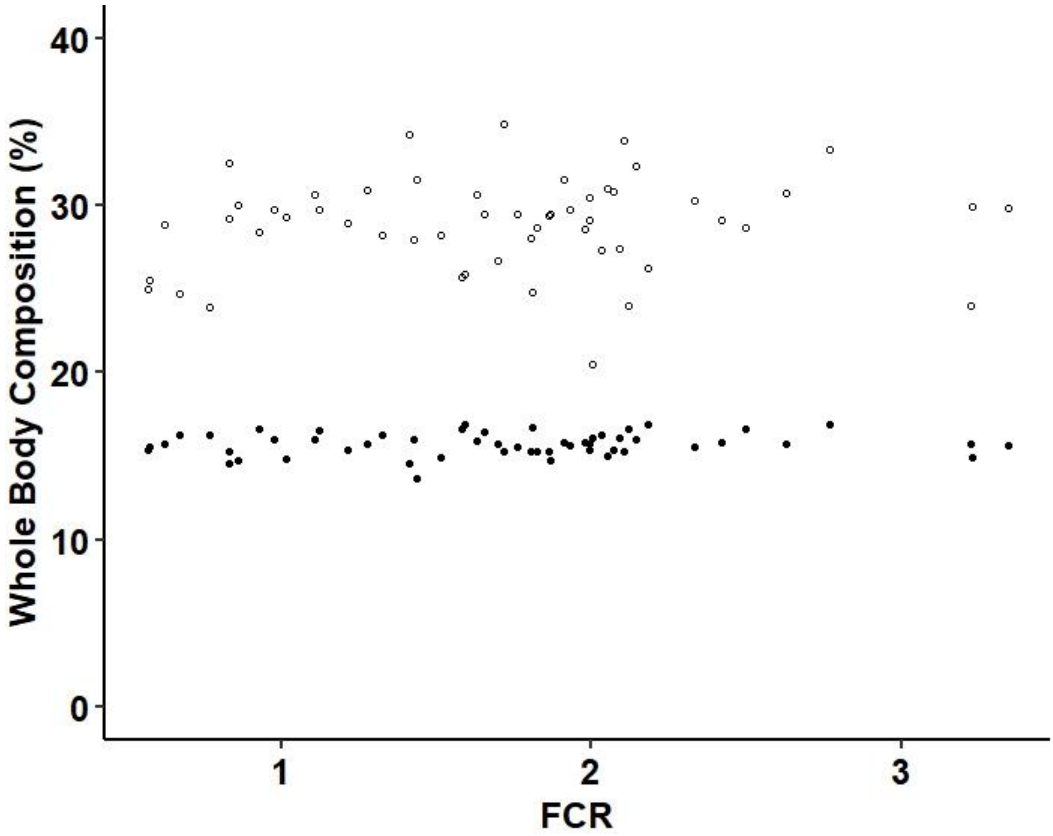

Figure 2.8. The relationship between whole body lipid (open circles) and protein (solid circles) composition (%) and feed conversion ratio (FCR).

2.3.3. Metabolism and FCR

Ten efficient and seven inefficient fish were used for swim flume respirometry. These fish covered a good range of FCR values (Fig. 2.5.). Two fish failed to swim during MMR measurement and, therefore, were excluded from the MMR and AS calculations. The average weights and lengths of fish used for respirometry did not differ significantly between the two FCR phenotypes (Table 2.4.).

Table 2.4. The metabolic rates of the efficient and inefficient feed conversion ratio (FCR) phenotypes: 1) minimum routine metabolic rate (RMR_{min}), 2) maximum metabolic rate (MMR), and 3) aerobic metabolic scope (AS), including the number of fish used and their average weight and length at the time of respirometry. Data are presented as mean values ± Standard error. $\dot{M}O_{2cor}$ is the metabolic rate corrected to that of a 2.5 kg fish. Values with different superscripts within a row are significantly different at $P < 0.05$, comparison is between efficient and inefficient phenotypes for each measure of metabolism. Rows without superscripts have no significant differences.

	RMR_{min}		**MMR**		**AS**	
	Efficient	*Inefficient*	*Efficient*	*Inefficient*	*Efficient*	*Inefficient*
$\dot{M}O_{2cor}$ ($mgO_2/kg/hr$)	107.01±4.84[a]	123.70±5.58[b]	301.62±18.45	301.93±17.20	192.55±20.84	180.78±17.66
Weight (g)	2604±126	2616±156	2522±106	2536±158	2522±106	2536±158
Length (mm)	484±7	489±12	480±8	486±14	480±8	486±14
Number of fish	10	7	9	6	9	6

Efficient fish had a significantly lower RMR_{min} than inefficient fish ($F = 5.03$, $P = 0.04$; Table 2.4.). MMR did not vary significantly between FCR phenotypes ($F = 0.0001$, $P = 0.99$). The resultant AS was slightly higher in the efficient fish ($F > 0.16$, $P = 0.70$), but it was not significantly different due to high variation in MMR.

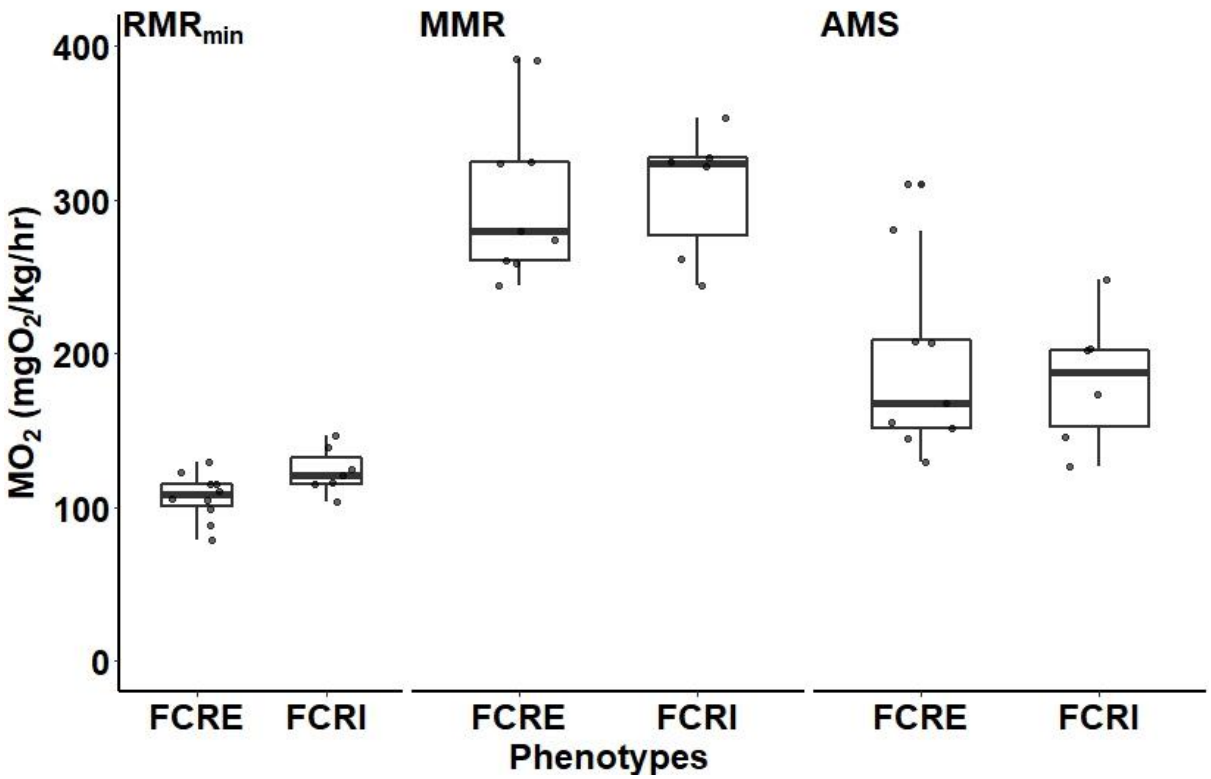

Figure 2.9. Comparison of minimum routine metabolic rate (RMR_{min}), maximum metabolic rate (MMR), and aerobic scope (AS) between feed conversion ratio (FCR) phenotypes. Dots represent individual fish values.

2.4. Discussion

Improving feed efficiency is imperative for reducing aquaculture feed costs and the environmental impacts of fish farming overall. Therefore, it is important to understand FCR variation and the underlying factors that could potentially influence FCR in Chinook salmon (*Oncorhynchus tshawytscha*) to be able to improve it, including via selective breeding. This study examined several key parameters that may be potential mechanisms underlying observed differences in feed efficiency in Chinook salmon. Feed-efficient Chinook salmon had higher growth rates, consumed smaller meals and retained more protein and lipids from feed than inefficient conspecifics. Efficient fish also had lower resting metabolism rates, indicating that some of the efficiency was gained via lower requirements for maintenance.

Using the Ballotini method, this current study showed FCR measures to be repeatable, and distinct feed-efficient and feed-inefficient phenotypes were identified. A positive relationship between growth rate and FCR has also been seen in rainbow trout

(*Oncorhynchus mykiss*; Eya et al., 2013; Henryon et al., 2002; Kause et al., 2016) and Atlantic salmon (*Salmo salar*; Thodesen et al., 1999), implying that this occurs across salmonid species. Typically, it is expected that animals with higher growth rates would consume larger amounts of feed (Allen et al., 2016; Kause et al., 2016; Rodde et al., 2020). However, Silverstein (2006) suggests that efficiency may be reduced at increased meal sizes as energy is wasted and not utilised for growth. This is also seen in Chinook salmon where growth, and FCR, did not differ despite differences in feed intake (Esmaeili et al., 2022b). Our results also support this hypothesis that larger meals may be wasted, as there was low correlation between growth rate and feed intake. In fact, the FCR_E fish ate smaller meals than their FCR_I conspecifics indicating that perhaps there are limits on growth rates that cannot be overcome, such as micronutrient limitation or limits on certain (currently unidentified) physiological processes that translate into wastage of excess nutrients. The idea that excess feed intake is wasted is supported by the nutrient retention results of the current study.

According to Fry et al. (2018), mean protein retention over nine aquatic species ranged from 14 – 28 %. They found the mean PRE of Chinook to be 27 % which is within and at the higher end of this range. Azevedo et al. (2004b) and Kolstad et al. (2004) observed higher PRE values of 40 % in Chinook salmon, and 44.3 % in Atlantic salmon respectively, but lower ERE values than the current study. However, there are limitations to comparing all our nutrient retention results with other studies as these previously mentioned studies often used much smaller fish, for example 24 – 28 g, representing a 100-fold difference in size compared to fish in this study. Size-related differences may offer some explanation of study-specific differences as ERE has been shown to decrease as body size increases (Azevedo et al., 2004a). A contributing factor may be that smaller fish often eat a larger amount of feed, which is higher in protein, in terms of % body weight and have a higher retention rate (Azevedo et al., 2004b; Fry et al., 2018).

Feed efficient Chinook salmon in the current study had higher PRE, which agrees with the results of rainbow trout, a closely related species (Eya et al., 2013; Silverstein et al., 2005). Higher protein retention efficiency may be due to differences in protein metabolism. Protein synthesis was shown to have no relation to feed efficiency in Atlantic salmon (Carter et al., 1993b), but the proteomics study of Esmaeili et al. (2021) recently demonstrated that proteins involved in Chinook salmon protein synthesis pathways were enriched in FCR_E fish.

This indicates that protein synthesis may be an important factor influencing FCR in Chinook salmon. Other FCR_E salmonids, and fast-growing fish, including Atlantic salmon, grass carp (*Ctenopharyngodon idella*), and rainbow trout, have been shown to have higher rates of protein retention and reduced protein degradation (Carter et al., 1993a; Carter et al., 1993b; Kolstad et al., 2004; McCarthy et al., 1994). Kolstad et al. (2004) observed that protein retention, but not lipid retention, was significantly related to feed efficiency in families of Atlantic salmon, and Esmaeili et al. (2021) also noted that inefficient fish had higher levels of proteins in white muscle associated with proteolysis. There is thus increasing evidence that FCR_E fish differ from FCR_I in terms of protein synthesis and degradation, which promotes higher levels of PRE.

The current study found that lipid retention efficiency was also significantly correlated with feed efficiency, and a similar result was found by Eya et al. (2013) and Silverstein et al. (2005) in rainbow trout. Proteomic analyses of Esmaeili et al. (2021) also showed that several pathways related to lipid metabolism and, in particular, fatty acid synthesis were increased in the livers of efficient Chinook salmon compared to FCR_I fish which all lends support to higher LRE of FCR_E fish. However, the study of Kolstad et al. (2004), found lipid retention efficiency was not significantly related to FCR in Atlantic salmon. One reason for the differences between these studies may be that as fish increase in size, growth rates decrease, and energy storage turns from predominantly protein to lipid (Denton and Yousef, 1976; Kolstad et al., 2004; McDonald et al., 1988). Thus, a large proportion of lipid deposition occurs later on in the grow-out phase (Kolstad et al., 2004). Therefore, lipid retention may be expected to be more important at later life stages.

As feed intake can be difficult to measure in a farm setting the use of a predictor or proxy for determining FCR would be more practical. It has been shown in rainbow trout that lipid as a proportion of whole-body composition can be used as a proxy for feed efficiency, where leaner fish have better feed efficiency (Kamalam et al., 2012; Kause et al., 2016; Kinghorn, 1983). Leaner individuals are thought to be more efficient due to a lower metabolic cost of growth, as lipid deposition has a higher energy cost than protein (de Verdal et al., 2017a). Kause et al. (2016) found that leaner fish also had improved PRE, utilising a higher proportion of dietary protein for growth. In the current study, despite finding differences in retention efficiency, whole-body composition (lipid and protein) did not differ between FCR

phenotypes and there was no relationship between relative lipid content and FCR, this is consistent with the findings of Esmaeili et al. (2021).

As well as having significantly higher energy retention, feed-efficient fish in the current study were also found to be more efficient in their energy expenditure. FCR_E Chinook salmon were found to have lower maintenance costs (RMR_{min}), which is consistent with the findings of Khan et al. (2014) in hapuku (*Polyprion oxygeneios*) and Rodde et al. (2021a) in European seabass (*Dicentrarchus labrax*). However, Rodde et al. (2021a) only saw this relationship at a population level not individually. Zeng et al. (2017) also found that Chinese crucian carp (*Carassius auratus*) with lower RMR were more feed efficient, when fed a restricted ration but when fed at ad libitum they were less efficient than their high RMR counterparts. FCR_E Chinook salmon therefore appear to require less energy for maintenance, potentially allowing more energy to be allocated to growth.

2.4.1. Conclusion

In summary, the results of this study indicate that selecting for growth in Chinook salmon may result in an indirect selection for improved feed efficiency as seen in other species such as rainbow trout (Gjedrem, 1983; Kause et al., 2006b). However, Chinook salmon farmers could achieve much greater gains if they select for feed efficiency directly, as feed efficiency appears to be related to both nutrient retention and metabolic efficiency rather than growth alone. This study also found that feeding to satiation may greatly increase feed costs but have no relative benefit for production gains, as the excess food eaten by some fish appears to be underutilised. Unfortunately, selection for individual fish with low DFI values or good feed efficiency would be difficult in a marine pen setting. Besson et al. (2019) suggested that feeding fish a restricted ration and selecting fish with improved growth rates under those conditions could indirectly select for feed efficiency. This could be the case for Chinook salmon, as we have shown that FCR_E fish consume smaller meals, even when fed to satiation. However, compared to feeding to satiation, feeding a fixed ration may affect behaviour and increase competition and aggression, so the impact of these factors would need further investigation and careful management. Another option is to include tank-based evaluation of individual DFI and FCR as part of the breeding programme. This is currently being analysed as an option for Chinook salmon.

Chapter 3

The relationships between specific dynamic action, nutrient retention, and feed conversion ratio in farmed freshwater Chinook salmon (*Oncorhynchus tshawytscha*)

3.1. Introduction

Feed can make up 30 – 70 % of fish farming operating costs due to the use of high-quality ingredients and specialised manufacture of a diet that needs to remain intact in water without losing nutrients (de Verdal et al., 2017a; Goddard, 1996). Feed conversion ratio (FCR) is a measure of feed efficiency calculated as the total feed consumed divided by growth within a set time period (de Verdal et al., 2017a). By improving FCR, the amount of feed required per kilogram of fish produced is reduced, as is the output of waste nutrients (e.g. ammonia and phosphorus) to the environment (de Verdal et al., 2017a; Eya et al., 2011; Kause et al., 2016). As it stands, Chinook salmon (*Oncorhynchus tshawytscha)* as a species is less feed efficient than other farmed salmonid species, such as Atlantic salmon (*Salmo salar*) which has a mean FCR of 1.1 – 1.2 (Cook et al., 2000; Mundheim et al., 2004) compared to ~1.8 for Chinook salmon (Araujo et al., 2021; NZKS Company, 2019; Walker et al., 2012). The relationship between growth, feed intake and feed efficiency is species-dependent and understanding the interactions between these three traits is needed to improve the FCR of target aquaculture species.

Improved FCR can be achieved directly and indirectly through selection for growth and feed intake. However, in tilapia (*Oreochromis niloticus*), indirect selection for weight gain accounted for only 36 % of the improvement seen compared to when FCR was selected directly (de Verdal et al., 2022). Selection for fast growth in Atlantic salmon has indirectly resulted in improved feed efficiency through decreased protein and energy intake per kilogram of growth (Thodesen et al., 1999). Variation in the feed efficiency of Atlantic salmon is also significantly related to family lineage, growth, and feed intake (Kolstad et al., 2004; Thodesen et al., 2001), supporting the idea that FCR can be improved through direct selection as well as indirectly through selection for growth and feed intake. Recent findings by Esmaeili et al. (2021) and Elvy et al. (2022) suggest that individual variation in feed efficiency of Chinook salmon is due in part to some fish feeding to excess without any

improvement in growth. Both studies suggest that overeating may be the cause of feed inefficiency, but more work is required to test and validate this finding in different size classes and growing environments (e.g., freshwater vs. saltwater).

Measuring the consumption and retention of protein, lipid and energy by individual fish can provide insights into the variability of FCR within a population. Individual nutrient retention efficiencies are measured by the amount of the nutrient consumed compared to the change in body composition over a set period of time (Azevedo et al., 2004b; Fry et al., 2018). A direct comparison of nutrient utilization by looking at which nutrients are deposited as growth can, therefore, be obtained (Esmaeili et al., 2021). Protein retention efficiency (PRE) appears correlated to feed efficiency in rainbow trout (*Oncorhynchus* mykiss; Eya et al., 2013; Overturf et al., 2013; Silverstein et al., 2005), Atlantic salmon (Kolstad et al., 2004) and Chinook salmon (Elvy et al., 2022). Kolstad et al. (2004) also measured lipid and energy retention efficiency (LRE and ERE, respectively) in Atlantic salmon, but detected no link to FCR in this species. However, European sea bass and Atlantic cod with poor feed efficiency had lower LRE (Du et al., 2005; Peres and Oliva-Teles, 1999). ERE was not found to differ greatly in rainbow trout (Overturf et al., 2013). This indicates that nutrient retention is species-specific but is likely to be an essential component of efficient growth in Chinook salmon and thus warrants further investigation when different size classes or growing conditions are considered.

McCarthy et al. (1994) and Tuzan et al. (2019) suggest that differences in feed efficiency are due to metabolic differences. Therefore, looking at the metabolism of differing FCR phenotypes could provide an understanding of FCR variability but also act as an indirect selection criteria (Rodde et al., 2021a). Metabolism is made up of a variety of chemical processes that are required for essential maintenance as well as non-maintenance processes. The rate at which fish consume oxygen is regularly used as an indirect proxy of total metabolic outlay. The minimum rate of oxygen consumption to support core body maintenance (i.e. protein turnover and repair, heart rate, ventilation, and blood flow) in an unfed, immobile, immature, post-absorptive ectotherm is usually termed the standard metabolic rate (SMR; Armstrong et al., 2011; Clark et al., 2013; Nelson, 2011). This study used the minimal routine metabolic rate (RMR_{min}) of fish, instead of SMR, because Chinook salmon cannot be held immobile in respirometers and some level of activity is both

necessary, and to be expected (Brett, 1972; Chabot and Ouellet, 2005; Elvy et al., 2022). Metabolic rates above RMR_{min} are generally thought to support growth and other non-maintenance processes, such as swimming etc., but are constrained by the maximum metabolic rate (MMR; Auer et al., 2017; Metcalfe et al., 2016; Norin and Clark, 2016). The difference between SMR and MMR defines the aerobic scope (AS; Claireaux and Lefrancois, 2007; Clark et al., 2013; Fry, 1957), within which non-maintenance processes, including specific dynamic action (SDA), are fuelled. SDA, also known as heat increment of feeding or feeding metabolism, is the metabolic cost of digesting, absorbing, and assimilating a meal (Bureau et al., 2003; Fu et al., 2005; Norin and Clark, 2017). Quantifying SDA can therefore provide an understanding of how individuals digest and utilise feed at a metabolic level. There are several contributors to the increased energy of SDA, including: 1) increased activity during feeding, 2) digestion and absorption, 3) formation and interconversion of substrates and their retention in tissues, and 4) the formation and excretion of metabolic waste products, in which the deamination of amino acids plays a major role (Bureau et al., 2003; Chabot et al., 2016a; Cho et al., 1982; Jobling, 1993; Nelson, 2011; Soofiani and Hawkins, 1985). Post-absorptive protein synthesis is considered a key contributor to SDA so, SDA is hypothesised to play a major role in growth and feed efficiency (Carter and Brafield, 1992; Khan et al., 2015; Li et al., 2013).

Understanding how SDA varies among individuals may help to understand variation in feed efficiency. SDA profiles could potentially vary between feed efficient and inefficient fish in a number of ways: 1) the duration of SDA; 2) the peak level of SDA; 3) the total energy required to process the meal; 4) the proportion of AS that the SDA peak takes up, known as the percentage reduction of AS (Jordan and Steffensen, 2007); 5) the proportion of the energy consumed via the meal that is then expended on SDA, known as the SDA coefficient (Dupont-Prinet et al., 2010; Fu et al., 2005; Khan et al., 2015); or a combination of the above. For example, Southern catfish (*Silurus meridionalis*) with higher maintenance requirements had a shorter SDA duration (Fu et al., 2018), indicating that the time to clear SDA and return to baseline level may be functionally linked with feed efficiency. The SDA coefficient estimates energy efficiency and is approximately 5 – 20 % in fish (Dupont-Prinet et al., 2010; Priede, 1985). Therefore, SDA coefficient, as well as SDA peak and the percentage reduction of AS, is likely to indicate that more energy is available for non-

maintenance processes (e.g. growth) and are proposed as important variables for the metabolic aspects of this study.

Given the species and context-dependent nature of feed efficiency in fish, this study investigated the potential cause of poor feed efficiency in a freshwater population of Chinook salmon. The approach examined the linkage of FCR, growth, feed intake, and nutrient retention efficiency in addition to differences in metabolism, RMR_{min}, MMR, AS and SDA parameters, between different FCR phenotypes. One of the aims of this study is to see the relationships between FCR, daily weight gain (DWG) and daily feed intake (DFI) and how these align with previous studies on Chinook salmon. It is hypothesised that nutrient retention efficiencies are negatively correlated with FCR in freshwater Chinook salmon as they were in larger saltwater Chinook (Elvy et al., 2022) where as FCR improved so did nutrient retention. This study also investigated how RMR_{min}, AS, SDA parameters or a combination of these, vary between FCR phenotypes as a contributor to FCR variation. These data formed a subset of a larger program looking at selective breeding and husbandry to improve the FCR of Chinook salmon in New Zealand. Understanding the complex relationship among traits contributing to feed efficiency in this species will ultimately allow for targeted selective breeding goals to be developed which can lead to economic and environmental gains for this sector of the salmon industry.

3.2. Materials and methods

3.2.1. Fish and trial set up

All-female Chinook salmon were sourced from a commercial hatchery (Salmon Smolt New Zealand) prior to transfer to the Cawthron Institute's Finfish Research Centre, Nelson, New Zealand. Fish were held in 3,000 L tanks containing oxygen-saturated freshwater at 14 °C on arrival. After 10 – 12 days of acclimation, the fish were anaesthetised in tricaine methanesulfonate (TMS; 65 mg/L) and were assessed for weight and length and individually tagged with passive integrated transponder (PIT) tags. The PIT tags (HIDGlobal, EM4305, 12 mm long and 2 mm diameter glass tags) were implanted by making a small incision (< 5 mm) between the pectoral and pelvic fin using a disinfected scalpel blade and inserting the tags into the abdominal cavity. Fish were then randomly distributed into six 3,000 L tanks with 91 fish per tank. Eighteen days after recovery, water temperature was increased 0.5 °C per day to 17 °C.

A recirculation system provided clean oxygenated freshwater at 17 °C to all tanks throughout the trial and photoperiod was set to 24-h artificial light. During acclimation, fish were hand-fed three times a day on a 4 mm commercial diet (Tasman Ocean; protein 43.6 %, lipid 23.7 %, ash 9.7 % and moisture 7.4 %). Initially fish were hand-fed to satiation twice daily. From day 35, when fish were ~460 g they were transitioned onto one daily feed and fed to satiation on the 4 mm commercial diet. Fish were then transitioned onto a 6 mm diet (Tasman Aoraki; protein 43.6 %, lipid 25.1 %, ash 7.6 % and moisture 5.8 %) from day 86. The feed bucket was weighed before and after the meal to determine the weight of feed delivered to each tank. Uneaten feed was recovered by a swirl collector and dried before being counted by an automated counter (Contardor2, PFEUFFER GMBH, Kitzingen, Germany). The number of uneaten pellets was multiplied by an average pellet weight and subtracted from the total feed delivered to the tank to calculate the total feed consumed by the tank for each meal.

3.2.2. Fish growth and feed intake assessments

Fish were assessed for growth, and individual feed intake using ballotini and X-radiography (Elvy et al., 2022; Esmaeili et al., 2021; Walker et al., 2012). Feed containing the ballotini beads were sourced from Ridley Corporation Ltd (Queensland, Australia) using the same ingredients as the commercial feeds used in the trial. Pellets of 4 and 6 mm containing ballotini were manufactured using a twin-screw extruder. The ballotini were 0.5 mm diameter ceramic zirconium silicate ("ZS type") SiLibeads® supplied by Sigmund Lindner GmbH. The beads were added to the feed during manufacture at an inclusion rate of 1.3 % in 4 mm feed and 1.0 % in 6 mm feed. A series of samples of known weights, ranging from one pellet to an amount higher than an expected meal size, were X-rayed for each diet to create calibration curves. The number of beads in each sample was counted using a semi-automated bead counting software "Bead Counter" developed by AgResearch Ltd, New Zealand (P Smale, Pers Comm). X-rays were manually checked to account for any beads the software missed. Final bead counts were then plotted against the weight of the sample to create a calibration curve. Curve intercepts were always forced to zero, and pellet-size-specific calibration curves were made. The same method was used to count the beads from the X-rays of the fish to determine feed intake.

For each feed intake assessment, fish were hand-fed to satiation with feed containing ballotini. Fish were then crowded in their rearing tank and groups of fish were netted, removed, and placed into 200 L bins containing TMS (65 mg/L) to be anaesthetised until they lost equilibrium and became unresponsive to touch. Fish and oxygen levels within the bins were monitored during handling. Fish were scanned using a microchip tag reader (Avid-Power TracKer VI, Avid Identification Systems, Inc. CA, USA), weighed using a digital balance (to 1 g), their fork length measured (to 1 mm) and then X-rayed. The external appearance of the fish was assessed. Only visually healthy fish that had gained weight since the last assessment were kept for the following assessment. Fish were assessed for feed intake three times at approximately 4-week intervals (Table 3.1.). This interval allowed the fish to recover from handling, which only impacted feed intake for 6 – 8 days before returning to normal levels. During assessment 3, the fish were re-distributed from 6 into 7 tanks to allow for further growth and to maintain density below 26 kg/m^3.

Table 3.1. The number of tanks and fish, including mean fish weight (± standard error), at each daily feed intake assessment (DFI 1–3). Number of fish measured is the total number of fish used while the number of analysed fish do not include the excluded fish as described in Section 2.3.

