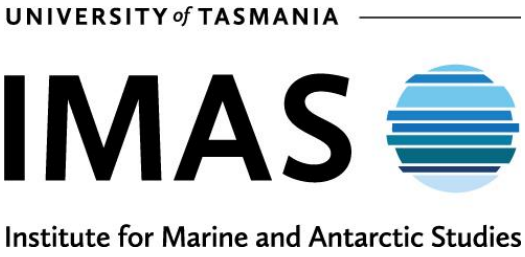

The effects of sustained swimming on long-term changes to Chinook salmon form and function

by

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Statements and Declarations

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General Abstract

The New Zealand salmon aquaculture industry is undergoing significant advances to underpin industry growth. These changes include expanding grow-out practices offshore and transitioning to recirculating aquaculture systems (RAS) with the inclusion of flow regimes to enhance production and to better prepare stocks for sea. To assist industry, research is required to understand how sustained swimming influences Chinook salmon form and function, which can be used to identify suitable flow regimes for hatchery tank-systems. This thesis first explored existing knowledge on the effects of sustained swimming on salmonids to identify how flow regimes can be used to prepare salmonids for offshore performance and identify any knowledge gaps (**Chapter 2**). Having identified that farmed Chinook salmon are unrepresented in studies investigating the effects of sustained swimming and recognising developments (i.e., expansion offshore) planned for the local aquaculture industry, this thesis created and used a large dataset to investigate and identify key parameters influenced by short and long periods of sustained swimming. An array of phenotypes and methods were used to achieve this, including measuring aspects of production performance at individual (**Chapter 3**) and tank levels (**Chapter 5**), genetic parameters, interactions, and correlations (**Chapter 4**), spinal health (**Chapter 3 and 6**), swimming performance and behaviour (**Chapter 3 and 6**), as well as internal changes in form: chemical composition (**Chapters 3, 5, and 6**), morphometrics (**Chapters 5 and 6**), and the transcriptome (**Chapter 5**). Lastly, this thesis brings together the knowledge gained within each data chapter and integrates it with oceanographic data collected in New Zealand's first proposed exposed offshore aquaculture site to construct bioenergetic models estimating energy consumption for individuals reared under different flow regimes, i.e., different levels of exercise-training (**Chapter 7**). The data generated in this thesis provides several advancements to the field of exercise physiology and to global aquaculture production, while providing key information to the New Zealand industry to make informed decisions for their farming developments and practices.

The experiments throughout this thesis were conducted using tank-based experiments, where post-smolt Chinook salmon were reared under low (0.3 body lengths per second; bl s^{-1}) and moderate (0.8 bl s^{-1}) flow regimes (LFR and MFR, respectively) from initial body size of 82.9 ± 0.3 g and 174.6 ± 0.2 mm to final metrics of 1870.5 ± 59.8 g and 439.4 ± 3.7 mm, over ten months. Each data chapter assessed different timepoints along this timeline to understand the short-term (three months under treatment; **Chapter 3 and 4**) and long-term (six and ten months; **Chapter 5 and 6,** respectively) impacts of sustained swimming on Chinook salmon form and function.

Chapter 3 investigated the influence of sustained swimming on Chinook salmon swimming behaviour, production performance, and spinal health under LFR and MFR. Chinook salmon reared under MFR had improved feed efficiency compared to LFR, but this was not reflected in individual feed intake rates or growth rates. Assessing the swimming behaviour of Chinook salmon between these two flow regimes revealed that the salmon were advancing around the tanks, swimming faster than the set flow regimes, and at similar speeds regardless of the flow regime. This study highlights the importance of measuring actual swimming speeds in exercise studies, as without these data, the interpretation of the results would have been substantially different. Spinal health was not different in individuals reared under the two flow regimes but unfavourable correlations between developing spinal curvature and higher condition factor existed. The results from this chapter suggest future research should use a new set of exercise regimes (with faster speeds) to adequately provide different exercise regimes for Chinook salmon between 82.9 g to 583.2 g. This chapter presents critical information on spinal curvature susceptibility for industry to consider when selecting for fast growing salmonids (i.e., higher condition factor).

Using the phenotypic data collected in **Chapter 3** and creating a genomic-relatedness matrix by genotyping 37 Chinook salmon families, **Chapter 4** assessed if any genotype-by-environment (G×E) interactions exist for production performance between LFR and MFR. To my knowledge, this is the first study to assess the interaction between genotypes and different flow regimes in finfish aquaculture. The analysis showed minimal evidence for G×E interactions based on genetic parameters and correlations. Genomic estimated breeding values (GEBVs) further supported this conclusion; however, when constructing re-ranking plots for feed efficiency GEBVs, some families did perform differently under LFR and MFR. The minimal G×E interactions between flow regimes could be associated with the similar swimming behaviour (i.e., speeds) that the fish exhibited when reared under LFR and MFR, as measured in **Chapter 3**. Nevertheless, the new information gained in this chapter is critical for the industry to consider when selecting candidate families as some families may not perform consistently across flow regimes, especially when using multiplier broodstock groups to select elite families.

Chapter 5 assessed the influence of sustained swimming on larger salmon (1056.0 ± 11.2 g and 374.1 ± 1.1 mm) by rearing Chinook salmon under LFR and MFR for six months. Several aspects of whole animal performance were explored and were matched with physical and chemical changes on lower biological organisation (i.e., tissue, cellular, and molecular levels). Production performance (growth, tank level feed intake and efficiency) was superior in Chinook salmon reared under LFR compared to MFR. Chemical composition (lipid and protein content), and muscle morphometrics did not differ between individuals reared under LFR or MFR, even though genes involved in lipid metabolism and muscle development and contraction were

upregulated in Chinook salmon reared under MFR. The lipid and protein content significantly different between the right and left fillets, supported by changes in muscle fibre morphology and gene expression. The right fillet presented higher lipid content in the lateral and visceral regions, lower protein content, denser white muscle fibres, and upregulation of genes involved in lipid metabolism and muscle development and contraction, revealing interesting lateral asymmetry between the left and right fillets. This study suggests continuous circular swimming can stimulate muscle blocks unevenly, revealing a new angle of questions associated with understanding the lateral influences of swimming in circular tanks on fish physiology.

Chapter 6 explored the use of rearing farmed Chinook salmon under LFR and MFR for ten months to improve swimming performance and assessed changes in physiological processes that underpin these enhancements. To better represent commercial stocks, physiological assessments also included individuals that developed spinal curvature. Critical swimming speeds and aerobic scope were significantly enhanced in individuals reared under MFR regardless of their spinal health, but individuals with spinal curvature required a larger amount of energy to recover from exhaustion. Chinook salmon reared under MFR presented leaner bodies and denser muscle fibres, while individuals presenting with spinal curvature showed greater protein content. Chemical composition also differed between the left and right fillets, in agreement with results obtained in **Chapter 5**. This chapter demonstrates how exercise-training provides a non-invasive method to enhance swimming performance in salmon for offshore farming and identifies a potential welfare concern for individuals with spinal curvature.

Combining the data generated in all chapters, **Chapter 7** discusses the integration of flow regimes and RAS into the New Zealand salmon aquaculture industry, it highlights where consideration might be given to condition salmon during early production stages. Bioenergetic models were constructed to demonstrate the importance of having exercise-trained fish for farming offshore, with relevance to a currently proposed offshore site: The Blue Endeavour. The analysis shows that exercise-trained fish are better equipped to sustain swimming speeds that could be encountered in the Blue Endeavour site and that oxygen consumption rates required to maintain these swimming speeds remain well below their aerobic ceiling. By having efficient cardiorespiratory and locomotory processes, less energy (feed) will be required to maintain activity, growth, and physiological processes in the Blue Endeavour, ensuring high fish performance and welfare. Lastly, this chapter discusses the next questions for future research to help pave the way for offshore developments in finfish aquaculture.

This thesis brings together several aspects of how sustained swimming influences farmed Chinook salmon form and function across a wide size range: providing valuable information on the benefits of integrating exercise regimes into RAS and early production stages to grow robust

and resilient salmon. Transitioning salmonid farming offshore into high energy environments remains a challenging task; however, this thesis provides additional support to an emerging solution of using exercise regimes to improve swimming phenotypes and maintain production performance in farmed salmonids to underpin offshore farming. Keeping high fish performance and welfare as the priority indicators when developing new farming endeavours will help lead to success and industry growth.

Chapter One General Introduction

1.1 Introduction to salmon aquaculture in New Zealand

The aquaculture industry is the fastest growing food sector worldwide (FAO, 2022). Driving this rapid increase is (i) overfished wild populations, (ii) demands for high-quality protein products, and (iii) exponentially increasing worldwide population (Tacon, 2022). The aquaculture industry supports food, nutrition and livelihood security, contributing to worldwide economies (FAO, 2020; Bax et al., 2021). In New Zealand (NZ), the aquaculture industry and the coastal blue economy are extremely important to the economy, Māori communities, and employment in regional and coastal areas, which generally have low job opportunities (Bax et al., 2021; FAO, 2023). Since 2005, the NZ aquaculture industry has had minimal growth in production (i.e., tonnes; Ritchie and Roser, 2021), despite these global trends in aquaculture. The NZ government announced an *Aquaculture Strategy* in 2020 to increase the sector to $3B in ten years. The industry in 2020 was valued at $650M and only consists of three main species; Pacific oyster *Crassostrea gigas*, Greenshell mussel *Perna canaliculus*, and King salmon (also known as Chinook salmon) *Oncorhynchus tshawytscha* (AQNZ, 2022). To reach the $3B goal, the NZ salmonid industry is expected to generate an extra $1.5B (64% of target; MPI, 2019). This means increasing salmon production from 15,500 tonnes to 80 - 90,000 tonnes per year: an additional 60 - 70,000 tonnes within the next six years.

Salmon farming in NZ predominantly occurs in nearshore sea pens located in the Marlborough Sounds, Stewart Island, and Akaroa Harbour. To a lesser extent, farming occurs in glacially fed hydrocanals at the base of Mt Cook: a unique freshwater farming system. Since the domestication of Chinook salmon (in NZ) in the mid-1970s, industry growth has been slow, and production in the last 10 - 20 years was stable at ~15,500 tonnes per year (FAO, 2020; NIWA, 2022). Industry growth is limited by available space for grow-out because of social and legislative constraints restricting development in new or expanding existing farm sites (Envirostrat, 2020; Morro et al., 2021). Many existing nearshore sites (predominantly in the Marlborough Sounds) are threatened by increasing sea surface temperatures and marine heatwaves (Oliver et al., 2018; Bulgin et al., 2020; Broekhuizen et al., 2021), both of which, can result in increased mortalities (Oliveira et al., 2021). Expanding farming offshore into open ocean is a promising and viable opportunity to maintain current production and enable industry growth.

Expanding finfish aquaculture into open ocean high energy environments is a large movement worldwide (Buck and Langan, 2017; Gentry et al., 2017; Novaglio et al., 2021). Open ocean provides a promising solution for industry growth by allowing access to more space, deeper waters, and improved water quality, as well as lower human impact and user competition.

However, open ocean farming can also be costly, as some sites are unprotected and susceptible to hostile conditions (Buck and Langan, 2017). See Buck et al. (2024) regarding precise terminology for various aquaculture sites. The Faroe Islands are already farming in exposed locations with substantial currents and wave action, but a period of bad weather, where current speeds of 60 cm s^{-1} measured within the sea pen led to mass mortalities (Johansson et al., 2014; Hvas et al., 2020). The Norwegian Atlantic salmon industry are also making significant advancements through the use of innovative technology (Moe Føre et al., 2022), where the first open ocean finfish farm was launched in 2021 (Watlestad, 2021). Norway is leading the transition to reinvent aquaculture and build the foundational framework necessary to successfully farm offshore (Klebert et al., 2023).

Open ocean farming in NZ is also progressing, where New Zealand King Salmon Co. Ltd. (NZKS; the largest producer of Chinook salmon worldwide) has received approval to build the first offshore open ocean farm site in NZ: The Blue Endeavour. Several other companies are now undergoing resource consent to attain ocean space and develop their own open ocean farms. Supporting the salmon industry's transition offshore are several major research platforms in Australia (AUS) and NZ that have active research programs underway:

- Blue Economy Cooperative Research Centre (CRC), Australian Government's CRC platform.
- *Experimental Platform for Aquaculture Production*, IMAS UTAS and Blue Economy CRC.
- *Enabling Open Ocean Aquaculture*, The Cawthron Institute.
- *Reimagining Aquaculture*, The New Zealand Plant and Food Research.
- *Fast-Tracking Finfish Climate Change Adaptation Research Programme*, The Cawthron Institute.

In combination with the knowledge being gained from these programs, the Blue Endeavour will also act as a pilot study to enable the success of offshore high energy farming for the entire salmonid industry in NZ.

The Blue Endeavour is in the Cook Strait, approximately 7 km from Picton, South Island. The site is twelve hectares (surface space) and will contain twenty circular pens (e.g., ScaleAQ Subsea System) that will harvest 10,000 tonnes per year, approximately 14% of the targeted 2030 aquaculture strategy (NZKS, 2020). The physical properties, defined by Newcombe et al. (2019) of the Blue Endeavour are:

- depth: 60 -110m

- tidal influence
- temperature: 12.5 – 17.0 °C
- salinity: 33 – 37.5 practical salinity unit (PSU)
- dissolved oxygen: >85%
- turbidity: 0 – 5 nephelometric turbidity unit (NTU)
- wave heigh: up to 1.5 m
- current speeds: mean = 40 cm s^{-1} and up to 124 cm s^{-1}

Increased wave height and environmental currents of the Blue Endeavour are arguably the largest concern for salmon production and welfare. Environmental currents in the Blue Endeavour site are more than double the environmental current speeds experienced in the existing nearshore farm sites, i.e., up to 30 cm s^{-1} (Gillespie, 2011; Campos et al., 2019), which raises several production-biology and welfare concerns for Chinook salmon.

1.2 Farming Chinook salmon: sustained swimming concerns and knowledge gaps

Profound swimming abilities are innate traits of salmonid species (Groot, 1991). Wild salmonids exhibit efficiency in aerobic and anaerobic metabolism, described by their high performance during large migrations and maintaining position in currents as well as escaping predators, capturing prey, and jumping waterfalls (Eliason and Farrell, 2016). However, domestication and selective breeding programs have changed the physiology of salmonids (Zhang et al., 2016). Comparisons between the swimming characteristics in farmed and wild salmonids show farmed salmonids exhibit poorer swimming abilities than their wild counterparts (Anttila et al., 2007; Anttila and Mänttäri, 2009; Zhang et al., 2016). Reduced swimming abilties in farmed salmonids is thought to be associated with feed formula, controlled environments, and genetically selecting families for fast growth. This has also led to changes in salmonids external morphology, where farmed salmonids are rounder, deeper bodied, and less streamlined than wild salmonids (Hard et al., 2000; Glover et al., 2017).

Despite the concerns surrounding how domestication has shaped salmonids, there is encouraging research that focuses on how flow regimes used to exercise-train salmonids can improve production performance and their aerobic efficiency (see reviews by Davison, 1997; Palstra and Planas, 2011; Davison and Herbert, 2013; McKenzie et al., 2020; Huang et al., 2021; Rodgers and Gomez Isaza, 2023). (This is discussed in more detail in **Chapter 2**.) Exercise-

trained salmonids show improvements in growth, feed efficiency, fillet quality, swimming performance, and welfare (Davison and Goldspink, 1977; Greer Walker and Emerson, 1978; East and Magnan, 1987; Totland et al., 1987; Jørgensen and Jobling, 1993; Totland et al., 2011; Solstorm et al., 2015; Nilsen et al., 2019). Much of this research, especially studies that document exercise-enhanced growth, were performed on Atlantic salmon and rainbow trout, as these two species are the leading salmonid species produced (FAO, 2022).

There are limitations in translating information generated on Atlantic salmon and rainbow trout to other salmonids species, i.e., Chinook salmon. The largest limitation is the contrast between Atlantic salmonids and Pacific salmonids, and how originating from different habitation, on an evolutionary scale, has influenced these species. This could be linked to the large differences evident between farmed Chinook salmon and farmed Atlantic salmon. Farmed Chinook salmon are significantly fattier, developing large fat deposits around the visceral organs and exhibiting higher intermuscular and whole-body lipid content compared with similar size Atlantic salmon (Johnsen et al., 2011; Araújo et al., 2022b). Farmed Chinook salmon exhibit poorer feed conversion efficiency than Atlantic salmon, where Chinook salmon can require up to double the amount of feed for equal fish mass (Petrell and Jones, 2000; Araújo et al., 2021; Elvy et al., 2022b; Elvy et al., 2024). While rainbow trout are more closely related to Chinook salmon (both belong in the *Oncorhynchus* genus), the information could be more applicable. However, rainbow trout are predominantly farmed inland and in freshwater (opposite to Chinook salmon; FAO, 2022), meaning research using rainbow trout is tailored towards this production strategy and is less relevant for Chinook salmon.

Farmed Chinook salmon also exhibit other health concerns that are not as significant in the Atlantic salmon and rainbow trout industry. Farmed Chinook salmon in NZ (particularly in the Marlborough Sounds) develop spinal curvature (lordosis, kyphosis, and scoliosis; LKS) as a late onset spinal deformity; typically appearing in the last eleven months leading up to harvest (Perrott et al., 2018), and can affect up to 40% of harvested Chinook salmon (Davie et al., 2018; Perrott et al., 2018; Lovett et al., 2020). Genetic and phenotypic correlations have identified that individuals with higher condition factor are more likely to develop LKS (Scholtens et al., 2023; Prescott et al., 2024), suggesting potential mismatches in the pace of somatic growth and bone mineralisation (Prescott et al., 2024). These species-specific differences make it difficult for information to be easily transferred from farmed Atlantic salmon and rainbow trout through to Chinook salmon, including the knowledge that has been developed around the influence of sustained swimming on salmonids.

A few studies have investigated the influence of sustained swimming on Chinook salmon, but most of this research was performed on wild Chinook salmon stocks (Thorarensen

et al., 1993; Kiessling et al., 1994; Kiessling et al., 2005; Hoffnagle et al., 2006), and the optimal flow regime to enhance production performance was unable to be identified (Kiessling et al., 1994; Hoffnagle et al., 2006). There are concerns that Chinook salmon have species-specific responses to flow regimes (Davison and Herbert, 2013; Prescott et al., 2024), but a wider range of flow regimes need to be tested to confirm this. As such, there is potential for exercise-enhanced traits to be applied to NZ farmed Chinook salmon. As the NZ industry evolves towards open ocean farming, there is greater urgency to explore the influence of flow regimes and sustained swimming on Chinook salmon production performance.

If the NZ Chinook salmon industry adopts flow regimes in hatchery settings and expands farming offshore, consideration into the genetic responses is required. Farmed Chinook salmon are genetically enhanced to perform under moderate to highly controlled farming conditions with minimal swimming requirements, therefore it is important to determine if a genotype-by-environment (G×E) interaction exists between these farming strategies (e.g., low and moderate flow; nearshore and offshore environments). Industry may need to reconsider selective breeding objectives, and therefore family performance should be assessed across multiple environments (i.e., nursery/recirculating aquaculture system (RAS), nearshore, and offshore environment) for each production stage to ensure the industry is selecting high-performing and consistent families. By identifying traits that can be enhanced by the environment (e.g., flow regimes), other traits may be of greater priority in the selective breeding program. This can alleviate pressures on genetic gains, improve environmental sensitivity, and reduce farming costs.

Understanding how production performance changes with exercise training will be critical for the industry to define the appropriate dietary ingredients and energy density needed to match the evolving farming strategy. Farmed Chinook salmon can have large variation in their feed intake rates (Elvy et al., 2022b; Scholtens et al., 2023; Elvy et al., 2024), where some individuals consume large amounts of feed with poor nutrient retention, which could be linked to sub-optimal feed formulations. Under increased sustained swimming, the nutrient demands, utilization, and deposition will change due to increases in energy expenditure (shares an exponential relationship with swimming speed) and different biochemical pathways (e.g., lipid metabolism) being up- or downregulated. In several fish and nearly all mammalian species, increasing aerobic activity is shown to directly increase metabolic rate and therefore nutrient demands (Houlihan and Laurent, 1987; Speakman and Selman, 2003). Proteins are essential for nearly all chemical reactions, through regulating physiological processes (e.g., gene expression and immune systems), giving structure to cells, and providing the primary component to muscles (NRC, 2011; Teles et al., 2020). Similarly, lipids and carbohydrates play important roles in aerobic metabolism (Alsop and Wood, 1997; Magnoni et al., 2013; Timmerhaus et al., 2021). Red muscle, the powerhouse for aerobically fueled sustained swimming, has a high capability of

utilizing free fatty acids, which increases with improved aerobic capabilities (Moyes et al., 1992; Moyes and West, 1995). As such, lipid composition has been documented to change with exercise regimes in several salmonid species (Houlihan and Laurent, 1987; Christiansen et al., 1989; Jobling et al., 1993; Arbeláez-Rojas and Moraes, 2017), but has yet to be described in NZ farmed Chinook salmon. By capturing the complete process from nutrient intake, assimilation, to deposition, responses due to exercise can be identified as well as assessing their implications for fish health and commercial product quality.