DFI Assessment	1	2	3
Total Number of Tanks	6	7	7
Number of Fish Measured	546	510	490
Number of Analysed Fish	400	400	400
All Fish Mean Weight ± SE (g)	300 ± 2.43	452 ± 4.25	612 ± 5.94
Analysed Fish Mean Weight ± SE (g)	305 ± 2.77	461 ± 4.52	619 6.47

3.2.3. Individual FCR calculation

FCR efficient and inefficient individuals were identified based on an individual's weight and estimates of daily feed intake (DFI) at the three time points using X-radiography. The share of the meal method, as described by Elvy et al. (2022) and Esmaeili et al. (2021) adds the DFI estimates of the tank together to calculate a tank DFI at each assessment. The

individual DFI values were then divided by the tank DFI to estimate a percentage share of the meal (SOM) for each fish at each assessment, as per the following:

SOM = (individual DFI (g) / tank DFI (g)) x 100

To estimate the amount eaten by an individual on the days between DFI assessments (e.g. the period between DFI 1 and DFI 2), the mean SOM from DFI 1 and DFI 2 was multiplied by the total feed consumed by the tank over the entire period between those two time-points.

The estimated total feed intake of each individual was then used to calculate FCR for each period between DFI measurements according to:

FCR = total feed eaten (g) / weight gain (g)

Fish were categorised into feed efficient (FCR_E), intermediate, inefficient (FCR_I), efficient outlier or inefficient outlier phenotypes using a novel method used by Elvy et al. (2022) and Esmaeili et al. (2021). Firstly, any fish with outlying SGR values (including values beyond 1.5 times the inter-quartile range of the data distribution or negative values) was removed from the analysis (Table 3.1.). Fish with DFI values that were less than the weight of one pellet were also excluded as the feed weight could not be accurately estimated. Only fish with complete data for all 3 DFI measurements were used in the analyses. Fish that showed outlying FCR values (values beyond 1.5 times the inter-quartile range of the data distribution) were also excluded.

For each period (FCR 1–2 and FCR 2–3), FCR was plotted as a box and whisker graph to determine the median and the first and third quartile of the distribution of FCR values (Fig. 3.1.). Values smaller than the first quartile were classified as FCR_E, values between the first and third quartiles were considered intermediate, and values greater than the third quartile were considered FCR_I. Fish that fell within the 1.5 interquartile range above and below the first and third quartile were classified as FCR_I outliers or FCR_E outliers, respectively. Fish that remained within the same category for both FCR 1–2 and FCR 2–3 were considered consistent. Fish that were intermediate at one period and either efficient or inefficient at the other were also categorized as FCR_E or FCR_I, respectively. Fish that moved between any

other categories between periods were classified as inconsistent and excluded from the analyses.

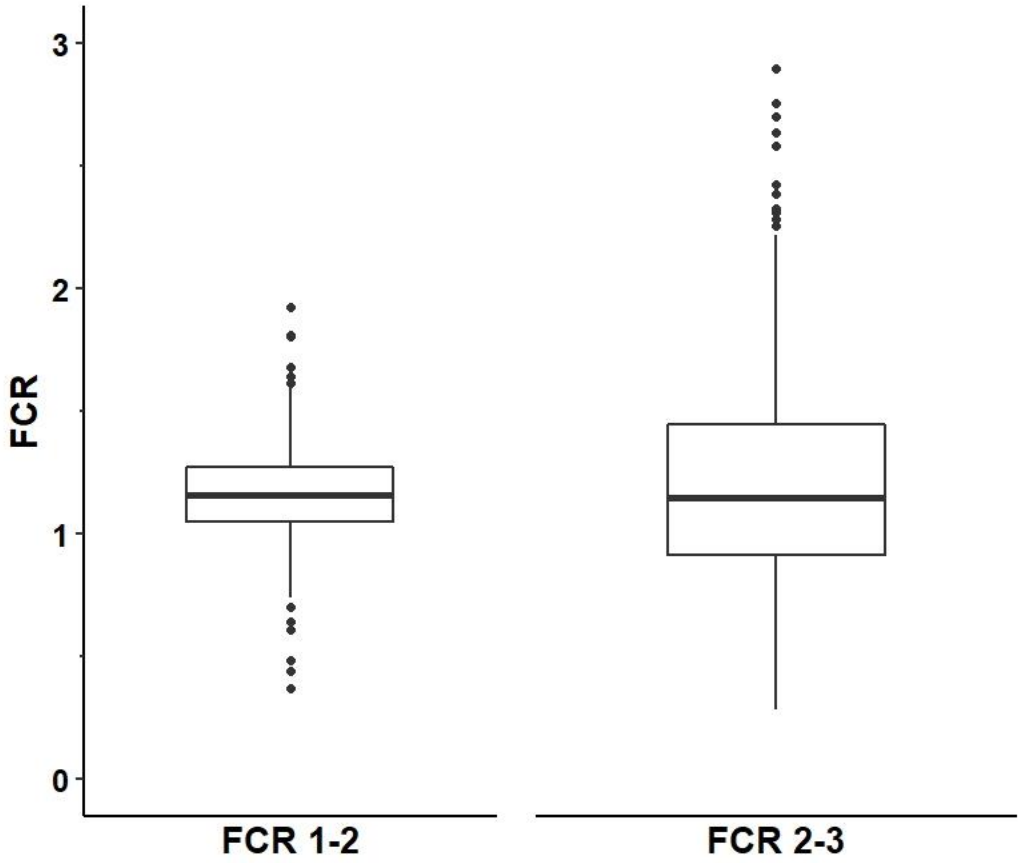

Figure 3.1. Box and whisker plot of estimated feed conversion ratio (FCR) across the FCR 1 – 2 and FCR 2 – 3 measurement periods. The box represents the inter quartile range ranging from the 25th percentile to the 75th percentile, the bold line is the median, the whiskers extend to the minimum and maximum values within 1.5 of the interquartile range, and the dots represent the outliers.

3.2.4. Fish trait calculations

3.2.4.1. Specific growth rate

Specific growth rate (SGR) was calculated with the following equation:

$$SGR = \frac{ln(w_f) - ln(w_i)}{days} \times 100$$

Where SGR is the specific growth rate (%/day), w_f is the final weight (g), w_i is the initial weight (g), and days is the number of days between measurements (Biswas et al., 2005).

3.2.4.2. Daily weight gain

Daily weight gain (DWG; g/day) was calculated according to:

$$DWG = \frac{w_f - w_i}{days}$$

3.2.4.3. Condition factor

Condition factor was calculated with the following equation:

$$CF = \frac{w}{L^3} \times 100000$$

Where CF is the condition factor, w is the weight (g), and L is the fork length (mm).

3.2.4.4. Specific feed rate

SFR = total feed eaten (g) / body weight (g)

3.2.5. Proximate composition and nutrient retention efficiency

Proximate composition of fish and feed was assessed in a commercial testing laboratory (Food Testing Laboratory of Cawthron Analytical Services; Nelson, NZ). Association of Official Analytical Chemists (AOAC) methods for crude protein (AOAC 981.10), total lipid (AOAC 948.15), moisture at 105 °C (AOAC 950.46) and ash (AOAC 920.153) were used. Energy was estimated by multiplying the total whole-body protein (g/100g) by 17 and adding this to the total whole-body lipid (g/100g) multiplied by 37 (Food Standards Australia and New Zealand, 2020). Whole fish (without skin) were blended using a food processor to form a homogenous mixture for sampling. A sample of 1 g of feed was used for protein, 2 g for lipid, 3 g for moisture and ash.

Twenty-four fish (4 fish per tank) were used for the composition analysis at DFI assessment 1. These fish were used to create calibration curves for lipid and energy by plotting nutrient against body weight. As composition sampling is terminal, these equations were used to determine what the initial nutrient concentration (C_i) of the fish used for final nutrient concentration (C_f) would have been at DFI 1 based on their body weight at this time. As there is little variation in protein composition between individuals on a percentage of body-weight basis (Knap and Kause, 2018), the mean protein composition of the initial fish was used as an initial level.

Following respirometry (see Section 2.5.3.), fish were euthanised with an overdose of AQUI-S® and frozen until analysis to obtain the final composition values. Composition and nutrient retention values were calculated using the following equation:

$$RE\ (\%) = 100 \times \left((C_f w_f - C_i w_i)(C_{Fe} \times TFI)^{-1} \right)$$

Where retention efficiency (RE) can be protein retention efficiency (PRE), lipid retention efficiency (LRE) or energy retention efficiency (ERE), C_f is the final protein, lipid, or energy concentration of the fish, C_i is the initial nutrient concentration of the fish, C_{Fe} is the selected nutrient concentration of the feed, and TFI is the total feed intake during the set period (g; Bendiksen et al., 2003; Biswas et al., 2005).

Nutrient retention was then compared to FCR using a single overall FCR value calculated using the mean of the three SOM values (as per section 2.3.). We estimated the total feed eaten by the tank's population between DFI 1 and DFI 3 and multiplied it by the mean SOM for those selected fish for this overall value. This value was divided by the total weight gain for each fish between DFI 1 and DFI 3.

3.2.6. Oxygen consumption rates

Intermittent flow respirometry was used to determine oxygen consumption rates. Only fish that were consistently FCR_E or FCR_I and that had gained weight since the last assessment were selected for respirometry. Individual fish selected for respirometry were removed from their tank and transferred to an empty 3,000 L holding tank to be starved for 66 to 68 h prior to being transferred to a swim flume respirometer.

The mass-specific rate at which fish consumed oxygen ($\dot{M}O_2$) was used as a proxy of metabolic rate and determined using intermittent-flow respirometry as described by Elvy et al. (2022) and Steffensen (1989). Various measures of metabolism were then estimated from $\dot{M}O_2$: 1) routine metabolic rate (RMR_{min}), a close approximation of basal metabolic rate in spontaneously, active fish, 2) maximum metabolic rate (MMR), 3) aerobic scope and 4) specific dynamic action (SDA). Chinook salmon are a stress sensitive species so RMR_{min} was used as a close approximation of SMR to allow for some activity (Brett, 1972).

Fish $\dot{M}O_2$ was measured using two separate but identical swim-flume respirometers set up per Elvy et al. (2022). Each respirometer consisted of a 38.4 L internal Perspex™ chamber that housed an individual fish and was surrounded by a reservoir tank supplied continuously with filtered, oxygenated fresh water from the recirculation system. A small Eheim™ pump connected the chamber and the reservoir tank (the 'flush pump') for water exchange. The internal chamber housed a temperature probe and a robust fibre-optic oxygen probe

(OXROB10-CL4, Pyroscience), connected to an oxygen meter (Pyroscience FirestingO_2, GmbH, Aachen, Germany). The oxygen probe was calibrated to 100 % and 0 % oxygen saturation using fully aerated freshwater and a sodium sulphite saturated solution, respectively. Calibration was carried out prior to adding a fish to the swim flume. A water current in the internal chamber was created using an impeller connected to a variable speed motor, the speed of which was manually controlled using a variable speed drive (WEG CFW500 Frequency Inverter, Georgia, USA).

$\dot{M}O_2$ was measured by sealing a fish in the respirometer and carrying out repeated measurement cycles. Each cycle consisted of a "measurement period" where the respiratory decline in O_2 was measured, a re-oxygenation "flush period" of internal chamber water, and a one-minute "wait period". The wait period ensured thorough mixing of the water within the internal chamber before $\dot{M}O_2$ was measured. The duration of the measurement period was set to ensure that O_2 saturation declined by at least 5 % but never below 80 %. Customised software (Leigh Resp), developed by The University of Auckland, was used to control the flush pump after manual setting of the required length of "flush", "wait", and "measurement" periods. The software then recorded oxygen saturation and temperature measurements in a MS Excel csv file. The decrease in chamber O_2 over time (α, $\Delta O_{2sat}/\Delta t$) was calculated for each measurement period by Leigh Resp. $\dot{M}O_2$ in mg O_2/kg/hr was calculated using the following formula:

$$\dot{M}O_2 = \left(\frac{\left(\frac{\alpha}{100} \text{x } PO_2 \text{ x } V_{resp} \text{ x } \beta \text{ x } 60 \right) \text{x-1}}{M} \right) \text{x } 60$$

Where PO_2 is the measured partial pressure of oxygen of 100% air saturated water, V_{resp} is the volume of the respirometer minus the volume of the fish (L), β is the oxygen solubility constant (mgO_2/L/kPa) in water at a specific salinity (0 ppt in this case), 60 converts seconds to minutes, -1 converts oxygen reduction to consumption, and M is the fish mass (kg). The overall equation is multiplied by 60 to convert minutes to hours.

3.2.6.1. Measuring minimal routine and maximum metabolic rates

Fish were placed in the flume, with a set speed of 0.5 body lengths (BL)/s, and oxygen consumption measurement cycles were initiated. The fish were left for 24 h to recover from

handling stress and for respiration rates to decline to a steady low rate. RMR_{min} was determined as the mean of the lowest 15th percentile of the first 24 h of measurements. MMR was then measured by gradually increasing the speed in the chamber by 0.5 BL/s up to 3 BL/s and then in steps of 0.25 BL/s until the fish could no longer maintain its swimming speed. Due to the behaviour of Chinook salmon in the swim flume, it was decided that each speed would be maintained for two measurement cycles. The $\dot{M}O_2$ values were averaged at each speed and MMR was taken as the highest averaged $\dot{M}O_2$ value. It was found that increased time spent at higher speeds resulted in fish moving rapidly around the chamber and losing their ability to remain upright and swim. Following MMR measurement, the fish were left to recover for 24 h.

$\dot{M}O_2$ was weight corrected to that of a standard 1 kg fish using the calculation in Schurmann and Steffensen (1997):

$$\dot{M}O_{2(cor)} = \dot{M}O_{2(meas)} \times \left(\frac{w}{w_{(cor)}}\right)^{1-b}$$

Where $\dot{M}O_{2(cor)}$ is weight corrected $\dot{M}O_2$, $\dot{M}O_{2(meas)}$ is the measured metabolic rate ($mgO_2/kg/h$), w is the weight (kg), $w_{(cor)}$ is the standard weight you want to correct all $\dot{M}O_2$ values to, and b is the metabolic scaling exponent. RMR_{min} was corrected using a scaling exponent (b) of 0.82 (Schurmann and Steffensen, 1997), while MMR was corrected using b = 0.918 (Glazier, 2009). The increase in b accounts for the increase in energetically expensive tissues used during swimming (Killen et al., 2010) and volume-related muscular power production (Glazier, 2009). Aerobic scope (AS) was calculated as the difference between MMR and RMR_{min}.

3.2.6.2. Specific dynamic action

Preliminary trials showed that Chinook salmon would not voluntarily feed in the swim flume, so a gavage feeding method was used instead (Thorarensen and Farrell, 2006). The gavage protocol did however increase metabolic rate due to stress, and this stress effect appeared to vary between individuals. To account for this, each individual was sham fed first, and $\dot{M}O_2$ was measured while the fish was allowed to recover fully. Subsequently, all fish were gavaged with a 1 % ration, based on body weight, and SDA was measured. The time taken for $\dot{M}O_2$ to return to RMR_{min} following the sham feeding was calculated. The $\dot{M}O_2$

values that fell within this time-period following the gavage were excluded from analysis, and the resulting $\dot{M}O_2$ is assumed to be due to SDA (Dupont-Prinet et al., 2010).

The sham and gavage techniques were carried out by removing the fish from the flume and placing it in an anaesthetic bin (AQUI-S®, 17 ppm) until it was suitably anaesthetised. The fish was then placed upside down in a V-shaped fish holder. A 1 % ration was inserted into the stomach using a ram rod pushed through a silicone tube. Prior to gavage, the food was soaked in water for approximately 30 s to soften the pellets (Frisk et al., 2013). In the case of the sham feeding, just the tube was inserted. The fish was then returned to the flume and $\dot{M}O_2$ measured.

Following the removal of the sham effect, the remaining $\dot{M}O_2$ values were smoothed (5-point moving average) to account for any spontaneous movement that may have occurred during measurement periods (Norin and Clark, 2017). SDA was considered complete when four $\dot{M}O_2$ values fell within 8 % of RMR (Dupont-Prinet et al., 2010).

3.2.6.2.1. SDA parameters

Differences in SDA between phenotypes were calculated using the following parameters described by Jobling (1981) and Secor (2009):

1) The maximum O_2 take-up during the SDA process (peak; $mgO_2/kg/h$)
2) Time from feeding to max O_2 (time to peak; h)
3) Total energy required for SDA, calculated as the total energy above baseline RMR_{min}, until $\dot{M}O_2$ returns to baseline or ± 8 % of baseline in this study (SDA total energy; mgO_2/kg)
4) Time taken for SDA to return to baseline or ± 8 % of baseline in this study (SDA duration; h)
5) SDA as a percentage of the energy content of the meal (SDA coefficient; %). To calculate the SDA coefficient, the total energy used in SDA was converted from mgO_2 to kJ by dividing the total SDA by 1000 and dividing by the weight of the fish to convert it from mg O_2/kg to g O_2. This value was then multiplied by an oxygen constant of 14.06 kJ/g O_2 (Dupont-Prinet et al., 2010; Gnaiger, 1983; Jordan and Steffensen, 2007). This was then divided by the total kJ consumed by the fish (a ration of 1 % body weight at 16.7 kJ/g) to determine the SDA coefficient (Secor and Faulkner, 2002).

6) AS reduction was calculated using the following calculation (Jordan and Steffensen, 2007):

AS reduction (%) = 100 x ($\dot{M}O_{2SDApeak}$ – RMR_{min}) x $(MMR\text{-}RMR_{min})^{-1}$

SDA of individuals given a constant meal size is considered independent of body size (Andrade et al., 2005). To ensure this applies to Chinook salmon, all parameters were compared to weight using a linear model and no significant relationships were found. Therefore, SDA parameters in this study have not been mass-corrected.

3.2.6.3. Solid blocking effect

Following the starvation period, the fish were anaesthetised using AQUI-S®, and weight, length, depth, and width measured for the $\dot{M}O_2$ and solid blocking corrections. Solid blocking is the effect of the fish itself 'blocking' the water, reducing the water velocity in the flume. The solid blocking calculation below corrects for this. All speeds were corrected for the solid blocking effects by calculating the corrected velocity (v_{cor}; Bell and Terhune, 1970) as follows:

$$v_{cor} = v \times \left(1 + \left(\left(\frac{L}{w+d}\right) \times 0.8\right) \times \left(\frac{0.25\pi wd}{S}\right)^{\frac{3}{2}}\right)$$

Where v is the velocity in the empty chamber (m/s) determined with a current meter in the empty swimming section of the flume, L is the fork length (m), w is the width of the fish (m), d is the depth of the body (m), and S is the cross-section of swim flume (m^2).

3.2.7. Statistical analysis

Mixed effect linear models (Zuur et al., 2009) were used to analyse the pairwise relationships between FCR, DWG and DFI. ANOVA and Tukey's HSD test were used to compare all phenotypes (efficient, inefficient, intermediate, efficient outlier, inefficient outlier and inconsistent) across all traits (Table 3.2.). Mixed effect linear models were also used to compare RMR, MMR, AS and SDA parameters between FCR_E and FCR_I fish. Data are presented as mean ± standard error.

We also performed linear mixed effect models to analyse the relationship between FCR, DWG and DFI and protein (PRE), lipid (LRE), and energy (ERE) retention efficiency. For this analysis, FCR values were natural log-transformed. We tested the log-transformed values for normality with an Anderson-Darling normality test (Thode Jr, 2002). We checked for any tank effect in all the models by using ANOVA to compare the mixed effect linear models with and without a random component.

All statistical analyses were performed using 'R' (R Core Team, 2020). We used the package lmerTest (Kuznetsova et al., 2017) for performing the mixed effect linear models. Significance was accepted at $P < 0.05$.

3.3. Results

3.3.1. Daily weight gain, FCR, and daily feed intake

Fish that did not grow or demonstrated negative growth during the study were excluded from the analyses. Daily weight gain (DWG) decreased over time from 5.68 g per day to 5.28 g per day whilst fish weight increased from a mean of 305 ± 2.77 g to a mean of 620 ± 6.56 g (Table 3.2.). Feed conversion ratio (FCR) also increased significantly over time from 1.16 ± 0.01 (FCR 1–2) to 1.21 ± 0.02 (FCR 2–3; $P < 0.01$, $F = 0.05$, Fig. 3.1.). Different feed conversion phenotypes were observed among the 400 fish measured; 28 % were classified as feed-inefficient (FCR_I), 31 % were feed-efficient (FCR_E), 28 % were intermediate, and 14 % of the fish were efficient outliers, inefficient outliers or inconsistent (Table 3.2.).

Daily feed intake (DFI) and specific growth rate (SGR) were positively correlated at FCR 1–2 ($P < 0.01$, $R^2 = 0.16$, $F = 78.92$) and at FCR 2–3 ($P < 0.01$, $R^2 = 0.21$, $F = 104.90$). DFI and DWG were positively correlated at FCR 1–2 ($P < 0.001$, $R^2 = 0.57$, $F = 532.6$; Fig. 3.2A.) and at FCR 2–3 ($P < 0.001$, $R^2 = 0.52$, $F = 425.2$; Fig. 3.2D.). DWG and specific feed rate (SFR) were positively correlated at FCR 1–2 ($P < 0.001$, $R^2 = 0.13$, $F = 60.66$) and FCR 2–3 ($P < 0.001$, $R^2 = 0.21$, $F = 105.3$).

Table 3.2. The growth performance of all FCR phenotypes at the daily feed intake (DFI) assessments. All data are mean values except the number of fish which is a total value. FCR: feed conversion ratio; SGR: specific growth rate; DWG: daily weight gain DFI: daily feed intake; CF: condition factor; 1–2: measured between DFI 1 and DFI 2; SFR: specific feed rate; 2–3: measured between DFI 2 and DFI 3; 1, 2 and 3 – measured at DFI 1, DFI 2 and DFI 3 respectively.

	Inefficient Outlier	Inefficient	Intermediate	Efficient	Efficient Outlier	Inconsistent	All Fish
Number of Fish	10	111	113	122	4	40	400
FCR 1 – 2	1.57^{a}	1.31^{b}	1.17^{c}	1.03^{d}	0.55^{e}	1.13^{c}	1.16
FCR 2 – 3	2.09^{a}	1.49^{b}	1.14^{c}	0.87^{d}	0.67^{d}	1.48^{b}	1.21
SGR 1–2 (%/day)	1.39^{ab}	1.45^{a}	1.49^{ab}	1.54^{b}	1.46^{ab}	1.52^{ab}	1.49
SGR 2–3 (%/day)	0.85^{ac}	0.94^{ac}	1.00^{ab}	1.01^{b}	1.01^{abc}	0.89^{c}	0.97
DWG 1–2 (g/day)	4.93	5.58	5.87	5.80	4.47	5.33	5.68
DWG 2–3 (g/day)	4.19^{ab}	5.14^{ab}	5.61^{a}	5.52^{a}	4.46^{ab}	4.40^{b}	5.28
Weight 1 (g)	291^{ab}	311^{a}	315^{a}	300^{ab}	243^{ab}	281^{b}	305
Weight 2 (g)	426^{ab}	465^{ab}	477^{a}	460^{ab}	369^{ab}	428^{b}	461
Weight 3 (g)	552^{ab}	619^{ab}	645^{a}	625^{ab}	503^{ab}	561^{b}	620
Fork Length 1 (mm)	254^{ab}	258^{a}	259^{a}	257^{ab}	243^{ab}	251^{b}	257
Fork Length 2 (mm)	280^{ab}	288^{ab}	290^{a}	288^{ab}	272^{ab}	281^{b}	288
Fork Length 3 (mm)	304^{ab}	314^{ab}	318^{a}	317^{b}	304^{ab}	306^{b}	315
DFI 1 (g)	6.01^{ab}	5.71^{a}	5.33^{ab}	4.88^{b}	2.15^{c}	5.00^{ab}	5.25
DFI 2 (g)	10.10^{ab}	9.61^{a}	8.89^{a}	7.43^{b}	3.01^{c}	7.30^{bc}	8.46
DFI 3 (g)	8.96^{ab}	8.68^{a}	7.97^{ab}	7.33^{ab}	3.46^{ab}	6.10^{b}	7.76
SFR 1 (%)	2.01^{a}	1.79^{ab}	1.66^{bc}	1.58^{c}	0.84^{d}	1.72^{abc}	1.68
SFR 2 (%)	2.30^{a}	2.03^{a}	1.85^{b}	1.59^{c}	0.75^{d}	1.68^{bc}	1.80
SFR 3 (%)	1.61^{ab}	1.35^{a}	1.21^{abc}	1.59^{bc}	0.75^{c}	1.68^{c}	1.21
CF 1	1.77^{ab}	1.79^{a}	1.79^{ab}	1.75^{b}	1.67^{ab}	1.76^{ab}	1.77
CF 2	1.92	1.92	1.92	1.90	1.79	1.89	1.91
CF 3	1.95^{ab}	1.97^{a}	1.98^{a}	1.95^{ab}	1.76^{b}	1.93^{ab}	1.96

Values with different superscripts within a row are significantly different ("all fish" column excluded). Rows without superscripts have no significant differences between any groups.

FCR and DWG were not significantly correlated at FCR 1–2 ($P = 0.56$, $R^2 = -0.002$, $F = 0.34$; Fig. 3.2B.) but had a negative relationship at FCR 2–3 ($P < 0.001$, $R^2 = 0.13$, $F = 60.04$; Fig.

3.2E.). FCR and SGR had a significant negative relationship at FCR 1–2 (R^2 = 0.05, P < 0.001, F = 19.78) and at FCR 2–3 (R^2 = 0.14, P < 0.001, F = 68.35) despite having low R^2 values.

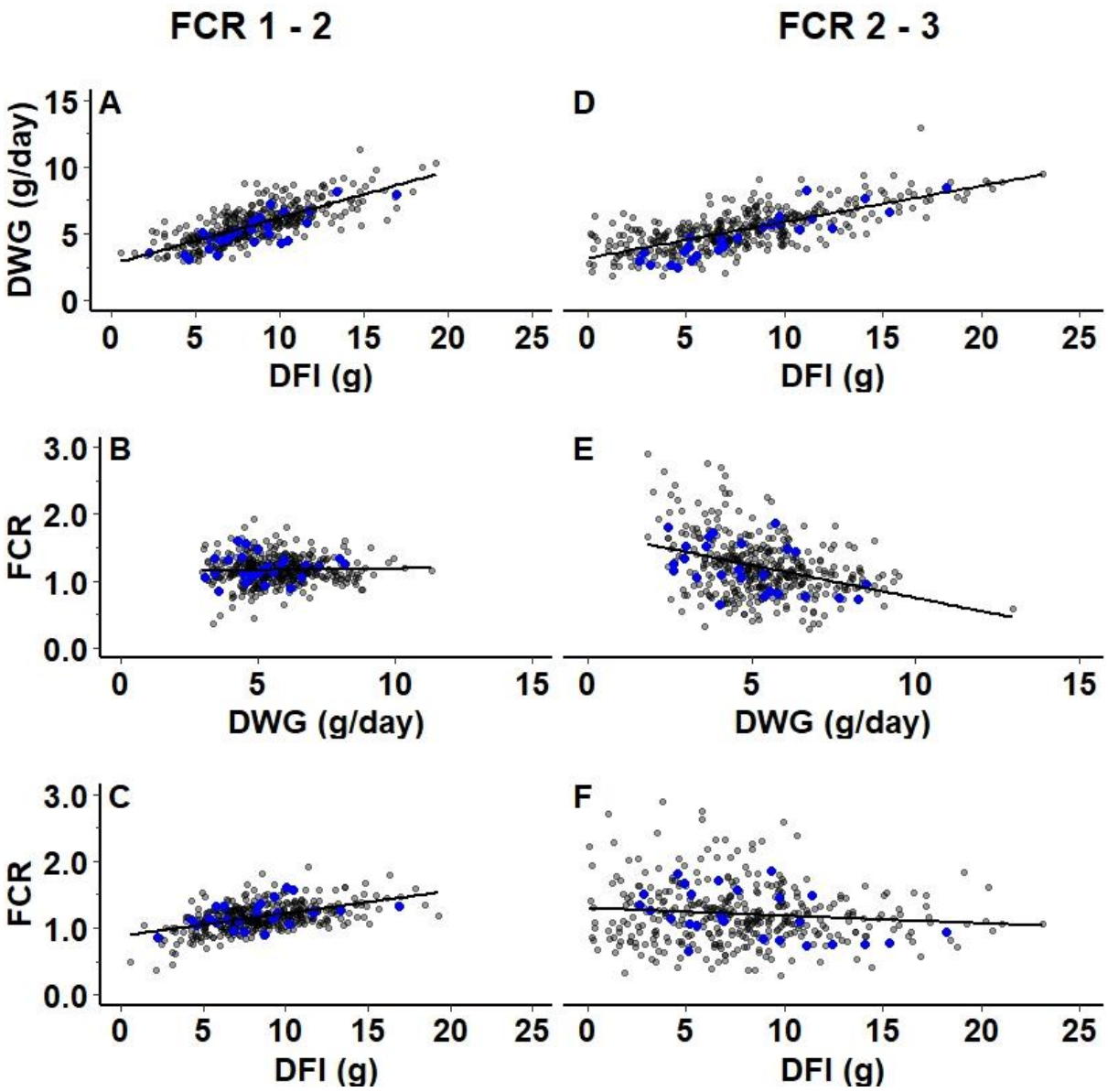

Figure 3.2. The relationship between daily weight gain (DWG), feed conversion ratio (FCR), and daily feed intake (DFI). A, B and C refer to time-period "FCR 1 – 2"; D, E and F refer to period "FCR 2 – 3". Blue dots represent the fish used for respirometry.

FCR had a significant positive correlation with DFI at FCR 1–2 (P < 0.001, R^2 = 0.26, F = 137.80; Fig. 3.2C.). However, at FCR 2–3 the relationship between FCR and DFI was negative (P = 0.02, R^2 = 0.01, F = 5.11; Fig. 3.2F.). FCR had a significant positive correlation with SFR at FCR 1–2 (P < 0.001, R^2 = 0.42, F = 286.3) but not at FCR 2–3 (P = 0.87, R^2 = -0.002, F = 0.03).

3.3.2. Nutrient retention efficiency, body composition and FCR

Protein, lipid, and total energy retention efficiencies were all significantly negatively correlated with FCR (FCR vs PRE: $P < 0.001$, $R^2 = 0.40$, $F = 16.87$; FCR vs LRE: $P < 0.001$, $R^2 = 0.51$, $F = 26.12$; FCR vs ERE: $P < 0.001$, $R^2 = 0.51$, $F = 26.08$), indicating that higher retention efficiencies were associated with feed efficiency (low FCR). DFI (mean of the three DFI's measured) did not correlate with PRE ($P = 0.15$, $R^2 = 0.05$, $F = 2.18$), LRE ($P = 0.29$, $R^2 = 0.01$, $F = 1.18$), or ERE ($P = 0.86$, $R^2 = -0.04$, $F = 0.03$). DWG did not correlate with PRE ($P = 0.23$, $R^2 = 0.02$, $F = 1.5$), LRE ($P = 0.27$, $R^2 = 0.01$, $F = 1.26$), or ERE ($P = 0.92$, $R^2 = -0.04$, $F = 0.01$). Protein, lipid, and energy retention efficiencies were all significantly higher in FCR_E fish compared to FCR_I fish (Table 3.3.). However, the whole-body composition of FCR_E fish was not significantly different to FCR_I fish in terms of percentage of protein (Table 3.3.; $P = 0.87$, $R^2 = -0.04$, $F = 0.03$) or lipid ($P = 0.52$, $R^2 = -0.02$, $F = 0.42$).

Table 3.3. The mean protein (PRE), lipid (LRE) and energy (ERE) retention efficiency, as well as whole body protein and lipid composition, for the two feed conversion ratio (FCR) phenotypes.

	Efficient	**Inefficient**
Number of Fish	12	13
PRE (%)	46.23 ± 1.97^a	33.41 ± 2.64^b
LRE (%)	114.76 ± 8.67^a	78.95 ± 5.59^b
ERE (%)	162.08 ± 8.04^a	117.67 ± 6.86^b
Protein Composition (%)	16.27 ± 0.20	16.22 ± 0.23
Lipid Composition (%)	19.90 ± 0.81	19.21 ± 0.70

Values with different superscripts within a row are significantly different. Rows without superscripts have no significant differences between groups.

3.3.3. Metabolism, SDA and FCR

The mean weights (Fig. 3.3B.), lengths and DWG (Fig. 3.3C.) of fish used for respirometry did not differ significantly between the two FCR phenotypes. RMR_{min} was measured in 12 FCR_E fish and 13 FCR_I fish with a mean value of 102.53 ± 4.97 $mgO_2/kg/h$ and 104.24 ± 4.90 $mgO_2/kg/h$ respectively ($P = 0.81$, $F = 0.06$; Fig. 3.4.). As three fish did not perform during the MMR protocol, MMR and AS were determined for 11 fish of each phenotype. MMR was found to be 338.49 ± 24.64 $mgO_2/kg/h$ in FCR_E fish and 320.70 ± 24.93 $mgO_2/kg/h$ in FCR_I and were not significantly different ($P = 0.62$, $F = 0.26$; Fig. 3.4.). Mean AS in FCR_E fish was found to be 233.51 ± 22.63 $mgO_2/kg/h$ which was not significantly different from FCR_I fish

with a mean of 218.28 ± 23.30 $mgO_2/kg/h$ (P = 0.64, F = 0.22; Fig. 3.4.). Only eight fish (four FCR_E and four FCR_I) returned to RMR following SDA and were therefore able to be used for the SDA calculations. SDA parameters were not mass corrected as no relationship was found between these values and weight, although this could be due to the narrow weight range of the fish used in this study. None of the SDA parameters were significantly different between the two FCR phenotypes (Fig. 3.5.), but statistical power was low due to a small sample size. Unfortunately, there were no more suitable fish available to increase the sample size.

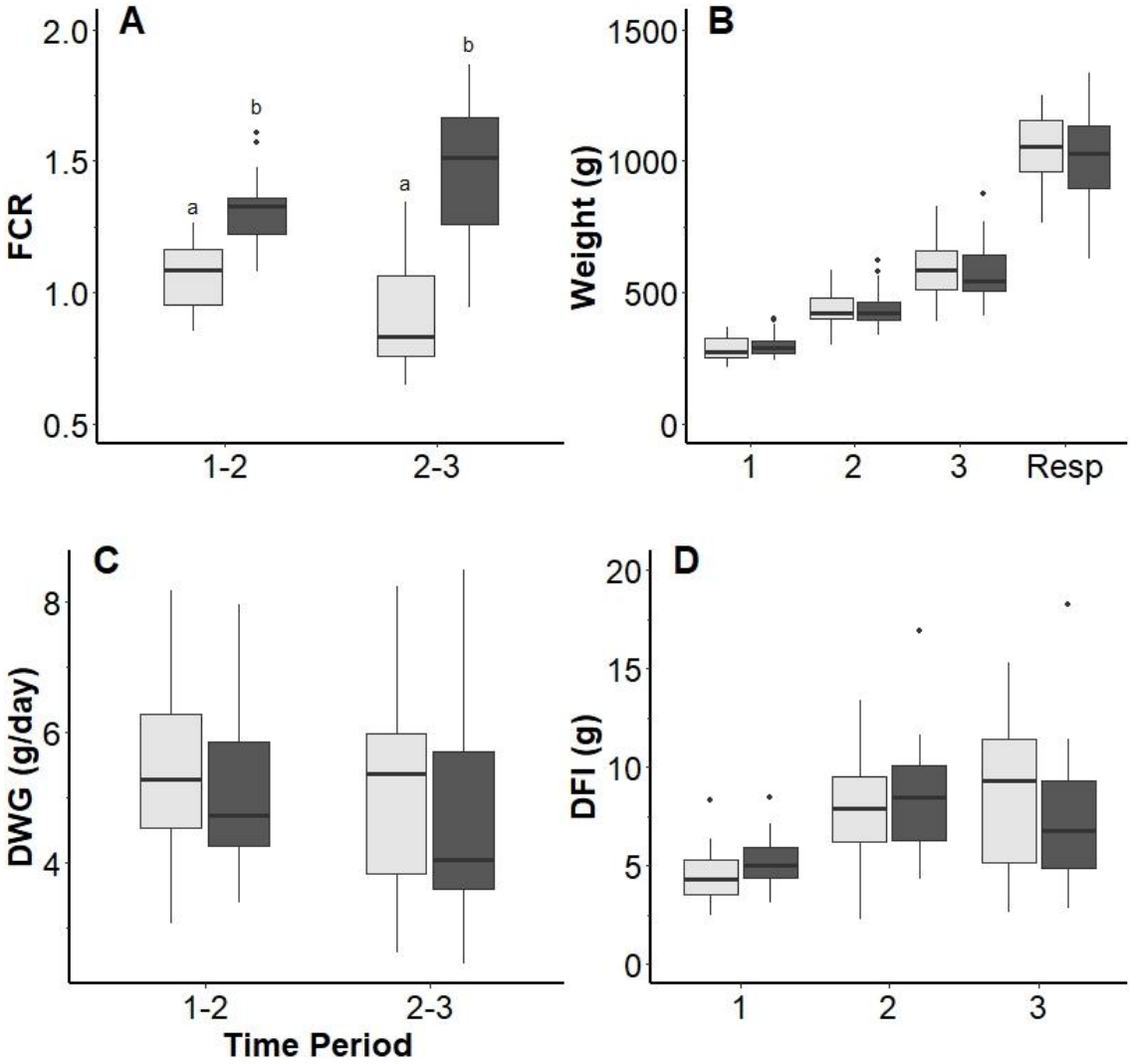

Figure 3.3. Box and whisker plots for feed efficient (light grey; n = 12) and feed inefficient (dark grey; n = 13) fish used in respirometry. A) Feed conversion ratio (FCR) at FCR 1 – 2 and FCR 2 – 3; B) Weight at assessment 1, 2, and 3; C) Daily weight gain (DWG) at FCR 1 – 2 and FCR 2 – 3; D) Daily feed intake (DFI) at assessment 1, 2, and 3. 'Resp' is the measurement of the fish at the time of respirometry. Superscripts indicate significant difference between the two FCR phenotypes at each time period. The lack of a superscript represents no significant differences between phenotypes. Data are presented as box and whisker plots, refer to Fig. 3.1. for interpretation.

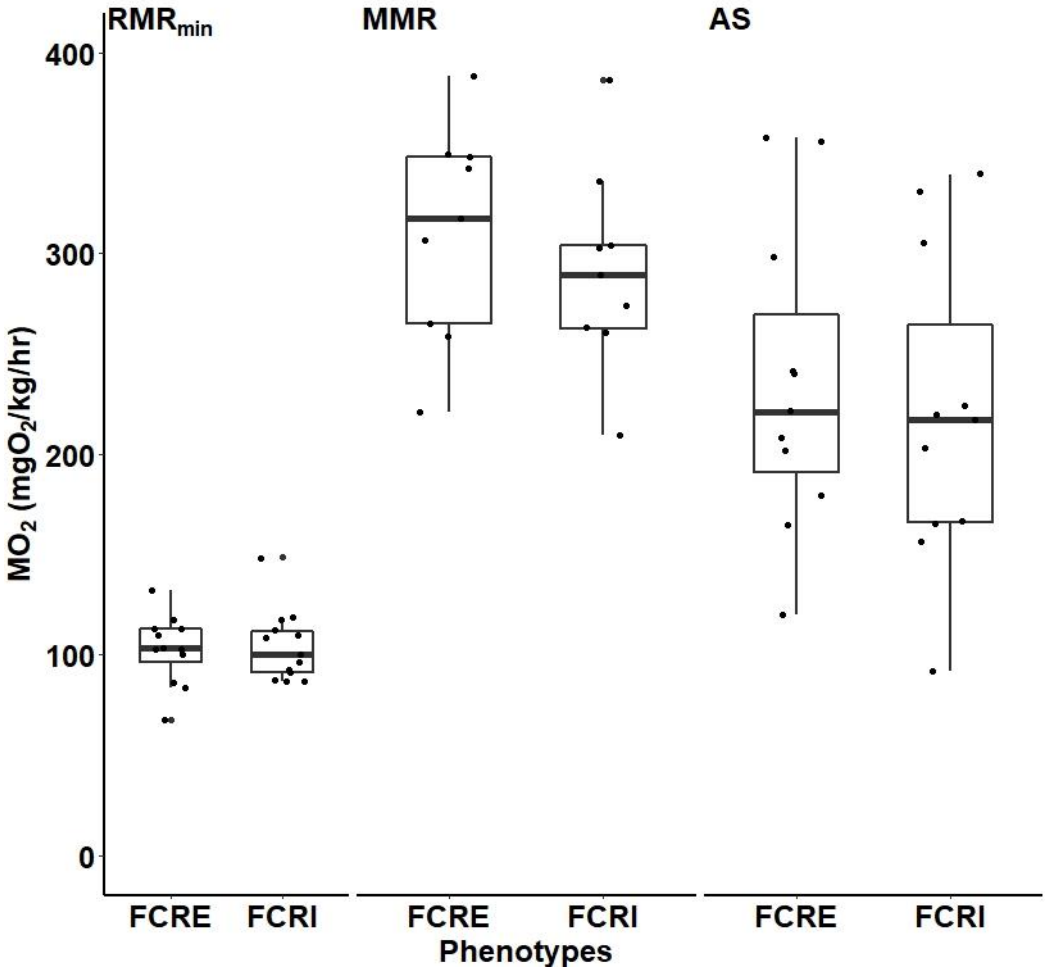

Figure 3.4. The minimal routine metabolic rate (RMR_{min}), maximum metabolic rate (MMR) and aerobic scope (AS) of FCR phenotypes: efficient (light grey) and inefficient (dark grey). No significant differences were observed between the FCR phenotypes for any of the measured metabolic rates. Data are presented as box and whisker plots, refer to Fig. 3.1. for interpretation.

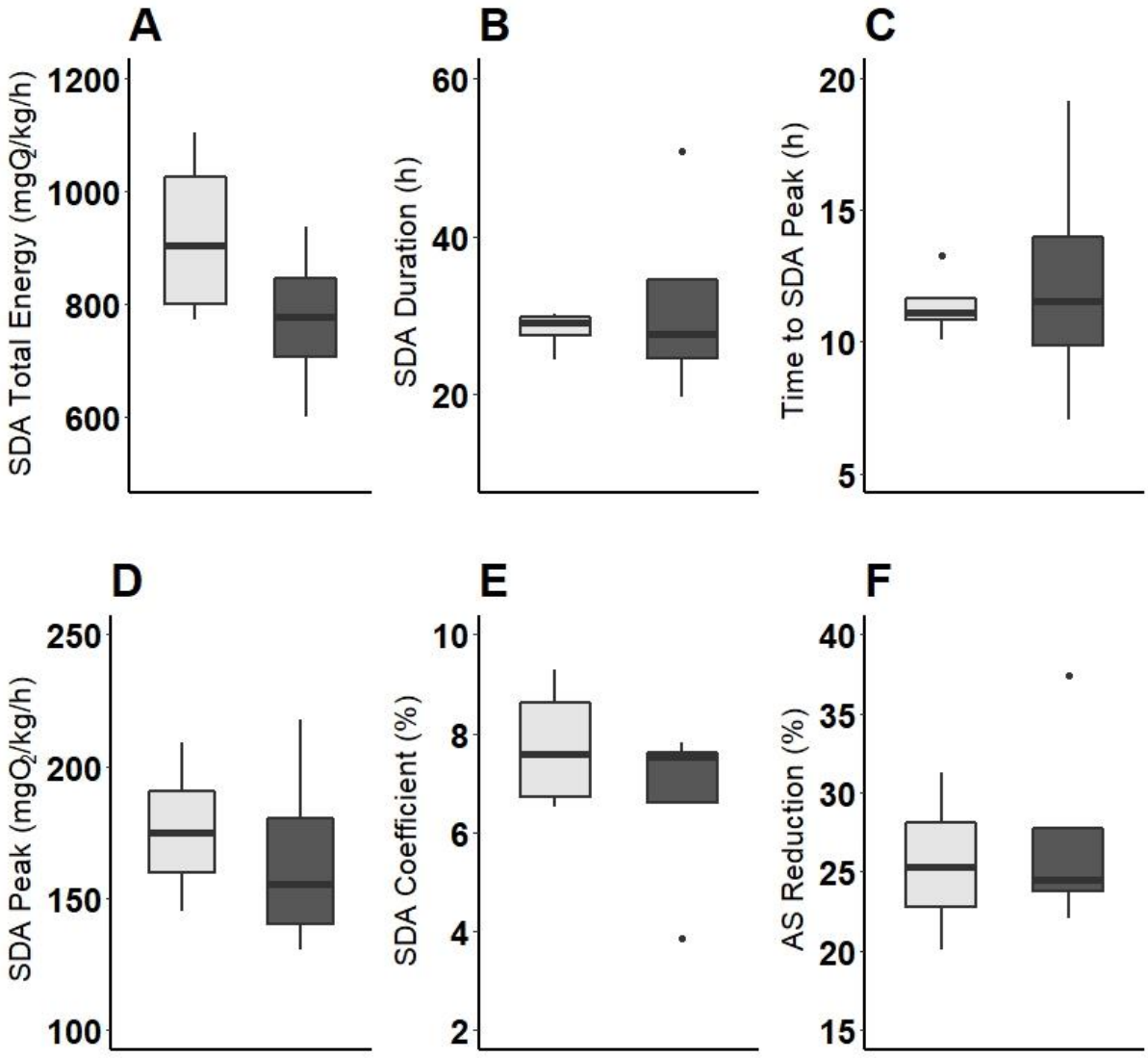

Figure 3.5. The specific dynamic action (SDA) parameters of FCR efficient (light grey) and inefficient (dark grey) phenotypes. Aerobic scope (AS) reduction. No significant differences were observed between the FCR phenotypes for any of the SDA parameters. Data are presented as box and whisker plots, refer to Fig. 3.1. for interpretation.

3.4. Discussion

Feed efficiency is a complicated trait that is influenced by multiple factors, including genetics, environmental and other physiological factors. This study presented a novel investigation of FCR variability in freshwater Chinook salmon (*Oncorhynchus tshawytscha*) and demonstrated how key factors, notably feed intake, growth, nutrient retention efficiency and feeding metabolism, are correlated with FCR as potential influencing factors. Understanding the complexity of feed efficiency in different life stages of Chinook salmon allows the implementation of targeted selective breeding programmes for a significant

improvement of Chinook FCR. This research helps to understand the complexity of salmon FCR and thus provides valuable mechanisms for economic and environmental gains to be made by the international Chinook farming industry.

It was expected that fast growth would correlate with feed intake, and DWG and DFI were indeed positively related in the current study and in line with a number of other studies (Allen et al., 2016; Elvy et al., 2022; Kause et al., 2016; Norin and Clark, 2017; Rodde et al., 2021b). A negative relationship between DWG and FCR, where fish with higher growth rates have better feed efficiency is often seen in salmonids, including rainbow trout (*Oncorhynchus mykiss;* Eya et al., 2013; Kause et al., 2016), Atlantic salmon (*Salmo salar;* Thodesen et al., 1999) and larger Chinook salmon in saltwater and freshwater (Elvy et al., 2022; Esmaeili et al., 2021) so the same was expected in this study. However, this relationship was different between the two time-periods and the expected negative relationship between DWG and FCR was only seen in the second time period. The same also followed for the relationship between FCR and DFI where a positive relationship only applied to the FCR 1–2 time period and was lost across FCR 2–3. Therefore, freshwater Chinook salmon do not appear to show consistent relationships between FCR, DWG and DFI and further research is required to fully understand the cause of this.

A potential reason why the relationship between FCR and DFI was lost in the last time period is a general loss of appetite across the course of this study, particularly within FCR_I fish. There was an especially noticeable decrease in feed intake and specific feeding rate (SFR) for FCR_I fish at DFI assessment 3 in the current study compared to the first two assessments. Obtaining repeated measurements from fish in this study meant that fish underwent multiple assessments, involving stressful crowding and handling (Kulczykowska and Sanchez Vazquez, 2010). Stress supresses the appetite of fish (Bernier, 2006; Bernier and Peter, 2001; Kulczykowska and Sanchez Vazquez, 2010) so a situation is hypothesised where some, if not all, individuals had a reduced appetite at the time of their final assessment. FCR_I appeared to be especially impacted by a stress-induced appetite reduction, seen by a reduced SFR at the time of the final DFI assessment while the SFR of FCR_E was constant across all three DFI assessments. This concurs with the study by Esmaeili et al. (2021) which found that inefficient Chinook salmon were more prone to stress compared to their efficient conspecifics. This in turn would have affected the growth of

Chinook salmon and potentially serves as an explanation to why the relationships between FCR and DWG, and FCR and DFI, differed between the two time periods. An important point to note, however, is that FCR_E Chinook salmon consumed smaller meals and had a lower SFR compared to FCR_I fish, which agrees with previous results of Esmaeili et al. (2021) for freshwater Chinook salmon and Elvy et al. (2022) for saltwater Chinook salmon. So, despite uncertainty in the linkage between FCR and DFI from the current sample population, it seems plausible to suggest that satiated feeding may not be the best feed management practise as it can lead to FCR_I fish, when they are not stressed, to overeat.

Nutrient retention efficiencies provide an indication of which nutrients an individual is utilising for growth. Feed efficient individuals appear to have higher levels of lipid retention as reported for grass carp (*Ctenophavyngodon Idella;* Du et al., 2005), European sea bass (*Dicentrarchus labrax*; Peres and Oliva-Teles, 1999) and Chinook salmon in saltwater (Elvy et al., 2022). This relationship was also seen in the current study for freshwater Chinook so is consistent with the current line of thinking. It needs to be pointed out, however, that the lipid retention efficiency (LRE) values for FCR_E fish in the current study were above 100%. LRE values exceeding 100 % has been seen in other salmonid studies (Dumas et al., 2018; Elvy et al., 2022; Weththasinghe et al., 2021; Weththasinghe et al., 2022). This is likely due to retention efficiencies only accounting for dietary lipid when glucose can be converted into fatty acids through *de novo* lipid biosynthesis pathways (Chen et al., 2019). Therefore, lipid synthesis is likely exceeding lipid catabolism (Dumas et al., 2018). Esmaeili et al. (2021) suggested that increased levels of fatty acid synthesis in the livers of FCR_E could be contributing to the increased LRE compared to FCR_I conspecifics. This process could therefore be contributing to the final whole body lipid composition which results in increased LRE, as well as ERE, because the energy content of the body was calculated from both protein and lipid composition. Despite differences in LRE, there were no differences in whole body lipid composition between FCR phenotypes in the current study as well as the studies of Elvy et al. (2022) and Esmaeili et al. (2021). FCR_E fish may achieve higher rates of growth from efficient muscle protein growth rather than through lipid deposition. This theory is compatible with the higher protein retention efficiency (PRE) of feed efficient rainbow trout (Eya et al., 2013; Overturf et al., 2013; Silverstein et al., 2005), European sea bass (Peres and Oliva-Teles, 1999), saltwater Chinook salmon (Elvy et al., 2022) as well as

the freshwater chinook in the current study. PRE is indeed expected to be higher in feed efficient fish because increased protein synthesis, protein retention, skeletal muscle growth and reduced protein degradation have all been linked to better feed efficiency in a number of studies (Carter et al., 1993a; Carter et al., 1993b; Esmaeili et al., 2021; Kolstad et al., 2004; McCarthy et al., 1994). Protein metabolism may therefore be an essential component of efficient growth and is potentially what contributes to better PRE in FCR_E Chinook in both freshwater and saltwater.

The study of Elvy et al. (2022) examined various aspects of oxygen consumption and observed some interesting differences between FCR phenotypes that were not observed in the current study. Feed efficiency was also shown to have no correlation with RMR in individual European sea bass (*Dicentratchus labrax*; Rodde et al., 2021a). Elvy et al. (2022) compared $\dot{M}O_2$ between FCR phenotypes in larger Chinook salmon (approx. 2.5 kg) in saltwater and found that the FCR_E fish had lower RMR_{min} values than FCR_I fish leading the authors to suggest that maintenance requirements of efficient fish are lower, potentially leaving more energy available for growth. As this was not seen for smaller Chinook in freshwater in the current study it is possible that RMR_{min} does not diverge between the two FCR phenotypes until a larger body mass is reached and/or FCR_I and FCR_E fish reared in saltwater differed in their energy requirements for osmoregulatory processes which was not a contributing factor in the current study. Also, as seen in Rodde et al. (2021a), there was a delay in when RMR_{min} was measured compared to DFI and the FCR values, especially by the time the last of the fish were used for respirometry. This delay in measurements could contribute to the lack of correlations as the repeatability of MO_2 and FCR values over a long-term period is unknown in Chinook salmon.

It was originally hypothesised that FCR_E fish would show heightened levels of SDA efficiency but no differences in SDA parameters were observed in the current study. However, in the current study, SDA was measured using a set ration, but DFI and SFR were highly variable among individuals when fish were fed to satiation. Indeed, SFR of FCR_E and FCR_I phenotypes varied at all three assessment points with FCR_E fish eating smaller meals. It is therefore possible that if fish were given a ration similar to what they would have chosen to eat under satiated feeding, the SDA parameters we measured may have differed. For example, in the event of larger voluntary meal sizes being consumed by FCR_I fish, it is

possible that the SDA duration may have been longer, the total energy required for digestion may have been higher, and AS reduction may have been larger. It is likely that these differences would have all contributed to less efficient growth. This line of thought is also supported by the species-specific effect of meal size on different SDA parameters. Ration size increased total SDA energy in plaice (*Pleuronectes platessa*; Jobling and Davies, 1980), barramundi (*Lates calcarifer*; Norin and Clark, 2017) and tilapia (*Oreochromis niloticus*; Skov et al., 2017) and SDA duration in barramundi (Norin and Clark, 2017) and cod (*Gadus morhua*; Jordan and Steffensen, 2007). Ration also increased SDA peak in barramundi (Norin and Clark, 2017) and AS reduction in cod (Jordan and Steffensen, 2007) and barramundi (Norin and Clark, 2017). Furthermore, ration size decreased SDA coefficient in barramundi (Norin and Clark, 2017). However, ration did not affect the total SDA energy, SDA peak or SDA coefficient of cod (Jordan and Steffensen, 2007), the SDA coefficient of tilapia (Skov et al., 2017), or the SDA peak of plaice (Jobling and Davies, 1980), further confirming that the response of SDA to ration size is highly species dependent. In addition, due to Chinook salmon being highly stress sensitive, only a small number of fish were able to provide SDA data. Therefore, before firm conclusions are reached further research on Chinook salmon is warranted to determine the effect of meal size on SDA parameters between individuals that voluntarily consume different meal sizes under satiated feeding.

3.4.1. Conclusion

This study looked at multiple factors potentially influencing FCR to understand which physiological traits could be selected to improve the overall feed efficiency of Chinook salmon. Understanding how FCR could be improved would greatly benefit the Chinook salmon farming industry because feed costs and environmental impact could be lowered. Contrary to the original hypotheses there were no significant differences between feed efficient and inefficient phenotypes in terms of metabolic rates and the measured SDA parameters. Differences in metabolism do not therefore appear to drive the performance of FCR phenotypes, but future studies should investigate the metabolic basis of feed efficiency with voluntary rates of feed intake. The most unequivocal result from the current study was that FCR_E had higher nutrient retention efficiencies compared to FCR_I individuals, and thus direct selection of FCR_E fish may be effective in improving nutrient retention and efficient growth. On the other hand, the relationships between FCR, DWG and DFI were all variable

throughout the study making it difficult to make clear conclusions on how these variables interact. Indirectly selecting for improved FCR via growth improvements has been achieved in other species (de Verdal et al., 2022; Thodesen et al., 1999) but the lack of any consistency between FCR, DWG and DFI in the current study means that the situation is more complicated for freshwater Chinook salmon. However, irrespective of how FCR_I fish performed in this study, FCR_E fish did consistently eat smaller meals. The current study has advanced our understanding, but additional investigations are clearly required to fully understand the intricacies of feed efficiency within the different life stages of Chinook salmon. For example, as FCR phenotypes' meal sizes varied, a future focus could possibly be placed on selecting fish showing lower rates of feed intake as a strategy to improve the FCR of freshwater Chinook salmon. Whether differences in feed intake are also potentially linked in with differences in feeding behaviours (i.e. competitive feeding hierarchies) under different rearing conditions (e.g. feeding regimes, stocking density) also warrant future research.