Alongside muscles, bones play a major role in locomotion (Videler, 1993a). Micronutrients, calcium (Ca) and phosphorus (P), form the primary foundation of bones, providing mechanical and physical strength to enable robust movements (Johns, 1977; Toppe et al., 2007). Bone mineral content can increase with exercise in fish (Totland et al., 2011), suggesting that exercise could alter micronutrient intake and usage. It is hypothesised that higher mineral content in bones can improve spinal structure and health (Deschamps et al., 2009; Totland et al., 2011). This is important for the NZ industry, as farmed Chinook salmon develop spinal deformities (previously discussed) that leads to financial consequences, welfare concerns, and potentially negative community perception (Lovett et al., 2018; Davie et al., 2019; Lovett et al., 2020; Wongprawmas et al., 2022). Therefore, understanding the relationship between sustained swimming and bone mineral content could provide an alternative avenue to improve spinal deformities in Chinook salmon and other finfish species.

On a functional level, increased exercise has been shown to enhance a fish's cardiorespiratory capacity. For instance, increases in swimming performance and aerobic capacity occur when fish are exercised or is superior in fish defined as good swimmers (i.e., fastest 30%; Thorarensen et al., 1993; Gallaugher et al., 2001; Claireaux et al., 2005; Anttila et al., 2014). Exercise regimes improve critical swimming speeds, endurance, and efficiency, as well as widening the aerobic window (Farrell et al., 1990; McKenzie et al., 2012). Improving salmon fitness is necessary to ensure they can withstand and recover from high energy hydrodynamic conditions of offshore farm sites. Locomotory costs can be expensive (Brett, 1964), therefore improving swimming efficiency and increasing the aerobic ceiling through exercise regimes may equip fish with the physiological abilities to sustain increased swimming speeds and production performance. Fueling these activities through aerobic processes is crucial for longevity. If activities become energetically expensive and greater than what is supplied through aerobic metabolism, partial transition to anaerobic metabolism will occur to maintain performance (Burgetz et al., 1998; Svendsen et al., 2010). Anaerobic metabolism is not sustainable and can lead to reduced health if constantly required and complete recovery is not met (restored creatine phosphate and glycogen; Boutilier et al., 1993; Peake and Farrell, 2004).

Linking whole-organism traits (e.g., swimming performance and aerobic capacity) to tissue, cellular and molecular functions can provide better understanding of fish's physiological abilities and could be used as a tool to maintain high fish welfare. Measuring markers (e.g., gene expression) involved in metabolism can be used to gain this insight. For example, sustained swimming stimulates aerobic pathways to sustain the increased energetic demands. This includes the upregulation of aerobic enzymes, blood-oxygen delivery properties, and cardiac function (Hochachka, 1961; Thorarensen et al., 1993; Anttila et al., 2008; Pettinau et al., 2022). However, if speeds are too high, beyond those that are supported by aerobic processes, upregulation of anaerobic pathways (e.g., lactate dehydrogenase) will occur. Consistently switching into anaerobic metabolism can reduce protein synthesis and degradation pathways, while upregulating amino acid catabolism (Nuez-Ortín et al., 2018). This would lead to significant muscle and growth reduction and potential muscle inflammation. Connecting responses across the biological organization levels (i.e., molecular through to whole-animal performance) can generate a deeper understanding of the underlying mechanisms associated with different levels of swimming and by understanding when these changes occur with respect to sustained swimming would ensure the safety and secure the productivity of farmed fish in high energy environments.

1.3 Thesis aims and outline

The overarching aims of this PhD are:

1. to understand the influence of sustained swimming on Chinook salmon form and function,
2. to determine if exercise can enhance production performance in Chinook salmon,
3. to assess if training can prepare Chinook with the respiratory and locomotory requirements for exposed offshore environments, and
4. to gain insight into physiological changes Chinook salmon may experience when farmed offshore in high energy environments.

Information on farmed Chinook salmon is sparse. Although available information (including the influence of exercise) from closely related species like rainbow trout and Atlantic salmon can offer guidelines, caution must be exercised as Chinook salmon have distinct characteristics.

This PhD investigated the short and long-term effects of sustained swimming on post-smolt Chinook salmon (83 g to 2000 g) using tank-based experiments with low (0.3 body lengths per second; bl s^{-1}; LFR) and moderate (0.8 bl s^{-1}; MFR) flow regimes. A low and moderate flow

regime was chosen because Chinook salmon (~387 g) have been estimated to exhibit an U_{opt} of 1.5 bl s^{-1} (Gallaugher et al., 2001; Davison and Herbert, 2013), but when exercised under similar flow regimes (i.e., 0.5, 1.0, and 1.5 bl s^{-1}) exercise-enhancements did not occur (Thorarensen et al., 1993; Kiessling et al., 1994; Kiessling et al., 2005; Hoffnagle et al., 2006). It was important to choose exercise regimes that can be maintained by larger fish and for long periods. This meant that the flow regimes during the beginning of the experiment were probably not very aerobically challenging.

Each data chapter within this thesis involved similar experimental setups, where various aspects were addressed at different timepoints (Figure 1.1). Therefore **Chapters 3, 4, 5, and 6** have similar Materials and Method descriptions. I have chosen to include similar Material and Methods within each chapter, despite the redundancy, because each data chapter represents a manuscript either published or in preparation for submission, and therefore have been included as they appear in publication.

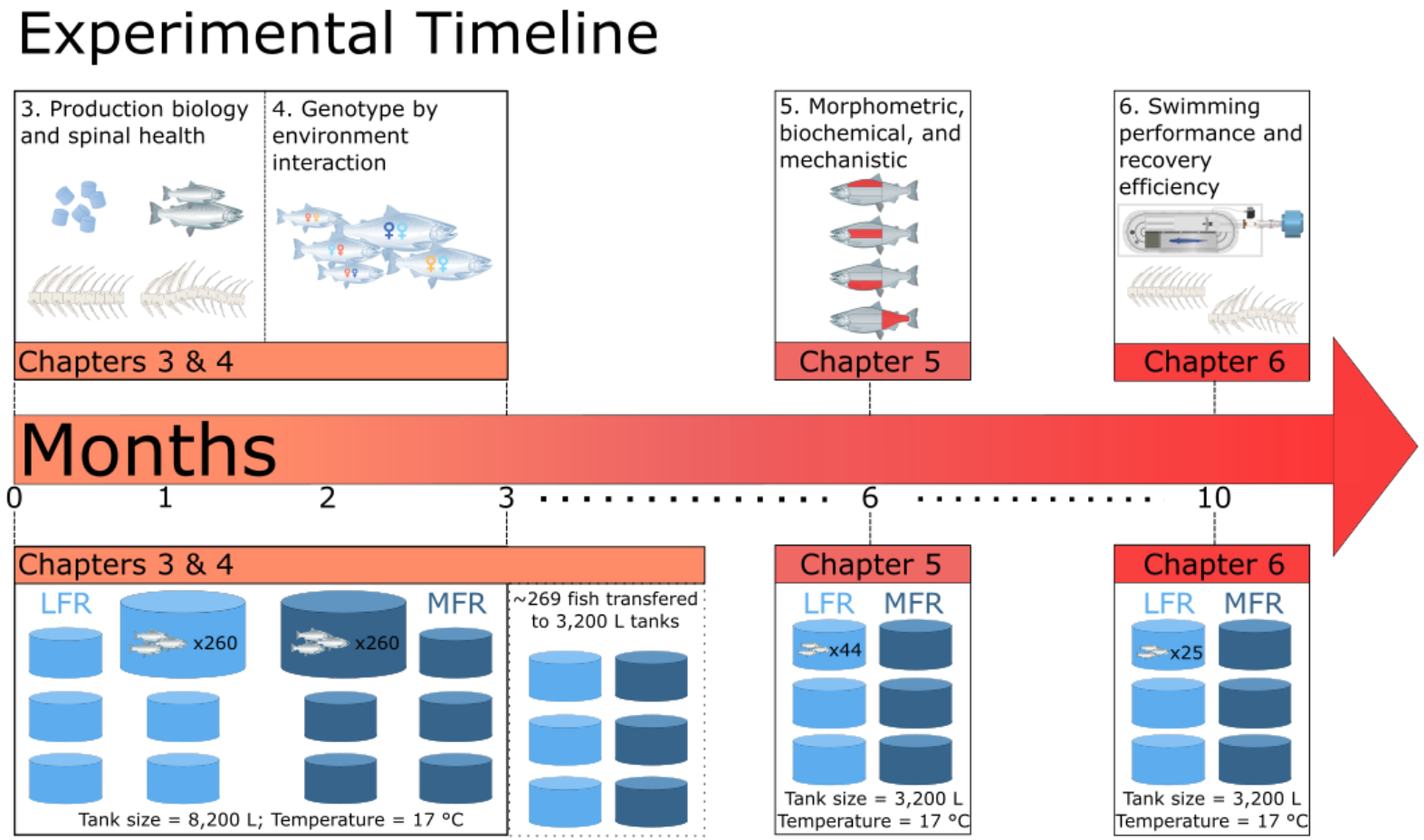

Figure 1.1 Experimental timeline depicting where each chapter was involved. LFR: low flow regimes; MFR: moderate flow regimes.

Chapter 2 uses a systematic review process to gain a thorough understanding of the complex relationships between sustained swimming and several aspects of salmon production. The information gathered in this review was evaluated to determine if exercise training through sustained swimming can build robust and resilient salmon in preparation for exposed offshore farming. Lastly, the review discusses limitations and knowledge gaps among the literature and provides future direction for research to develop and optimise farming practices for exposed offshore success. **Chapters 3 and 4** present individual performance data from the first three months under low and moderate flow regimes. Specifically, **Chapter 3** investigates the influence of LFR and MFR on swimming behaviour, production-biology (i.e., feed intake, growth, and feed efficiency), and spinal health. Using this data, **Chapter 4,** generates genetic parameters for each production-biology trait measured in **Chapter 3**, to determine if a G×E interaction exists between the LFR and MFR and to identify any unfavourable genetic or phenotypic correlations among production traits. This information is critical to ensure that commercial selective breeding programs can continue performing efficiently as the industry integrates exercise and evolves offshore. **Chapter 5** investigates the long-term influence of LFR and MFR on production performance, and provides detailed morphometric, biochemical, and mechanistic responses of the white muscle to sustained swimming. The white muscle tissue was only focused on as it provides majority of the primary product marketed in salmonid aquaculture and represents approximately 90% of muscle mass (Kiessling et al., 2006). **Chapter 6** uses swimming respirometry to determine if long-term sustained swimming under LFR and MFR can enhance swimming performance and recovery efficiency in individuals with and without spinal curvature. Providing insight into the locomotory performance of farmed Chinook salmon and how training can enhance these traits for exposed offshore farm sites. This chapter further explores changes in nutrient composition and morphology across an array of tissues. Lastly, **Chapter 7,** in the format of a general discussion, summarises the main findings across all chapters and integrates the data generated in the context of the NZKS Blue Endeavour site by creating bioenergetic models. **Chapter 7** also highlights points of interest for future research to enhance finfish farming in exposed offshore sites and in the Anthropocene.

This PhD was part of a larger research and development program ("Feed-efficient salmon for the future") led by Dr. Jane Symonds (Cawthron Institute) working closely with the NZ chinook salmon aquaculture industry, and a collaboration across multiple research platforms: Institute for Marine and Antarctic Studies (IMAS) University of Tasmania, Blue Economy Cooperative Research Centre, and the Cawthron Institute. Results produced in this PhD thesis were directly communicated (from myself and the team) to the industry through presentations, reports, and advisory meetings and are now being used to improve farming practices.

The “Feed-efficient salmon for the future” program was a NZ Government Ministry of Business, Innovation, and Employment Endeavour Fund that ran from 2019 to 2021. The program included several experiments (Figure 1.2) led by Dr. Jane Symonds in collaboration with scientists from other research organisations (AgResearch, University of Auckland, IMAS University of Tasmania) and with the NZ salmon industry that aimed to improve feed efficiency in Chinook salmon and enable industry growth. My PhD research was part of trial 4, the final research package of the program, which was supported by a wealth of research and knowledge that had previously been developed throughout trials 1 – 3 of this program. Research questions generated for each data chapter were developed during the PhD as a collaboration between the Cawthron Institute, IMAS University of Tasmania, and the Blue Economy Cooperative Research Centre. For more information about the research undertaken during the “Feed-efficient salmon for the future” program, please visit: Salmon Feed Conversion Efficiency Research Programme.

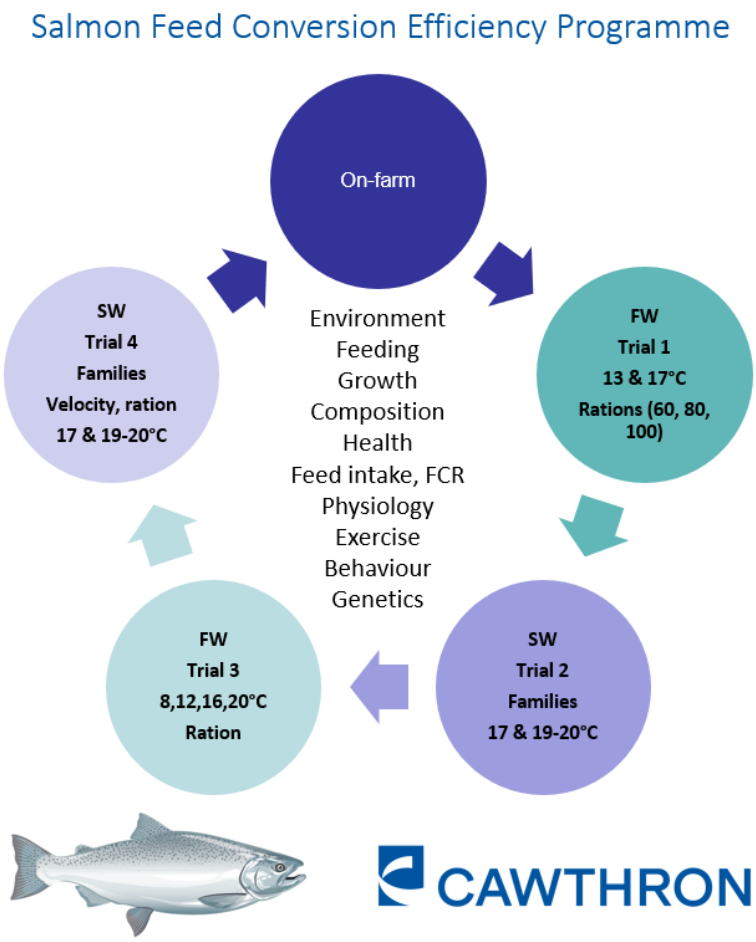

Figure 1.2 Salmon feed conversion efficiency research programme led by Dr. Jane Symonds, Cawthron Institute. FW: freshwater; SW: seawater; FCR: feed conversion ratio. Created by Jane Symonds.

Chapter Two Systematic Review: Is exercise training during early rearing stages the key to building robust and resilient salmonids for high energy offshore farming?

Part of the research contained within this chapter is in preparation as Prescott, L.A., Symonds, J.E., Walker, S.P., Miller, M.R., Semmens, J.M., Carter, C.G., 2023. Exercise training as an early life shaping tool for enabling high-energy offshore salmonid farming.

2.1 Abstract

The salmonid industry and other finfish aquaculture are expanding production offshore into high energy environments, as an avenue to grow the industry. Offshore open-ocean farming increases the available farming space and reduces user competition but can present several challenges associated with their physical hydrodynamic conditions, such as strong currents. Ensuring efficient and economically viable salmonid production in exposed offshore locations, as well as maintaining good fish welfare, is critical. To assist the industry's transition to exposed offshore sites, a systematic literature review was conducted to understand how salmonids respond when exercised and to define suitable hydrodynamic conditions and appropriate size ranges. Most studies describe results relating to salmonid production, swimming and aerobic capacity, and health, with a notable gap in breeding and genetics. This analysis provides insights into how salmonids may respond when farmed under conditions that promote sustained swimming while identifying knowledge gaps and inconsistencies in the existing literature. This review discusses the viability of using exercise training as a tool to build robust and resilient salmonids and provide direction for future research to facilitate the industry's transition offshore.

2.2 Introduction

Sustainable aquaculture is key to securing future supplies of seafood as stocks from wild capture fisheries have remained static since the mid-1990s, and human population and seafood consumption continues to grow (FAO, 2020). To meet increasing seafood demands, the aquaculture production has increased over the same period and this growth in demand is predicted to continue for the next decade (Delgado et al., 2003; Kobayashi et al., 2015; FAO, 2020). New, sustainable, and more efficient farming strategies are needed to match these increasing seafood demands. Existing farming strategies, such as on land- tank, pond, or recirculating aquaculture systems (RAS), as well as nearshore operations are restricted in terms of available farming space with limited potential of significant expansion, can be resource expensive (e.g, RAS), and are

increasingly affected by heatwaves and anthropogenic climate change, where RAS is an exception (Crawford, 2003; Crawford et al., 2003; Di Trapani et al., 2014; Froehlich et al., 2017; Gentry et al., 2017; Envirostrat, 2020; Broekhuizen et al., 2021; Morro et al., 2021), and therefore are unlikely to match the increasing demands. An alternative, which has been recognised globally, that could underpin the growth of aquaculture, is utilising the offshore marine environment as a multi-use platform, such as the integration of offshore renewable energy and seafood production (Froehlich et al., 2017; Morro et al., 2021). However, offshore aquaculture is still in its infancy because there is uncertainty surrounding how existing farming infrastructure, operations, and stocks will perform if these sites are characterised challenging high energy conditions. In this review, we adopt terminology defined in Buck et al. (2024) for different aquaculture sites, and focus on the challenge associated for farming in exposed offshore sites.

Identifying suitable species to farm in exposed offshore sites is a priority and considering the hydrodynamic conditions that can dominate, such as strong currents and waves, research on species that can withstand these challenging conditions is of main focus. A key characteristic for finding suitable finfish species is that they must exhibit profound sustainable swimming abilities like thunniform (e.g., tuna and kingfish) and carangiform (e.g., salmonids and cobia) swimmers. Current speeds in some of the existing (e.g., Faroe Islands, Denmark) and proposed offshore sites (e.g., Cook Strait, New Zealand) rarely fall below 20 cm s^{-1}, which is typical of existing nearshore sites (Johansson et al., 2007; Gillespie, 2011), and can reach up to 150 cm s^{-1} (0.5 to 3.8 body lengths per second; BL s^{-1}, for a 370 mm finfish; 0.3 to 2.25 BL s^{-1} for a 620 mm finfish) (Johansson et al., 2014; Campos et al., 2019; Newcombe et al., 2019; Chu et al., 2020). Thus, being able to swim against currents in this range for substantial periods and continue to feed and grow is pivotal for the success of offshore finfish farming.

Currently, the salmonid industry is at the forefront of developing exposed offshore farming strategies. The reason for this is not because they are necessarily regarded as the most suitable species, but because of the well-established large scale global salmonid industry (FAO, 2022) and the extensive knowledge base that exists on wild and farmed salmonids (Scheer, 1939; Dittman and Quinn, 1996; Sargent and Tacon, 1999; Torrissen et al., 2013), including their cardiorespiratory physiology (Fry, 1947, 1957; Brett et al., 1958; Brett, 1964; Fry et al., 1971), bringing together expertise from scientists and industry. From this body of knowledge, there are well-defined and optimised methods for understanding salmonids physiological performance, especially in the context of swimming and energetics, which is highly relevant and beneficial for gaining essential knowledge to assist the development of exposed offshore farming. The pioneering work of understanding the energetics of wild migratory salmonid populations (Brett et al., 1958; Brett, 1964; Brett, 1965, 1967) has led to an active fish energetics and kinematics research area. This includes standardised methods and understanding of the relationships between

swimming performance (e.g., reported in cm s^{-1} and BL s^{-1}) and energetic costs (e.g., oxygen consumption rates; $\dot{M}O_2$) (Steffensen et al., 1984; Steffensen, 1989; Steffensen, 2005), and the effect of body size (Videler and Weihs, 1982; Videler, 1993a; Clarke and Johnston, 1999; Chabot et al., 2016a).