Chapter 4

Can dual ballotini combined with X-radiography resolve differences in the timing of fish feed intake? A case study using Chinook salmon feed conversion ratio phenotypes.

4.1. Introduction

For some farmed fish species, feed costs can be up to 70 % of the total cost of production (de Verdal et al., 2017a). Improving feeding efficiency within a species can reduce costs and maximise growth. Understanding feeding behaviour in a cultured setting could be used to improve feed efficiency. The feeding behaviour of fish is challenging to analyse as it can be influenced by a variety of factors including 1) the nutritional composition of the feed and an individual's feed preferences, 2) the amount of feed available, e.g. rations, meal frequency; 3) food detection; 4) modes of feeding, e.g. ambush, foraging, bottom feeders; 5) the timing of feeding within the meal, e.g. the total time spent feeding, how long it takes for fish to begin feeding and how long they feed for; 6) how active a fish is during the meal, e.g. speed and turning angle; 7) social hierarchies, competition and aggression (Andrew et al., 2004b; Martins et al., 2011b; Øverli et al., 1998; Volkoff and Peter, 2006).

Most methods for analysing feed behaviour in fish use single fish held in individual aquaria, or at low densities, and do not always have relevance in an aquaculture setting. Chinook salmon (*Oncorhynchus tshawytscha*) is the main finfish species farmed in New Zealand, but it generally has poor and variable feed conversion ratio (FCR), the total feed consumed divided by growth within a set time period (de Verdal et al., 2017a), compared to other cultured salmonid species, such as Atlantic salmon (*Salmo salar*). Currently, Chinook salmon have a FCR of ~1.8 (Araujo et al., 2021; NZKS Company, 2019; Walker et al., 2012), compared to a mean FCR of 1.1 – 1.2 in Atlantic salmon (Cook et al., 2000; Mundheim et al., 2004). Understanding how feed efficient and inefficient fish behave during feeding under commercially relevant densities would provide insight on feed efficiency in an aquaculture setting.

Feeding behaviour, including feed intake, is commonly studied by rearing individual fish in aquaria (Martins et al., 2005), in pairs (Øverli et al., 1999) or by assessing externally

tagged fish from video footage (Øverli et al., 1998; Smith and Houlihan, 1995). However, these methods have their limitations; rearing fish individually or paired excludes social interactions and behaviours that would occur at densities used under commercial farming conditions, < 15 kg/m^3 (High Country Salmon, 2023). Video analysis is time consuming (de Verdal et al., 2017a; Jobling et al., 2001a; Rodde et al., 2021b) and limited by multiple factors including water clarity, tank size and fish density. Rearing at commercial densities results in fish obscuring one another and prevents accurate visual-based estimates of feed intake.

Another commonly used method to measure feed intake is X-radiography of fish that have consumed feed containing known levels of indigestible X-ray opaque beads ('ballotini') of a single size. This method has the major advantage that feed intake can be estimated in individual fish reared in larger groups than alternative methods (Bégout et al., 2012; Difford et al., 2023; Jørgensen and Jobling, 1992; McCarthy et al., 1992; McCarthy et al., 1993). The ballotini method has also been used to compare daily and seasonal effects on feed intake, feeding location within the water column, effects of lighting regime, gut evacuation time, and feed wastage from chewing (Busti et al., 2022; Jørgensen and Jobling, 1990, 1992; Mock et al., 2022; Talbot and Higgins, 1983). However, the ballotini method in its current form, i.e., the use of one bead size, provides a limited amount of detailed information on fish feeding behaviour.

The timing of feeding is one behaviour that may influence the FCR of farmed fish. For example, previous studies in Nile tilapia (*Oreochromis niloticus)* and African catfish (*Clarias gariepinus*) have shown that more feed efficient individuals are slower to begin feeding and ate their meals faster (Martins et al., 2011a; Martins et al., 2005). At the beginning of a meal fish are hungry and feed tends to be delivered at a faster rate to meet the demand of the fish. This increased feed delivery rate increases the swimming speed and the frequency of sharp angled turns of fish (Andrew et al., 2004a). As the meal progresses the feed rate decreases, and swimming activity is also expected to reduce. Therefore, fish that start feeding later into the meal, like the efficient fish in Martins et al. (2011a) and Martins et al. (2005), skip this period of increased competition and activity. This in turn potentially lowers their energy expenditure during the meal compared to inefficient individuals that feed

earlier in the meal. Therefore, the less energy expended to obtain the meal, the more potential for improved feed efficiency.

Most studies applying the ballotini method use one bead size to determine the feed intake of individual fish from X-ray. The use of multiple bead sizes has been used far less commonly but a few studies show that multiple bead sizes can be offered together and differentiated from X-rays (Amundsen et al., 1995; Christiansen and George, 1995; Damsgard and Dill, 1998; Thorpe et al., 1990; Toften et al., 2003; Toften et al., 1995). Thorpe et al. (1990) compared two feeding strategies (hand feeding and automatic feeder) using one ballotini bead size per method, to allow comparison of feed intake and feed wastage. Damsgard and Dill (1998) compared feed intake in different locations of a tank using different bead sizes. This 'dual ballotini' method has also been used to look at differences in feed selection based on diet composition. For example, the addition of mineral oil concentration in polar cod (*Boreogadus saida*; (Christiansen and George, 1995), the use of a feeding stimulant on a regular diet for Atlantic salmon (Toften et al., 2003) and in a medicated feed for Atlantic salmon (Toften et al., 1995). However, to our knowledge the dual ballotini method has never been used to examine the timing of feeding within a meal and could be used to understand feed efficiency.

This study aimed to adjust the traditional ballotini method to provide extra information on fish feeding behaviour by determining whether the 'dual ballotini' method is effective in examining differences in the timing of Chinook salmon feeding. By feeding fish one bead size in the first half the meal followed directly by the second bead size for the remainder of the meal, it was hypothesised that both the total feed intake of individual fish and the timing of fish feeding within the meal can be estimated. The proportion of bead sizes in the meal should give an indication of whether fish were feeding earlier, later or during both halves of the meal. As FCR_I Chinook salmon have been seen to gorge and overeat compared to efficient (FCR_E) fish (Elvy et al., 2022; Elvy et al., 2023; Esmaeili et al., 2021; Esmaeili et al., 2022a) and efficient fish are slower to begin feeding and eat their meals faster (Martins et al., 2011a; Martins et al., 2005) it is hypothesised that the FCR_I and FCR_E fish would show a difference in the timing of feeding. Understanding how the timing of feeding differs between FCR phenotypes provides much needed information on the feeding behaviour of

farmed Chinook salmon and also offers scope to manipulate the feeding husbandry of this species in commercial operations as a means of improving commercial FCR.

4.2. Materials and methods

4.2.1. Ballotini feed

Feed containing opaque ballotini beads was made by CSIRO Australia's feed extrusion laboratory using commercial feed mash with the same ingredients as the commercial feed used in the rest of the trial (46.5 % protein, 23 % fat, 11.1 % carbohydrates, and 8 % moisture on a wet weight basis). The 9 mm diet was manufactured using a twin-screw extruder. The ballotini were 0.5 mm (small) and 1.0 mm (large) diameter ceramic zirconium silicate ("ZS type") SiLibeads® supplied by Sigmund Lindner GmbH. The beads were added to the feed after manufacture at an inclusion rate of 1.25 % for both the 0.5 mm beads and the 1.0 mm feeds. The pellets were coated in tallow during the oil coating stage of the pellet manufacture to allow the ballotini to adhere to the pellet's surface. This was done to determine if ballotini beads could be added to the pellets after extrusion to make the inclusion of the beads more practical and reduce the risk of damaging the extruder or fragmenting the beads (a risk for the larger 1.0 mm beads).

A series of samples of known weights of both feeds containing the small and large ballotini, ranging from one pellet to an amount higher than the expected meal size, were X-rayed for each diet (Fig. 1). The number of beads in each sample was counted using a semi-automated bead counting software "Bead Counter" developed by AgResearch Ltd, New Zealand (P. Smale, Personal communication), following the methods of Elvy et al. (2022). Final bead counts were then plotted against the weight (zero intercept) of the sample to create a calibration curve for each diet (Fig. 2). The same method was used to count the beads from the X-rays of the fish to determine feed intake. To test the feasibility of the 'dual ballotini' method some samples of a known mix of the two ballotini sizes were made to test whether the Bead Counter program could differentiate between the bead sizes within the same X-radiograph.

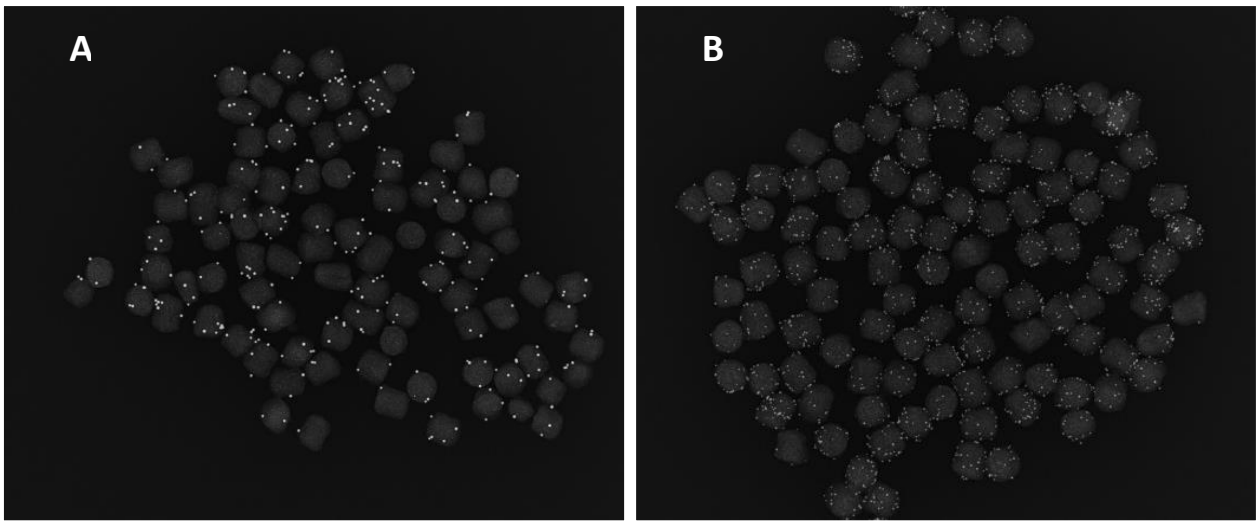

Figure 4.1. An X-radiograph of the diets top coated with A) the large 1 mm ballotini beads B) the smaller 0.5 mm ballotini beads.

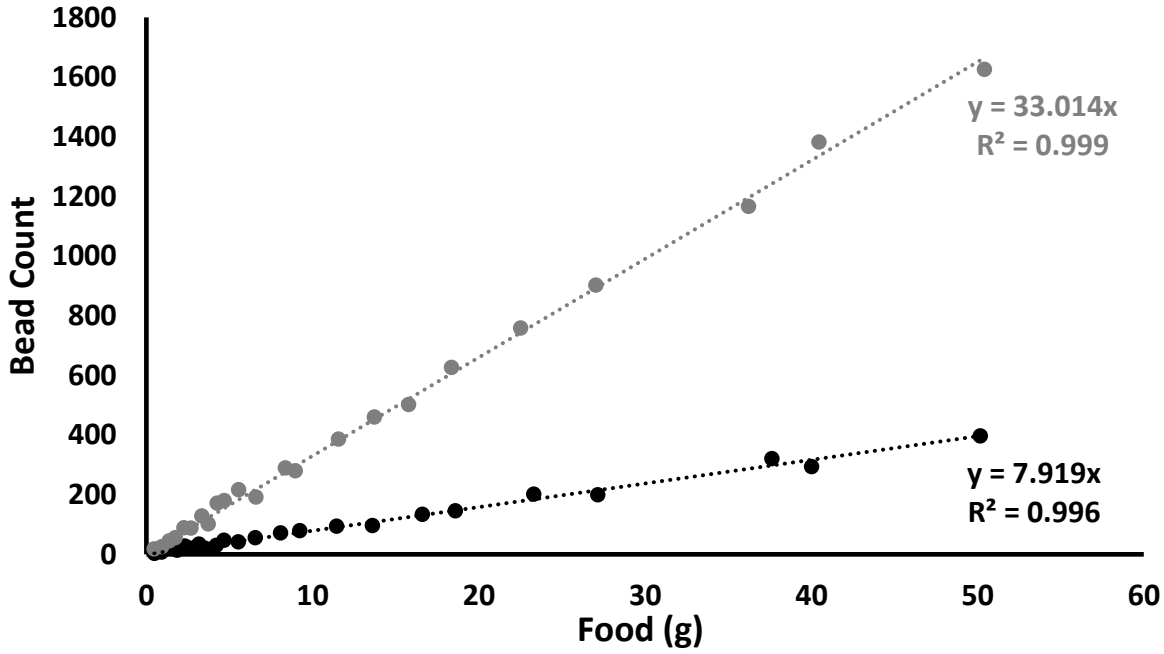

Figure 4.2. Calibration curves for 0.5 mm (grey) and 1 mm (black) ballotini diets.

4.2.2. Case study setup

All-female Chinook salmon, individually tagged with passive integrated transponder (PIT) tags, were transferred to the Cawthron Institute's Finfish Research Centre, Nelson, New Zealand from a commercial hatchery (Salmon Smolt New Zealand). Fish had been on site for approximately 9 months prior to commencement of the 'dual ballotini' feed intake study. 144 Chinook salmon (1601 ± 36 g and 424 ± 3 mm) were held at a stocking rate of 16 – 21

fish per tank across eight 3,000 L tanks, giving a stocking density of 7 – 12 kg/m^3 across the eight tanks.

The eight 3,000 L tanks were part of a recirculation system which provided disinfected oxygenated freshwater at 17.20 ± 0.01 °C to all tanks throughout the trial. Photoperiod was set to a commercial standard of 24 h artificial light. Fish were transitioned to 9 mm commercial feed (Skretting Alpine) 2 weeks prior to the dual ballotini (DB) assessments. They were hand-fed to satiation once a day throughout the experiment. Uneaten feed was recovered by a swirl collector and removed from the total feed fed to the tank to determine the actual feed consumed by the fish in the tank as described by Elvy et al. (2022).

4.2.3. Dual ballotini method

On the two days that the dual ballotini assessments were carried out, the pellets containing the 0.5 mm (small) beads were fed for the first half of the meal. The pellets containing 1.0 mm (large) beads were then fed for the second half of the meal. Prior to feeding, the meal size was estimated based on the average amount consumed by the tank for the previous five days. This estimated meal size was divided by two and equal amounts of 0.5 mm and 1.0 mm ballotini diets were weighed out. The meal was determined to be half-way through when half of the estimated meal was consumed as well as feeding behaviours exhibited by the fish. The feeding of the 0.5 mm diet was stopped, and the 1.0 mm diet was then fed an equal amount to the 0.5 mm diet that had been fed. Uneaten feed was recovered by a swirl separator as above, the recovered food was then subtracted from the amount of feed added to the tank.

For each assessment, within an hour after feeding, fish were crowded and anaesthetised in tricaine methanesulfonate (TMS; 65 mg/L), X-rayed (Fig. 4.3.) and then assessed for weight and length. The beads were counted using the same method as described above for the diets. The second dual ballotini assessment (DB2) was repeated 22 days after the first (DB1), allowing time for the fish to recover from the handling stress. Fish that did not eat at one (49 fish), or both dual ballotini measurements (39 fish), were excluded from analysis leaving 62 fish: 29 FCR_E, 26 FCR_I, and 7 of Intermediate FCR. Fish that ate less than 0.46 g were also excluded as this was less than the weight of one pellet and is assumed to be an error in the method.

Individual feed intake was broken down in to three categories:

1) Daily feed intake (DFI) - the total feed eaten by an individual at that dual ballotini assessment.

DFI (g) = 0.5 mm Diet (g) + 1.0 mm Diet (g)

2) Percentage of small beads - how much of the meal was made up of the 0.5 mm diet.

Percentage of small beads = 0.5 mm Diet (g) / DFI (g) × 100

3) Percentage of large beads - how much of the meal was made up of the 1.0 mm diet.

Percentage of large beads = 1.0 mm Diet (g) / DFI (g) x 100

The percentage of each bead type the fish ate per meal was then used to estimate when the fish was eating. For example, a higher percentage of 0.5 mm beads indicated an individual ate mainly in the first half of the meal, whereas conversely a higher percentage of 1.0 mm beads indicated the fish had eaten later in the meal.

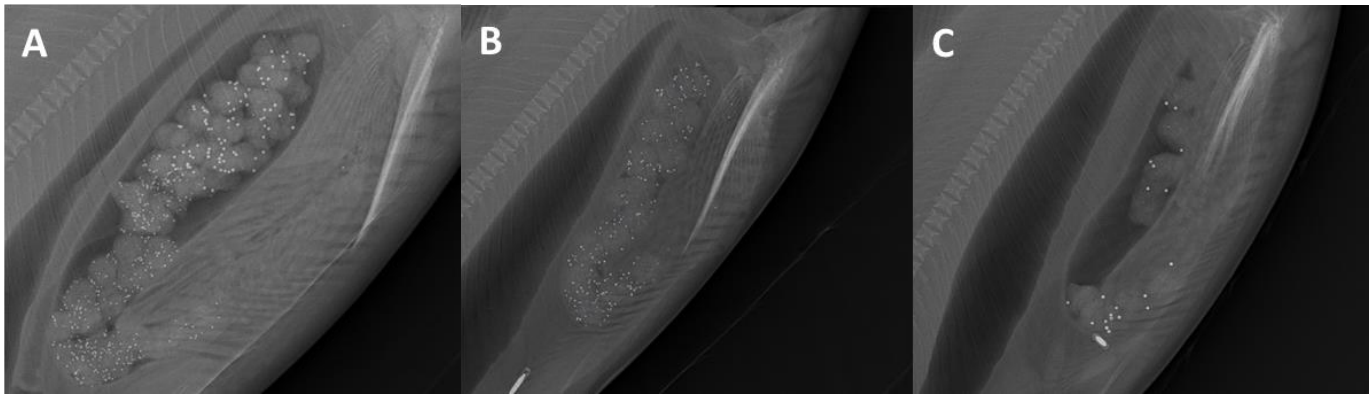

Figure 4.3. X-rays of fish following feeding with dual ballotini during the same meal. A) Fish that ate throughout the meal with a mixture of both bead sizes (0.5 mm and 1 mm) in their stomach, B) Fish that ate early with only small (0.5 mm) beads in their stomach, C) Fish that ate late with only (1.0 mm) beads in their stomach.

4.2.4. FCR phenotype determination

Using the two feed intake measurements from DB1 and DB2 and the weight gain during this period, a FCR was calculated for each fish using the following equation:

FCR = total feed eaten (g) / weight gain (g)

Total feed eaten needs to account for the total feed intake between DB1 and DB2. This was determined using the share of the meal (SOM) method where each individual's feed

intake on the day was calculated as a proportion of the tank's total consumption (Elvy et al., 2022; Elvy et al., 2023; Esmaeili et al., 2021).

SOM (%) = (individual DFI (g) / tank DFI (g)) x 100

An average SOM of DB1 and DB2 was used to determine the individual's total feed intake between assessments using the following equation:

Individual total feed intake (g) = Mean SOM (%) x total tank feed intake (g)

FCR values were then used to categorise fish into feed efficient (FCR_E), intermediate and inefficient (FCR_I) phenotypes based on their distribution within the quartiles of a box and whisker graph as per Elvy et al. (2022).

4.2.5. Other fish trait calculations

4.2.5.1. Specific growth rate

Specific growth rate (SGR) was calculated with the following equation:

$$SGR = \frac{ln(w_f) - ln(w_i)}{days} \times 100$$

Where SGR is the specific growth rate (%/day), w_f is the final weight (g), w_i is the initial weight (g), and days is the number of days between measurements (Biswas et al., 2005).

4.2.5.2. Specific feed rate

Specific feed rate (SFR) was calculated using the following equation:

SFR = total feed eaten (g) / body weight (g)

4.2.5.3. Condition factor

Condition factor was calculated with the following equation:

$$CF = \frac{w}{L^3} \times 100000$$

Where CF is the condition factor, w is the weight (g), and L is the fork length (mm).

4.2.6. Statistics

We tested if feed palatability was affected by the tallow coating by performing a linear regression between the average feed intake of the tanks for the previous 7 days and the

feed intake on ballotini assessment days (at both DB1 and DB2). The assumptions of the linear model were tested on the residuals of the model performing a Shapiro-Wilk test of normality, a Breusch-Pagan test of homoscedasticity, and a Durbin-Watson test of auto-relationship of the residuals.

To confirm that the estimate of feed from the X-ray represented the amount of feed consumed by the tank, we fit a generalised linear model with a Poisson family error distribution between the estimate of feed intake from the X-rays (count of beads) and the amount hand fed to the tank. We also use a linear regression to compare the total amount of feed eaten by each tank at both DB assessments.

We fit a generalised linear model with a quasibinomial family error distribution to test the relationship between the percentage of large beads eaten by individuals at both DB1 and DB2. A quasibinomial distribution was used to account for the overdispersion of the data.

Linear models were used to test if timing of feed intake was related to individual fish size (weight and length), condition factor, FCR, growth (SGR), and feeding performance estimates (SFR, SOM and total meal size). The assumptions of the linear model were tested on the residuals of the model performing a Shapiro-Wilk test of normality, a Breusch-Pagan test of homoscedasticity, and a Durbin-Watson test of auto-relationship of the residuals.

We compared the percentage of large beads eaten by the different FCR phenotypes (FCR_E and FCR_I) using a generalized linear mixed model with a binomial distribution for the family error distribution, and the tank as a random effect, to account for the non-independence of the fish at each tank. An ANOVA was used to compare the models with and without a random effect and assess the effect of the tank on the results.

All statistical analyses were performed using 'R' (R Core Team, 2020). Significance was accepted at $P < 0.05$.

4.3. Results

4.3.1. The feasibility of the dual ballotini method

A total of 188 X-rays of fish containing the two ballotini beads were analysed, 97 at DB1 and 91 at DB2. The X-rays showed that the two ballotini bead sizes could be differentiated

by human eye (Fig 3.) and the bead counting program. However, some of the smaller beads were missed by the image analysis program and needed to be detected manually and added to the count, on average 6 % ± 10 % (± standard deviation) of large beads were missed by the program and 13 % ± 12 % of small beads were missed. The program parameters for the larger beads accurately counted the beads except if beads were overlapping. This source of error was also subsequently corrected by visual quality control. This was easily completed by the human operator without adding significant time to the analysis.

Feed palatability was not affected by the tallow coating as total feed consumed by the tank on the day of the dual ballotini assessment was comparable to the feed intake of the tanks fed the non-tallow coated feed. A linear regression showed that the average feed intake of the tanks for the previous 7 days was comparable with the feed intake on ballotini assessment days at both DB1 ($P < 0.001$, $R^2 = 0.65$, $F = 113.50$) and DB2 ($P < 0.001$, $R^2 = 0.33$, $F = 31.56$). However, tallow coating did impact pellet performance in the water as the pellets were more likely to float on the water's surface. The tallow also caused the pellets to clump together but as long as the person feeding separated the pellets before distributing to the tank, the fish's ability to feed was not impaired. This did slow the feed delivery rate compared to a commercial diet or a standard ballotini diet.

4.3.2. Comparison of feed intake assessments

As total feeding time was not known from the outset of DB1 and DB2 it was difficult to establish the midway point of the meal, hence it was not always possible to accurately deliver an even split of small and large ballotini beads. At DB1 most of the tanks fed roughly 50 ± 5 % of each of the bead sizes (Table 1) but tanks 1, 2, and 8 were skewed towards the large beads (66.6 – 69.8 %). However, the average distribution of the ballotini sizes at DB1 was close to 50 % (Table 1). At DB2, all the tanks fed within 50 ± 10 %. The percentage distribution of bead sizes for DB1 and DB2 in Table 1 are based on the bead counts from the X-rays, so this only accounts for what was eaten by the fish and not the uneaten feed. Linear regression analysis indicated there was a significant correlation between the estimate of feed intake from the X-rays and the amount hand fed to the tank, that accounted for uneaten feed at DB1 ($P < 0.001$, $R^2 = 0.96$) and DB2 ($P < 0.001$, $R^2 = 1.00$; Table 1). Linear regression analysis also indicated there was a significant correlation between the total amount of feed eaten by each tank at both DB assessments ($P = 0.001$, $R^2 = 0.21$, $F = 12.95$).

4.3.2.1. Individual performance

A total of 62 fish ate at both DB1 and DB2. There was a weak but significant correlation between the percentage of large beads eaten by individuals at both DB1 and DB2 (Fig. 4; P = 0.04, R^2 = 0.06, F = 4.56). Forty five of the 62 fish were assigned FCR phenotypes and were subsequently used for the analysis of the dual ballotini method (see Table 2 for a summary of growth and feeding performance) and the differences among the phenotypes.

Table 4.1. Summary of the dual ballotini feeding data. For both DB1 and DB2 the following is provided: the total percentage of small beads and large beads eaten by all the fish in the tanks; the time taken for the tank to eat each bead size; the total feed consumed by the fish based on the wet weight fed to the tank minus the recovered uneaten feed; the total amount eaten by the tank estimated from all the X-rays; and the total number of fish in each tank. The number of fish that ate at both DB1 and DB2 in each tank are listed under DB2.

	% small beads	**% large beads**	**Time to eat small beads (mm:ss)**	**Time to eat large beads (mm:ss)**	**Total feed wet weight (g)**	**Total feed X-ray (g)**	**Tank total number of fish**	**Fish that ate at DB1 and DB2**
	DB1							
Tank 1	30.4	69.6	05:31	09:32	44.0	39.0	19	

Tank 2	30.2	69.8	05:57	10:46	54.6	68.8	17	
Tank 3	52.5	49.8	13:24	11:19	105.5	96.5	18	
Tank 4	49.6	50.4	10:58	09:32	163.6	145.0	21	
Tank 5	50.0	50.0	10:38	9:36	141.8	126.6	20	
Tank 6	50.2	49.8	15:53	10:34	151.8	151.3	18	
Tank 7	53.0	47.0	10:28	08:29	141.5	121.6	21	
Tank 8	33.4	66.6	06:07	07:33	39.8	36.8	16	
Mean of all tanks	43.7	56.6						
	DB2							
Tank 1	58.8	41.2	10:53	07:55	105.9	99.0	19	3
Tank 2	56.6	43.4	08:50	08:15	70.7	63.2	17	3
Tank 3	49.7	50.3	09:56	09:36	50.2	48.2	18	5
Tank 4	53.3	46.7	09:45	12:25	159.0	143.9	21	11
Tank 5	56.9	43.1	10:50	11:18	174.8	160.3	19	8
Tank 6	46.4	53.6	15:19	09:37	103.1	94.0	17	6
Tank 7	45.0	55.0	07:05	06:47	72.5	64.6	20	7
Tank 8	52.5	47.5	06:53	06:17	79.2	67.8	16	2
Mean of all tanks	52.4	47.6						

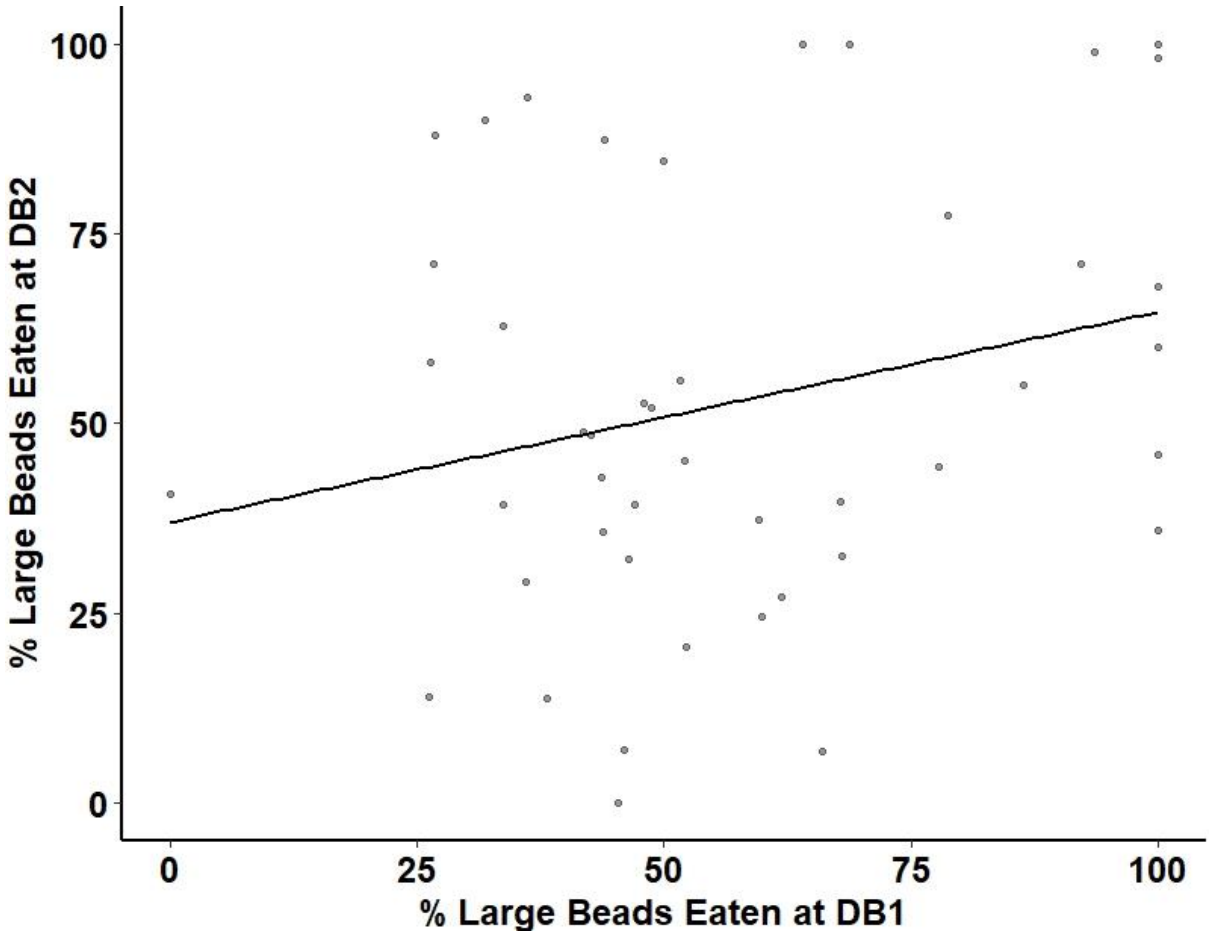

Figure 4.4. The relationship between the percentage of large beads eaten by individual fish at DB1 and DB2.