Wild salmonids are known to exhibit profound swimming abilities (Ellis, 1966; Colavecchia et al., 1998), demonstrated by their large migrations (Groot, 1991), but since domestication, the aquaculture environment and applied genetic selection have shaped salmonids differently to the natural environment. There is evidence to suggest that farmed salmonids exhibit poorer swimming abilities than their wild counterparts (Davison, 1989; Anttila et al., 2007; Anttila and Mänttäri, 2009; Zhang et al., 2016). As such, redefining the swimming range farmed salmonids are capable of is important for the industry's transition to exposed offshore sites. A priority is to determine what size salmonids can withstand the offshore current speeds and what locations are most suitable. This information for the Norwegian Atlantic salmon (S*almo salar*) aquaculture industry has recently been evaluated (Hvas et al., 2017; Hvas and Oppedal, 2017; Hvas et al., 2020, 2021b, 2021a; Hvas, 2022), see figure 3 in Hvas et al. (2020). The swimming speeds farmed Atlantic salmon are capable of matching the current speeds measured in offshore sites, but knowing if these speeds can be maintained for substantial periods of time and potentially repeated throughout a day (if tidally influenced) is of interest (Hvas and Oppedal, 2017; Hvas et al., 2021a; Athammer et al., 2024). It is also important to consider that the swimming speeds farmed Atlantic salmon predominantly swim at in nearshore sea pens (0.2 - 0.9 BL s^{-1} or ~12.6-56.7 cm s^{-1} for >3kg salmonid) (Korsøen et al., 2009; Oppedal et al., 2011) are considerably lower, and it is unknown if fish that have been genetically selected for performance at these slower swimming speeds, can continue to maintain performance under exposed offshore conditions. Although, this area is gaining attention (Athammer et al., 2024; Barbier et al., 2024).

There is a large knowledge gap around the energetic requirements associated with high levels of sustained swimming speeds and whether salmonids can continue to be produced efficiently, safely (in terms of fish welfare), and economically in exposed offshore locations. However, there is a substantial body of knowledge on salmonids and other commercial fish species about how culture in flowing water that encourages sustained swimming can enhance key phenotypes, such as, growth, feed efficiency, swimming performance, and health (Davison and Herbert, 2013; Huntingford and Kadri, 2013; McKenzie et al., 2020; Huang et al., 2021). These studies can provide baseline understanding and insights into how salmonids may respond to currents, such as those found in exposed offshore farm sites, and provide guidance on what the most appropriate maximum current speeds are for farming salmonids. Future studies can build from this research and determine if exercise training during pre- and post-smolt stages is essential

in preparing robust and resilient salmonids for high energy environments (e.g., oscillating currents, wave action, tides etc.).

A systematic literature review was conducted to outline the relationships between exercise training and aspects of salmonid production, fitness, health, and genetics, and to identify knowledge gaps within these categories. Further, this review discusses the limitations of using exercise training to build robust and resilient salmonids and provide direction for future research to assist finfish aquaculture's transition to exposed offshore locations.

2.3 Methods: Search strategy

Using ISI Web of Knowledge (Clarivate Analytics, Core collection search on 12.12.2021, revisited 19.10.2023) and relevant search terms (Title: salmon* OR trout OR char AND exerc* OR train* OR active* OR velocity* OR prolong* OR sustain* OR swim*), 1,453 papers were found, which were further checked for relevance. Studies were included if a salmonid species (other fish species were excluded) was subjected to an exercise training program implemented for longer than seven days and provided comparison to another treatment(s) (e.g., control group (no flow) or minimal flow (i.e., to maintain water quality). This resulted in 81 suitable studies. Subsequently, results from most of these papers (62 of the refined 81) were sorted into sub-topics and deemed as having 'positive', 'negative' or 'no difference' outcomes, based on statistical significance, in response to exercise training. Positive effects were based on improved fish performance and not an increase in value (e.g., a lower feed conversion ratio (FCR) was deemed as a positive outcome). The three sub topics evaluated were; (1) feeding and growth: results associated with feed intake, growth, and their interaction i.e., feed efficiency, (2) swimming and aerobic capacity: results associated with swimming performance and respiratory properties (e.g., metabolic rates; blood oxygen transport and delivery properties), and (3) health: results associated with survivability, stress, and disease resilience, thermal and hypoxia tolerance, external condition (e.g., skin health), and spinal- and tissue health. Breeding and genetics are discussed in this review but was not included as one of the sub-topics for evaluation due to the limited number of studies.

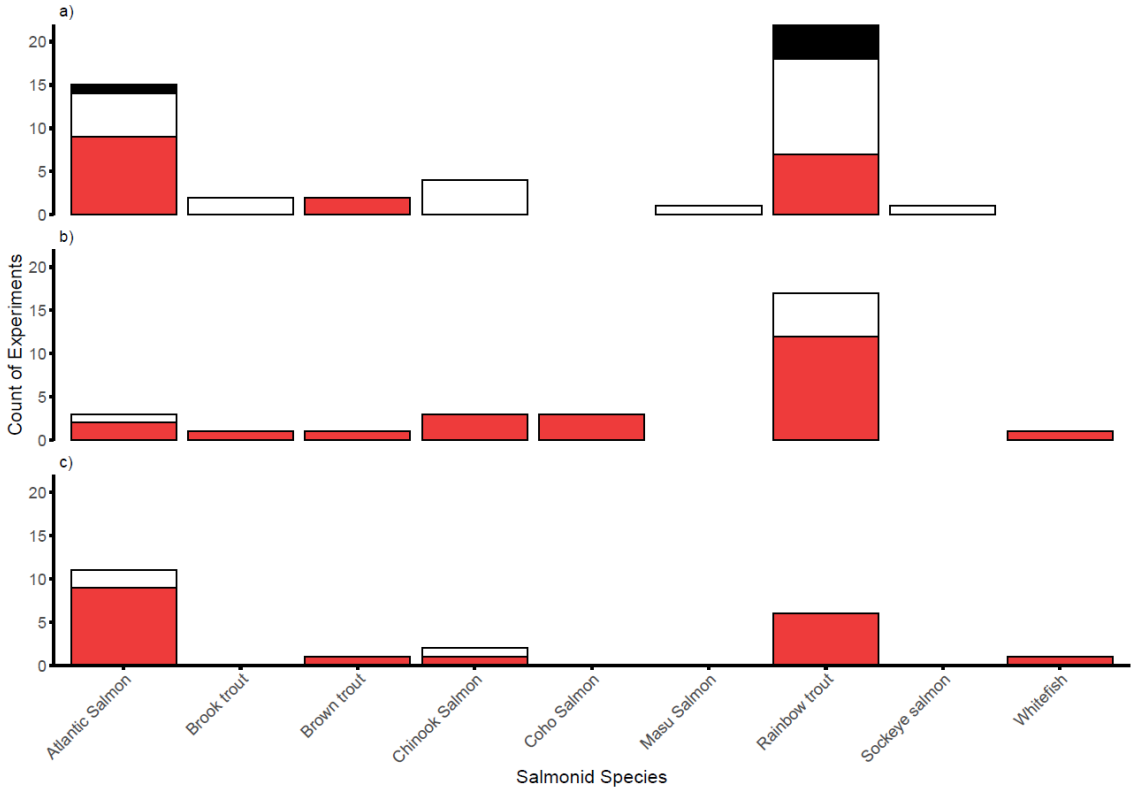

Figure 2.1 Number of publications investigating exercise-training in salmonid species, presenting results in a) feeding and growth, b) swimming and aerobic capacity, and c) health. Red = significantly enhanced; white = not significantly different; black = significantly reduced. Note: the studies included vary in training regimes, water temperatures, and sample size.

2.4 Influence of exercise training on production-biology, swimming and aerobic capacity, and health in salmonids

Studies investigating the influence of exercise training have predominantly focused on salmonids, where most studies reveal positive outcomes occurred when fish were reared under environments that encourage sustained swimming (Davison and Herbert, 2013; Huntingford and Kadri, 2013; McKenzie et al., 2020; Huang et al., 2021). Studies that aim to exercise-train salmonids typically use low (< 0.5 BL s^{-1}), moderate (0.5 – 1 BL s^{-1}) and/or high (> 1 BL s^{-1}) flow regimes with small salmonids (< 500 g) for short durations (< five months) (Solstorm et al., 2016b; Timmerhaus et al., 2021; Rodgers and Gomez Isaza, 2023). Among these studies, Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) were the main species used (~74% of studies conducted), likely owing to these species dominating the salmonid aquaculture industry (Figure 2.1) (FAO, 2020). Most studies report results relating to feeding, growth, and feed efficiency, swimming and aerobic capacity, and/or health (62 papers from the refined 81; Figure 2.1; summarised in Table 2.1), indicating there is substantial understanding on how salmonids respond to sustained swimming in a finfish production context. However, there are knowledge gaps and inconsistencies needing to be addressed, and now that the industry is expanding offshore, there is a new set of questions requiring answers. The gaps and new questions are highlighted throughout this review.

Table 2.1 Observed exercise-enhanced traits in salmonid species. Exercise-enhanced traits are separated into categories representing production performance, swimming and aerobic capacity, and health. Note: references were included if some level of exercise was reported to elicit positive changes for a specified trait, not necessarily the highest exercise level tested.

Exercise-enhanced trait	Species	Salinity	Swimming speed (BL s^{-1})	References
Production performance:				
Growth	Atlantic salmon	SW	0.5 – 1	(Totland et al., 1987; Nilsen et al., 2019; Timmerhaus et al., 2021)
		FW	1 – 2	(Jørgensen and Jobling, 1993; Castro et al., 2011; Waldrop et al., 2018a; Mes et al., 2020)
	Brown trout	FW	1 – 2	(Davison and Goldspink, 1977; Bugeon et al., 2003)
	Rainbow trout	FW	0.5 – 1.5	(Nahhas et al., 1982b; Davie et al., 1986; Houlihan and Laurent, 1987; Farrell et al., 1990; Nielsen et al., 2000; Martin and Johnston, 2005)
Condition factor	Atlantic salmon	SW	0.5 – 2.5	(Nilsen et al., 2019; Ytrestoyl et al., 2020; Timmerhaus et al., 2021)
		FW	0.8 – 1.5	(Jørgensen and Jobling, 1993; Castro et al., 2011; Ytrestoyl et al., 2020)
	Brown trout	FW	1 – 2	(Bugeon et al., 2003)
	Rainbow trout	FW	0.5	(Nielsen et al., 2000)
Feed efficiency	Atlantic salmon	FW	1 – 1.5	(Jørgensen and Jobling, 1993)
	Brown trout	FW	1.5	(Davison and Goldspink, 1977)
	Chinook salmon	SW	0.8	(Prescott et al., 2024)
	Rainbow trout	FW	0.5	(Nielsen et al., 2000)
Fitness and aerobic capacity:				
Swimming performance	Atlantic salmon	FW	1.5	(Anttila et al., 2011)
	Brook trout	FW	0.8 – 1	(Leon, 1986)
	Brown trout	FW	1 - 2	(Anttila et al., 2008)
	Chinook salmon	SW	0.8	(Prescott et al., 2023)
	Rainbow trout	FW	1 – 5	(Hochachka, 1961; Nahhas et al., 1982a; Houlihan and Laurent, 1987; Farrell et al., 1990; Pearson et al., 1990; Holk and Lykkeboe, 1998)
	Whitefish	FW	1 – 2	(Anttila et al., 2008)
Aerobic performance	Atlantic salmon	FW	2 – 2.8	(Zhang et al., 2016)
	Chinook salmon	SW	0.8 – 2.5	(Gallaugher et al., 2001; Prescott et al., 2023)

	Rainbow trout	FW	0.9 – 1.4	(Farrell et al., 1991; Larsen et al., 2012)
Blood oxygen transport properties	Chinook salmon	SW	1.5 – 2.5	(Thorarensen et al., 1993; Gallaugher et al., 2001)
	Coho salmon	FW	1 – 3	(Zbanyszek and Smith, 1984)
		SW	1 – 3	(Zbanyszek and Smith, 1984)
	Rainbow trout	FW	1.2 – 5	(Hochachka, 1961; Holk and Lykkeboe, 1998; Parker and Barnes, 2015)
Health:				
Tissue health (e.g., muscle, heart, gill, brain, skeletal, skin, fins, etc.)	Atlantic salmon	SW	0.5 – 2	(Totland et al., 1987; Totland et al., 2011; Solstorm et al., 2016b; Timmerhaus et al., 2021)
		FW	0.5 – 3	(Jørgensen and Jobling, 1993; Anttila et al., 2011; Castro et al., 2013a; Ytteborg et al., 2013; Mes et al., 2020)
	Brown trout	FW	1 – 2	(Anttila et al., 2008)
	Chinook salmon	SW	0.8	(Prescott et al., 2023)
	Rainbow trout	FW	1	(Deschamps et al., 2009)
	Whitefish	FW	1 – 2	(Anttila et al., 2008)
Thermal resilience	Rainbow trout	FW	1 – 1.7	(Papadopoulou et al., 2022; Pettinau et al., 2022)
Disease resistance	Atlantic salmon	FW	0.8 – 1	(Castro et al., 2011)
Stress tolerance and survivability	Rainbow trout	FW	1 – 3	(Walker and Emerson, 1978; Woodward and Smith, 1984; Hernandez et al., 2002)

FW, freshwater; SW, seawater; BL s^{-1}, body lengths per second.

2.4.1 Feed intake

Feeding fish is among the most important tasks associated with finfish aquaculture, as the feed provides the primary nutrient and energy source that underpins daily activities and growth, while being the greatest costs in production (Rana et al., 2009; Goddard, 2012). Feed intake rates are highly dependent on feed recognition and feeding behaviour (Assan et al., 2021; Elvy et al., 2024). Even though these factors are primarily stimulated through pathways within the nervous and endocrine system (Kuz'mina, 2019), extrinsic factors (abiotic and biotic) can also have large impacts, including how the feed is delivered and how often (Sun et al., 2016). Forced swimming could influence feed recognition and feeding behaviour if the fish are focusing more on maintaining their swimming speed, holding their position, and avoiding collisions (Solstorm et al., 2016b). In contrast, forced swimming may stimulate feeding behaviour because of increased energy demands associated with higher swimming speeds, i.e., energy expenditure increases as a function of swimming speed (Roche et al., 2013).

In this literature search, most studies showed feed intake increased with increasing swimming speed (Nahhas et al., 1982b; Totland et al., 1987; Jørgensen and Jobling, 1993; Thorarensen et al., 1993; Castro et al., 2011; Felip et al., 2012; McKenzie et al., 2012), likely as a product of increased energy expenditure. But some studies reported higher feed intake in the control treatment when compared to sprint training and higher swimming speeds (Gamperl et al., 1988; Nielsen et al., 2000), or no difference between control and exercised treatments (Hernandez et al., 2002; Larsen et al., 2012). This suggests that as swimming speeds exceed moderate levels, sensory awareness for feed recognition and available energy for feeding behaviour may become limited. Investigating relationships between feed recognition, appetite regulation, and feeding behaviour under increasing swimming speeds could provide key information for the salmonid industry to include in their advanced feeding technology (e.g., automated detection systems) for exposed offshore environments.

Future studies should include more accurate feed intake methods (e.g., using ballotini feed methods (McCarthy et al., 1992; McCarthy et al., 1993; Walker et al., 2012; Elvy et al., 2024) to better understand relationships between exercise training and feeding. Assessing feed intake on an individual level (McCarthy et al., 1992) can provide greater detail than feed intake values on a tank level, as there can be a large variation in individual daily feed intake (McCarthy et al., 1992; McCarthy et al., 1993; Elvy et al., 2022b; Scholtens et al., 2023). In Prescott et al. (2024), individual feed intake measurements, used to construct feed consumption-growth curves, revealed significant relationships between low and moderate exercise training that were not reflected when comparing group means. In Jørgensen and Jobling (1993), individual feed intake

measurements revealed that exercising Atlantic salmon reduced intraspecific feed intake variation and improved feed utilization, which would not be captured when comparing feed intake on a tank or group level. Together, these studies show the value in using individual feed intake measurements to minimise error and alleviate possible inconsistencies within exercise studies.

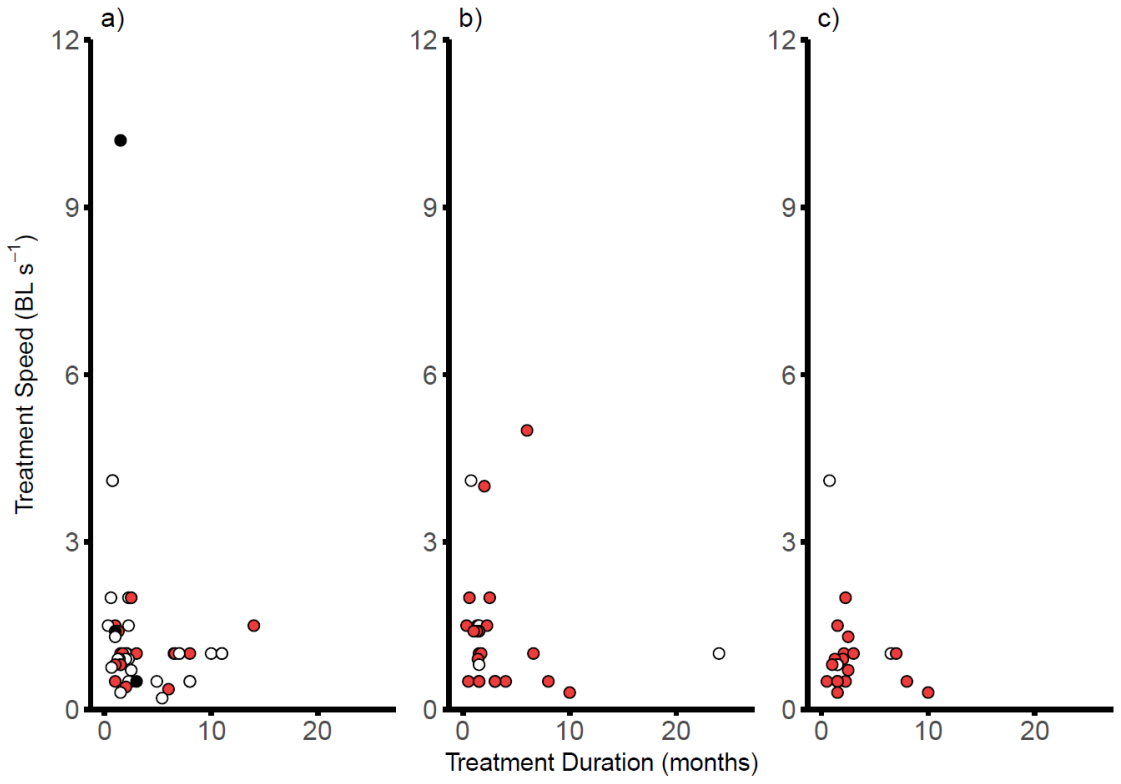

Figure 2.2 Treatment speed by treatment duration in publications exploring traits related to a) feeding and growth, b) swimming and aerobic capacity, and c) health. Red = significantly enhanced; white = not significantly different; black = significantly reduced.

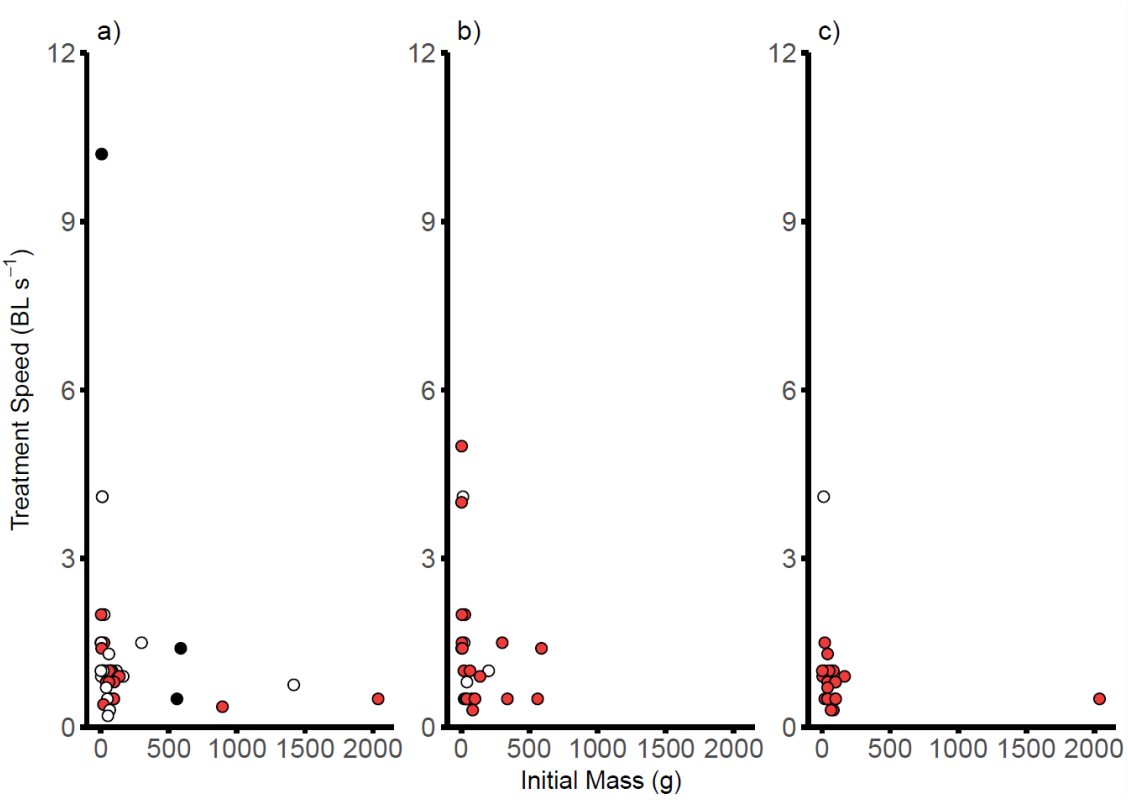

Figure 2.3 Treatment speed by initial mass (g) in publications related to a) feeding and growth, b) swimming and aerobic capacity, and c) health. Red = significantly enhanced; white = not significantly different; black = significantly reduced.

2.4.2 Growth

Improved growth is considered among the most desired traits and is a prioritised breeding objective when farming fish because it decreases rearing time and reduces exposure to uncontrollable conditions when at sea. Swimming-enhanced growth has been reported in Atlantic salmon (Totland et al., 1987; Jørgensen and Jobling, 1993; Skilbrei and Holm, 1998; Castro et al., 2011; Solstorm et al., 2016a; Waldrop et al., 2018b; Nilsen et al., 2019; Mes et al., 2020; Ytrestoyl et al., 2020; Timmerhaus et al., 2021), rainbow trout (Walker and Emerson, 1978; Nahhas et al., 1982b; Davie et al., 1986; Houlihan and Laurent, 1987; Farrell et al., 1990; Nielsen et al., 2000; Martin and Johnston, 2005), and brown trout, *Salmo trutta* (Davison and Goldspink, 1977; Bugeon et al., 2003), but in other salmonids, such as the Brook trout, *Salvelinus fontinalis* (Atkins et al., 2001), Chinook salmon, *Oncorhynchus tshawytscha* (Thorarensen et al., 1993; Kiessling et al., 1994; Kiessling et al., 2005; Prescott et al., 2024), masu salmon, *Oncorhynchus masou* (Azuma et al., 2002), and sockeye salmon, *Oncorhynchus nerka* (Patterson et al., 2004), growth was similar (i.e., not significantly different) under exercise training. These studies suggest that exercise training can have a positive relationship with growth; but this is mostly based on short exercise training durations, < 5 months (Figure 2.2) with small fish, < 500 g (Figure 2.3) (Rodgers and Gomez Isaza, 2023). Mixed responses between salmonids exhibiting exercise-enhanced growth and those that are not affected by exercise suggests there are some inconsistencies in the literature, and it is not clear why some salmonids exhibit enhanced growth and others do not. There are several possible reasons exercise-enhanced growth was not achieved in some species, including the methods used to implement the exercise regimes, fish size, and variation in species responses to the exercise regime, such as behaviourally. Inconsistencies within the literature could be explained and interpreted through additional analyses such as meta-analysis approach, as described in Rodgers and Gomez Isaza (2023).

The most supported hypothesis identifying suitable exercise training to promote exercise-enhanced growth is when fish are encouraged to swim at their most cost-efficient swimming speed. A review by Davison and Herbert (2013) outlined that, in most species, swimming-enhanced growth aligned closely to the optimal swimming speed (U_{opt}) or preferred swimming speed for the given species. This is because U_{opt} is the most cost-efficient swimming speed (i.e., lowest cost of transport), allowing maximum amounts of energy to be allocated to other processes, such as growth (Videler, 1993a). Some studies have shown that when speeds are below U_{opt}, spontaneous activity can occur (e.g., aggressive behaviour) and when speeds are above U_{opt}, more energy is directed to sustain swimming (Jørgensen and Jobling, 1993; Solstorm et al., 2015; Solstorm et al., 2016b; Waldrop et al., 2018b). However, providing exercise training

that is thought to align closely to the species U_{opt} does not always enhance growth in salmonids (Thorarensen et al., 1993; Kiessling et al., 1994) or in other finfish species (Graziano et al., 2018; Palstra et al., 2020).

A reason explaining why some salmonid may not exhibit exercise-enhanced growth could be associated with inaccurate reporting of the swimming speed being performed (Rodgers and Gomez Isaza, 2023). Most studies compare different flow environments and assume that the swimming speed and effort being performed reflect the set flow environment. However, recent evidence suggests that some species could be training themselves by swimming faster than the set current, e.g., Prescott et al. (2024). This could also be the reason why no trends emerge when presenting relationships between results with fish size, treatment speed, and duration (Figure 2.2 and Figure 2.3). Actual swimming speeds, behaviour, and/or effort (e.g., tail beat frequencies; tbf) are rarely reported within exercise training studies, but see Herbert et al. (2011) and Prescott et al. (2024). It is expected that fish would match their swimming speed to their optimal or preferred swimming speed, unless the treatment speed exceeds this. Interestingly, in Herbert et al. (2011) and Prescott et al. (2024), measured swimming speeds of Atlantic and Chinook salmon were higher than their previously estimated U_{opt}, and in Prescott et al. (2024), Chinook salmon were swimming substantially faster than the set flow regimes. If Prescott et al. (2024) did not provide measurements of the actual swimming speeds being performed, the interpretation of their study would be largely different and misleading. Future studies are urged to provide more accurate estimates of the swimming speeds their subjects are performing under different flow regimes to gain a clearer understanding of the relationship between exercise and enhanced growth.

Of the studies that document exercise-enhanced growth, increased skeletal muscle mass is suggested to be the main driver, but the mechanisms involved in this process are not yet fully understood. Several studies have shown different mechanisms to drive increased growth in exercised salmonids. These include up-regulation of growth- and insulin-like growth factor hormones (Davison, 1997; McKenzie et al., 2020), and increases in carbohydrate and lipid metabolism providing a protein-sparing effect (Huang et al., 2021; Timmerhaus et al., 2021), which in turn increases muscle mass, and therefore whole-animal growth. In rainbow trout and Atlantic salmon, as well as in a non-salmonid species the gilthead sea bream, gene expression and proteomics revealed exercise training improved protein synthesis capacity, energy (glucose or lipids) use, and aerobic enzyme capacity within the white muscle, while protein turnover was not affected (Martin-Perez et al., 2012; Magnoni et al., 2013; Pengam et al., 2021; Timmerhaus et al., 2021). However, Palstra et al. (2020) did not find the insulin growth factor hormone gene to be key in a follow up study with the gilthead sea bream, despite exhibiting exercised-enhanced growth. In rainbow trout, exercise increases carbohydrates metabolism in the liver (converted to

lipids) and in the muscles (oxidation) after feeding a high-starch diet (Felip et al., 2012). Developing a detailed sequence of changes across the lower biological organisations (e.g., -omics, metabolic pathways) in multiple tissues when exercising salmonids will improve understanding of the underpinning mechanisms involved in exercise-enhanced growth. This information will aid other farming aspects that may need optimising as exercise training and offshore farming become a reality, such as making the necessary adjustments to diets to ensure sufficient energy density and nutrient demands are met.

Varying methods of exercise training can result in drastically different outcomes, highlighting that attention should be directed to the design of exercise programs to maximise output. In studies that utilize exhaustive chase protocols (i.e., acute periods of exercise that result in fatigue), fish tend to exhibit reduced growth, condition factor, and feed intake, as energy is monopolised by the demands of swimming (Gamperl et al., 1988; Gamperl et al., 1991; Hernandez et al., 2002). This is because, during an exhaustive chase protocol, the subjected fish are encouraged to swim by a moving object or through light touching of their tail. This triggers an escape response powered by white muscles (Webb, 1994; Bone and Moore, 2008). White muscles are supported by anaerobic metabolism (phosphocreatine, adenosine triphosphate; ATP, and glycogen utilization) (Sänger and Stoiber, 2001; Gibb and Dickson, 2002), and consequently, leaves a buildup of lactate (Boutilier et al., 1993; Peake and Farrell, 2004). After exhaustion is reached, the subjected fish undergoes a 'recovery' process to restore the depleted energy (glycogen) stocks and clear the built-up lactate (Wood, 1991; Milligan, 1996; Kieffer, 2000; Svendsen et al., 2010). The recovery process results in large portions of the metabolic scope redirected to the associated tissues, a process termed excess post-exercise oxygen consumption (EPOC). At the same time, increased expression of stress-related hormones occurs (e.g., cortisol, norepinephrine etc.) that require additional energy to clear (Gamperl et al., 1994; Milligan, 1996; Kieffer, 2000). During these high swimming speeds, the maintenance cost of digestible amino acids is also elevated (Grisdale-Helland et al., 2013), which could indirectly reduce protein synthesis, as limited energy is available to support these processes. Together, this highlights the importance of avoiding extreme swimming speeds, and that additional measures (e.g., pen skirts) should be implemented in exposed offshore farm sites to avoid conditions that near or result in fatigue.

2.4.3 Feed efficiency

Rapid growth rates are highly desired in farmed fish, but the amount of feed needed to support these growth rates also need to be considered concomitantly, as fish feed contributes to some of the largest costs in aquaculture (Rana et al., 2009; Goddard, 2012). Of the studies showing swimming enhanced-growth, nearly half also reported improved or equal feed efficiency

when compared to control fish (Davison and Goldspink, 1977; Leon, 1986; Totland et al., 1987; Jørgensen and Jobling, 1993; Nielsen et al., 2000; Castro et al., 2011; Waldrop et al., 2018b; Ytrestoyl et al., 2020). Improving growth rates with similar feed efficiency as control fish is still a positive result, as an increased growth rate reduces the time to reach market size and therefore reduces exposure when at sea. Conversely, poor feed efficiency was associated with studies involving exhaustive chase protocols (Gamperl et al., 1988; Hernandez et al., 2002), likely linked to the high costs of exhaustion, and in one other study using low exercise, but the underlying cause for this result could not be explained (Farrell et al., 1991). This highlights the importance of the type of exercise training being implemented, and the high costs of recovery when exhaustion and fatigue are reached.

2.4.4 Chemical composition and body shape

Under exercise regimes, improved growth and feed efficiency are linked to adjustments in chemical composition and body shape. Understanding these changes can help define the appropriate dietary ingredients and energy density needed to match changing body composition under evolving farming practices, see review by McKenzie et al. (2020). For instance, increased energy expenditure from exercise training can influence nutrient demands, utilization, and deposition, because of changes to biochemical pathways. Changes in rates of catabolism and anabolism can lead to large changes in fish size and skeletal muscle mass (McCarthy and Esser, 2010). Since swimming is powered by the activation of red (aerobic) and white (anaerobic) muscles, where red muscle is fuelled predominantly by lipids (complemented with carbohydrates), and white muscle mainly by glycogen and to an extent protein (e.g., cytosolic phosphocreatine and ATP) (Sänger and Stoiber, 2001; Kiessling et al., 2006; Bone and Moore, 2008), different swimming states may have large influences on the fish's primary energy source, and therefore can modify the chemical composition. It is pivotal to know if changes in chemical composition at specific locations e.g., skeletal muscle or visceral fat, are responsible for increases in growth, as this can provide an indication into the mismatches between primary energy demands and feed intake (diet composition), and indicate if nutritional profiles, taste, and fillet yield require further investigation (Rasmussen, 2001).

4.4.1 Protein content

In salmonids, under low swimming speeds, protein content appears to be unaffected, remaining similar to control fish (Nahhas et al., 1982b; White and Li, 1985; Totland et al., 1987; Kiessling et al., 1994; Patterson et al., 2004; Castro et al., 2011; Rasmussen et al., 2011; Grisdale-Helland et al., 2013). This could be due to exercise training speeds aligning closely to the fish's U_{opt}, which is supported by aerobic metabolism and thus, predominately red muscle. As red muscle makes up <10% of the skeletal muscle mass, and skeletal muscle represents about 60%

of the fish's mass (Sänger and Stoiber, 2001; Kiessling et al., 2006; Grunow et al., 2021), changes in protein content in the red muscle incurred by exercise training may not be shown on a whole animal scale because of their small representation. This is further supported by comparisons of protein synthesis among tissues in salmonid species, where red muscle contributes far less than white muscle (Carter and Houlihan, 2001). However, in other finfish species (i.e., non-salmonids), studies have found that higher swimming speeds, activating larger proportions of white muscle, lead to increases in protein content and skeletal muscle mass, reviewed by Huang et al. (2021). Changes in protein content (even if small) within the white muscle are likely to drive differences on a whole-body scale because of their large representation relative to the fish's mass. Increased protein content was only reported in one paper within this search. Pre-smolt rainbow trout (15-20g) exercise trained at 1 BL s^{-1} in a swim tunnel and fed 1% body weight day^{-1} were reported to have greater protein content compared to control trout (Lauff and Wood, 1997).

4.4.2 Muscle cellularity

Even though changes in protein content remained similar in exercise trained and control salmonids, many studies still reported exercise trained salmonids to develop larger skeletal muscle mass. Increased skeletal muscle mass is described by changes in muscle cellularity (the state of a tissue with regard to the degree, quality, or condition of cells present in it). Histological techniques reveal that muscle fibres when exercised, in both red and white muscle, enlarge (hypertrophy) reducing fibre density (hyperplasia), and increasing the total area occupied and the number of capillaries per fibre (Walker and Emerson, 1978; Johnston and Moon, 1980; Davie et al., 1986; Bugeon et al., 2003; Martin and Johnston, 2005; Rasmussen et al., 2011; Timmerhaus et al., 2021). These changes are not only thought to increase the skeletal muscle mass, but also improve swimming efficiency.

Improved red and white muscle cellularity occurs at low to moderate exercise training speeds, even though these swimming speeds are thought to be supported by red muscle and thus, predominately aerobic metabolism. The proportion of white muscle contraction increases with increasing swimming speeds (Burgetz et al., 1998; Martin-Perez et al., 2012; Alfonso et al., 2021; Hachim et al., 2021; Huang et al., 2021), which could provide some explanation of why white muscle morphology responds when exercise trained under low and moderate levels. Developing detailed measurements of the relationship between increasing swimming speeds with red and white muscle involvement is yet to be thoroughly described (Hachim et al., 2021). Yet, this information could provide valuable details and explanation of the inconsistencies found in exercise-studies and potentially improve accuracy in achieving optimal speeds for exercise-enhanced traits.

4.4.3 Lipid content

Unlike protein, lipid content has a more complex relationship with exercise. Lipids are more energetically dense than proteins (38.5 kJ g^{-1} verse 23.6 kJ g^{-1}), often making them the primary source for energy production (Bureau et al., 2002; Glencross, 2009). Because of this, lipid content is highly influenced by energy metabolism and therefore, is indirectly affected by other factors that are closely linked to metabolism, such as size, exercise training intensity, and duration (Huang et al., 2021). Changes in lipid content under exercise is not completely clear among the salmonids, but most exercise training programs with low speeds often find lipid content to be similar to control fish (Walker and Emerson, 1978; Johnston and Moon, 1980; Nahhas et al., 1982b; White and Li, 1985; Totland et al., 1987; Jørgensen and Jobling, 1993; Kiessling et al., 1994; Lauff and Wood, 1997; Castro et al., 2011; Rasmussen et al., 2011; Grisdale-Helland et al., 2013; Nilsen et al., 2019); whereas, in exercise training programs with higher swimming speeds (including exhaustive chase protocols) or for long durations, lipid content often decreases (Gamperl et al., 1988; Bugeon et al., 2003; Simpkins et al., 2003a, 2003b; Patterson et al., 2004; Kiessling et al., 2005; Rasmussen et al., 2011; Prescott et al., 2023). Sustained swimming at higher speeds and for longer likely influenced lipid composition through increased energy demands needed to sustain swimming, whereas the lack of differences between control fish and fish exercised at low levels could indicate similar nutrient demands or that fish feeds are too energy dense and are not optimized for the respective rearing environment.

In other finfish species (non-salmonids), studies have found lipid content to initially increase and then decrease, before remaining stable (Davison, 1997; Li et al., 2013; Huang et al., 2021). In this search, an increase in lipid content was only found in one study, where the mesenteric fat of rainbow trout significantly increased in the higher swimming group compared to the control and moderate swimming groups (Nielsen et al., 2000). Wild salmon exhibit profound energetic efficiency to survive large unfed migrations in order to reproduce (Hillestad and Johnsen, 1994; Johnsen et al., 2011; Li et al., 2011; Araújo et al., 2022b), which could be why large changes in lipid content aren't shown, and why farmed salmonids are much fatter than their wild counterparts (Sprague et al., 2016); high energetic efficiency and reduced exercise load under farm settings.

4.4.4 Body shape

Exercise training influences the location and retention of protein and lipids, which can shape salmonids (Pakkasmaa and Piironen, 2000). Using exercise to reduce visceral fat content, a more streamlined body shape could be achieved, which would minimise drag and enable more efficient swimming performance (Webb, 1982; Webb, 1984; Pakkasmaa and Piironen, 2000). Therefore, exercise training could provide a valuable tool to improve swimming performance

and health of farmed fish through improvements in body shape. This is extremely relevant as farmed fish, including salmonids, typically have a deeper and rounder body shape, and exhibit large fat deposits around the visceral organs and heart compared to their wild counterparts (Hard et al., 2000; Poppe et al., 2003; Anttila et al., 2007; Sprague et al., 2016; Saavedra et al., 2017). These changes in body shape and nutritional composition, alongside changes in biochemical pathways have been associated with poorer swimming performance and reduced health, e.g., increased susceptibility to spinal deformities (Claireaux et al., 2005; Anttila et al., 2007; Anttila and Mänttäri, 2009; Zhang et al., 2016; Robinson et al., 2017; Scholtens et al., 2023; Prescott et al., 2024). Thus, improving body shape towards a leaner more naturally appearing salmon could lead to a cascade of health improvements.

In this literature search, changes in condition factor, a proxy for body shape, was most commonly reported, where the majority of studies report condition factor to increase (Jørgensen and Jobling, 1993; Nielsen et al., 2000; Bugeon et al., 2003; Castro et al., 2011; Parker and Barnes, 2015; Nilsen et al., 2019; Ytrestoyl et al., 2020; Timmerhaus et al., 2021) or remain similar (Davie et al., 1986; Gamperl and Stevens, 1991; Kiessling et al., 1994; Skilbrei and Holm, 1998; Azuma et al., 2002; Hernandez et al., 2002; Martin and Johnston, 2005; Anttila et al., 2006; Anttila et al., 2010; Waldrop et al., 2018b; Prescott et al., 2023) when compared to control fish. Similarly, exercise training can influence fin morphology, where studies have shown exercise training to increase dorsal-, caudal-, and pectoral fin size in Arctic char (Grünbaum et al., 2007), brown trout, and Atlantic salmon (Pakkasmaa and Piironen, 2000), but not in Chinook salmon (Prescott et al., 2023). Changes in fin morphology under exercise training is likely to support swimming performance. Fin morphology differs drastically among fishes and are specialised structures for specific lifestyles, swimming modes, and environments (Westneat and Wainwright, 2001; Fulton et al., 2005; Hunt et al., 2023).

2.4.5 Swimming and aerobic capacity

4.5.1 Swimming and aerobic capacity

Under exercise, a suite of physiological mechanisms respond that underpin improvements in fitness and aerobic capacity of salmonids. Exercise training intensity positively corresponds to increased swimming performance, where exercise improves preferred swimming speeds (Brett et al., 1958), stamina (Hochachka, 1961; Nahhas et al., 1982b, 1982a; Leon, 1986; Houlihan and Laurent, 1987; Pearson et al., 1990; Anttila et al., 2008; Anttila et al., 2011), and critical swimming speed; U_{crit} (Besner and Smith, 1983; Farrell et al., 1990; Holk and Lykkeboe, 1998; Prescott et al., 2023). Enhanced swimming performance is supported by several functional, morphological, and cellular changes within the cardiorespiratory system, and has been associated

with an increase in aerobic enzyme activity, upregulating metabolic pathways, and increased aerobic-related gene expression.