Only a few fish ate pellets with one bead size exclusively. At DB1 two fish ate 100 % small beads (1 FCR_I, 1 Int) and six ate 100 % of their meal as large beads (4 FCR_E, 2 Int). At DB2 one fish ate 100 % small beads (1 FCR_E) and three fish ate 100 % large beads (1 FCR_E, 2 Int). More FCR_E fish appeared to feed late with only large beads in their stomachs across DB1 and DB2 (n=5). However, due to the low numbers of fish that ate only in one half of the meal it was not possible to determine if one FCR phenotype had a consistent preference for early or late feeding. A more detailed approach was therefore taken to analyse the FCR phenotypes (see Section 3.3.2.).

Table 4.2. Growth and feeding performance of the FCR phenotypes across the dual ballotini assessments. All data are mean values (± SE) except the number of fish which is a total value. Rows without superscripts have no significant differences between the FCR phenotypes. FCR: feed conversion ratio; SGR: specific growth rate; DFI: daily feed intake; SFR: specific feed rate. 1 represents measurements at DB1 and 2 represents measurements at DB2.

	FCR_E	Intermediate	FCR_I	All Fish
Number	14	16	15	45
FCR	0.81 ± 0.05^{a}	1.18 ± 0.02^{b}	1.67 ± 0.06^{c}	1.23±0.06
SGR	0.34±0.03	0.40±0.03	0.38±0.3	0.37±0.02
Weight (g) 1	1797±110	1666±94	1738±119	1730±61
Weight (g) 2	1929±117	1809±98	1877±123	1869±64
Length (mm) 1	427±5	444±13	428±5	431±4
Length (mm) 2	437±5	454±12	439±5	442±4
DFI (g) 1	5.98 ± 1.19^{a}	10.07 ± 1.10^{b}	8.59 ± 0.97^{ab}	8.30±0.67
DFI (g) 2	5.58 ± 1.24^{a}	7.46 ± 01.11^{a}	13.53 ± 1.32^{b}	8.90±0.86
SFR 1	0.38±0.09	0.64±0.0.08	0.52±0.07	0.52±0.05
SFR 2	0.27 ± 0.05^{a}	0.40 ± 0.05^{a}	0.75 ± 0.08^{b}	0.48±0.05

4.3.3. FCR phenotypes and performance

4.3.3.1. Fish performance traits

Individual fish size (weight and length) and condition factor were also compared with the timing of feed intake with the % of large beads used as a representation of when fish were eating (i.e. a higher % of large beads shows that individuals ate later in the meal). There was no correlation between when fish were eating and fish weight at DB1 ($P = 0.23$, $R^2 = 0.01$, $F = 1.51$) or at DB2 ($P = 0.73$, $R^2 = -0.02$, $F = 0.12$). Fish length and the % of large beads eaten were also not correlated at DB1 ($P = 0.36$, $R^2 = -0.004$, $F = 0.84$) and DB2 ($P = 0.93$, $R^2 = -0.02$, $F = 0.01$). Fish condition factor and the % of large beads eaten was also not correlated at DB1 ($P = 0.15$, $R^2 = 0.03$, $F = 2.13$) or at DB2 ($P = 0.47$, $R^2 = -0.01$, $F = 0.53$).

Individual fish growth (SGR) and FCR was also compared against the timing of feed intake. A significant correlation was found between FCR and the % of large beads eaten at DB1 (Fig. 5A; $P = 0.004$, $R^2 = 0.16$, $F = 9.07$) but not at DB2 (Fig. 5A; $P = 0.29$, $R^2 = 0.003$, $F = 1.14$). There was also no correlation between SGR and the % of large beads eaten at DB1 (Fig. 5B; $P = 0.09$, $R^2 = 0.04$, $F = 2.97$) or at DB2 (Fig. 5B; $P = 0.10$, $R^2 = 0.04$, $F = 2.84$) indicating that time of eating was not correlated with fish growth rate.

Feeding performance such as SFR, SOM and total meal size was also compared with when in the meal fish were feeding. There was a significant negative correlation between

SFR and the % of large beads eaten at DB1 (Fig. 5C; P = 0.002, R^2 = 0.18, F = 10.54) and at DB2 (Fig. 5C; P = 0.01, R^2 = 0.11, F = 6.66) indicating that fish eating smaller meals (as a percentage of their body weight) ate later in the meal (or vice versa). There was no correlation between SOM and the % of large beads eaten at DB1 (P = 0.54 R^2 = -0.01, F = 0.38) or at DB2 (P = 0.11, R^2 = 0.04, F = 2.68). Total feed intake was also significantly but weakly correlated to the % of large beads eaten at DB1 (P = 0.001 R^2 = 0.22, F = 13.38) and at DB2 (P = 0.04, R^2 = 0.08, F = 4.70) indicating that fish that ate later in the meal ate smaller amounts of feed.

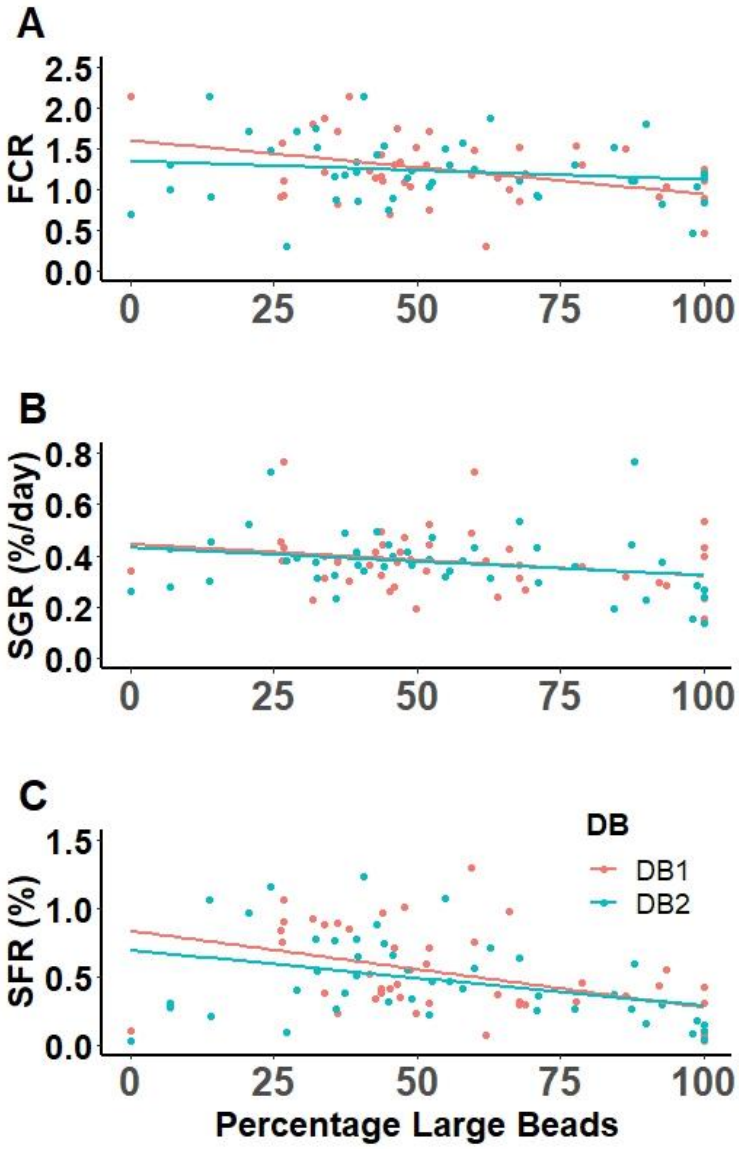

Figure 4.5. Performance traits vs percentage of meal consisting of large beads (i.e. feeding in the last half of the meal) at DB1 (red) and DB2 (blue). A) FCR from DB1 to DB2; B) SGR from DB1 to DB2; C) SFR from DB1 to DB2.

4.3.3.2. FCR phenotypes

The percentage of large beads eaten did not differ between FCR_E, Int and FCR_I fish at DB1 (P = 0.09) or DB 2 (Fig. 6 and Fig. 7; P = 0.87). This indicates there was no difference in when the two FCR phenotypes were feeding as they were not consistently eating earlier (higher percentage of small beads) or later (higher percentage of large beads) in the meal. There was no tank effect on the percentage of large beads eaten by the different FCR phenotypes at DB1 (P = 1) or DB2 (P = 1).

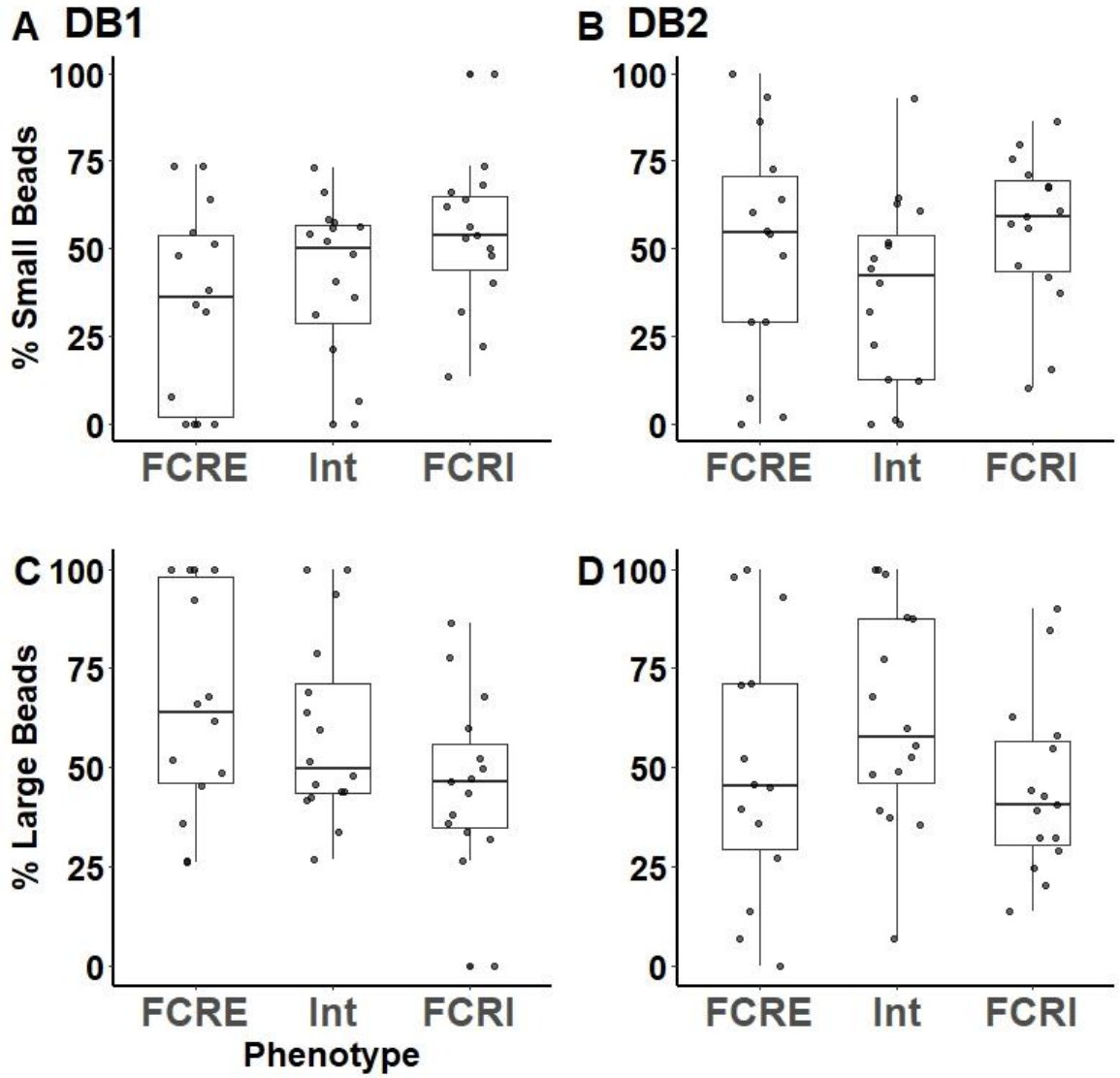

Figure 4.6. The percentage share of feed intake between FCR phenotypes and the two ballotini sizes (top and bottom) for DB1 (left) and DB2 (right). Small (0.5 mm) beads were fed for the first half of the meal (top plots) and large (1.0 mm) beads were fed for the second half of the meal (bottom plots). Dots over the box and whisker plots represent individual fish. The box represents the inter quartile range ranging from the 25th percentile to the 75th percentile, the bold line is the median, the whiskers extend to the minimum and maximum values within 1.5 of the interquartile range. No significant differences among the FCR phenotypes were observed.

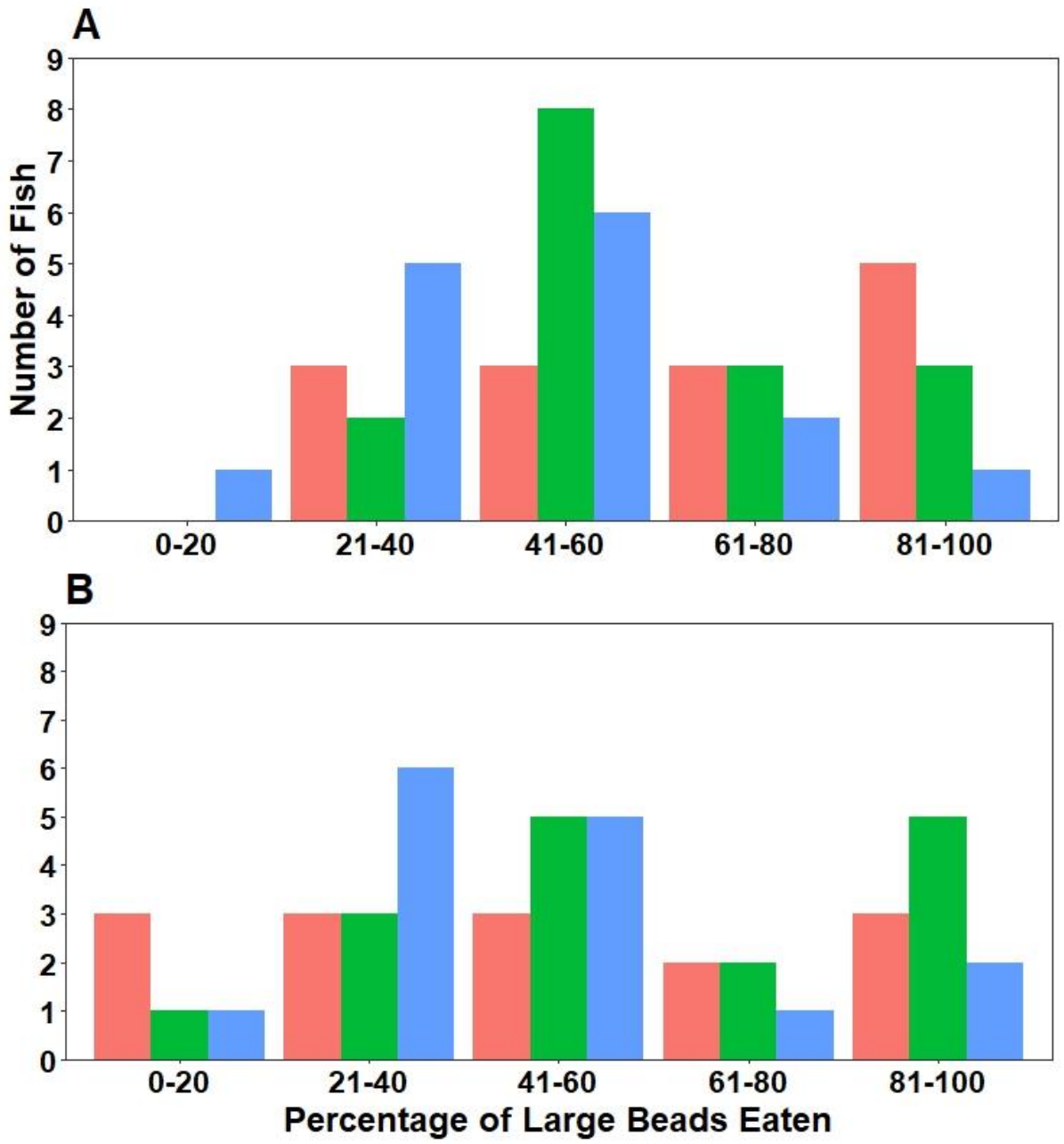

Figure 4.7. Fish groups based on the percentage of large beads eaten and the number of FCR_E (red), Intermediate (green) and FCR_I (blue) fish at DB1 (A) and DB2 (B) in each group.

4.4. Discussion

This study shows that the novel use of two different ballotini bead sizes in feed can be used to assess when fish eat within a meal, defined as early, later, or in both halves of the meal. Using portable digital X-ray equipment the dual ballotini method can be incorporated into fish handlings events allowing simultaneous measures of feed intake, weight, growth, FCR and for the first time, the timing of feed intake during a meal. This method provides an alternative way to analyse feed timing in groups of fish when cameras are not applicable,

such as when individual fish cannot be easily identified and tracked, visibility in tanks is poor, and/or there are large numbers of fish to be analysed. There was a significant correlation between when a fish ate and the size of their meal, with fish that ate smaller meals eating later at both DB1 and DB2. During the first dual ballotini assessment (DB1) there was also a significant correlation between the percentage of large beads (feeding later) and FCR, with more efficient individuals feeding later in the meal. However, this relationship was not observed at the second assessment (DB2), and there was no significant difference in the feeding timing when the fish were assigned to FCR_E and FCR_I groups. Whilst the method offers great promise for understanding feeding behaviour of different fish species in aquaculture, feed efficient or feed inefficent Chinook salmon (*Oncorhynchus tshawytscha*) do not appear to differ in their time to feed during meals. The following sections discuss the results of this research with respect to advancing the methodological approaches for the field of fish feeding behaviour.

4.4.1. Dual ballotini method development

4.4.1.1. Diet manufacture

One of the biggest challenges for the application of this method is the manufacture of the ballotini diets. In some circumstances incorporation of the beads during extrusion (the standard method used), can result in damage to extrusion equipment and larger ballotini beads can be crushed (1.0 mm; D. Forte, personal communication, 2021). To avoid damaging the extruder and the risk of bead fragmentation which makes counting different bead sizes difficult, the ballotini in this study were adhered to the external surface of pellets with tallow during the oil coating process after extrusion. This increased the potential for ballotini beads to dislodge from pellets during feeding. However, despite this potential error, there was a significant correlation between what was eaten by all the fish in the tank (determined by recovery of the uneaten feed) and the calculation of tank total feed intake estimated from the X-ray/ballotini data. This external application also makes the beads easier to use and a similar approach could be used to adhere ballotini to any commercial diet allowing for a wide range of uses in other culture species.

4.4.1.2. Diet characteristics

Under ideal scenarios, the same mash should be used for the ballotini feed to keep feed composition and palatability consistent with the normal daily feeding and not affect feed intake quantification (Toften et al., 1995). We achieved this, but subsequently added tallow externally to the feed. Given this addition, it was important to understand the effect of tallow on the palatability of the diet and the behaviour of the pellets in the water. The addition of tallow to the external surface of feed pellets would have changed the lipid composition of the feed slightly. A change in feed composition can act as a feed deterrent and reduce feed intake, the effect of which can be species-specific (De La Higuera, 2001). However, other studies have found that the inclusion of tallow did not reduce the palatability of diets fed to Chinook salmon (Mugrditchian et al., 1981) or Atlantic salmon (*Salmo salar*; Hardy et al., 1987), which was confirmed in this study. This study assumed that there was little effect on the pellet's stability when added to water based on visual assessments and that the amount of feed fed to the tank was equivalent to the amount of feed estimated from the X-radiographs. However, it would be beneficial to perform a stability test in future studies by agitating the pellets in a beaker to mimic feedings and determine the likelihood of ballotini loss during feeding.

Tallow did affect the physical characteristics of the pellets in the current study. When added to water the tallow coated pellets tended to float, whereas the commercial diet fed in this study had slow sinking characteristics. Feed intake is affected by various characteristics of the pellet such as shape, density (sinking rate), and size (De La Higuera, 2001; Jobling et al., 2001b). Therefore, it is possible that the tallow coating might have affected the feeding behaviour of Chinook salmon in this study, especially if they were reluctant to eat the floating pellets (Jobling et al., 2001b). However, the intake of tallow coated ballotini pellets in the current study was similar to the feed intake of the non-tallow coated diet, suggesting that the tallow did not affect the palatability or feed intake of the Chinook salmon. Nevertheless, a comparison of individual feed intake using feed incorporating ballotini added during the extrusion process versus externally tallow coated ballotini feed on the same fish would confirm this.

The method potentially has wide applicability as a range of ballotini bead sizes can be fed and detected using this method (0.23 to 1.0 mm). For example, Christiansen and George

(1995) fed beads sized 0.23 – 0.32 mm, 0.40 – 0.46 mm and 0.65 – 0.75 mm to polar cod (*Boreogadus saida*) to determine the effect of crude oil on feed intake. Similarly, Toften et al. (1995) and Toften et al. (2003) used 0.23 – 0.32 mm and 0.49 – 0.70 mm beads in diets of Atlantic salmon to assess feed intake with the use of feeding stimulants. Thorpe et al. (1990) fed 0.40 – 0.45 mm and 0.65 – 0.75 mm beads and compared hand feeding and an automatic feeder in an Atlantic salmon sea cage. The current study also showed that the bead counting image analysis software program used was accurate and efficient as it could differentiate and count the two bead sizes with only a slight error in counting. This error was also easily and quickly corrected manually. This counting method provided a major advantage compared to the methods used in previous studies, where beads were counted manually on developed X-ray plates (Christiansen and George, 1995; Damsgard and Dill, 1998; Toften et al., 2003; Toften et al., 1995). Difford et al. (2023) found that an image analysis programme was 1 – 6 times faster than manual counting. Manual counting is time consuming and, therefore, restricts the number of fish that can be analysed.

4.4.2. Method application in Chinook salmon

Fish performance in the current study was comparable to the results obtained for Chinook salmon of a similar sizes in Chapters 2 and 3. In particular the FCR and daily feed intake data we obtained matched the results observed in previous studies, even though these studies used ballotini feed manufactured using the standard method of ballotini incorporation during extrusion (Elvy et al., 2022; Esmaeili et al., 2022a). FCR_I ate more in the current study compared to FCR_E especially at DB2. This agrees with previous studies that showed that inefficent Chinook salmon ate in excess to their nutritional requirements (Elvy et al., 2022; Elvy et al., 2023; Esmaeili et al., 2022). There was no significant difference between the weight and length of FCR phenotypes measured in the current study.

This study found that the fish that ate a larger proportion of their meal in the first half of the meal (a higher % of small beads) were more likely to eat a larger meal, in terms of total meal size and specific feed rate (SFR). As fish growth and feed intake are positively correlated in many studies (Allen et al., 2016; Elvy et al., 2023; Kause et al., 2016; Norin and Clark, 2017; Rodde et al., 2021b) it would be expected that fish eating earlier in the meal would show higher rates of growth (weight gain). However, no such relationship between weight gain and the timing of fish eating in the meal was found in the current study. Based

on this, it is possible that fish feeding at the beginning of the meal could be engaging in competitive behaviours and higher levels of swimming activity at the point of food addition and are therefore, exerting more energy (Andrew et al., 2004b; Hart, 1986; Zhao et al., 2017). If so, these fish would use more energy during feeding, leaving less energy available for growth. This is further supported by more efficient Chinook salmon with lower FCR values feeding later in the meal at DB1 (Fig. 4.5.). This was also the case for individually reared African catfish and tilapia, where more efficient individuals were slower to begin feeding and ate their meals in a shorter amount of time (Martins et al., 2011a; Martins et al., 2005).

Another way to assess the relationship between the timing of feeding and FCR is to classify the individuals based on their FCR phenotypes and analyse their differences as per the approach used in previous studies (Elvy et al., 2022; Elvy et al., 2023). However, when the fish were grouped by their FCR phenotype no difference was observed in when the three FCR phenotypes fed throughout the meal (Fig. 6). This was despite there being a significant relationship, albeit weak, between FCR and the % of large beads at DB1, based on the individual fish FCR data (Fig. 5). Further research, which ideally includes more repeated measures, is needed to understand the effect of feeding timing on both individual FCR values and FCR phenotypes.

Whilst the current study has successfully implemented the dual ballotini method for this specific purpose (feed timing) for the first time, past studies have also successfully adapted the use of dual ballotini to compare feed preference, feeding locations and feeding strategies (Christiansen and George, 1995; Damsgard and Dill, 1998; Thorpe et al., 1990; Toften et al., 2003; Toften et al., 1995). Therefore, this method could be used to understand other aspects of Chinook salmon feeding behaviour, such as the inclusion of a third ballotini bead size to further differentiate timing differences. It is also possible that the use of video recording and externally tagged fish could be used to determine a more in-depth understanding of behaviours exhibited by Chinook salmon (see Chapter 5).

4.4.3. Limitations of the method

As well as the limitations of the physical manufacturing of the pellets mentioned above and the use of tallow there were other limiting factors to this method, which are common

limitations to the X-ray ballotini method in general. In the current study, repeated X-rays were limited by Chinook salmon being sensitive to accumulated stress. Ideally, a minimum of three repeated measures are required to improve the reliability of this method as it is often assumed to have low repeatability (Grima et al., 2008; Kause et al., 2006a), due to the variable daily feed intake of salmonids (Kause et al., 2006b; McCarthy et al., 1992). The number and frequency of measurements is limited by the need to anaesthetise the fish, handle them, and allow complete evacuation of ballotini from previous meals (de Verdal et al., 2017b; Grima et al., 2008) before assessing again. Feeding techniques need to be selected carefully as they can also limit the reliability of the method. Feeding multiple meals per day, but recording only one, can underestimate the individual feed intake. To minimise this error fish ideally should be fed once a day throughout the trial, as was done in this study. This may limit the size of fish this method can be used for as smaller fish require more frequent meals. However, it is possible to feed ballotini across multiple meals within a day, as long as the fish are X-rayed prior to gut evacuation (McCarthy et al., 1993). Therefore, understanding the gut evacuation of the species in the experimental conditions is required, though the time frame of this will vary with species, age, and other contributing factors such as temperature and feed type (Busti et al., 2022; Mock et al., 2022).