Salmonid's aerobic capacity improves under exercise allowing for better swimming performance. Exercise trained salmonids have been shown to exhibit an increased maximum metabolic rate; MMR, larger aerobic scope; AS (Nahhas et al., 1982b; Gallaugher et al., 2001; Larsen et al., 2012; Prescott et al., 2023), improved cardiac function, e.g., cardiac output, i.e., volume of blood per min; Q, power, rate, and cardiac size (Davison, 1989; Farrell et al., 1991; Gallaugher et al., 2001; Nilsen et al., 2019), improved blood oxygen carrying properties, e.g., hemoglobin (Hb) and hematocrit (Hct) (Hochachka, 1961; Zbanyszek and Smith, 1984; Thorarensen et al., 1993; Holk and Lykkeboe, 1998; Gallaugher et al., 2001; Parker and Barnes, 2015), and improved oxygen delivery properties, e.g., arterial oxygen partial pressures, carbonic anhydrase activity i.e., enzymes involved in removing CO_2 from tissues (Zbanyszek and Smith, 1984; Holk and Lykkeboe, 1998; Gallaugher et al., 2001). Greater aerobic capacity allows higher amounts of oxygen and faster delivery rates to the muscles and various tissues, allowing fish to swim faster and for longer. These traits are essential for salmonids to withstand current speeds in exposed offshore farm sites.

4.5.2 Tissue remodelling

Changes in tissue morphology of exercised fish have been documented to underpin enhanced fitness. Both white and red muscles have received the most attention when documenting morphological responses to exercise training, where muscle fibres typically increase in size (previously discussed in section 2.4.2) (Davie et al., 1986; Postlethwaite and McDonald, 1995; Bugeon et al., 2003; Rasmussen et al., 2011; Palstra et al., 2014; Huang et al., 2021; Timmerhaus et al., 2021). Other highly plastic and important tissues involved in aerobic processes, such as the heart and gills, have received less attention in regards to their morphological responses to exercise training (Prescott et al., 2023).

Poor heart shape (i.e., rounded ventricle and misaligned bulbus arteriosus) is a large concern for farmed salmonids (i.e., Atlantic salmon and rainbow trout), causing inefficient heart function often leading to mortality (Poppe et al., 2003). Links between poor heart shape in rainbow trout (Claireaux et al., 2005) and thinner compact myocardium in Atlantic salmon (Anttila et al., 2014) were associated with individuals that exhibited poorer swimming abilities. As exercise can improve swimming performance and heart function, it is hypothesised that exercise would also improve heart shape.

Similarly, the teleost gill is capable of remodelling in response to several environmental cues (Sollid et al., 2003; Sollid et al., 2005; Sollid and Nilsson, 2006), including in fish that

inhabit environments with high aerobic metabolic pressures (e.g., low-latitude locations) (Bowden et al., 2014; Hess et al., 2015; Hess et al., 2017; Johansen et al., 2021). Gill remodelling has been documented in Atlantic salmon parr with good swimming abilities, where superior swimming Atlantic salmon parr had taller lamellae (Anttila et al., 2014), likely increasing the available surface area for oxygen diffusion and therefore enhancing oxygen uptake. This suggests that the gills of exercise trained fish may also undergo structural remodelling to enhance oxygen uptake processes (e.g., improving oxygen diffusion distance and surface area). However, only small morphological changes in the gills have been documented in exercise trained Chinook salmon, even though improved swimming performance and aerobic capacity were found (Prescott et al., 2023).

4.5.3 Gene regulation and biochemical pathways

With the development of advanced biochemical techniques (e.g., genomics, transcriptomics, and proteomics), more studies are reporting changes at the cellular and molecular levels that provide valuable insights into mechanisms underpinning changes reported on tissue and whole-animal levels (i.e., growth, aerobic metabolism, and swimming). Upregulation of oxidative enzyme activity occurs in both red and white muscle of exercised fish, while anaerobic enzyme activity decreases or remains similar to control fish (Johnston and Moon, 1980; Anttila et al., 2006; Anttila et al., 2011; Zhang et al., 2016). Muscle excitation-contraction coupling mechanisms increase in activity in red and white muscles with exercise training (Anttila et al., 2006; Anttila et al., 2008), which have been reported to be reduced in domestic salmonids (Anttila et al., 2007; Anttila and Mänttäri, 2009). Gene expression and proteomic techniques revealed upregulation of pathways related to glycolysis metabolism and growth (Timmerhaus et al., 2021), underpinning enhancements presented on a functional organism level. Using ‘omic approaches to understand mechanisms across different tissues could be an extremely powerful tool (Esmaeili et al., 2021; Anderson et al., 2022; Esmaeili et al., 2022; Young et al., 2023) and would greatly improve understanding.

2.4.6 Health

Fish health is considered among the highest priority in fish production. Healthy fish exhibit optimised physiological performance that maximises product output and quality (Assefa and Abunna, 2018). Producing healthy and robust salmonids is extremely relevant under current-day scenarios, where anthropogenic climate change is rapidly changing environments, including existing farming sites which are becoming unsuitable to rear salmonids (e.g., warming waters, heatwaves, disease outbreaks, and harmful algal blooms) (Oliver et al., 2018; Broekhuizen et al., 2021; Maulu et al., 2021). Using exercise training to grow healthy and more robust fish could safeguard finfish farming in the Anthropocene.

4.6.1 Survivability, stress, and disease resilience

Under exercise training, survivability can improve on a population level (Walker and Emerson, 1978) and during disease challenges (Castro et al., 2011), which could be linked to improved stress resilience (Woodward and Smith, 1984; Hernandez et al., 2002; Ytrestoyl et al., 2020). Castro et al. (2011), found that fish exposed to interval exercise training exhibited the highest survivability during a disease challenge, followed by the continuous exercised fish and then the control fish. Enhanced disease resistance was linked to stimulated protective mechanisms within the cardiac transcriptome, which is thought to broadly improve immunity (Castro et al., 2011). In other studies, exercise training is suggested to improve stress resilience. Plasma cortisol levels measured in fish after a period of exercise training were similar (Hernandez et al., 2002; McKenzie et al., 2012; Timmerhaus et al., 2021) or higher (Ytrestoyl et al., 2020) between exercise treatments, but some studies showed exercise trained fish to have improved mechanisms in clearing plasma cortisol levels following a stressful event, e.g., crowding, exhaustive exercise (Hernandez et al., 2002; McKenzie et al., 2012). Exercise training could provide an alternative tool to strengthen farmed salmonids and provide protection against some of the largest mortality events the salmonid aquaculture industry faces, e.g., seawater transfer, salmon louse, and amoebic gill disease (Nilsen et al., 2020; Oliveira et al., 2021). These improvements could lead to large industry advancements, as it has been estimated that more than 50 million Atlantic salmon are lost late in production (Oliveira et al., 2021).

4.6.2 Thermal and hypoxia tolerance

The salmonid aquaculture industry is vulnerable to the devastating impacts caused by anthropogenic climate change, where mass mortalities have been reported in the summer months (Oliveira et al., 2021; Rosewarne, 2022) because of the increasingly warming waters and potentially reduced oxygen saturation (Broekhuizen et al., 2021). It is necessary for the aquaculture industry to implement adaptation frameworks to ensure they can continue farming in the Anthropocene. It has been proposed that exercise training could improve thermal and hypoxia tolerance through improvements associated within the cardiovascular system (AS, heart function, and oxygen transport properties), since correlations between larger AS with hypoxia and thermal tolerance have been documented (Zhang et al., 2018a).

Using exercise training to improve thermal tolerance has only recently been explored. Exercise training can widen the thermal scope for cardiac function in rainbow trout (e.g., temperature at maximum heart rate, arrhythmia), but only under moderate exercise training regimes (Papadopoulou et al., 2022; Pettinau et al., 2022). Thermal tolerance and upper thermal limits are bounded by the organism's AS, which in salmonids is dictated by the cardiac thermal scope and thus, temperatures that cause heart failure (Eliason and Anttila, 2017; Ern et al., 2023).

Improving the thermal scope for cardiac function could improve thermal tolerance on a whole-animal level.

Linking exercise with whole animal thermal resilience was investigated by Gomez Isaza and Rodgers (2022) using Chinook salmon. Gomez Isaza and Rodgers (2022) exercise trained Chinook salmon at 4.1 BL s^{-1} (60% of U_{crit}) in a Blazka-type swimming tunnel for one hour per day for three weeks before measuring the critical maximum temperature (CT_{max}). No statistical differences were reported for CT_{max} or oxygen carrying capacity between controlled and exercise trained Chinook salmon (Gomez Isaza and Rodgers, 2022). Cardiac thermal performance was not measured in this study. Minimal evidence supporting links between exercise training and thermal tolerance could be due to the acute exercise regime implemented and that Chinook salmon are yet to show exercise-enhanced growth (Gallaugher et al., 2001; Kiessling et al., 2005; Prescott et al., 2024).

4.6.3 External condition and aesthetics

A fish's integument (i.e., skin) is a continuous structure covering the entire fish and is in direct contact with the external environment, therefore it acts as the first defensive barrier to parasites and pathogens (Hawkes, 2004; Elliott, 2011a; Elliott, 2011b; Ángeles Esteban, 2012; Jensen et al., 2015; Sveen et al., 2016). To function efficiently, the skin must remain intact and uncompromised, and it has been hypothesised that a thicker skin would reduce susceptibility to lacerations and pathogen entry (Svendsen and BØGwald, 1997; Madetoja et al., 2000). The skin is also involved in locomotory properties, acts as a secondary site for respiratory and ion-osmo-regulatory processes (Elliott, 2011a; Elliott, 2011b) and holds a large aesthetic value in terms of a commercial product (Colihueque et al., 2011; Colihueque and Araneda, 2014), further underscoring its importance.

Salmonids skin condition and aesthetic value often improves under exercise training regimes, through reductions in agonistic behaviours and enhanced skin morphology compared to controls. The prevalence and severity of fin damage, eye bites, and skin wounds were all reduced in salmonids reared under moderate exercise levels compared to control fish (Totland et al., 1987; Jørgensen and Jobling, 1993; Solstorm et al., 2016b). In Prescott et al. (2023), Chinook salmon reared under low and moderate exercise training showed no difference in the skin epidermis (outermost layer) thickness, but individuals raised under moderate flow reduced dermis thickness (layer under epidermis). Ytrestoyl et al. (2020) found skin condition in low exercised fish to be superior to those in higher exercise regimes, where morphological features negatively correlated with increased exercise and was further exacerbated by increased salinity levels. In another study, skin morphology showed a similar response, where the skin epidermis was thickest in low and medium exercise regimes compared to the high exercise regime, and the dermis thickness

increased with increasing exercise speeds (Timmerhaus et al., 2021). In agreement, swimming speeds above moderate levels begin to compromise the skin but are optimised at lower sustainable speeds.

4.6.4 Spinal health

It is not clear how the skeletal system, another integral part of locomotion, is impacted by exercise training (Videler, 1993a). This is extremely important in salmonid aquaculture as 30-70% of harvested salmonids can exhibit skeletal deformities (Fjelldal et al., 2012; Perrott et al., 2018). Micronutrients, calcium (Ca) and phosphorus (P) form the primary make up of bones, where they provide mechanical and physical strength, enabling more robust movements. Bone mineralization has been shown to increase alongside exercise (Deschamps et al., 2009; Totland et al., 2011; Ytteborg et al., 2013; Solstorm et al., 2016a), but no study has shown bone mineralisation to increase with exercise and therefore improve locomotive abilities or reduce spinal deformities (Deschamps et al., 2009; Solstorm et al., 2016a; Prescott et al., 2023; Prescott et al., 2024). In non-salmonid species, exercise training under higher water currents increased the prevalence of vertebral lordosis (Divanach et al., 1997; Kihara et al., 2002; Palstra et al., 2020), but this was not the case for Chinook salmon (Prescott et al., 2024).

4.6.5 Tissue health

Another area that has been studied when exercising salmonids is the energetic burden placed on respiratory and muscular tissues. Fish under low exercise regimes display healthy functioning tissues, whereas signs of compromised respiratory and muscular tissues observed through histopathological assessments have been associated with higher water velocities, i.e., speeds exceeding optimal (Timmerhaus et al., 2021). Inflamed muscle fibres and gill diseases become prevalent in fish under high aerobic pressure (Timmerhaus et al., 2021; Jones and Price, 2022). During these periods of high aerobic pressure, energy would be redirected to affected areas to recover the damaged tissues, which would reduce oxygen to pathways involved in growth and feed efficiency, ultimately having negative impacts on their production-biology phenotypes and could lead to mortality if not reprieved. However, as in mammalian species, acute muscle inflammation is a key parameter involved in muscle growth, and maintaining a balanced cycle of inflammation and repair is important (Yang and Hu, 2018). Muscle growth in fish needs to be tested against this model to further understanding and enable appropriate swimming regimes to be determined, which was also suggested in Palstra et al. (2020).

2.4.7 Breeding and genetics

Selective breeding programs are a pivotal component in nearly all farming industries (plants and animals), as selection for performance, disease resistance, and product quality directly

improves industry profitability, sustainability, and efficiency (Hill, 2001). Within a breeding program there is a trade-off between the number of traits included in the selection criteria (traits you are selecting for) and the response to selection. A high number of traits can reduce genetic gains, but could improve individual robustness and resilience under varying conditions, while inclusion of a low number of traits can improve genetic gains of selected traits but may increase environmental sensitivity (Hershberger, 1990; Gjedrem and Baranski, 2010). As such, traits that can be manipulated and cost-effectively improved through husbandry (e.g., vaccines) or by changing environmental conditions, may not need to be included in the breeding goal. However, these traits and environments need to be assessed appropriately (e.g., testing for genotype by environment interactions).

An emerging challenge associated with expanding salmonid farming offshore, is the ability to select performance and product quality traits that are suitable for nursery/RAS, nearshore, and exposed offshore environments, and whether swimming performance needs to be included as a trait in the breeding objectives. Swimming performance is heritable in some fish species (Blanc and Toulorge, 1981; Hurley and Schom, 1984; Rogers et al., 2002; Falica and Higgs, 2013; Vandeputte et al., 2016; Wakamatsu et al., 2019; Mengistu et al., 2021), thus providing scope for genetic gains to be achieved through breeding programs. If swimming performance is to be included within the selection criteria, it is important to understand how swimming performance traits genetically and phenotypically correlate with other selected production performance traits (Hershberger, 1990; Gjedrem and Baranski, 2010). Very few studies have investigated genetic and phenotypic correlations between swimming- and production performance, and in these studies, genetic correlations between weight and swimming performance were low in Atlantic salmon (Hurley and Schom, 1984) and moderate in Nile tilapia (Mengistu et al., 2021), which could be problematic for the industry.

It is important to ensure that the selected traits perform equally across the entire production cycle (e.g., hatchery, nearshore, and offshore environments). To assess whether exposed offshore performance needs to be selected as a different trait to nearshore performance, a measure of the genetic by environment (G×E) interaction for performance in these two locations should be used. A G×E interaction is commonly found when comparing two rearing environments, e.g., tank vs sea pen (Winkelman and Peterson, 1994; Kause et al., 2003; Mas-Muñoz et al., 2013; Sae-Lim et al., 2013), but when different rearing environments that are influenced by multiple factors, identifying the key factor(s) driving the G×E becomes difficult, and therefore so is the ability to manage the factor. However, testing two rearing environments with many possible factors is important when there is limited ability to control these environmental influences and therefore identifying the factor becomes irrelevant, e.g., geographically separated farm sites. Testing single environmental factors that are being

implemented by industry in control setting, such as flow regimes, is highly beneficial for industry. Determining if G×E interactions exist between lower and higher flow regimes has only been investigated in one study in salmonids (**Chapter 4**) and none in any other finfish species. This presents a large knowledge gap in understanding how farmed fish may perform when industry implements exercise regimes in tank settings and to gain insight for when salmon are farmed in offshore high energy environments. The limited genetic information restricts the industry's ability to optimise their selective breeding programs appropriately.

Another aspect of animal breeding that is yet to be thoroughly explored in exercise training or to achieve robust salmonids for exposed offshore farming, is the inter-, transgenerational plasticity from parental exercise training. Only one study has investigated the intergenerational effect on offsprings from exercise training of the maternal parent (Pettinau, 2023). Pettinau (2023) showed that brown trout offspring from exercise trained mothers had larger body mass and length, but no improvements in thermal performance was transferred to the offspring, as measured in the mothers. However, the heritability of thermal performance traits were greater in offspring with exercise trained mothers than those without exercise trained mothers (Pettinau, 2023), suggesting that exercise training could lead to improved gains in selective breeding programs through higher heritabilities, thus enabling greater and faster genetic gains.

2.5 Limitations and future directions for building robust and resilient salmonids for exposed offshore farming conditions

Robust and resilient salmonids for exposed offshore farming may be defined as a salmonid exhibiting proficient growth rates and efficient feed conversion without compromising any organs, as well as presenting high athleticism, stress resilience, and disease resistance (Knap, 2005; Castro et al., 2011). The studies evaluated throughout this review show that exercise training can improve traits that define a robust and resilient salmonid (Table 2.1), offering reassurance and encouragement for exposed offshore salmonid farming success. However, uncertainty remains, as there are inconsistencies among traits, species, and exercise training regimes, as well as these studies involving highly controlled experimental setups that lack many of the physical dynamics that occur in exposed offshore farming environments (e.g., hydrodynamics, temperature, oxygen, salinity). Also, many of these studies focus on fish < 500 g (Figure 2.2) (Rodgers and Gomez Isaza, 2023), thus mainly including size classes that are reared on land in freshwater and under controlled settings (Table 2.1), and not across size classes that are grown out at sea. Therefore, it is uncertain if these exercise-enhanced traits will persist

in high energy environments and can only be used to give insight into how salmonids may respond when farmed offshore.

Our literature search shows that there are clear differences in flow environments that encourage sustained swimming versus swimming to exhaustion, where sustained swimming generally shows positive attributes that would benefit exposed offshore farming. However, pinpointing what level of sustained swimming or aspects of exercise training that improve these traits is not clear among salmonid species. In contrast, exhaustive exercise reduces production performance, and that the industry can be advised to avoid offshore locations or infrastructure that would lead to these scenarios. The inconsistency within the literature suggests there could be some flexibility in the range of flows encountered in offshore locations, as the salmon can respond by swimming faster than the current, or by modifying their swimming behaviour (i.e., maintaining position) to avoid fast speeds. Determining this range remains a challenge.

A key factor used to inform guidelines for finding suitable farming locations is the maximum swimming speed of the subject species. Determining the upper swimming limit in fish is mainly based on U_{crit} tests, which estimates the maximum sustainable swimming speed and is commonly measured alongside $\dot{M}O_2$ in a swim tunnel (Brett, 1964; Steffensen et al., 1984). However, this protocol uses fish in a post-absorptive state with quick incremental periods, meaning these experiments can overestimate the maximum sustainable (i.e., > 4 hours) speed that fish can endure (Wilson and Egginton, 1994; Hvas et al., 2020). A more reliable swimming range would be 70-90% of the U_{crit}, as these speeds can be maintained for long periods of time (Burgetz et al., 1998; Hvas and Oppedal, 2017; Hvas et al., 2021a). Under exercise, swimming performance can be improved, meaning offshore environments with greater current speeds could be selected. However, it is important to determine how exercise trained salmonids will respond to a range of swimming speeds across a day and in combination with daily activities, such as feeding and digestion.

Exposed offshore farming environments present many challenging features that are yet to be investigated. For instance, offshore currents vary both spatially and temporally (Danielssen et al., 1997; Johansson et al., 2014), where research has only focused on currents at low to moderate speeds either at constant rates or incrementally increased to accommodate growth in either circular tanks, raceways, or flumes. Future studies need to consider the magnitude, duration, and frequency of differing currents, and evaluate how salmonids respond under these simulated conditions. Several other physical factors that will influence the success of farming salmonid offshore include the impact of net movement and confining space, recovery after colliding with other fish and the net, wave height and variation, tidal movements, wind speed and surface currents, as well as stratification and variation of abiotic factors, e.g., temperature,

dissolved oxygen, and salinity (Klebert et al., 2015; Moe-Føre et al., 2016; Froehlich et al., 2017; Morro et al., 2021). Physical factors of exposed offshore sites need to be investigated individually and in combination, as these factors will provide pivotal information for finding suitable locations and designing appropriate cage infrastructure (e.g., mobile, submersible etc.).

Anthropogenic impacts are already affecting several salmonid industries (Broekhuizen et al., 2021; Maulu et al., 2021; Oliveira et al., 2021; Rosewarne, 2022). Understanding the dynamics between exercise training and anthropogenic resilience, and how these factors may impact exposed offshore farming is essential. Higher acute temperatures and reduced oxygen saturation are the most imminent anthropogenic threats currently impacting nearshore salmonid farming, leading to mass-mortality events (Broekhuizen et al., 2021; Maulu et al., 2021). Offshore farms are not exempt from these threats, and in combination with higher energy demands offshore (i.e., maintaining higher swimming speeds), the impacts of these threats to the salmon could be greater. Temperature and oxygen saturation dictates metabolic rates in ectothermic organisms (Fry and Hart, 1948; Pörtner and Knust, 2007; Farrell and Richards, 2009; Rummer et al., 2014; Claireaux and Chabot, 2016), where deviations from the optimal conditions can cause catastrophic impacts, i.e., death (Callaway et al., 2012; Jensen et al., 2015; Wade et al., 2019; Calado et al., 2021; Oliveira et al., 2021). Defining temperature and hypoxia thresholds of exercise trained salmonids where performance can be maintained will improve the success of farming in exposed offshore locations.

There is also a need for studies to determine if exercise training can prepare salmonids for exposed offshore farm sites, and if exercise-enhanced traits can be maintained or continue to improve when farmed offshore. Exercise training salmonids until they reach a sea transfer size and undergoing a simulated offshore environment could help determine if exercise training is important to prepare salmonids for exposed offshore environments. If exercise training prepares salmonids for exposed offshore environments, ensuring the longevity of these exercise-enhanced traits is critical. There are positive signs that exercise-enhanced traits can persist offshore, as flow environments offshore can provide steady swimming states (mimicking exercise training regimes), provided the current speeds do not elicit burst and coast swimming speeds and/or rely on anaerobic metabolism. However, the current speeds will oscillate over the course of the day (Campos et al., 2019; Newcombe et al., 2019), and seldom studies have investigated the influence of oscillating current speeds on salmonid performance (Castro et al., 2011; Esbaugh et al., 2014; Athammer et al., 2024; Barbier et al., 2024). Determining the longevity of exercise-enhanced traits could be simulated in controlled environments, but implementing a field pilot study may provide better insight, as exercise trained salmonids will experience all the influencing factors simultaneously. Generating this knowledge is critical for the industry's transition offshore and the welfare of the animals.

2.6 Conclusion

Matching exposed offshore conditions to the optimal conditions for farming salmonids remains a challenging task. It is known that exercise training improves several key phenotypes that benefits aquaculture production in controlled environments, but knowing if these traits are enhanced in variable environments is unknown. Research should now focus on whether exercise training can better prepare salmonids for exposed offshore environments and whether these exercise-enhanced traits can persist offshore and are heritable. This information is pivotal to enable the aquaculture industry and marine engineers to develop improvements to farming infrastructure and ensure sea pen conditions promote optimal farming conditions. Even so, maintaining culture conditions within the realm of physical parameters tolerated by salmonids will be increasingly difficult in the face of exacerbating anthropogenic impacts (e.g., marine heatwaves and environmental hypoxia) (Hoegh-Guldberg, 2014; Oliver et al., 2018; IPCC, 2022), and these changing abiotic factors require further investigation. Therefore, it is essential to broaden the scope of optimal conditions for farming fish offshore to lessen the impact of such events. The use of exercise training in combination with enhanced breeding programs may provide an avenue forward in achieving robust and resilient salmonids for exposed offshore farming.

Chapter Three The mismatch between swimming speeds and flow regimes when optimizing exercise regimes to improve Chinook salmon, *Oncorhynchus tshawytscha*, performance

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3.1 Abstract

Exercise regimes provide a promising opportunity to enhance production performance during hatchery rearing, but exercise-enhanced traits are presently poorly understood in Chinook salmon. In addition, spinal health can be a concern in some farms in New Zealand and unfavourable correlations between higher condition factor and spinal curvature have been detected. Exercise regimes, in other salmonids, have improved bone mineralisation and lowered condition factor. Therefore, it is hypothesised that sustained swimming could be used as a tool to improve spinal health. This study tests the influence of low (0.3 bl s^{-1}) and moderate (0.8 bl s^{-1}) flow regimes on post-smolt (initial size: wet weight = 82.9 ± 0.3 g, fork length = 174.6 ± 0.2 mm) Chinook salmon feed efficiency and growth performance, as well as spinal health. This study first measured the actual swimming speeds of Chinook salmon under low and moderate flow regimes to determine differences in exercise levels. Swimming speeds were not different between the flow regimes and were much higher than the set flow, because the Chinook salmon were advancing around the tank, swimming faster than the set flow at their own chosen swimming speed. Moderate flow regimes improved feed efficiency by 2.5% but did not influence other feed and growth metrics or spinal curvature and vertebral anomalies prevalence or severity. Production performance significantly differed between individuals with normal spinal health and those that developed spinal anomalies (vertebral compression, −fusion, and/or -vertical shift) or spinal curvature (lordosis, kyphosis, and/or scoliosis). Chinook salmon that developed spinal anomalies were smaller, grew slower, and exhibited poorer feed efficiency, whereas individuals that developed spinal curvature were significantly heavier, longer, and had higher condition factor. The probability of individuals developing spinal curvature increases with higher condition factor. These results provide critical information for industry to consider in their selective breeding objectives to curb the prevalence and severity of spinal curvature incidences.

3.2 Introduction

Exercising salmonids, such as Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), has shown to improve growth efficiency (Ytrestoyl et al., 2020), swimming (Anttila et al., 2008), cardiac performance (Nilsen et al., 2019), muscular capacity (Walker and Emerson, 1978), brain function (Mes et al., 2020), as well as thermal- (Pettinau et al., 2022), stress- (McKenzie et al., 2012) and disease tolerance (Castro et al., 2013b). Exercise is being integrated into commercial aquaculture settings to promote exercise-enhanced traits and aid industry growth and success (Palstra et al., 2015a). For example, the production performance and survival of commercial Atlantic salmon and rainbow trout have been improved using exercise regimes, prior to sea transfer (e.g., CtrlAQUA, 2022; MOWI, 2022; Huon, 2023). However, forming a consensus as to what is the optimal flow regime to promote exercise-enhanced traits in salmonids (and in commercial stocks) remains challenging.

Most exercise studies on salmonids show positive results when conducted under low to moderate flow regimes, e.g., optimal exercise for < 500 g Atlantic salmon and rainbow trout is 0.5-1.5 body lengths per second; bl s^{-1} (Davison, 1997; Palstra and Planas, 2011; Davison and Herbert, 2013). These swimming speeds align closely to the optimal swimming speed, U_{opt} (Davison and Herbert, 2013; McKenzie et al., 2020; Timmerhaus et al., 2021), which is thought to allow for more available energy to be allocated to other physiological processes by minimising the amount of energy spent on swimming (Tucker, 1970; Ware, 1978; Videler, 1993a; Videler, 1993b). Nevertheless, not all exercise studies using salmon have shown improvements in production performance under flow regimes (Leon, 1986; Azuma et al., 2002; Patterson et al., 2004; Deschamps et al., 2009). These inconsistencies could be explained by fish seeking pockets of slower/higher flows and swimming slower/faster than the set flow (suggested in McKenzie et al., 2020). Improvements to the experimental design (i.e., mesh screens to restrict fish from advancing faster than the water flow; Palstra et al., 2015b) and/or additional methods to measure the absolute swimming speed (Herbert et al., 2011) is needed to accurately determine the optimal flow regime for promoting exercised-enhanced traits.

Exercise training could also improve spinal health in farmed salmonids. Skeletal deformities can be prevalent and severe in farmed salmonids (Sadler et al., 2001; Lijalad and Powell, 2009; Powell et al., 2009; Perrott et al., 2018), such as developing spinal curvature (lordosis, kyphosis, or scoliosis; LKS) and/or vertebral anomalies (vertebral fusion, -shift, or -compression) (Lovett et al., 2018; Perrott et al., 2018; Lovett et al., 2020). Bone mineralisation is recognised as a key factor in building and maintaining skeletal health, where several mechanisms behind skeletal deformities are linked to bone demineralisation (Baeverfjord et al., 2019). In some circumstances, an unfavourable relationship exists between spinal curvature and

higher condition factor, potentially linked to genetically selecting for faster growth (Perrott et al., 2020; Scholtens et al., 2023). Previous studies have demonstrated that exercise can increase bone mineralisation (Deschamps et al., 2009; Totland et al., 2011; Ytteborg et al., 2013; Solstorm et al., 2016a) as well as alter body shape (e.g., condition factor, fineness ratio) in salmon (Webb, 1982; Webb, 1984; Pakkasmaa and Piironen, 2000) and in other fish species (Alcaraz and Urrutia, 2008; Lu et al., 2020), but using exercise to improve spinal health is yet to be documented. A reduction in condition factor, without compromising fillet growth, could therefore potentially improve spinal health, while maintaining efficient production performance.

The New Zealand (NZ) Chinook salmon aquaculture industry presents a promising opportunity to integrate exercise training into their production strategy. The industry is moving their hatchery production from raceways to circular tanks, which allows flow regimes to be set. In addition, the industry is exploring the option to use recirculating aquaculture systems (RAS), also with circular tanks, during the pre-smolt stage and considering extending the hatchery phase to transfer larger fish (post-smolts) to nearshore sea pens as a management strategy to avoid peak summer water temperatures (Waddington, Z., pers. comm. 2023). The industry also has plans for expansion by developing new farms in open ocean locations with high water currents, up to 1.5 m s^{-1} (i.e., increased swimming speeds; Newcombe et al., 2019; NZKS, 2020). Farmed Chinook salmon (*Oncorhynchus tshawytscha*) have received little research attention on optimising hatchery conditions, including the influence of exercise regimes on Chinook salmon, but see Thorarensen et al. (1993), Kiessling et al. (2005), and Gallaugher et al. (2001). In these studies, there is little indication that exercising Chinook salmon can improve their production-performance traits (Gallaugher et al., 2001; Kiessling et al., 2005). Yet, there is an urgent need to further explore how flow regimes influence Chinook salmon production and skeletal health, and to identify suitable flow regimes for hatcheries to advance performance, but also, to provide insight into production expectations when the industry expands offshore.

In this study, a low (0.3 bl s^{-1}; LFR) and moderate (0.8 bl s^{-1}; MFR) flow regime was used to investigate the influence of sustained swimming on Chinook salmon swimming speed, feed efficiency, growth performance, and spinal health. Flow regimes were chosen for several reasons:

1. Little is known of the swimming characteristics in Chinook salmon, and based on previous studies it is predicted Chinook salmon are poorer swimmers than Atlantic salmon (Gallaugher et al., 2001; Remen et al., 2016; Hvas et al., 2017; Hvas and Oppedal, 2019; Prescott et al., 2023).
2. Chinook salmon (~387 g) have been estimated to exhibit an U_{opt} of 1.5 bl s^{-1} (Gallaugher et al., 2001; Davison and Herbert, 2013), but when exercised under

similar flow regimes (i.e., 0.5, 1.0, and 1.5 bl s^{-1}) exercise-enhancements did not occur (Thorarensen et al., 1993; Kiessling et al., 1994; Kiessling et al., 2005; Hoffnagle et al., 2006).

3. Previous exercise studies were performed on wild strains of Chinook salmon, but based on what is known in other salmonid species (e.g., Atlantic salmon; Anttila et al., 2007; Pedersen et al., 2008; Anttila and Mänttäri, 2009; Zhang et al., 2016), farmed Chinook salmon are expected to exhibit reduced swimming performance due to domestication and breeding programs predominantly selecting for growth.

Therefore, additional investigation into the influence of enforced exercise under lower flow regimes (i.e., < 1.0 bl s^{-1}) is warranted, as speeds > 1.0 bl s^{-1} become difficult to achieve in high volumes (e.g., commercial RAS) without large modifications.

3.3 Materials and Methods

All protocols performed on animals followed animal ethics protocols through Nelson Marlborough Institute of Technology Animal Ethics Committee (AEC2018 CAW01).

3.3.1 Fish husbandry and experimental conditions

Sourcing and acclimation to experimental conditions followed protocols outlined in Prescott et al. (2023). All-female Chinook salmon were sourced from Sanford's Kaitangata commercial salmon hatchery, where they were tagged with passive integrated transponder tags (HIDGlobal, EM4305, 12 mm long and 2 mm diameter glass tags) and transferred to the Finfish Research Centre at Cawthron Aquaculture Park, Glenduan Nelson, NZ on 7th December 2020. The fish were placed in 8,000 L tanks at 14-15 ppt salinity and 13 ± 0.2 °C upon arrival. Fish were then acclimated to 35 ppt (seawater) and 17 ± 0.2 °C across a seventeen-day period and supplied with filtered recirculating seawater on a 24 h light photoperiod.

Fish (n = 3119) were then sorted across twelve 8,000 L treatment tanks (top diameter: 2602 mm; bottom diameter: 2400 mm; depth: 1750 mm; ~260 fish per tank) and tank water velocities were set to 4.93 ± 0.08 cm s^{-1}. Prior to exercise regimes commencing, all fish were measured for mass (M; 82.9 ± 0.30 g, mean ± SE) and fork length (174.6 ± 0.17 mm, mean ± SE) and following recovery, flow regimes were adjusted across seven days to achieve the LFR (initial: 6.20 ± 0.01 cm s^{-1}; final: 8.47 ± 0.01 cm s^{-1}; relative to fish length: 0.27 ± 0.0003 bl s^{-1}) and MFR (initial: 17.32 ± 0.01 cm s^{-1}; final: 22.47 ± 0.01 cm s^{-1}; relative to fish length: 0.75 ± 0.0007 bl s^{-1}; six tanks per treatment). Flow regimes were measured daily and adjusted monthly to account for fish growth. Flow regimes across the tank profile were not uniform and was found to decrease towards the centre standpipe (diameter: 100 mm). The variation across the tank profile on average

differed by ~10% in respect to the mean speed, e.g., a mean flow speed of 21.77 ± 0.26 cm s^{-1} with an average flow speed of 29.02 ± 0.16 cm s^{-1} near the outer wall and 21.42 ± 0.44 cm s^{-1} near the centre standpipe. The flow speed was measured at the same spot in all tanks (i.e., 40 cm from edge of tank and 50 cm tank depth) to attain representative and consistent speed values.

Fish were hand fed (diet composition: protein 37.5 g 100 g^{-1}, lipid 24.2 g 100 g^{-1}, energy 1705 kJ 100 g^{-1}) with a commercial feed (Skretting, Tasmania Australia) to satiation daily (AM) and pellet size was increased with fish growth, as per manufacturer's recommendation. Daily tank feed intake (tank DFI) was measured by subtracting final feed bucket weight including uneaten pellets (retrieved by swirl separator) from the initial feed bucket weight. Uneaten pellets were counted using an automated counter (Contardor2, PFEUFFER GMBH, Kitzingen, Germany) and multiplied by the average pellet weight.

3.3.2 Swimming speeds

Surveillance cameras (HIKVISION, Network Camera, V5.6.2) were positioned above four treatment tanks (two per treatment) to capture swimming behaviour under LFR and MFR. Footage was recorded three times a day (7:00, 15:00, and 23:00) for six minutes. From these recordings, the distance a fish swam in body lengths (estimated from the mouth to tail distance) was estimated across one second and expressed as bl s^{-1} (Herbert et al., 2011). The camera's field of view captured approximately ¾ of the tank allowing for fish near the edge, middle, and centre of the tank to be measured. This was repeated weekly (from week 2 to 9), where five measurements of swimming speed from five fish were estimated for each time of day and tank. Tank location for each measurement was recorded, ranging from the centre of the tank to the edge. Tank locations were estimated across rings of ~185mm wide and categorised as standpipe, inner, middle to inner, middle, middle to outer, outer, edge, or cross tank (swimming diagonally across tank). Swimming speed was adjusted to accommodate for the speed of water moving in the opposite direction of the swimming fish by adding 0.3 or 0.8. Variation of the flow speeds across the tank profile, in relation to the position of the fish, was unable to be accounted for because it was not possible to judge the fish's depth with the camera view and the fish did not always remain in a single section of the tank (sections presented in Figure 3.1d).

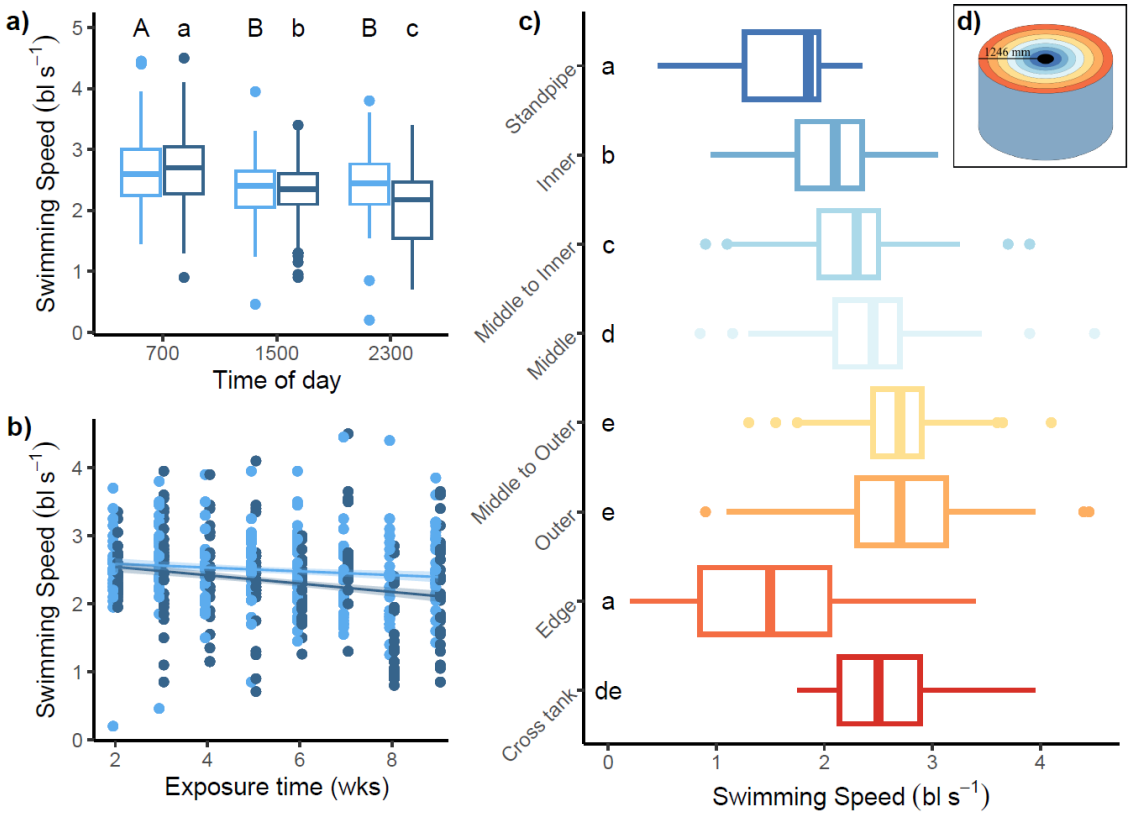


Figure 3.1 Swimming speeds of Chinook salmon under low (light blue) and moderate (dark blue) flow regimes by time of day a) and exposure time b). Lines and shading represent fitted linear model with 95% confidence interval. Significant differences in swimming speeds across time of day are indicated by uppercase for low flow regime and lowercase for moderate flow regime. Swimming speeds of Chinook salmon with respect to tank position c), and significant differences between tank locations are indicated by letters. Boxplots present the median (middle bar), first and third quartiles (upper and lower bars), and the largest and smallest value within 1.5 interquartile range (IQR; vertical bars). Points represent outliers determined as values beyond the vertical bars (i.e., > third quartile +1.5*IQR, < first quartile +1.5* IQR). Boxplot colours correspond to tank locations depicted in d) (not to scale).*

3.3.3 Fish feed and growth measurements

At eight and twelve weeks under flow regimes, all fish were fed pellets (composition previously described) incorporating X-ray opaque ballotini beads (~1 mm). Ballotini beads used were ceramic zirconium silicate type ZS (9305, 0.4-0.6 mm and 9309, 0.8-1.0 mm) SiLibeads, supplied by Sigmund Lindner GMbH. Beads were added to the feed during manufacturing at 1% of the total mass of the feed. Immediately after fish were fed with ballotini feed, all fish were anaesthetised (tricane methanesulfonate, Syndel, Canada; 65 ppm) and their mass, fork length, and girth were measured over a two-week sampling period (~520 fish per day). Later, condition factor (K) was calculated as:

$$\mathrm{K} = 100000 \times \frac{M}{FL^3}, \quad (1)$$

where M is the mass of fish (g) and FL is the fork length (mm). Specific growth rate (SGR; (% day^{-1}) was calculated as:

$$SGR = \frac{\ln(M_f) - \ln(M_i)}{days} \times 100, \quad (2)$$

where M_f is the final mass (g), M_i is the initial mass (g), and days is the number of days between measurements.