4.4.4. Future considerations

This study demonstrated that the dual ballotini method is a useful method to determine when in the meal individual fish are feeding. In addition, the results showed promising relationships between the timing of feed intake and feed efficiency in fish. This is in agreement with previous results in rainbow trout (Pouil et al., 2023), African catfish (Martins et al., 2006) and Nile tilapia (Martins et al., 2011a) that show phenotypic correlations between feed efficiency and feeding behaviours indicating that feeding behaviours are an important consideration when looking at feed efficiency. The dual ballotini method has a unique advantage as multiple feed intake measurements can be obtained on the same fish. To further improve the reliability of the dual ballotini method, as many ballotini measurements as possible should be included in the experimental design (Grima et al., 2008). Adaptations of this method include the inclusion of an alternative feed marker, such as yttrium and ytterbium oxide which are inert markers that can be distinguishable from one another at relatively low concentrations (Storebakken et al., 1999) and may be more readily

available than the addition of ballotini beads and the required X-radiography equipment. However, this would require terminal sampling and does not allow for repeated measures on the same fish, a major advantage of the dual ballotini method. However, this method could be used to validate the dual ballotini method further.

4.4.5. Conclusion

To determine the timing of feeding in commercial densities of farmed fish, the dual ballotini method of the current study provides a novel and validated alternative to time-consuming video analysis. This study showed that the use of different ballotini bead sizes in feed could be used to differentiate whether fish ate earlier, later or during both halves of the meal and that individual fish feeding patterns were similar across two repeated meals. Whilst the results of this study did not detect differences between Chinook salmon feed efficient and feed inefficient phenotypes, there was a significant albeit weak relationship between feeding later in the meal and FCR during one assessment. The dual ballotini method has the potential to be used as an alternative method to video recording to understand fish feeding behaviour and can be applied under a range of different conditions, e.g., higher rearing densities, that video recording could not be used for. While the timing of feeding is not associated with specific FCR phenotypes in farmed Chinook salmon, future studies could look at other feeding or competitive behaviours and how they influence FCR in this species.

Chapter 5

Differences in group feeding behaviours of feed efficient and inefficient Chinook salmon (*Oncorhynchus tshawytscha*)

5.1. Introduction

Feed can make up to 30 – 70 % of fish farming operating costs (de Verdal et al., 2017a; Goddard, 1996). Therefore, optimising the feed conversion ratio (FCR), a measure of feed efficiency calculated as the feed consumed divided by growth (de Verdal et al., 2017a), is important as more fish can be produced with the same resources and production costs (Besson et al., 2014; de Verdal et al., 2017a; Eya et al., 2011; Gjedrem et al., 2012; Kause et al., 2016). Chinook salmon (*Oncorhynchus tshawytscha*) is a high-value salmonid species which is currently less feed efficient than other farmed salmonid species. For example, Atlantic salmon (*Salmo salar*) has a mean FCR of 1.1 – 1.2 (Cook et al., 2000; Mundheim et al., 2004) compared to ~1.8 for Chinook salmon (Araujo et al., 2021; NZKS Company, 2019; Walker et al., 2012). To improve FCR it is important to understand the multiple factors that influence FCR, including physiology, genetics, the environment, and behaviour (Emmerson, 1997), and that the interactions of these can be highly species-specific.

Previous studies differentiated Chinook salmon based on their feed conversion ratio (FCR) and identified feed efficient (FCR_E) and inefficient (FCR_I) individuals for further investigation (Elvy et al., 2022; Elvy et al., 2023; Esmaeili et al., 2021). These studies found that FCR_E Chinook salmon tended to consume less food compared to their FCR_I conspecifics. This, combined with information on nutrient retention efficiency, indicated that FCR_I fish were eating more than they were biologically benefiting from. It was also found that when the different FCR phenotypes were fed the same sized ration, the energy required to digest the meal did not differ (Elvy et al., 2023). However, this was measured in individually held fish which were gavaged, so energy use may not have been sufficiently estimated as social interactions and feeding behaviours, such as manoeuvring, that normally occurs during feeding (Boisclair and Tang, 1993; Hughes and Kelly, 1996) were not accounted for. Feeding behaviour is an important factor to consider because as fish compete for available feed, activity levels and social interactions increase (Zhao et al., 2017). Therefore, the oxygen consumption of gavaged fish in swim flumes is likely underestimating energy use for the

entire feeding process. Further research is required to understand the contribution of behaviour to the feeding process.

Feed intake is one of the two variables driving FCR, and understanding the behaviours used to obtain feed may therefore help explain why FCR differs among individuals. Examination of the feeding behaviours an individual chooses, allows a comparison of the relative energy expenditure used in food acquisition versus the amount of energy obtained from the food and, therefore, the efficiency of food acquisition itself. In addition, there is an increasing interest in the consistency of behaviours over time (Castanheira et al., 2017; Castanheira et al., 2013; Ferrari et al., 2015; Martins et al., 2011a). Understanding which behaviours are used by fish in aquaculture environments and whether they remain consistent over time can prove beneficial for the production and welfare of farmed fish (Andrew et al., 2004a; Brännäs and Johnsson, 2008; Huntingford and Adams, 2005; Martins et al., 2012).

Locomotion is an important component of feeding for fish (Rice and Hale, 2010) as successful feeding begins with locating and moving towards the feed item (Jobling et al., 2012a; Stradmeyer, 1989). For an individual fish to reach food after it has been identified, fish often make angular turns before travelling a given distance at speed (Andrew et al., 2004b). Once the individual has reached the food it must then obtain and ingest the item before swallowing (Jobling et al., 2012a; Stradmeyer, 1989; Stradmeyer et al., 1988). However, food items are not necessarily eaten straight away as they can be rejected at multiple time points. For example, once food items are approached, they can be ignored or, if ingested, be rejected and spat out (Jobling et al., 2012a; Stradmeyer, 1989). Understanding how fish are obtaining food and the success rate of their manoeuvres can provide a good indication of the level of effort required, hence the efficiency of energy use during food acquisition.

Other feeding behaviours that could feasibly correlate with feed efficiency include the size of the meal and the time taken to consume it. Behavioural feed intake can be determined by recording the total number of pellets ingested by individual fish during the meal (Adams et al., 1995; de Verdal et al., 2017a; Øverli et al., 1998). It is also possible to measure when feeding begins and finishes and therefore, the total time spent feeding. This is important as Martins et al. (2011b) found that feed efficiency was significantly correlated

with feeding behaviours, such as time to start feeding and total time spent feeding. In Chapter 4, no significant difference was found between when FCR_E and FCR_I Chinook salmon were feeding within the meal but there was a significant correlation between individual FCRs and when a fish was feeding. The current study therefore set out to examine when FCR phenotypes are feeding in more detail using video images to assess feeding. It is presumed that fish spending less time feeding use less energy to feed, sparing more energy for metabolism and growth, and thus are probably more feed efficient (Hart, 1986). This was observed in tilapia (*Oreochromis niloticus;* Martins et al., 2011b) and African catfish (*Clarias gariepinus;* Martins et al., 2005) where more efficient fish took longer to begin eating but ate for less time and were less active. Thus, differences in feeding activity result in variation in energy expenditure that contributes to differences in feed efficiency (Martins et al., 2011b). Therefore, feeding behaviour could be used to predict and select for FCR which would lead to financial and welfare benefits in aquaculture.

This study aimed to observe and quantify the feeding behaviours of captive, group-reared Chinook salmon to understand: 1) what feeding behaviours are expressed during feeding, 2) the consistency of these behaviours, and 3) how they differ between different FCR phenotypes. It was hypothesised that FCR_I Chinook salmon are less efficient than FCR_E salmon because they exhibit more costly behaviours in the acquisition of feed. On the basis that FCR_I Chinook eat larger meals, likely in excess of their nutritional requirements (Elvy et al., 2022; Esmaeili et al., 2021), it was hypothesised that FCR_I salmon use more effort to consume larger meals which comes at a cost to growth. For example, FCR_I individuals are expected to carry out more energy-expensive behaviours than their efficient conspecifics, such as swimming larger distances at greater speed, turning greater distances and/or repeatedly spitting out food without eating. By classifying and quantifying feeding behaviours in detail, it may be possible to determine if behaviour contributes to FCR variation in Chinook salmon, and if it can be modified to improve the overall FCR of this species.

5.2. Materials and methods

5.2.1. Fish and trial set up

All-female Chinook salmon sourced from a commercial hatchery (Salmon Smolt New Zealand) were individually tagged with passive integrated transponder (PIT) tags prior to

transfer to the Cawthron Institute's Finfish Research Centre, Nelson, New Zealand. Fish had been on site for several months prior to commencement of this study (see Chapter 3 for husbandry up until this point). 174 fish were held in three 3,000 L tanks at 56 – 61 fish per tank. Each tank was supplied by a recirculation system which provided clean oxygenated freshwater at 17.2 ± 0.01 °C to all tanks throughout the trial. Photoperiod was set to 24 h artificial light. Fish were fed by hand to satiation once a day in the morning on a commercial diet (Tasman Aoraki; 6 mm).

5.2.2. Fish growth and feed intake assessments prior to the behaviour study

All fish were assessed for individual feed intake using ballotini and X-radiography according to the methods described in Elvy et al. (2022). Fish were weighed and daily feed intake (DFI) measured across three DFI assessments. DFI measurements were used to estimate the total feed intake between assessments using the share of the meal (SOM) method, as described previously by Elvy et al. (2022) and Esmaeili et al. (2021). The individual DFI values were divided by the tank DFI (the sum of individual DFI estimates within the tank), to estimate a percentage SOM for each fish at each assessment, as per the following:

SOM (%) = (individual DFI (g) / tank DFI (g)) x 100

The total feed eaten by an individual was calculated from the mean SOM from two feed assessments multiplied by the total feed consumed by the tank between feed assessments. FCR was then calculated for individuals in the time-points between DFI 1 and DFI 2 (FCR 1–2) and DFI 2 and DFI 3 (FCR 2–3) using the following equation:

FCR = total feed eaten (g) / weight gain (g)

Fish were classified by their feed efficiency phenotypes based on their distribution within a box and whisker plot (Elvy et al., 2022). Only feed efficient (FCR_E) or feed inefficient (FCR_I) phenotypes were used for the behavioural analysis.

5.2.3. Feeding behaviour assessment

Twenty-seven days after the last feed intake assessment, 10 FCR_I and 10 FCR_E fish were randomly selected from the pool of fish and externally tagged through the dorsal fin with a T-bar anchor tag (Hallprint, Australia) and moved into a single 3,000 L tank. The orientation

of the tag on the fish was used to identify phenotype: A tag extending on the left-hand side for FCR_I fish and a tag on the right-hand side for FCR_E fish (Fig. 5.1.). These fish were left to recover from the tagging and handling and acclimatise to their new tank. During this period, fish were fed the 6 mm commercial diet once a day to satiation. The first video recorded meal was carried out 74 days after the fish were tagged.

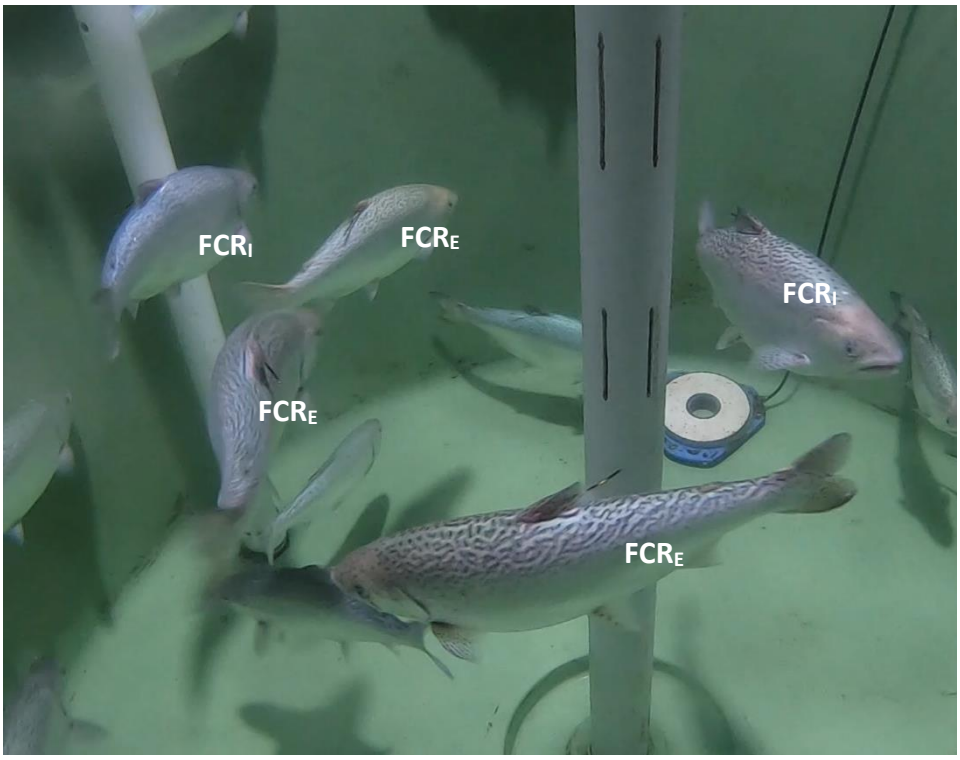

Figure 5.1. Image of tagged FCR_E and FCR_I Chinook salmon during a meal.

One month prior to recording the meals, three suction cup camera holders, with the camera frames (to simulate the camera) attached, were placed in the tank, allowing fish to acclimatise to their presence. Ninety minutes prior to filming, three GoPro Hero 8 cameras were carefully placed on the camera holders within the tank to minimise disturbance. Two of the cameras were angled downwards towards the bottom of the tank. The third camera in the middle faced straight ahead to view the upper portion of the water column. This allowed approximately 95 % of the tank to be included in the video frame but excluded the space directly below the cameras. However, it is thought that little feeding happened in this area due to how pellets were thrown into the tank. However, a very small proportion of turning angles were unable to be determined when they occurred in this portion of the tank.

Feed behaviour assessments were aligned with normal feeding schedules with fish fed to satiation in the morning with feed delivered across two 10 min rounds of feeding with a gap of 20 min in between. Three of these meals were recorded a week apart. One tank was used for the behaviour analyses using the three meals as temporal replicates. One FCR_I fish died prior to the recordings so the one observation tank contained 10 FCR_E and 9 FCR_I individuals. The video recordings were analysed using VidSync software (Fig. 5.2.) which allows for behaviours (Section 5.2.3.1.) to be annotated on screen and stored (Neuswanger et al., 2016). As tags only identified the phenotype rather than individuals, behaviours were combined and grouped by FCR phenotype.

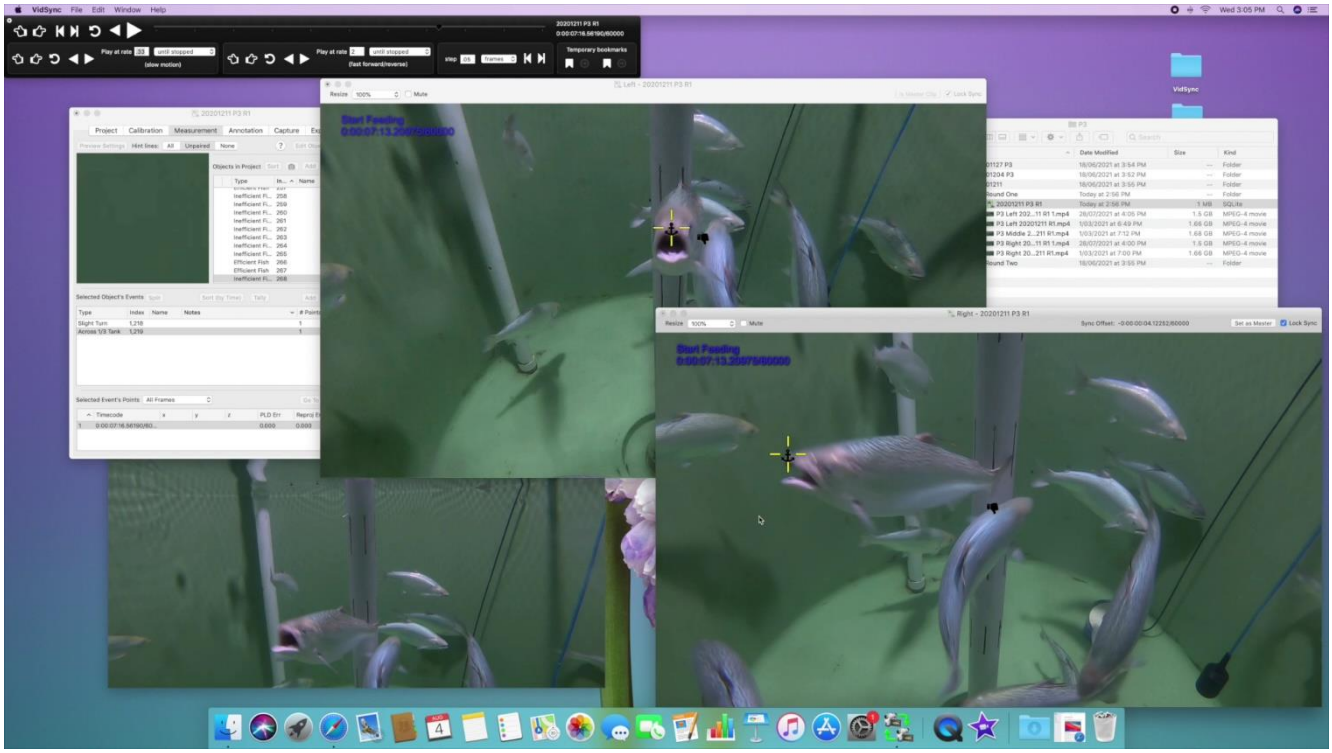

Figure 5.2. A screenshot of the VidSync program with the three camera angles and the behavioural annotation.

5.2.3.1. Behaviour variables

A total of 8 different behaviour types were assessed as defined below (summarised in Appendix One):

1) Turn angle to approach feed: Fish movements away from their initial travel direction to approach feed were assigned 3 categories for turn angle: 0 – 45° (slight turn), 45 – 90° (moderate turn) and 90 – 360° (extreme turn; Andrew et al., 2004b).

2) Distance travelled to approach feed: Fish were given a score based on how far they travelled in the tank to approach a feed pellet. The movement started when the fish finished its turn (behaviour 1 above) and ended when the fish had reached the food item (behaviour 3). The tank was split into thirds vertically up the tank and thirds horizontally to provide boundaries. Fish could move within boundaries, or they could cross these boundaries (Fig. 5.2.). It was also possible for fish to move in a combination of across and up/down movements and cross none, one or multiple boundaries. The number of boundaries that were crossed were counted and it was noted if they travelled across and/or in an up/down direction (Table 5.1.). It is important to note that fish could move in any direction within a boundary and not cross a boundary. As the tank was deeper than it was wide, across boundaries were considered a smaller distance compared to up or down, and therefore, the ranked movements were split into five movement levels as an estimation of the distance travelled.

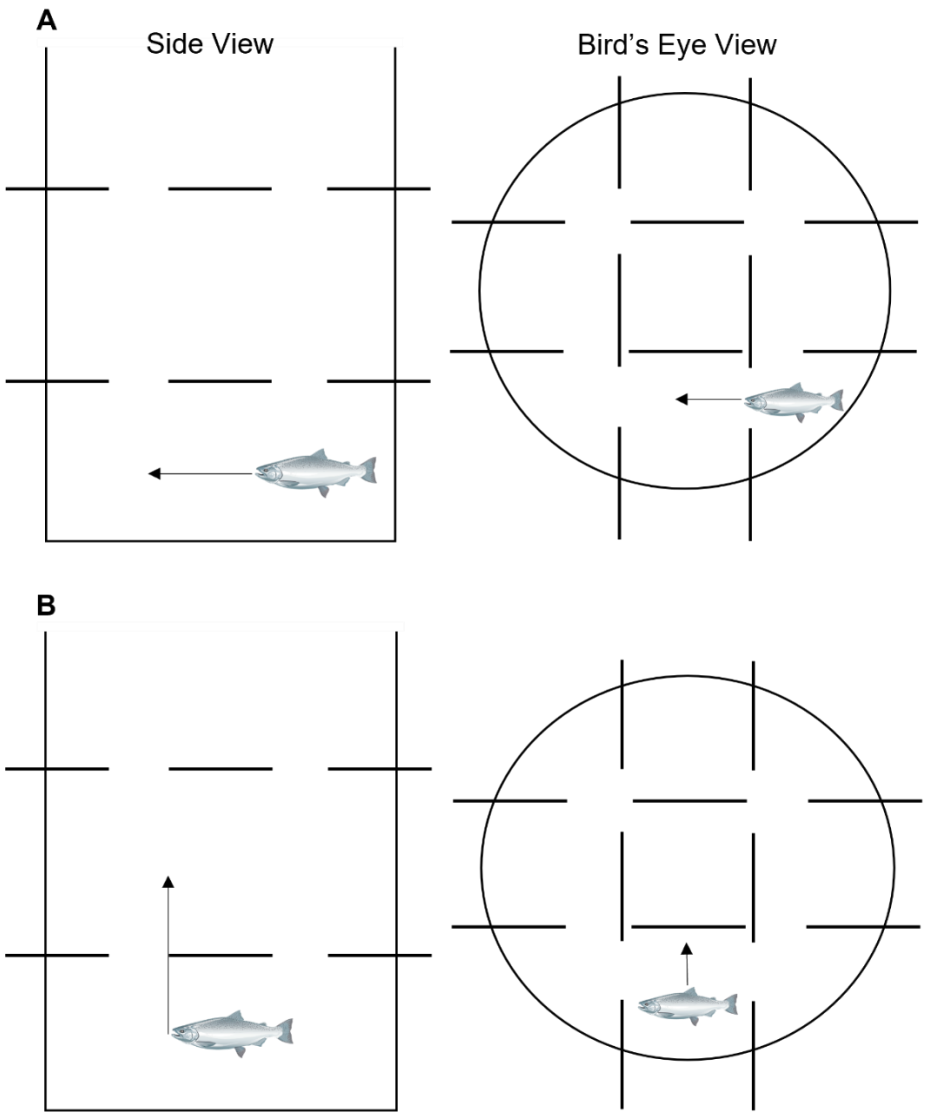

Figure 5.3. Examples of distance levels travelled by fish within the tank (Table 5.1.). A) Level 2, fish moved across one boundary, but no boundaries were crossed going up or down; B) Level 3, moved across none and up across one boundary.

Table 5.1. Distance levels based on the number of boundaries crossed by individual fish. Each line is a separate combination to make up the level.

Distance Level	Across		Up/Down	Description
L1	None		-	Moved horizontally across without crossing boundaries, no up/down movement
	-		None	Moved vertically up or down without crossing boundaries, no horizontal movement
L2	One		-	Moved horizontally across and crossed one boundary, no up/down movement
	None	AND	None	Movement occurred horizontally and vertically (up or down) within a boundary
L3	-		One	Moved straight up or down and crossed one boundary, no horizontal movement
	Two		-	Moved horizontally across and crossed two boundaries, no up/down movement
	One	AND	None	Movement occurred horizontally and vertically crossing a horizontal boundary but no vertical boundary
	None	AND	One	Movement occurred horizontally and vertically crossing a vertical boundary but no horizontal boundary
L4	-		Two	Moved straight up or down and crossed two boundaries, no horizontal movement
	Two	AND	None	Movement occurred horizontally and vertically crossing two horizontal boundaries but no vertical boundary
	One	AND	One	Movement occurred horizontally and vertically crossing a horizontal boundary and a vertical boundary
	None	AND	Two	Movement occurred horizontally and vertically crossing two vertical boundaries but no horizontal boundary
L5	Two	AND	One	Movement occurred horizontally and vertically crossing two horizontal boundaries and one vertical boundary
	One	AND	Two	Movement occurred horizontally and vertically crossing one horizontal boundary and two vertical boundaries
	Two	AND	Two	Movement occurred horizontally and vertically crossing two horizontal boundaries and two vertical boundaries

3) Success of feeding attempt: All observed feeding attempts were defined as a movement towards a pellet that ended with either a) the pellet being refused (not taken into the mouth) or b) accepted (taken into the mouth).
4) Feed consumed or rejected: Once the pellet was taken into the mouth it was either consumed (swallowed and eaten) or subsequently rejected (spat out).
5) Time taken to consume/reject feed: This behaviour was quantified as the time (s) from when the pellet was taken into the mouth to when it was either rejected (spat) or eaten (swallowed). The pellet was considered rejected when the pellet was seen leaving the mouth. The pellet was considered eaten when jaw movement was observed after acceptance as this represented the pellet being chewed and swallowed. The time was classified into 5 groups described in Table 5.2.

Table 5.2. Decision timing groups for the time the fish took to decide whether to eat or spit the pellet.

Group	Timing (s)
1	0 – 1
2	1 – 2
3	2 – 3
4	3 – 4
5	>4

6) Interval in which fish fed within a meal: It was recorded at what time during the meal (min:sec.millisec) each pellet was eaten, and which phenotype ate the pellet. The whole 20 min feeding period each day was then split into ten 2 min intervals labelled feeding intervals 1 – 10.
7) Vertical positioning of fish in the tank: Still images of the tank were taken one second before feed entered the tank and every minute thereafter throughout the meal. The tank was divided into three vertical sections (see behaviour 2 above) and the number of each phenotype in each section of the water column was determined from each image.
8) Aggressive interactions: Video recordings were observed for aggressive behaviours, including chasing and biting (Adams et al., 2007; Pottinger and Carrick, 2001).

5.2.4. Statistical analysis

Linear mixed effect models, with "meal" as a random factor, were used to analyse the relationship between FCR phenotypes and behaviours 1 – 6. Because the data from each meal were not independent from each other (the observations were performed on the

same fish on three successive occasions), this approach allowed the effect of "meal" on behaviours to be accounted for. For each case, an ANOVA was used to compare the linear models with and without a random component. A binomial general linear model was used to compare the total counts of behaviours 3 and 4 between phenotypes. For behaviours 1, 2, 5 and 6 an ordinal regression was used in the linear mixed effect models to compare the total counts between phenotypes. The total counts for each behaviour were divided by the number of fish in each phenotype group for each of the three meals. The counts per fish were then compared using a linear model for each behaviour. As there were only 3 values per phenotype, the results are an estimation of individual performance. All statistical analyses were performed using 'R' (R Core Team, 2020). Significance was accepted at $P < 0.05$.

5.3. Results

Only fish that had gained weight and were in good condition were used in the study (Table 5.3.). The amount of feed fed on the days of the behavioural analysis was similar to the amount of feed fed on the days prior to the cameras being added to the tank, indicating that the addition of cameras to the tank did not disturb the fish and put them off their feed.

Table 5.3. Fish data (mean ± SE) for fish used in the behavioural study. Initial values were measured 2 months prior to the behavioural study (at time of external tagging) and final values are 2 months after the behavioural study. Values with significance difference (P < 0.05) are marked with superscripts.

	FCR_E	**FCR_I**
Number of fish	10	9
FCR	1.06 ± 0.9^a	1.59 ± 0.10^b
Initial		
Weight (g)	704±31	711±36
Length (mm)	331±5	329±6
Condition Factor	1.94±0.03	1.98±0.03
SGR (%/day)	0.57±0.05	0.48±0.08
Final		
Weight (g)	1430±78	1282±110
Length (mm)	409±6	397±12
Condition Factor	2.09±0.08	1.99±0.08
SGR (%/day)	0.47±0.03	0.35±0.07

Feeding behaviours were compared between the three replicate meals to determine how repeatable the behaviours were over time. None of the recorded behaviours analysed varied significantly between the three replicate meals. This was determined by comparing models with meal as a random factor to models without this random factor for each behaviour. The models were not significantly different to one another, indicating that meal had no influence on how behaviours were expressed. Therefore, at least at a group level, behaviours were assumed to be repeatable and consistent over this timeframe. The FCR phenotypes were evenly distributed vertically within the water column throughout the whole meal indicating that neither phenotype monopolised any specific area of the tank and therefore, did not have a spatial advantage when feed entered the tank.

5.3.1. Turn angle to approach feed

Overall, 2266 turns were made during the study period. The total number of turns (regardless of phenotypes) classified into the three categories were significantly different from one another i.e., the number of slight turns (0 – 45 °) was higher than that of moderate turns (45 – 90 °; P = 0.001) which was higher than the number of extreme turns (>90 °; Fig. 5.4.A; P = 0.001). FCR_I individuals had more total turns in all categories, but this was not significantly different to the FCR_E individuals (P = 0.32; Fig. 5.4.A). However, when the turns were analysed per fish (Fig 5.4.C.), FCR_I made significantly more slight turns than FCR_E (P < 0.001, F = 75.57, R^2 = 0.94) while moderate (P = 0.05, F = 7.69, R^2 = 0.57) and extreme turns (P = 0.29; F = 1.5; R^2 = 0.09) did not differ between FCR phenotypes. Slight turns were the most common category, making up approximately 50 % of all turn activity (Fig. 5.4.B). However, the proportion of the three turns observed (Fig. 5.4.B) did not significantly differ between FCR phenotypes. FCR_I fish as a group carried out significantly more turns than FCR_E. The type and proportion of turns were not different between FCR phenotypes when analysed as a group but per fish FCR_I fish carried out significantly more slight turns compared to FCR_E fish.

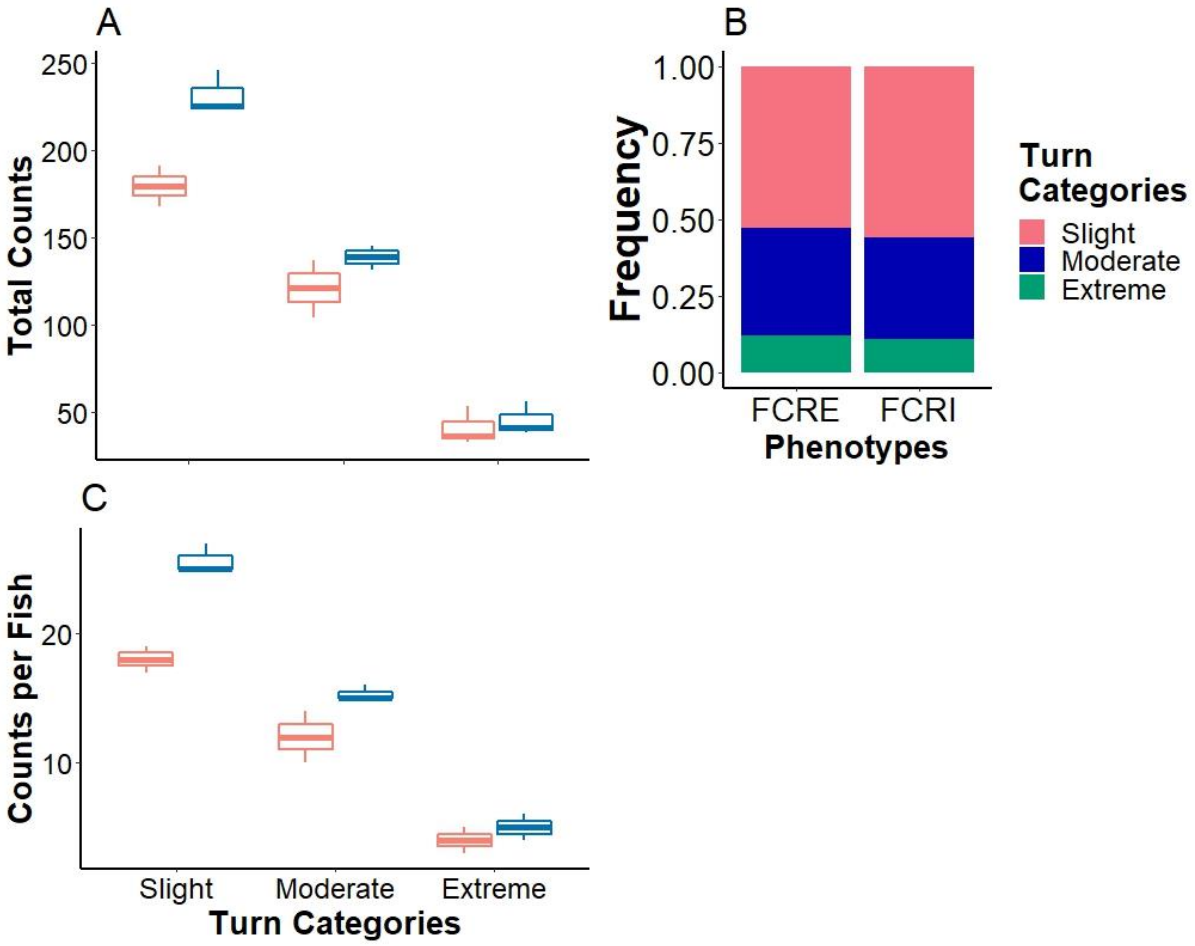

Figure 5.4. The turning angles of fish moving to orientate towards the food item. A) The total counts of observed behaviours of FCR_E (red) and FCR_I (blue) fish. B) The proportion of turn angle categories for each phenotype as a function of total counts. C) The counts of the observed behaviours per fish of FCR_E (red) and FCR_I (blue) fish, letters represent a significant difference between FCR phenotypes within the behaviour category.

5.3.2. Distance travelled to approach feed

After initiating a turn the fish then travelled within the tank to reach the pellet. The total number of observations at each of the 5 distance levels (ordered from 1 to 5) were all significantly different from each other. i.e., the total number of observations at L1 was higher than that of L2, which was higher than L3 etc (Fig. 5.5.A). FCR_I fish carried out more movements overall (1313), compared to the FCR_E fish (1091). FCR_I fish carried out significantly more movements at L1 compared to FCR_E fish in total (Fig 5.5.A; $P < 0.001$) and per fish (Fig 5.5.C; $P < 0.001$, $F = 38.44$, $R^2 = 0.88$). The number of counts did not differ between the other distance levels. L1 was the most common category for both phenotypes with over 50 % of distance travelled falling into this category. L5 was the least common category for both phenotypes (Fig. 5.5.B).

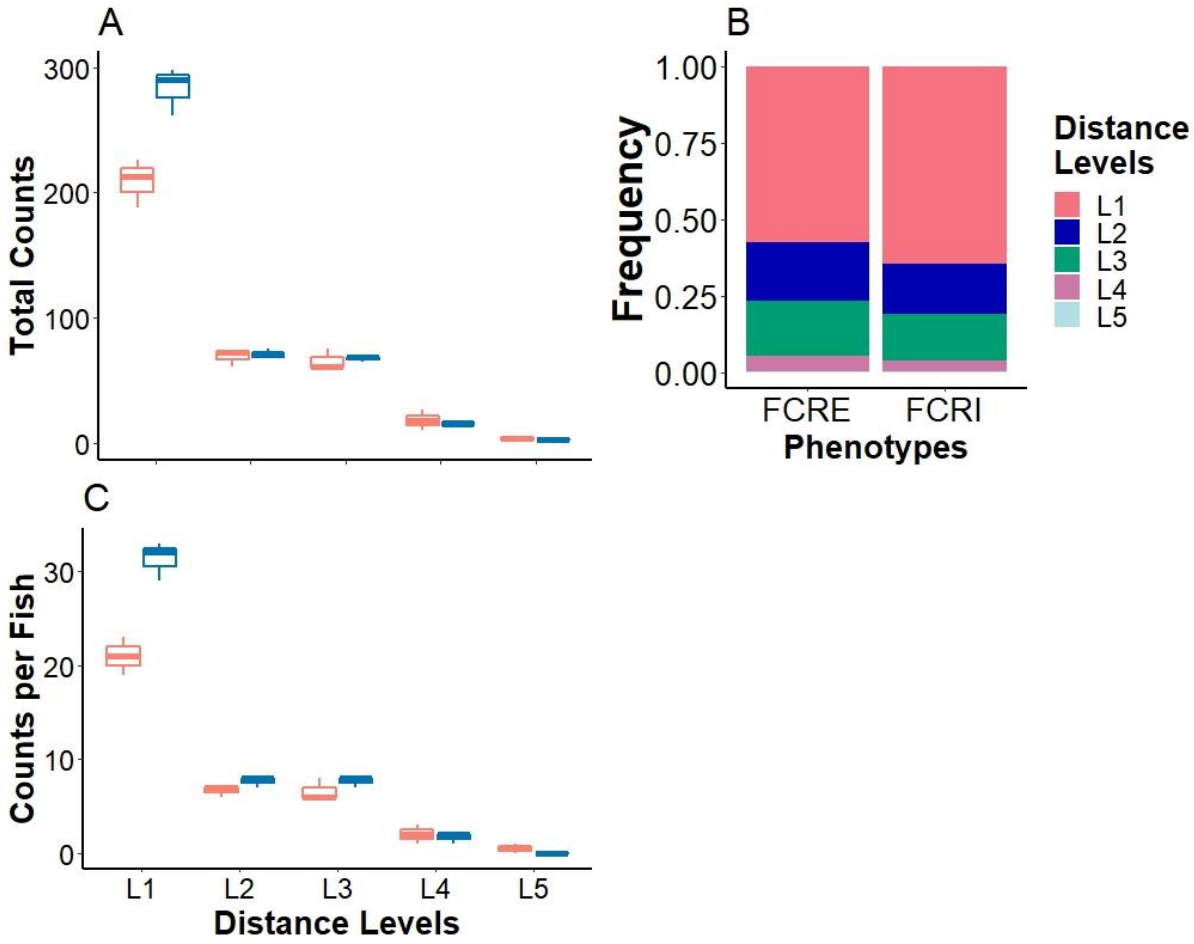

Figure 5.5. The distance levels the fish moved towards the feed. The distance levels represent the number of boundaries crossed by a fish to reach the feed, see Section 5.2.3.1 for more information. A) The total counts of observed behaviours of efficient (red) and inefficient (blue) fish. B) The frequency of distance levels for each phenotype as a function of total counts. C) The counts of the observed behaviours per fish of FCR_E (red) and FCR_I (blue) fish, letters indicate a significant difference between FCR phenotypes within a behaviour level.

5.3.3. Success of feeding attempt

Once fish reached the pellet, they either took the pellet into their mouth or not. Fish that began a feeding movement (completed a turn and moved around the tank) took a pellet into their mouth > 80 % of the time, and this did not differ between phenotypes ($P = 0.05$). However, when compared on a per fish basis, FCR_I fish took more pellets into their mouths than FCR_E fish ($P < 0.001$, $F = 94.23$, $R^2 = 0.95$). There was no significant difference between the FCR phenotypes in the number of pellets not taken into their mouth ($P > 0.05$).

5.3.4. Pellet consumed or rejected

The fish that took a pellet into their mouths either ate or rejected it. Overall, fish were more likely to eat the pellet than reject it. FCR_I fish ate 694 pellets in total across the three

meals while FCR_E fish ate 687 pellets in total, so total meal sizes were not significantly different between the two FCR phenotypes. The estimated meal size per fish also did not differ between the FCR phenotypes (Fig 5.6.; $P > 0.05$, $F = 0.64$; $R^2 = -0.08$). However, FCR_I fish interacted with 839 pellets while FCR_E fish interacted with only 790 pellets in total. Thus, FCR_I fish were significantly more likely to spit a pellet than FCR_E fish ($P = 0.02$). FCR_I fish rejected 17 % of pellets they captured compared to 13 % rejected by FCR_E fish. Interestingly, spitting occurred throughout the duration of the meal.

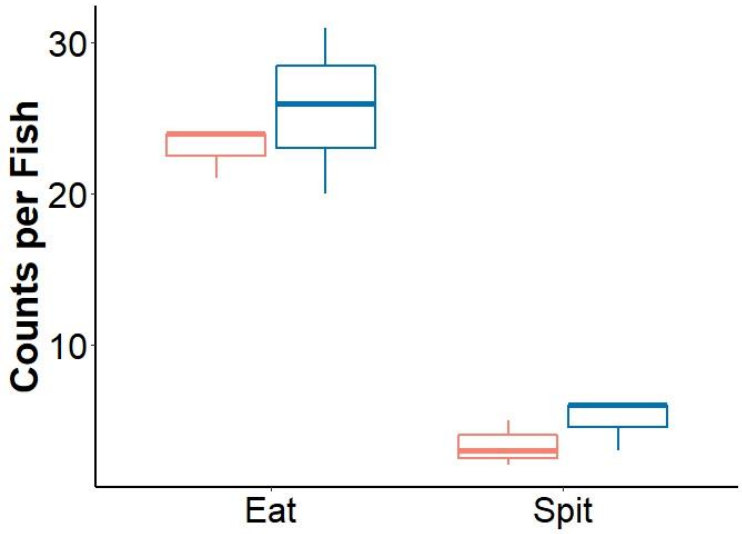

Figure 5.6. The number of pellets eaten or spat behaviours per fish of FCR_E (red) and FCR_I (blue) fish.

5.3.5. Time taken to consume or reject feed

The time to make the decision to accept or reject the pellet was quantified into groups of 1 second intervals. The total counts of all five categories were significantly different to the level above i.e., the number of observations in group 1 was higher than that of group 2 which was higher than group 3 etc (Fig. 6A). The time to decide did not differ between phenotypes in any of the timing groups ($P = 0.2$; Fig. 5.7.A) with over 75 % of decisions, regardless of FCR phenotype, being made in the first 2 seconds (Fig. 5.7.B). However, in group 2 (1 – 2 sec) FCR_I fish made significantly more decisions, per fish, than FCR_E fish (Fig. 5.7.C).

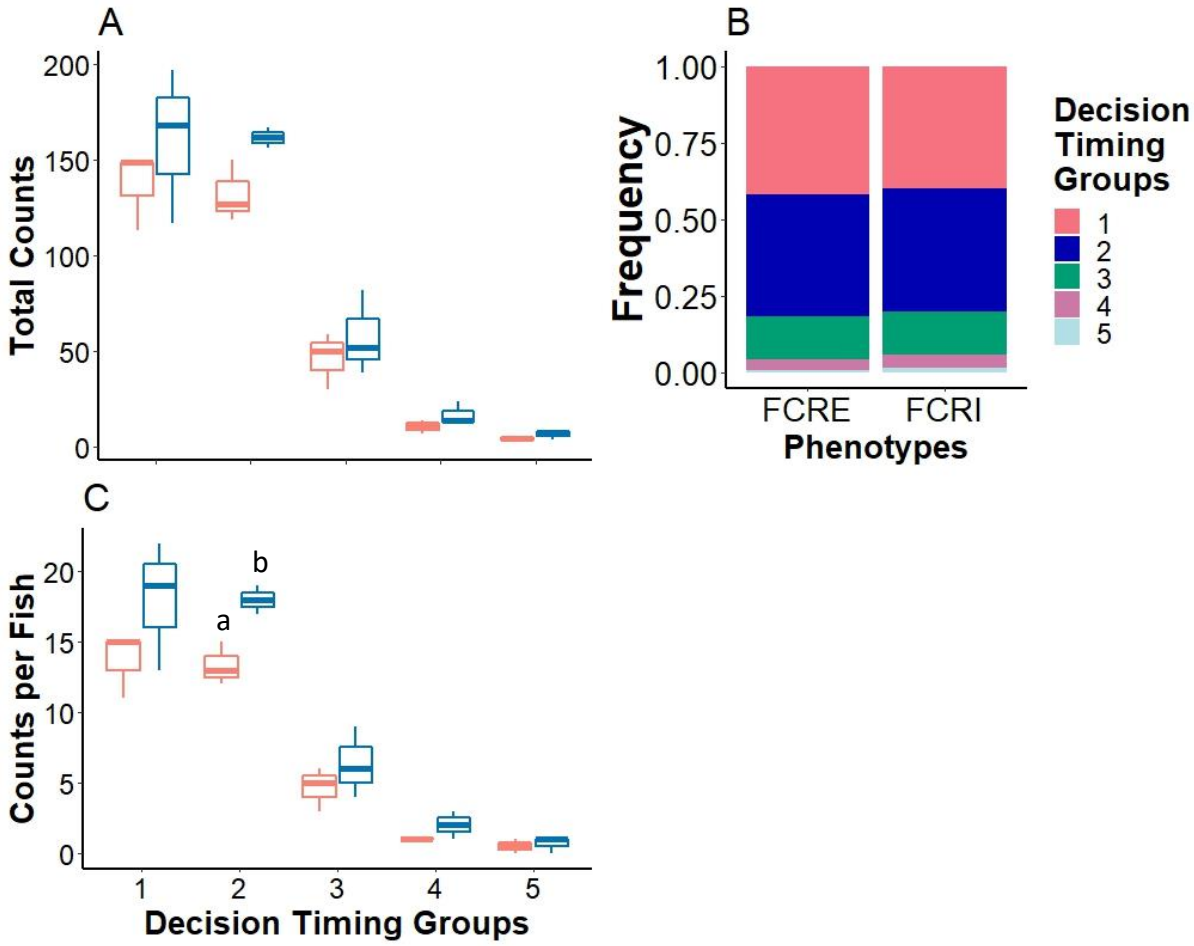

Figure 5.7. The time taken by fish to decide whether to eat or reject the food item once taken into their mouth. The decision timing groups represent 1 second intervals, see Section 5.2.3.1 for more details. A) The total counts of observed behaviours of efficient (red) and inefficient (blue) fish. B) The proportion of decision timing groups for each phenotype as a function of total counts. C) Counts per fish of observed behaviours of efficient and inefficient fish, letters represent a significant difference between FCR phenotypes within a group.

5.3.6. Interval in which fish fed within the meal

The feeding interval represents when the FCR phenotypes were eating pellets within the meal (Fig. 5.8.). The 2-minute feeding intervals were ordered from 1 – 10 and the total counts of each group differed significantly from the next group up (P = 0.001). i.e. the total collective counts of group 1 differed significantly from the total counts occurring at group 2 which in turn differed from group 3 etc (Fig. 5.8.A). The exception to this was for groups 5 and 6 which were not significantly different from each other (P = 0.53). Overall, the phenotypes differed in terms of when they ate within the 20 min feeding period (P = 0.03). During the initial part of the meal, and up to interval 7 (13 – 14 min of the meal) the mean number of pellets eaten did not differ between FCR phenotypes. However, after feeding

interval 7 (from 15 minutes onwards) the mean number of pellets eaten by FCR_E fish was less than that of their FCR_I conspecifics until the end of the meal (Fig. 5.8.B). At interval 7 FCR_I fish ate more pellets compared to FCR_E fish (Fig. 5.8.C; P = 0.047, R^2 = 0.58, F = 8). The number of pellets consumed by FCR_I fish did not decrease until the last 2 min interval of the meal.

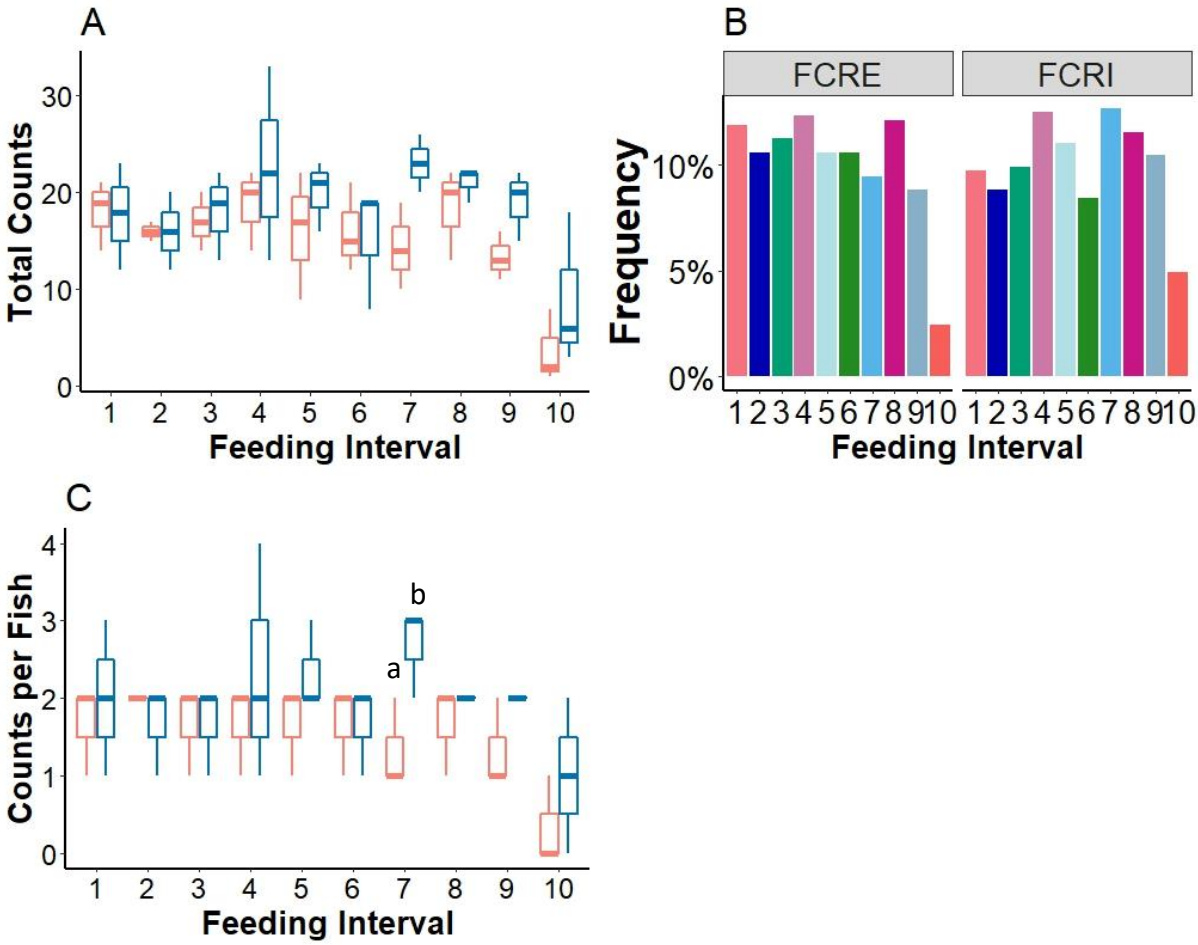

Figure 5.8. The number of pellets being eaten at each feeding interval. Each feeding interval represents a 2-min interval within the 20 min meal, see Section 5.2.3.1 for more details. A) The total counts of pellets consumed in each feeding interval efficient (red) and inefficient (blue) fish. B) The frequency proportional of pellets eaten in each feeding interval compared to the total pellets eaten for each phenotype. C) The counts of pellets consumed per fish in each feed interval for feed efficient (red) and inefficient (blue) fish.

5.3.7. Aggressive behaviours during feeding

Aggression was analysed during all three meals. However, as limited aggressive behaviour was observed, the sample size was too small for statistical analysis. Only one bite was observed during all three meals where a FCR_I fish bit another FCR_I fish. This appeared to be an accident when the first fish was going for a pellet and the other fish's tail was next to

the pellet. There were also two incidents where a fish was chased by another, one was an FCR_I fish chasing a FCR_E fish, and the other incident was the reverse, which occurred when the FCR_E 'chaser' fish was going for a pellet close to the 'chased' FCR_I fish.

5.4. Discussion

This study used video imaging to observe and analyse the feeding behaviour of a tank of captive reared Chinook salmon (*Oncorhynchus tshawytscha*). This is the first time this type of analysis has been applied to study Chinook salmon feeding behaviour to investigate the link between these behaviours and different FCR phenotypes. The behaviours were also analysed across three time points which showed that, in the short-term (over a three-week period), feeding behaviours were consistent in Chinook salmon. This pilot study showed that aggression was rarely seen under the conditions of the trial and that FCR_I fish exerted more effort during feeding through an increased number of movements and an increased likelihood of spitting feed compared to their FCR_E conspecifics, which may be contributing to their poor FCR values.

5.4.1. Feeding behaviour and a comparison of FCR phenotypes

5.4.1.1. Getting to the feed

Regardless of phenotype, Chinook salmon appeared to minimise their effort in obtaining food by predominantly using narrow turning angles (0 – 45 °) and travelling only relatively short distances (Level 1) to get to food items. However, the FCR_I fish did perform significantly more total movements, especially slight turns, to obtain food and completed a significantly higher number of short-distance movements, whilst consuming a similar total amount of food as FCR_E fish. This contrasts with previous studies where FCR_I consistently ate significantly larger meals than FCR_E (Elvy et al., 2022; Elvy et al., 2023; Esmaeili et al., 2021). Therefore, if FCR_I ate as much as they would normally be expected to, they would be performing even more movements than FCR_E fish. This behaviour was consistent with Martins et al. (2011b) who also found that higher feeding activity (more feeding acts) were seen in less feed efficient fish. FCR_I fish were therefore likely using more energy during feeding while ingesting the same amount of feed as the FCR_E fish; as suggested by Martins et al. (2011b) for tilapia (*Oreochromis niloticus*), these behaviours could be directly influencing overall FCR.

5.4.1.2. Feed rejection

FCR_I fish were significantly more likely to reject and spit the food, thus leading FCR_I fish to interact with even more pellets, whilst also wasting the extra effort involved in food capture by not actually consuming the pellet. The increased level of interaction with feed without benefitting from it also likely contributes to the poor feed efficiency of FCR_I fish. One potential reason for this behaviour is that spitting is thought to be part of 'tasting' the feed, contributing to whether food is accepted or rejected (Andrew et al., 2004b; De La Higuera, 2001). As most of the feed was consumed, it is assumed that the palatability of the diet was not a contributing factor to the spitting seen in the current study. Another potential reason for spitting is the fish 'playing with' the pellet, which is thought to be linked to satiation (Andrew et al., 2004b). Playing with food may also indicate that the fish are bored (Burghardt et al., 2014), and are taking the daily feeding as an opportunity for play. In the current study spitting occurred throughout the meal, therefore it was assumed that spitting was not explained by fish becoming satiated during the meal. Instead spitting may be due to fish being fed to satiation daily, so fish were never very hungry or constrained by food availability during the trial. Daily feeding to satiation may therefore be promoting spitting behaviours, as fish became habituated to receiving plentiful feed on a regular schedule. Therefore, feeding to slightly less than satiation could minimise spitting and potentially reduce feed wastage. This agrees with Elvy et al. (2022) and Araújo et al. (2023) who also suggest that feeding Chinook salmon a reduced ration would be beneficial for production without compromising FCR.

5.4.1.3. Aggression

The presence of social hierarchies and aggression are commonly reported in the literature for salmonids (Boujard et al., 2006; Damsgård and Huntingford, 2012; Heydarnejad and Purser, 2010; Huntingford et al., 2012; McCarthy et al., 1992; Moutou et al., 1998), and it was expected that this would be observed in Chinook salmon. However, aggressive behaviours were not observed in the current study. There were occasional occurrences where a fish was chased or bitten, though this appeared to be accidental when two fish were swimming towards the same pellet and got in each other's way. The lack of aggression and social hierarchies in the current study may be due to the low numbers in the trial tanks and the satiation regime (Adams and Huntingford, 1996). Esmaeili et al. (2022a)

and Esmaeili et al. (2021) suggest that inefficent Chinook salmon are more dominant than their efficient conspecifics based on having a larger share of the meal. Aggression and dominance are often associated with monopolisation of territory and food (Adams and Huntingford, 1996; Adams et al., 1995; Andrew et al., 2004b) and may not be as evident if space and food are not limited. The studies by Esmaeili et al. (2022a) and Esmaeili et al. (2021) were performed at higher densities than the current study, perhaps giving a more accurate representation of farm conditions. Another example of dominance is holding a midwater position and carrying out more movement around the tank, as seen in rainbow trout (Pottinger and Carrick, 2001). While FCR_I fish carried out more movements during feeding, there was no evidence that they held their position in a particular part of the water column any more than FCR_E fish. Further research into the effects of density, feeding regime and rations is required to determine if Chinook salmon develop social hierarchies under specific circumstances and, if so, how they are maintained.