After fish were weighed and measured, they were laterally radiographed (completed within three hours after feeding; Difford et al., 2023) at 60kV and 0.1mAs^{-1} 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; image area = 430 × 420 mm, resolution = 3408 x 3320 pixels, pixel pitch = 125 μm) set at 50 cm distance. The X-ray was used to estimate daily feed intake (DFI_b; using ballotini method) by counting the number of beads present in the X-ray and using a standard curve to convert into grams of food ingested (McCarthy et al., 1992; McCarthy et al., 1993; Walker et al., 2012). Beads were counted using semi-automated "Bead Counter" software developed by AgResearch, NZ (Elvy et al., 2022b; Elvy et al., 2022a). The DFI was corrected for by multiplying the share of the meal (SOM) against the tank DFI. Share of the meal was calculated following McCarthy et al. (1992):

$$SOM = \frac{DFI_b}{tank\ DFI}\ . \quad (3)$$

Subsequently, specific feed rate (SFR; % g^{-1}) was calculated following McCarthy et al. (1992):

$$SFR = \frac{DFI}{M} \times 100\ , \quad (4)$$

Feed conversion ratio (FCR) was calculated as:

$$FCR = \frac{TFI}{MG} \ , \tag{5}$$

where *TFI* is the mean share of the meal ($\overline{SOM}$) multiplied by the total tank feed intake between the two assessments, and MG is the fish's mass gained between the two sampling assessments.

3.3.4 Sampling assessments

At eight and twelve weeks under treatment, five and six fish per tank, respectively, were euthanised with a lethal dose of AQUI-S (100 ppm). Whole viscera, heart, liver, and gonads were weighed and later analysed relative to body weight. For fish euthanised at twelve weeks belly-flap thickness was measured at three locations: in line with pectoral, pelvic, and anal fins. Blood was drawn from the caudal blood vessel and haematocrit (Hct) was measured by centrifuging blood in a HemoCue Microhematocrit tube at 9000 rpm for seven minutes. Approximately 300-400 µl of whole blood was assessed for hemoglobin (Hb; g L^{-1}), white blood cell count (10^9 L^{-1}), neutrophils (%), lymphocytes (%), and monocytes (%) by Gribbles Veterinary, Christchurch, NZ.

3.3.5 FT-NIR proximate composition

Euthanised fish at eight and twelve weeks under treatment, were used to estimate lipid, protein, ash, and moisture composition using Fourier transform – near infrared reflect (FT-NIR) spectroscopy the following day after feed and growth assessments. Following protocols from Miller et al. (2019), FT-NIR was used to estimate total lipid in whole-body, fillet, liver and viscera. Whole-body and fillet were also assessed for total protein, ash, and moisture. The viscera and liver were homogenised using an IKA T18 Ultra turrax for 30-60s, whereas the fillet was homogenised using a food processer for 30-60 s. Uniform samples for testing were placed in a 50 mm rotating cup and scanned in reflectance mode using a Bruker MPA FT-NIRs (Bruker, Ettlingen, Germany). The remaining carcass was broken down using a meat mincer, before being placed into the food processer, where it was further homogenised along with the fillet, viscera, and liver to obtain a whole-body reading. All samples were scanned (FT-NIR spectroscopy) using models developed and validated by Miller et al. (2019). The model was validated against proximate composition values assessed through a commercial testing laboratory (Food Testing Laboratory of Cawthron Analytical Services; Nelson, NZ). Whole-body, fillet, liver, and viscera samples were collected from six fish per treatment and sampling assessment (i.e., 12 fish) as well as diet samples and were assessed following the methods from Association of Official Analytical

Chemists (AOAC) for crude protein (AOAC 981.10), total lipid (AOAC 948.15), moisture at 105˚C (AOAC 950.46), and ash (AOAC 920.153). FT-NIR lipid and protein values were < 10% (R^2 = 0.99) and < 4% (R^2 = 0.78) different from values obtained from wet chemistry, respectively.

3.3.6 Spinal health assessments

Spinal health was assessed using X-rays obtained during individual feed intake assessments. Radiographs were assessed by following previous studies evaluating spinal health in farmed Chinook salmon (Munday et al., 2016; Perrott et al., 2018; Lovett et al., 2020; Araújo et al., 2022a), which were originally characterised in Witten et al. (2009). Scores (0-3) were assigned for severity level, where spinal curvature (lordosis-kyphosis-scoliosis; LKS) was graded on degree angle of curvature (severity 0, no deviation 0˚; severity 1, mild deviation 1-20˚; severity 2, moderate deviation 20-40˚; severity 3, severe deviation >40˚) and vertebral anomaly (VA; vertebral compression, -fusion, and -vertical shift) was graded on number of vertebrae affected (severity 0, no VA; severity 1, mild VA 1 vertebra involved; severity 2, moderate VA 2-5 vertebrae involved; severity 3, severe VA >5 vertebrae involved). The spinal region where LKS or vertebral anomaly presented was recorded as either region 1 (postcranial: vertebra V1:V8), region 2 (posterior truncal: V9:V31), region 3 (caudal: V32:50), or region 4 (ural: V51:V63±) as described in Munday et al. (2016) and Perrott et al. (2018). Where LKS or vertebral anomalies overlapped between regions, a combined region was assigned, for example occurring in region 1 and region 2, 1.5 was assigned.

3.3.7 Statistical Analysis

All statistical analysis was performed using the R statistical language and lme4, MuMin, nnet, and MASS packages. Data handling and figures were produced using tidyverse and ggplot2 packages. Model selection was assessed using Akaike's information criterion (AIC), following selection criteria from Richards (2005). Model parameters (normal distribution and equal variances) were assessed visually through Q-Q plot and residual versus fitted plot. Comparisons between exercise treatments were evaluated using general and generalised linear mixed effects model analysis, where interactions between predicator and fixed variables were investigated. Interactions were removed if not significant and the model was assessed for main effects. Tank and fish identification were included as random effects, where appropriate, and removed if not significant. For the model investigating the relationship between swimming speed with time of day and exposure time, tank location was included as a random effect, and the model investigating the relationship between swimming speed and tank location, exposure time was included as a random effect. A significance level of $P < 0.05$ was used for all statistical tests. Linear mixed effects models with significant interactions or main were compared using estimated marginal means (*P*-value adjusted using tukey method) when three or more variables were

specified (e.g., spinal health). For assessing where LKS and vertebral anomalies appear on the spinal region, visual inspection and descriptive statistics were used because models could not converge based on the low counts across each region. Multinomial models (nominal and ordinal) were used to assess the probability of developing spinal curvature and vertebral anomalies and their severity with increasing condition factor.

3.4 Results

3.4.1 Swimming speeds under low and moderate flow regimes

Measured swimming speeds were not significantly different between the LFR (2.44 ± 0.04 bl s^{-1}) and the MFR (2.33 ± 0.05 bl s^{-1}; LMER; $F_{1,4} = 0.41$, $P > 0.05$). Standard deviation and coefficient of variation did not differ between flow regimes (LM; $F_{1,94} = 2.21$, $P = 0.07$; LM; $F_{1,94} = 0.81$, $P > 0.05$, respectively). Swimming speeds had a significant interaction between flow regimes against time of day (LMER; $F_{2,2318} = 17.47$, $P < 0.001$; Figure 3.1a) and exposure time (LMER; $F_{2,2323} = 15.52$, $P < 0.001$; Figure 3.1b; Table A.1). Post-hoc comparisons showed that swimming speed was highest during the early morning and reduced towards midnight, and similarly, swimming speed was the highest towards the beginning of the trial and declined with weeks under treatment. Swimming speeds significantly differed between tank locations (LMER; $F_{7,2314} = 6.63$, $P < 0.001$), where swimming speeds progressively increased from the centre of the tank towards the outer, but swimming speeds measure on the edge of the tank did not differ from those near the standpipe (Figure 3.1c).

3.4.2 Individual feed and growth performance

Feed and growth performance from fish raised under LFR and MFR are presented in Table 3.1. Mass, fork length, girth, and condition factor did not statistically differ between LFR and MFR among any assessment period (Table A.2). Similarly, specific growth rate and specific feed rate did not differ between LFR and MFR at any given period (Table A.2), and variation around these parameters were not different between flow regimes. In contrast, FCR was significantly different between LFR and MFR, where fish raised under MFR had a lower (1.16 ± 0.01) FCR than fish raised under LFR (1.19 ± 0.01; LM; $F_{1,2948} = 5.65$, $P < 0.05$). Significant differences in linear regressions existed between specific growth rate and specific feed rate (LM; $F_{1,6124} = 31.1$, $P < 0.0001$; Figure 3.2a), where the relationship was different at eight weeks only. The relationship between FCR and mean specific feed rate (LM; $F_{1,2946} = 24.72$, $P < 0.0001$; Figure 3.2c) was significantly different between LFR and MFR. Linear regression between specific growth rate and condition factor was significantly correlated (LM; $F_{1,6125} = 67.04$, $P < 0.001$; Figure 3.2b), but the relationship did not differ between flow regimes (LM; $F_{1,6125} = 0.0025$, $P > 0.05$).

Table 3.1 Short-term effects of low and moderate flow regimes on growth and feed performance1 in Chinook salmon.

Parameters	Low tank velocity			Moderate tank velocity			*P*-value
	4 weeks	8 weeks	12 weeks	4 weeks	8 weeks	12 weeks	
Sample size	1586	1582	1494	1590	1575	1492	
M (g)	229.7 ± 1.1	397.1 ± 1.9	591.5 ± 3.1	226.5 ± 1.1	383.9 ± 1.9	575.0 ± 3.0	*P>0.05*
FL (mm)	234.5 ± 0.3	271.4 ± 0.4	305.6 ± 0.4	233.6 ± 0.3	269.4 ± 0.4	303.1 ± 0.4	*P>0.05*
G (mm)		199.9 ± 0.4	229.7 ± 0.5		197.7 ± 0.4	227.5 ± 0.5	*P>0.05*
K	1.76 ± 0.003	1.96 ± 0.005	2.05 ± 0.004	1.76 ± 0.003	1.94 ± 0.003	2.04 ± 0.004	*P>0.05*
	0-4 weeks	4-8 weeks	8-12 weeks	0-4 weeks	4-8 weeks	8-12 weeks	
SGR (% day^{-1})	2.42 ± 0.005	1.96 ± 0.005	1.42 ± 0.005	2.40 ± 0.005	1.88 ± 0.005	1.42 ± 0.005	*P>0.05*
SFR (% day^{-1})		2.15 ± 0.02	1.60 ± 0.02		2.18 ± 0.02	1.59 ± 0.02	*P>0.05*
FCR			1.19 ± 0.01[a]			1.16 ± 0.01[b]	***P=0.04***

Values are means ± S.E.M.

Letters indicate significant differences between experimental treatments.
[1] Performance: M = mass; FL = fork length; G = girth; K = condition factor; SGR = specific growth rate; SFR = specific feed rate; FCR = feed conversion ratio.

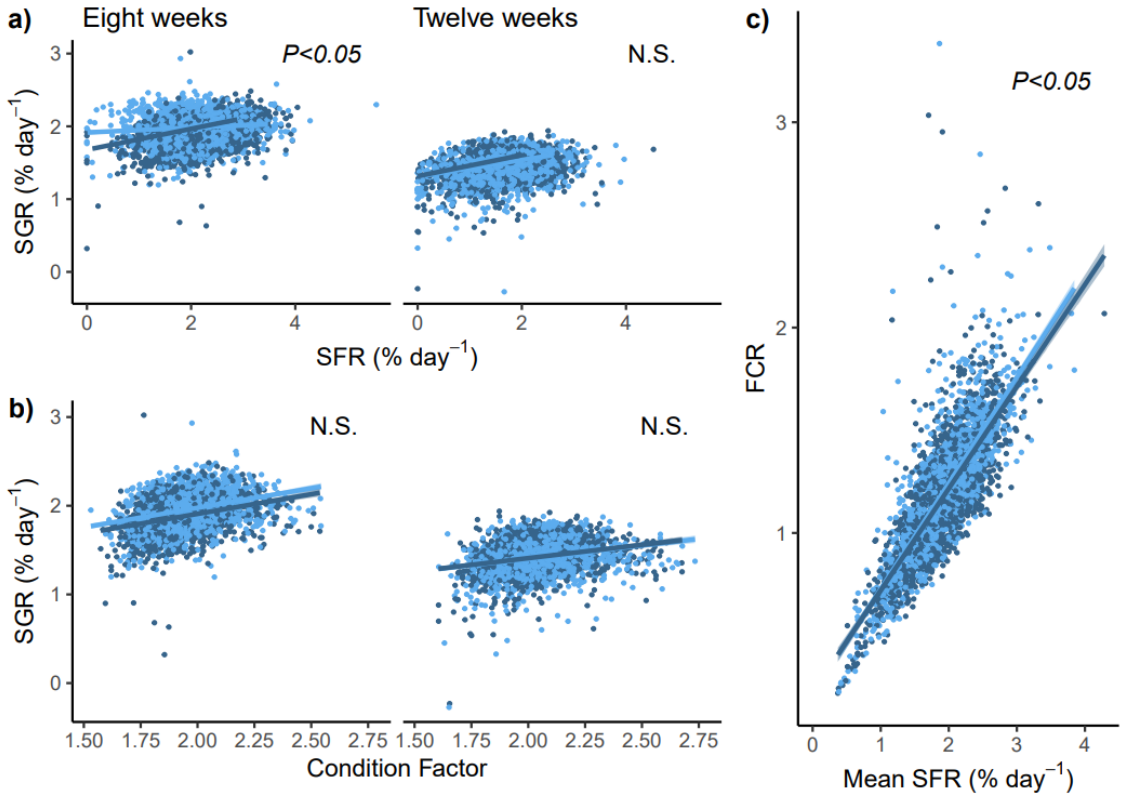


Figure 3.2 Linear relationships between a) specific growth rate (SGR; % day-1) and specific feed rate (SFR; % day-1), b) specific growth rate (SGR; % day-1) and condition factor at two and three months under treatment, and the relationship between feed conversion ratio (FCR) against mean specific feed rate (SFR; % day-1) between eight and twelve weeks under treatment c), in Chinook salmon raised under low (light blue) and moderate (dark blue) flow regimes. Points represent individual fish. Line and shading represent linear relationship with 95% confidence interval.

3.4.3 Sampling assessment

Hepatosomatic index (HSI) statistically differed between LFR and MFR after twelve weeks under treatment, where the mean HSI of fish raised under MFR ($\bar{x}$ =1.54 ± 0.01) were higher than in fish raised under LFR ($\bar{x}$ =1.41 ± 0.02; Table 3.2; Table A.3). Other tissue weights, relative to mass did not significantly differ between LFR and MFR (Table 3.2; Table A.3). Belly-flap thickness and blood properties did not significantly differ between LFR and MFR (Table 3.2; Table A.3).

3.4.4 FT-NIR proximate composition

Proximate composition within liver, viscera, fillet, and whole-body is presented in Table 3.3. Proximate composition did not significantly differ between LFR and MFR, except for liver lipid content (LMER; $F_{1,465}$ = 5.87, P < 0.05) and whole-body ash content (LM; $F_{1,465}$ = 18.71, P < 0.001; Table A.4). Whole-body ash content in fish raised under MFR ($\bar{x}$ =1.443 ± 0.005 and $\bar{x}$ =1.434 ± 0.002, respectively) was greater than in fish raised under LFR ($\bar{x}$ =1.431 ± 0.002 and $\bar{x}$ =1.420 ± 0.001, respectively) after two and three months under treatment.

3.4.5 Spinal health

The prevalence of spinal curvature and vertebral anomalies did not differ between LFR and MFR (LRT; G_1 = 2.17, P > 0.05), and number of cases at the end of the trial in each treatment were low (8.3% low, 9.1% moderate). Between two and three months under treatment, prevalence of spinal curvature and vertebral anomalies significantly increased (LRT; G_1 = 13.86, P < 0.001) by 2%, which predominantly included the development of LKS (54%), followed by 20% developing both vertical shift and vertebral compression, while 17% developed vertebral fusion only. The remaining 9% developed either vertical shift or vertebral compression.

Overall, when comparing the prevalence of spinal curvature and vertebral anomalies at three months under treatment, vertebral fusion was the most common in the cohort, followed by spinal curvature, where both cases mostly presented in the absence of other spinal anomalies (32.7% and 25.5%, respectively, excluding individuals with normal spinal health). Vertebral compression (7.2%) and shift (8.7%) were less common but, when present, frequently occurred in combination (8%). Severity levels of vertebral anomalies and spinal curvature were mainly mild, with only a small number of fish (< 4%) having spinal curvature and vertebral anomalies at moderate and severe levels. The occurrence of LKS and vertebral anomalies occurrence differed across spinal regions but were similar between flow regimes (Figure A.1). The occurrence of LKS was predominantly in region two (77%, 60% in LFR and 92% in MFR), and nearly all cases occurred anteriorly (97% between V1 and V31; 100% in LFR and 94% in MFR between V1 and V31; Figure A.1). Vertebral anomalies were common across all spinal regions

(V1 = 24%, V2 = 25%, V3 = 17%, V4 = 29%, and ~5% occurring across two adjacent regions; Figure A.1).

Individuals with spinal curvature had significantly higher mass ($\bar{x}$ = 684.4 ± 12.4), fork length ($\bar{x}$ = 309.7 ± 1.6), and condition factor ($\bar{x}$ = 2.29 ± 0.025) than individuals with normal spinal health (mass $\bar{x}$ = 583.1 ± 2.2, fork length $\bar{x}$ = 304.6 ± 0.3, condition factor $\bar{x}$ = 2.04 ± 0.003; Figure 3.3a,b,c,d; Table A.5). These differences, however, were not reflected in SFR, SGR or FCR, where individuals with spinal curvature were not significantly different from individuals with normal spinal health (Figure 3.3e,d; Table A.5). On the contrary, individuals with vertebral anomalies had significantly lower mass ($\bar{x}$ = 544.3 ± 10.0) and fork length ($\bar{x}$ = 300.8 ± 6.9) than individuals with normal spinal health (mass $\bar{x}$ = 583.1 ± 2.2, fork length $\bar{x}$ = 304.6 ± 0.3; Figure 3.3b,c; Table A.5). Individuals with vertebral anomalies also had slower SGR ($\bar{x}$ = 1.32 ± 0.024) and higher FCR ($\bar{x}$ = 1.25 ± 0.03) than individuals with normal spinal health (SGR $\bar{x}$ = 1.42 ± 0.003, FCR $\bar{x}$ = 1.17 ± 0.01; Figure 3.3e,f; Table A.5).

Using a multinomial model, these data show that the probability of an individual developing spinal curvature was more likely if they had higher condition factor, whereas for vertebral anomalies, the probability was relatively unaffected by increasing condition factor (Figure 3.4a). Severity of spinal curvature was also more likely to increase in individuals with higher condition factor (Figure 3.4b).

Table 3.2 Short-term effects of low and moderate flow regimes on tissue metrics in Chinook salmon.

Parameters	Low tank velocity		Moderate tank velocity		*P*-value
	8 weeks	12 weeks	8 weeks	12 weeks	
Viscerosomatic Index	9.46 ± 0.12	8.07 ± 0.06	9.14 ± 0.19	8.37 ± 0.06	*P>0.05*
Heart Index	1.60 ± 0.03	1.39 ± 0.01	1.68 ± 0.03	1.48 ± 0.02	*P>0.05*
Hepatosomatic Index	1.56 ± 0.01	1.45 ± 0.01[a]	1.52 ± 0.01	1.54 ± 0.01[b]	***P<0.01***
Gonadosomatic Index	0.09 ± 0.003	0.08 ± 0.001	0.09 ± 0.004	0.08 ± 0.001	*P>0.05*
Belly-flap thickness:					
Pectoral (mm)		7.26 ± 0.07		7.46 ± 0.07	*P>0.05*
Pelvic (mm)		8.12 ± 0.05		8.39 ± 0.06	*P>0.05*
Anal (mm)		3.43 ± 0.06		3.39 ± 0.06	*P>0.05*
Hct (%)		40.24 ± 0.17		40.90 ± 0.19	*P>0.05*
Hb (g L^{-1})		98.56 ± 0.42		99.50 ± 0.05	*P>0.05*
White Blood Cell Count (10^9 L^{-1})		30.57 ± 0.42		32.83 ± 0.41	*P>0.05*
Neutrophils (%)		5.17 ± 0.20		3.61 ± 0.11	*P>0.05*
Lymphocytes (%)		94.31 ± 0.21		95.72 ± 0.13	*P>0.05*
Monocytes (%)		0.53 ± 0.03		0.67 ± 0.03	*P>0.05*

Values are means ± S.E.M.

Letters indicate significant differences between experimental treatments at given timepoint.

Table 3.3 Short-term effects of low and moderate flow regimes on FT-NIR proximate composition in Chinook salmon.