5.4.2. Consistency of behaviours

In the current study, the same tank of fish was analysed across three meals, and results indicated that feeding behaviours were consistent at a group-level across meals. Due to the constraints of this pilot study, we were not able to determine whether behaviours would have been consistent at an individual level. Similar research has found that, depending on the species, behaviours are consistent or inconsistent at an individual level. For example, Castanheira et al. (2013) observed that risk-taking and escape behaviours were consistent over time and under different situations in individual Gilthead seabream (*Sparus aurata*). Similarly, Martins et al. (2011a) found that time to start feeding and the number of feeding acts were repeatable in individual tilapia. However, it is also possible that behaviours are consistent at a group level, even if the behaviour of individuals within the group are not. This is not an unreasonable suggestion as this phenomenon has been observed elsewhere. For example, the behaviour of European seabass (*Dicentrarchus labrax*), including feeding recovery, aggression, and exploratory behaviours, was not consistent at an individual level but was at a group level over short- and long-term experiments (Ferrari et al., 2015).

5.4.3. Limitations

The results of the current study were limited by the use of repeated measures on groups of fish in only one tank due to camera and time restrictions. Ideally, this study would have

been carried out on multiple tanks of fish that were able to be identified individually. Initially, during data collection, three tanks of fish (10 FCR_E and 10 FCR_I in each tank) had multiple meals recorded. However, due to time constraints and camera issues, such as batteries dying, murky water and bubbles forming on the camera lens, which obscured the view of the fish and their feeding behaviours, only one tank was able to be analysed. Individual fish were also unable to be identified as the video quality was not sufficient to distinguish between enough combinations of colours on tags to identify all fish individually. Despite these constraints, three meals were able to be analysed for the same group of fish, allowing feeding behaviours to be identified in Chinook salmon, and these were determined as being consistent in the short-term. Therefore, future studies can build on the learnings of the current study to further understand the individual behaviour of Chinook salmon in larger groups.

5.4.4. Conclusion

This study is the first in-depth characterisation of the feeding behaviours exhibited by captive farmed Chinook salmon. The results showed that behaviour was consistent within the same group of individuals in the short-term (over a three-week period). Feeding behaviours were used as an indicator of relative energy expenditure in the current study to help understand FCR variation. Inefficient fish exerted more effort during feeding without energetically benefitting from their behaviour as their feed intake was not increased. It is also possible that feeding Chinook salmon to satiation may promote spitting behaviour through daily overfeeding. To further understand Chinook salmon behaviour, it is important to understand which behaviours remain consistent in the long-term and see how these feeding behaviours are expressed across multiple groups of fish. However, the current study provides a good start to understanding the short-term group behaviours of farmed Chinook and their possible impact on FCR.

Chapter 6

General Discussion

6.1. Main findings

Feed is an expensive component of finfish farms, making up to 70 % of total production costs. Feed conversion ratio (FCR), the proportion of feed intake to weight gain, is a measure of feed efficiency. Currently, FCR in Chinook salmon (*Oncorhynchus tshawytscha*) is poor compared to other salmonid species farmed worldwide. This thesis aimed to look at FCR variation between individuals and the factors that influence this variation. Factors investigated in this thesis included growth, feed intake, nutrient retention efficiencies, metabolic rates, and behaviour. Understanding the influence of these factors can help farmers implement husbandry techniques and/or select for certain traits in breeding programs, all with the goal of improving FCR in Chinook salmon. By improving FCR overall, finfish farm productivity could be enhanced without increasing feed input, with the added benefit of reducing the amount of nutrients excreted into the environment.

Several physiological variables that could influence FCR in Chinook salmon were selected for examination in this thesis, including growth, feed intake, nutrient retention, and oxygen consumption (as a proxy for metabolic rates). This study supported the hypothesis that feed efficient fish would have higher growth rates, higher nutrient retention, and lower routine metabolic rate (RMR_{min}) than inefficient fish, at least in fish reared in saltwater (Chapter 2). In freshwater Chinook salmon (Chapter 3), feed intake and growth followed a similar trend to saltwater fish, with FCR_I consuming larger meals, having lower growth rates and nutrient retention rates, however, there was no difference in RMR_{min} between FCR phenotypes. Maximum metabolic rate (MMR) and aerobic scope (AS) did not vary between FCR phenotypes in both fresh and saltwater fish. Specific dynamic action (SDA) was also investigated in freshwater fish, but after being fed the same ration size, no part of SDA varied between FCR phenotypes. These similarities and differences between Chinook salmon reared in freshwater and saltwater emphasise the importance of understanding the physiology of this species in both fresh and saltwater environments and over a range of body sizes.

In addition to the physiological parameters investigated, behaviours between the FCR phenotypes were compared. The ballotini method (Chapter 4) was used to assess the timing of feeding at commercially relevant densities. The results went against the hypothesis that feed efficient fish ate later in the meal and no difference was seen when FCR phenotypes ate within the meal. Video analysis was used to assess feeding behaviours in more depth (Chapter 5). This pilot study was carried out on a small group of Chinook salmon which were externally tagged according to their FCR phenotype. This study supported that FCR_I fish would be more active within a meal (performed more turns and travelled more distance) compared to FCR_E fish. The FCR_I fish were also more likely to spit out food pellets compared to FCR_E fish, indicating that they were likely to exert more energy on feeding during a meal without benefitting from it nutritionally. It also appeared that feeding behaviours were repeatable in Chinook salmon over a three-week period. The outcomes of this research contribute important new information to the current body of knowledge regarding feed conversion ratio, metabolism, and behaviour in Chinook salmon.

6.2. Implications and application of the research

A major finding of Chapters 2 and 3 was that FCR_E fish consumed significantly smaller meals compared to their FCR_I conspecifics. Other studies have seen a similar negative relationship between feed intake and FCR, indicating that efficient individuals consumed smaller meals (Peterson and Small, 2006; Rodde et al., 2020; Silverstein, 2006; Silverstein et al., 2005). In addition to smaller meals, FCR_E Chinook salmon also had higher growth rates compared to feed FCR_I fish, consistent with findings in other species (Eya et al., 2013; Henryon et al., 2002; Kause et al., 2016; Rodde et al., 2020; Silverstein, 2006; Silverstein et al., 2005; Thodesen et al., 1999). It was hypothesised that FCR_E fish would have higher growth rates; however, the difference in feed intake amongst FCR phenotypes was an unexpected result but agreed with previous research by Silverstein (2006), who suggested that efficiency decreases at larger meal sizes as energy is not fully utilised for growth and therefore wasted. This appears to be the case in FCR_I Chinook salmon as they eat larger meals without improving their growth rates and have decreased retention of nutrients (protein, lipid, and energy). Thus, nutrients from the feed are being wasted.

It was hypothesised that, in addition to faster growth, FCR_E fish would have higher nutrient retention as seen in other species (Carter et al., 1993a; Carter et al., 1993b; Eya et

al., 2013; Kolstad et al., 2004; McCarthy et al., 1994; Overturf et al., 2013; Peres and Oliva-Teles, 1999; Silverstein et al., 2005). It is thought that differences in protein metabolism contributed to the improved growth rates and therefore, increased retention of nutrients observed in FCR_E. In a proteomic study on these same two phenotypes of Chinook salmon, Esmaeili et al. (2021) found that protein synthesis pathways were enriched in FCR_E fish, indicating that FCR_E individuals have higher rates of protein synthesis. It has also been shown in other species that feed efficient individuals have lower rates of protein degradation (Carter et al., 1993a; Carter et al., 1993b; Kolstad et al., 2004; McCarthy et al., 1994). Higher rates of protein synthesis combined with lower rates of protein breakdown likely contribute to higher growth rates in FCE_E fish. In contrast, FCR_I fish had enriched proteolysis pathways in the liver (Esmaeili et al., 2021). These individuals appear to be eating more than they can process and incorporate into their body tissue but are also breaking down proteins in their bodies at a higher rate than feed efficient fish. FCR_I fish ate larger meals, had lower growth rates, and retained less nutrients, so these fish are eating more, increasing expensive feed requirements on the farms, while also taking longer to reach harvest size.

Chapters 2 and 3 found that lipid retention efficiency (LRE) also differed significantly between FCR phenotypes, with FCR_E fish having higher LRE values. A similar result was seen in rainbow trout (*Oncorhynchus mykiss*; Eya et al., 2013; Silverstein et al., 2005). Proteomic findings of Esmaeili et al. (2021) on Chinook salmon FCR phenotypes suggest that this was likely due to several pathways related to lipid metabolism being upregulated in FCR_E fish. In particular, fatty acid synthesis was increased in the livers of FCR_E Chinook salmon compared to FCR_I fish. The LRE values for FCR_E fish in Chapters 2 and 3 were also above 100%. This is likely due to retention efficiencies accounting for dietary lipid as the only lipid source contributing to lipid deposition. However, glucose can be converted into fatty acids through de novo lipid biosynthesis pathways (Chen et al., 2019). Therefore, it appears that lipid biosynthesis may contribute significantly to the lipid composition of Chinook salmon, and more so in FCR_E, according to both the LRE findings from Chapters 2 and 3 as well as the findings of Esmaeili et al. (2021). Further research into the de novo lipid synthesis to understand their capacity for these processes could be beneficial in terms of diet composition, especially if current lipid inclusion rates are in excess to requirements.

As feed intake is difficult to measure directly in fish, a proxy could be beneficial for providing data to help improve FCR in fish. Nutrient composition has been hypothesised to be a useful tool for estimating FCR in mammals, poultry, and fish (Knap and Kause, 2018). Lipid composition has been shown to be useful proxy for estimating FCR in rainbow trout (Kamalam et al., 2012; Kause et al., 2016; Kinghorn, 1983). However, whole-body lipid composition did not vary between individuals (Chapter 2), nor was there any correlation between lipid composition and FCR values, consistent with the findings of Esmaeili et al. (2021). Therefore, whole-body lipid composition cannot be used as a proxy for FCR in Chinook salmon as hypothesised in this thesis.

Chapters 2 and 3 examined the relationship between oxygen consumption rates (RMR_{min}, MMR and AS) and FCR phenotypes in Chinook salmon reared at 17 °C. It was hypothesised that FCR_E Chinook salmon would have lower RMR_{min} compared to their FCR_I conspecifics. However, it turned out the relationship between metabolic rates and FCR in this species is highly complex. Chapter 2 assessed larger Chinook salmon (approx. 2.5 kg) in saltwater and found that the FCR_E fish had lower RMR_{min} values than FCR_I fish, while MMR and AS did not differ between the FCR phenotypes. Chapter 3 assessed smaller freshwater Chinook salmon (approx. 1.0 kg) and in these RMR_{min}, MMR and AS did not vary between FCR phenotypes. Variable relationships between metabolic rates and feed efficiency have also been observed in European seabass (*Dicentrarchus labrax*), where more feed efficient fish had lower RMR_{min} values at a population level but not at an individual level (Rodde et al., 2021a).

There were two main differences between the experimental conditions of Chapters 2 and 3: salinity and body size. One of the potential causes of differences between the relationship with RMR_{min} between the phenotypes in the two studies is the influence of osmoregulatory processes. Salinity has been shown to increase the maintenance metabolism associated with homeostasis (Claireaux and Lefrancois, 2007; Febry and Lutz, 1987). FCR phenotypes may differ in their ability to carry out this process resulting in the significant difference in RMR_{min} between phenotypes in saltwater (Chapter 2). This difference in metabolism may not be seen in freshwater fish as these pathways are not required. The second factor influencing the difference between the studies is body size. It is possible that variation in RMR_{min} becomes apparent at a larger body mass, either due to body mass itself and/or an additional complication seen when these fish got bigger, which was stress. Stress appears to

be more of an influence in larger Chinook salmon (Pers. Obs). It was originally planned to incorporate SDA measurements into Chapter 2. However, these larger fish in saltwater were unable to be kept in the swim flumes for longer than three days as their metabolic rates remained elevated or the fish died. This was not the case for smaller fish in freshwater, as those fish could stay in the swim flume for seven days. In addition, the larger fish examined in Chapter 2 were handled an additional two times (a total of five times; Fig. 2.1.) compared to the smaller fish, which were only handled three times in total for the three ballotini assessments. This additional handling stress may have had an impact on their metabolism, especially if one phenotype was more prone to stress than the other. Esmaeili et al. (2021) suggested that FCR_I Chinook salmon were more stressed than FCR_E fish based on the detection of elevated stress and inflammatory response proteins in muscle and indicators of endoplasmic reticulum stress in the liver. Stressed fish tend to produce higher levels of cortisol (Gilmour et al., 2017; Gilmour et al., 2005; Sloman, 2011), which has been shown to increase metabolic indices (Trenzado et al., 2003) and standard metabolic rate (SMR; Sloman et al., 2000). Stress has also been shown to decrease nutrient assimilation, FCR, and growth, while also increasing routine energy expenditure and the cost of growth (Pfalzgraff et al., 2021). These impacts of stress would all be consistent with the differences observed, and not observed, between FCR_I and FCR_E fish in Chapters 2 and 3, i.e., if FCR_I fish are more stressed in the swim flumes when they are larger, this could contribute to the RMR_{min} differences seen in Chapter 2.

It was hypothesised that the energy required to digest a meal would vary between FCR phenotypes. However, it was found that there was no statistical difference between the SDA response of FCR_E and FCR_I fish when fed the same ration. This is consistent with the findings of Li et al. (2016), who found no difference in SDA variables in southern catfish (*Silurus meridionalis*) with differing FCR values, indicating that SDA was not an important mechanism underlying differences in FCR. This was also observed in hapuku when fish were fed a constant ration (Khan et al., 2015). However, to measure SDA in this thesis, fish needed to be gavage fed. Daily feed intake is highly variable in Chinook salmon, and when feeding normally, FCR_I individuals ate larger meals than FCR_E individuals (Chapters 2 and 3). It is well known that ration influences SDA, with a larger meal likely to result in an elevated metabolism for a prolonged amount of time (Jobling, 1993; Khan et al., 2015; Skov et al.,

2017) although variation in the components of SDA is species specific. Therefore, if allowed to feed normally, the SDA would likely have been higher in FCE_I fish, and thus, the effect of ration on SDA needs to be further researched in Chinook salmon to determine how the daily energy expenditure attributable to SDA varies between FCR phenotypes.

Using a dual ballotini method proved to be a more practical way of analysing feeding behaviour on a larger number of fish compared to other methods, i.e., video recording and individual rearing. It was also easy to incorporate a second ballotini bead into feed intake assessments that were already being carried out. The dual ballotini method has previously been successfully used to provide information on feed preference choices in several species including polar cod (*Boreogadus saida*), Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus;* Amundsen et al., 1995; Christiansen and George, 1995; Thorpe et al., 1990; Toften et al., 2003; Toften et al., 1995). In Chapter 4, a novel application of using dual ballotini feeding was developed to reveal the timing of feeding within a meal. Previous studies have shown good correlation between ballotini feed estimates indicating that the method has good repeatability over time (Difford et al., 2023; Elvy et al., 2022). This was also seen in the dual ballotini method.

Video analysis was used in Chapter 5 to provide a more in-depth analysis of when FCR phenotypes were feeding. This allowed for confirmation of whether the FCR phenotypes did vary feed intake throughout the meal. Video analysis found that the feed intake of FCR_I fish remained consistent throughout the meal and only started to drop off in the last couple of minutes. In comparison, in FCR_E fish, feeding began to decrease slightly earlier in the meal, therefore overall, these fish were feeding for a shorter amount of time. This is consistent with studies of African catfish (*Clarias gariepinus*) and tilapia (*Oreochromis niloticus*), where more efficient individuals ate their meals in a shorter amount of time (Martins et al., 2011a; Martins et al., 2005). There was a slight difference in how long FCR phenotypes fed for within the meal, and this potentially could be detected using the ballotini method if a third bead was incorporated near the end of the meal.

It is hypothesised that one factor contributing to the inefficiency of FCR_I fish may be their excess effort to obtain the meal. This agrees with activity levels in Chapter 5, where FCR_I fish expended more effort overall during feeding by engaging in more swimming activity during feeding, in the form of turns and distance travelled during the meal. As well as completing

more moves these fish were also more likely to spit pellets, meaning more activity occurred without the result of pellet consumption. In addition, these feeding behaviours were observed repeatably during the three meals studied and were consistent, at least in the medium-term (3 weeks), in the same group of fish. Sole (*Solea solea*) were also reported to have consistent feeding strategies over time (Mas-Muñoz et al., 2011). There is also some indication that group-based behaviours are consistent in the short- and long-term, even if individual behaviours are not (Ferrari et al., 2015). If these observed behaviours remain consistent over the long-term, then it could be a permanent factor influencing FCR, in particular, FCR_I are consistently and regularly exerting more energy than necessary. This then opens the potential for moderation via husbandry changes such as feeding methodology or selective breeding.

Understanding which phenotypic traits have a strong correlation with FCR can assist in selecting traits to improve FCR indirectly. Chinook salmon have been farmed in New Zealand for 40 years and have been selectively bred, particularly for growth, since the mid-1990s (Stenton-Dozey et al., 2020; Symonds et al., 2019). Despite this, FCR is still relatively poor compared to other salmonids. The relationships in this thesis indicated that feed intake is likely a strong influencer of FCR, far more than growth. Therefore, feed intake, specifically selecting for individual that eat smaller meals as a percentage of their body weight, would be a good trait to select for to improve FCR in this species. This agrees with the recently published work of Scholtens et al. (2022a) on Chinook salmon families, which also concluded that selecting for feed intake will promote better gains in FCR than selecting for growth.

There was no significant difference between when the FCR phenotypes were feeding when examined using dual ballotini, but this only differentiated the meal into two temporal halves (Chapter 4). A more in-depth look at feed timing, broken into 2-minute intervals, using videography (Chapter 5) indicated that FCR_I fish were more likely to keep eating at the end of the meal when FCR_E had slowed down their eating. Feeding a reduced ration (slightly below satiation) or finishing feeding slightly earlier, rather than when the last fish stops eating, may prevent FCR_I fish from overeating, particularly at the end of the meal. Feeding a restricted ration has been observed to reduce feed efficiency variation (Silverstein, 2006) without negatively affecting FCR in Chinook salmon (Araújo et al., 2023). Feeding a

restricted ration could therefore be an immediate implementable and high-impact husbandry change that is easily incorporated on farms.

6.3. Limitations and improvements

The study of specific dynamic action (SDA) in this thesis was limited by the number of replicate fish per FCR phenotype, so it would be useful to repeat the SDA experiment to get more data on this trait in Chinook salmon. Unfortunately, Chinook salmon are highly stress-sensitive, and only four fish per phenotype completed the swim flume measurement protocol. Due to the high level of individual variability observed, it is hard to determine if the lack of differences between FCR phenotypes is in fact due to a lack of differences or the low sample size. Power analysis suggests that if there were significant differences, this might have been detected with a larger sample size ($n > 7$). In addition to the low numbers, stress was likely a contributing factor to the results of fish completing the SDA protocol, as in many of the individuals, the oxygen consumption remained elevated for a prolonged period and never returned to within 8 % of RMR_{min}. In the future, as a way of minimising stress, SDA could be carried out as a separate experiment, not in conjunction with MMR as was done in this thesis.

Chapter 5 provided novel insight into the feeding behaviours of Chinook salmon and revealed that the same group of fish would perform the same behaviours over multiple meals. This was a good starting point for understanding Chinook salmon feeding behaviours, but this study was restricted to using only one tank. Future research would benefit from a trial using replication across multiple tanks. Identifying individual fish, rather than by FCR phenotype, would also provide a better understanding of the repeatability of behaviours. Another limiting factor for the experiment carried out in Chapter 5 was that low numbers of fish were used due to the restrictions of the method, meaning they were not necessarily relevant to farming densities. A larger number of fish per tank could also provide more information, but this is difficult to achieve while preventing fish from blocking one another and being able to identify individuals. Cameras were strategically positioned in this study in an attempt to limit blind spots; however, some blind spots were unavoidable, and there was a section of the tank that was not observed in the video footage. However, this was estimated to be a small proportion of the tank, ~10 %, and very little feeding behaviour seemed to occur in this section of the tank.

6.4. Future research

It would be useful to understand why feed intake varies across FCR phenotypes. One potential cause is an imbalance of feed regulating hormones in FCR_I fish, resulting in them eating more than biologically required. Leptin, ghrelin, and insulin are key hormones in feed regulation by stimulating or depressing appetite and feed intake. Leptin and insulin are involved in appetite suppression as they give an indication of internal energy stores, while ghrelin stimulates appetite and increases feed intake (Jobling et al., 2012b). Leptin is produced by fat cells, so as lipid stores increase, more leptin is produced, and feed intake reduces in turn (Jobling et al., 2012b; Silverstein, 2002). However, fish with higher levels of muscle adiposity have been shown to have impaired leptin production, as seen in rainbow trout (Gong et al., 2016; Johansson et al., 2016; Kamalam et al., 2012). These fish also had lower growth rates and feed efficiency (Johansson et al., 2016; Kamalam et al., 2012). While whole body lipid composition did not vary in Chinook salmon in this thesis, lipid composition of the muscle was not investigated and may still vary between FCR phenotypes. Another hormone of interest is ghrelin. The addition of ghrelin to the diet of sea bream (Sparus aurata) increased growth and feed efficiency (Rodríguez-Viera et al., 2022). Therefore, it is possible that FCR_I fish could also have impaired ghrelin production. As leptin and ghrelin have been shown to influence growth and feed efficiency performance in fish, it stands to reason that the genes related to the production of these hormones are up-regulated (ghrelin) or down-regulated (insulin, leptin) in FCR_I fish, causing these fish to eat larger meals than are biologically required. Further research is needed to fully understand the effect of appetite regulating hormones on feed intake and FCR in Chinook salmon.

It is hypothesised from the findings of this thesis that Chinook salmon farmers could improve FCR by feeding a restricted ration or limiting the time fish are able to feed, which is easily applied as a feed management strategy. However, further research is needed to determine if regulating feed intake can improve the FCR of feed inefficient individuals without impairing performance and to confirm that they are eating excess nutrients that are being wasted. A risk of applying this method without determining the impact of ration/timed feeding is that FCR_I fish could change their feeding behaviours, such as feeding more at the beginning of the meal, as an adaptation to a perceived resource limitation. Another way of reducing overfeeding is by determining when feeding can be stopped based

on a feeding behaviour. Many Chinook salmon farms in New Zealand already use cameras during feeding to determine when the last fish has stopped eating and stop feeding accordingly. By determining a stop signal to avoid over-feeding the FCR_I fish, the overall FCR of the group could potentially be improved through feed management practices without the need to breed for improved feed intake.

The results of this study led to the hypothesis that FCR_I fish were overeating due to boredom. These fish may be using feeding time as a form of enrichment and spitting out pellets was a form of 'play' (Andrew et al., 2004b), especially as spitting occurred throughout the entire meal. In this thesis, fish were fed once a day, with their single daily meal being their only form of enrichment. Potential enrichment additions include the addition of overhead cover, submerged structures, underwater feeders, and live feed, which have been shown to mitigate behaviours that occur in hatchery rearing, promoting more natural behaviours (Berejikian et al., 2001; Brown et al., 2003; Roberts et al., 2011). Research into enriching the tank environment for Chinook salmon could indicate if this is a possible solution to preventing overfeeding or spitting. Theoretically, this could improve fish welfare and possibly FCR if energy expenditure and overeating were increased due to boredom.

The effect of ontogeny on factors such as FCR consistency and potential metabolic variation would be beneficial to understand how energy requirements may change over time. Metabolism has been shown to change with ontogeny (Moran and Wells, 2007; Oikawa et al., 1991). In contrast, FCR in smaller fish is thought to represent FCR across the whole rearing period, as observed in tilapia (Rodde et al., 2020). Chapters 2 and 3 indicated that FCR is consistent over time in 1000 – 2000 g and 300 – 620 g Chinook salmon, respectively, as FCR was significantly correlated between FCR 1 – 2 and FCR 2 – 3 in each instance. If fish remain feed efficient or inefficient over the long-term, the trait could be measured in smaller fish when they are easier to handle and are less prone to stress (Pers. Obs.). In addition to body size, the effect of salinity on different aspects of metabolism would be beneficial to examine in more detail as this is an under researched area in this species.

Another potential area for future research would be examining the effect of ration sizes on the SDA of Chinook salmon. Ration size has been shown to increase the total SDA energy

(Jobling and Davies, 1980; Norin and Clark, 2017; Skov et al., 2017) while also increasing SDA duration (Jordan and Steffensen, 2007; Norin and Clark, 2017). A larger ration is likely to increase some SDA parameters in Chinook salmon, but which ones and to what extent is currently unknown in this species. This would be relevant to industry as Chinook salmon have been shown to consume a wide range of meal sizes, especially when fed to satiation, and would allow a more in-depth understanding of individual daily energy expenditure by the different FCR phenotypes.

Chapter 4 provided a good introduction to the dual ballotini method but repeating the experiment on a larger number of fish would be useful. The study executed in this thesis saw a significant but slight correlation in the first dual ballotini assessment between FCR and the percentage of large beads consumed. However, this relationship was not evident at the second assessment. Carrying out the experiment again with a larger number of fish would determine if the relationships between feed intake timing and FCR become more or less apparent. It would also be useful to have a slightly longer time between dual ballotini assessments and include at least one more assessment to reduce any potential feed intake variation that occurs across meals. The addition of a third bead could also be added to the method to provide a more in-depth look at when individuals feed within a meal. If the method does continue to show a relationship between timing of feeding and FCR values, as seen in this initial study, it could also be compared between families to see if the timing of feed intake is heritable and could be beneficial to incorporate into a breeding program.

6.5. Conclusions

Overall, feed inefficient Chinook salmon appeared to be both physiologically and behaviourally inefficient: they ate larger meals but were more likely to spit out captured pellets. They ate for longer during meals, carried out more feeding activity, and did more turns but retained a smaller proportion of nutrients from those meals in their body tissue. In addition, in saltwater, they had higher minimal resting metabolic rates (RMR_{min}). Aspects of physiology and behaviour that did not differ between the FCR phenotypes included: RMR_{min} (in freshwater), MMR, AS, timing of feeding within the meal, the likelihood of a pellet being rejected or taken into the mouth. Overall, it was seen that feed intake and feeding behaviour are important factors in FCR. The benefit of this is that industry can influence these, to a certain extent, through husbandry techniques such as how and when they feed

as well as the length of the meal. Feeding for a slightly shorter time or to a ration slightly below satiation could prevent Chinook salmon otherwise prone to overfeeding from doing so. This could improve their FCR and would likely do so without impacting growth due to the results found from nutrient retention efficiencies where protein and lipid from the feed were not being used fully. Further research is required to better understand optimal feeding regimes for Chinook salmon, especially across the wide range of environmental conditions they experience and at different life stages. However, this thesis provides a good introduction to Chinook salmon physiology, metabolism, and behaviour, which is beneficial to the industry as, prior to this thesis, FCR in this species was largely understudied.

Appendix One – Feeding behaviour classifications from Chapter 5

	Behaviours	Categories/Level	Description
1	**Turn angle**	Slight	0 – 45°
		Moderate	45 – 90°
		Extreme	90 – 360°
2	**Distance travelled**	See table 5.1. for details	Boundaries crossed by the fish to reach the pellet
3	**Feeding attempt**	Refused	Pellet is approached but not taken into the mouth
		Taken	Pellet is taken into the mouth
4	**Feeding result**	Accepted	The pellet is eaten
		Rejected	The pellet is spat out
5	**Time taken to decide the fate of the pellet after taken into the mouth (eat or spit)**	1	0 – 1 sec
		2	1 – 2 sec
		3	2 – 3 sec
		4	3 – 4 sec
		5	> 4 sec
6	**When pellet was eaten - time from the start of the meal**	1	0 – 2 min
		2	2 – 4 min
		3	4 – 6 min
		4	6 – 8 min
		5	8 – 10 min
		6	10 – 12 min
		7	12 – 14 min
		8	14 – 16 min
		9	16 – 18 min
		10	18 – 20 min
7	**Vertical positioning in tank**	Top	Number of each phenotype in top third of the tank
		Middle	Number of each phenotype in middle third of the tank
		Bottom	Number of each phenotype in bottom third of the tank
8	**Aggression**	Biter	Fish that bite another fish
		Bitten	Fish that got bitten
		Chasing	Fish chased another fish
		Chased	Fish that got chased

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