Response Variable		Low tank velocity		Moderate tank velocity		*P-value*
		8 weeks	12 weeks	8 weeks	12 weeks	
Lipid (g 100 g^{-1})	Liver	4.71 ± 0.14[a]	5.40 ± 0.10[a]	4.07 ± 0.07[b]	4.85 ± 0.10[b]	***P < 0.05***
	Viscera	27.33 ± 0.83	28.15 ± 0.41	27.40 ± 0.81	30.44 ± 0.42	*P > 0.05*
	Fillet	16.21 ± 0.14	18.15 ± 0.12	15.98 ± 0.19	17.83 ± 0.14	*P > 0.05*
	Whole-body	14.93 ± 0.16	18.06 ± 0.11	14.47 ± 0.19	17.91 ± 0.11	*P > 0.05*
Protein (g 100 g^{-1})	Fillet	18.18 ± 0.04	18.0 ± 0.03	18.29 ± 0.05	18.07 ± 0.03	*P > 0.05*
	Whole-body	16.36 ± 0.03	16.01 ± 0.02	16.44 ± 0.03	16.0 ± 0.01	*P > 0.05*
Ash (g 100 g^{-1})	Fillet	1.129 ± 0.003	1.129 ± 0.002	1.130 ± 0.003	1.125 ± 0.001	*P > 0.05*
	Whole-body	1.431 ± 0.002[a]	1.420 ± 0.001[a]	1.443 ± 0.005[b]	1.434 ± 0.002[b]	***P < 0.001***
Moisture (g 100 g^{-1})	Fillet region	63.24 ± 0.12	61.85 ± 0.10	63.20 ± 0.18	61.81 ± 0.12	*P > 0.05*
	Whole-body	63.10 ± 0.17	60.97 ± 0.12	63.35 ± 0.19	61.14 ± 0.13	*P > 0.05*

Values are means ± S.E.M.

Letters indicate significant differences between experimental treatments at given timepoint.

3.5 Discussion

The present study measured the absolute swimming speed of Chinook salmon under LFR and MFR in attempt to provide an accurate indication of the optimal exercise regime needed to enhance production performance in farmed Chinook salmon. Mismatches between swimming speed and flow regimes occurred, where chinook salmon under LFR and MFR were swimming faster than the set flow regimes and at similar speeds. A similar level of exercise being performed under LFR and MFR was most probably the reason why feed intake and growth performance did not differ between flow regimes. However, significant differences in feed consumption and growth curves between LFR and MFR were shown, leading to improved feed efficiency in individuals under MFR. Spinal health did not improve under exercise, disproving the hypothesis that exercise, when comparing LFR and MFR, can reduce the incidence or severity of spinal curvature or vertebral anomalies. In contrast, it was found that individuals presenting with spinal curvature exhibited enhanced feed and growth performance in comparison to individuals with normal spinal health and that the probability of individuals developing spinal curvature increases with higher condition factor. These results provide critical information for industry to consider in their selective breeding objectives to curb the prevalence and severity of spinal curvature incidences.

3.5.1 Mismatch between swimming speed and flow regimes

LFR and MFR did not reflect the exercise regime experienced by post-smolt Chinook salmon (82.9 ± 0.3 to 583.2 ± 2.1 g). Chinook salmon in both flow regimes advanced around their tank, choosing their own swimming speeds, which were similar under both flow regimes and did not form regimented schools in which individuals held position into the current. Mismatches between measured swimming speeds and respective flow regimes may provide some explanation of the inconsistencies among the literature on Atlantic salmon and rainbow trout when defining optimal flow regimes (Davison and Herbert, 2013; Solstorm et al., 2015; McKenzie et al., 2020) and why studies are yet to document Chinook salmon benefitting from exercise (Thorarensen et al., 1993; Kiessling et al., 1994; Kiessling et al., 2005). The latter could be linked to Chinook salmon demonstrating species-specific responses to flow regimes (also suggested in reviews by Davison, 1997; Davison and Herbert, 2013), and choosing to swim faster than the previously estimated U_{opt} for 387 g Chinook salmon, i.e., 1.5 bl s^{-1} (Gallaugher et al., 2001; Davison and Herbert, 2013). However, in wild Chinook salmon smolts of similar size (~30 cm), the most common swimming speeds were estimated to be between 2.0 – 2.7 bl s^{-1} (Holleman et al., 2022). This emphasises the importance of using pilot studies to determine appropriate set ups and validating the technology needed to record and measure swimming speeds accurately.

By having measured the absolute swimming speeds of Chinook salmon in this study, future studies can use these data to choose more appropriate flow regimes for exercise studies.

Measuring the absolute swimming speed in exercise studies would be highly advantageous, as many exercise studies suggest that exercise-enhanced traits do not occur at low speeds (i.e., < 1 bl s^{-1}) because of the increased spontaneous activity and/or agonistic behaviours (Jørgensen and Jobling, 1993; Solstorm et al., 2015; Solstorm et al., 2016b; Waldrop et al., 2018b). By measuring the absolute swimming speed in exercise studies, exploration of correlations between variation in swimming speeds under different flow regimes that do- and do not promote exercise-enhanced traits can occur. In the current experiment, variation (standard deviation and coefficient of variation) in swimming speed under low and moderate flow regimes were not statistically different between flow regimes, although the standard deviation in swimming speed under LFR was larger (*P*-value = 0.07) than swimming speeds under MFR. This provides some indication that individuals under LFR may behave more erratically than individuals under MFR and could be linked to differences found in some production performance metrics (discussed in the section below). Additional methods are required to measure absolute swimming speed to better predict optimal flow regimes, since the set flow regime clearly does not provide an accurate representation of true swimming speed. This fact has largely been overlooked in most exercise studies on fish.

3.5.2 Swimming behaviour and production performance responses to low and moderate flow regimes

Under LFR and MFR, swimming speeds of Chinook salmon changed across the time of day and the length of the trial. Chinook salmon were recorded to swim the fastest during the early morning and reduced swimming speeds after feeding and towards midnight. This diurnal variation in swimming speed is likely to be associated with anticipatory behaviour in response to early morning feeding. Anticipatory behaviour prior to feeding is common among fishes (Martins et al., 2012; Palstra et al., 2021; Jimenez Rivera et al., 2022). Similarly, the Chinook salmon's swimming speeds decreased as the trial progressed, also shown in Herbert et al. (2011), that measured swimming speeds of Atlantic salmon in response to light stimulus across a 28 day period. The underlying reason for the decline in swimming speed across the length of the trial is unknown. The most probable reason could be because the fish increased their absolute swimming speed as they grew, but not at equal rates, where fish size increased faster than the absolute swimming speed. This explanation could be linked to the negative relationship between increasing fish size and U_{opt}, i.e., in bl s^{-1} (Videler, 1993a; Palstra et al., 2015b).

Swimming speeds of Chinook salmon were slower when positioned near the centre of the tank compared to the outer regions, likely associated with a longer distance in the outer

regions and tighter turning circle in the closer regions. The decline in swimming speed towards the centre of the tank matches the variation in flow across the tank (i.e., slower near the centre, faster near the outer regions). There is possibility that fish are seeking slower/faster flows within the tank, which could be important for welfare aspects, as it might be beneficial to swim at different speeds depending on the fish's physiological state (e.g., reduced aerobic scope due to digestion, diurnal fluctuations). Interestingly, swimming speeds measured closest to the tank edge showed the slowest swimming speed, which may indicate some fish to be showing signs of thigmotaxis behaviour (Schnörr et al., 2012). Thus, gaining accurate measurements of swimming speeds with multiple aspects (e.g., time, duration, tank location) is a valuable method to understand the influence of exercise, and provides additional information in swimming behaviour and preferences that can improve fish husbandry, identify suitable offshore environments, and define welfare standards (Martins et al., 2012; Wiese et al., 2023).

Rearing Chinook salmon under LFR and MFR does not elicit different exercise levels and are not sufficient to promote exercise-enhanced growth at an initial body size of 82.9 ± 0.3 g growing to 583.2 ± 2.1 g in post-smolt Chinook salmon. Comparisons of specific feed rate and specific growth rate between flow regimes were not found to differ; however, when using individual data to construct feed consumption-growth relationships, significant differences between the flow regimes were revealed. Chinook salmon exposed to MFR showed an improved consumption-growth relationship, leading to improved FCR (0.03 increase; 2.5%) and FCR as a function of mean specific feed rate. It is unknown whether this result is linked to the flow regimes, but similar results are documented in other salmonids under similar flow regimes (Jørgensen and Jobling, 1993; Nielsen et al., 2000; Ytrestoyl et al., 2020) and the level of spontaneous activity being performed under each flow regime may have differed (i.e., standard deviation in swimming speed under LFR was larger than under MFR). These results warrant further investigation as small differences in FCR translate to large financial gains for the industry.

In individuals raised under MFR, lipid content in the liver was significantly less than in individuals raised under LFR, leading to significantly larger HSI values (also found in Kiessling et al., 1994). The liver is a vital organ involved in metabolic homeostasis and can rapidly respond to intrinsic and extrinsic factors (Champe et al., 2005; Alberts et al., 2015; Esmaeili et al., 2021, 2022). The plasticity shown in relative liver weight and liver lipid content in individuals under MFR (especially at the later timepoint) may indicate that MFR becomes challenging in larger salmon. Metabolic processes, at this timepoint, may respond by predominantly depending on lipid and carbohydrate metabolism and suppressing protein metabolism (Alsop and Wood, 1997; Felip et al., 2012; Magnoni et al., 2013), as suggested in Chinook salmon that were exercised for ten months (Prescott et al., 2023). Changes in nutrient usage and deposition in salmonids under exercise warrants further investigation to understand the physiological demands on the animal

but also for commercial application (McKenzie et al., 2020). Existing feeds may not provide optimal nutrition (protein to energy ratios) to fuel increased aerobic metabolism under exercise (leading to increased feed intake) and that energy and nutrient requirements in lower and higher energy farming environments may be different, requiring optimised nutrient profiles for these production locations.

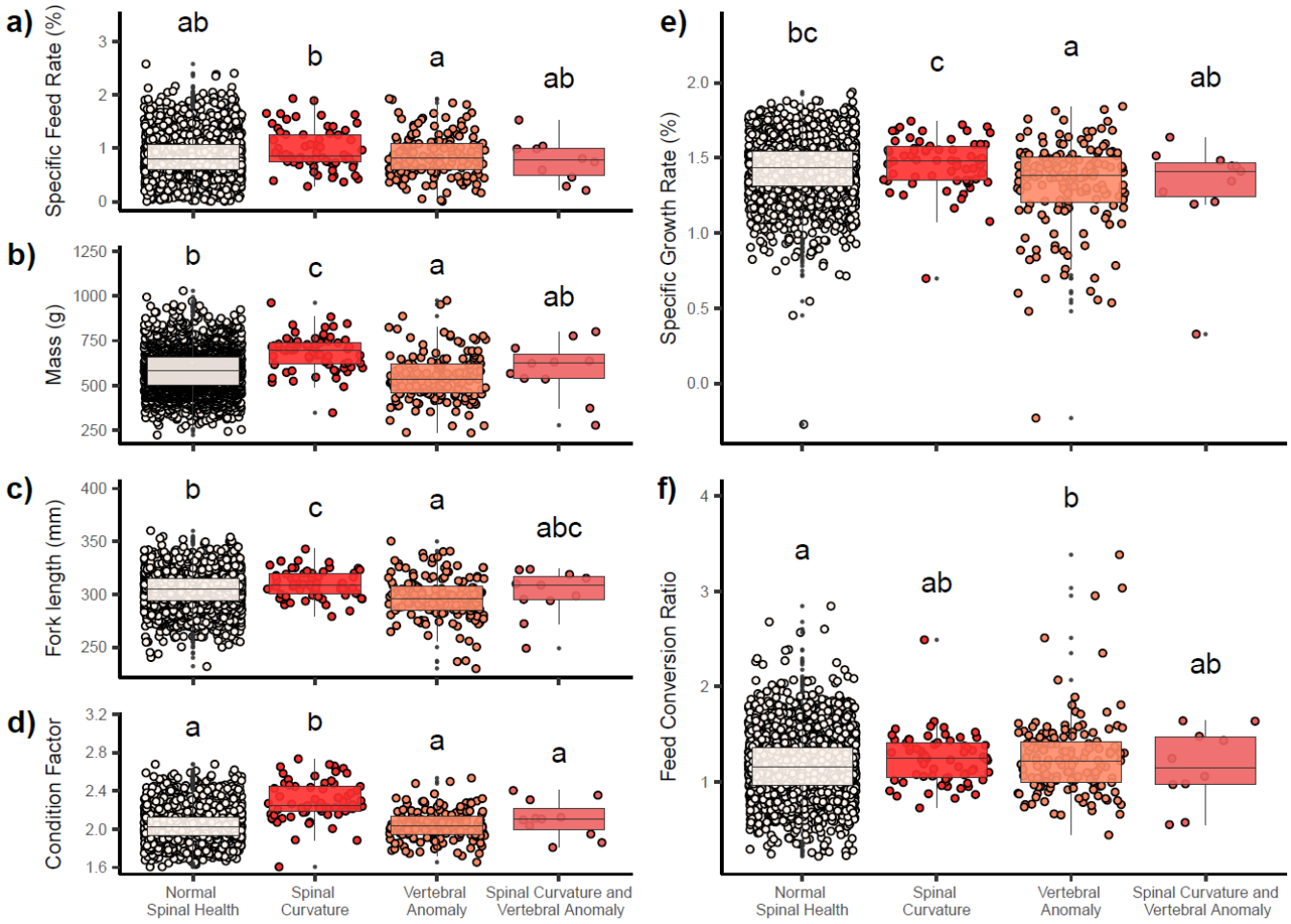


Figure 3.3 Influence of spinal health on feed and growth performance in Chinook salmon raised under low and moderate flow regimes. Specific feed rate (%) a), mass (g) b), fork length c), condition factor d), specific growth rate (%) e), and feed conversion ratio f) against spinal curvature and vertebral anomalies. Letters indicate significant differences in performance trait against spinal curvature and vertebral anomalies.

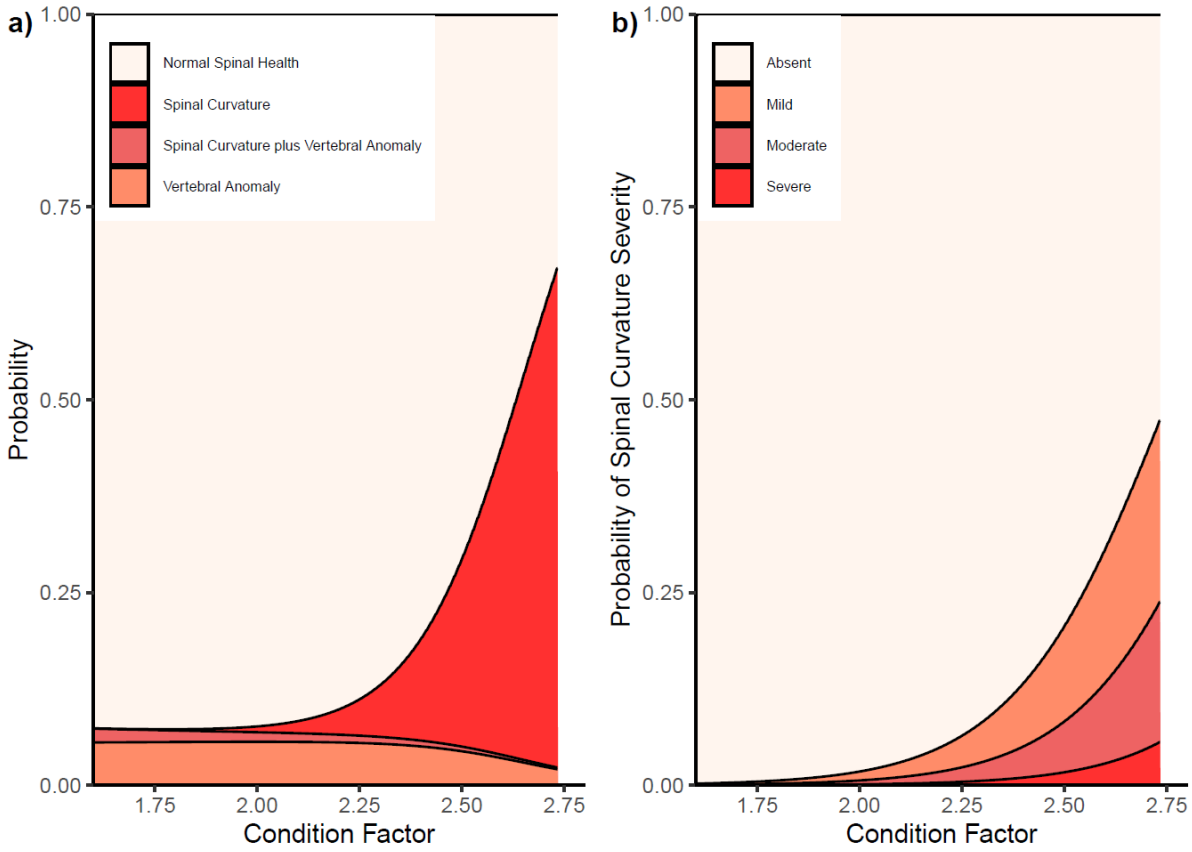


Figure 3.4 Multinomial models showing probability of developing spinal curvature and vertebral anomalies a) and spinal curvature severity against increasing condition factor b).

3.5.3 Spinal health under low and moderate flow regimes and the influence on feed and growth performance

We hypothesised that exercise regimes could be used as a tool to improve spinal health in Chinook salmon. In the current experiment, flow regimes did not positively influence spinal health, and that, contrary to the initial hypothesis, flow regimes and continuous swimming in the same direction increased spinal curvature and influenced location, severity, and curvature direction. The percentage of fish with spinal curvature (~2.2%) in the current experiment was higher than those presenting with spinal curvature in Perrott et al. (2018; ~0.5%), who evaluated spinal health from commercial sea pens in NZ. In previous studies on European seabass (*Dicentrarchus labrax* L.), gilthead seabream (*Sparus aurata*), and red seabream (*Pagrus major*), higher water currents increases the prevalence of vertebral lordosis (Divanach et al., 1997; Kihara et al., 2002; Palstra et al., 2020). The immediate factor(s) causing spinal curvature and vertebral anomalies is yet to be fully understood, and the role of exercise in abnormality prevalence may play a synergistic role, where rates of myogenesis exceed bone formation and mineralisation (Palstra et al., 2020). This experiment evaluated spinal health in post-smolt Chinook salmon up to ~1 kg ($\overline{x}$ = 583.24 ± 2.14 g), yet spinal curvature is a late onset trait in sea pens and Chinook salmon are harvested mainly in the 4 - 5 kg range (Lovett et al., 2020; NZKS, 2022), thus determining long-term trends in spinal health under LFR and MFR, especially starting at smaller sizes (e.g., fry and parr) would provide greater detail.

In Chinook salmon there is an unfavourable relationship between higher condition factor and spinal curvature (Perrott et al., 2018; Scholtens et al., 2023), which presents several health and welfare concerns. In this study, unfavourable relationships were also found between spinal curvature and vertebral anomalies with feed and growth performance. Individuals with spinal curvature had increased mass, fork length, and condition factor, while SFR, SGR and FCR were comparable to individuals with normal spinal health. This suggests that enhanced growth performance increases susceptibility to developing spinal curvature, and that spinal curvature could be linked to myogenic drivers and genetically selecting for enhanced growth. It is suspected that feed efficiency in individuals with spinal curvature will deteriorate with time and severity due to the higher locomotory costs associated with spinal curvature (Lijalad and Powell, 2009; Powell et al., 2009; Perrott et al., 2018; Prescott et al., 2023). Vertebral anomalies had the opposite response, where individuals with vertebral anomalies presented lower mass (also found in Davie et al., 2019) and fork length, which led to poorer SGR and FCR than in individuals with normal spinal health. It is important to mention that the number of individuals with normal and reduced spinal health were not equally balanced (most fish exhibited normal spinal health);

however, linear mixed effects models were used to account for these differences (Lenth et al., 2023). Therefore, the models present interesting perspectives on the relationships between spinal health and production performance and emphasises the need for farmers to consider the impacts of selecting for enhanced growth without considering other traits such as spinal health.

3.5.5 Conclusion

Improving performance traits by manipulating flow regimes is a highly desired outcome, as it provides a non-invasive avenue that can deliver relatively immediate (in comparison to selective breeding) results and potentially improve environmental resilience. This study shows that low to moderate flow regimes do not elicit different exercise regimes in 82.9 ± 0.3 to 583.2 ± 2.1 g post-smolt Chinook salmon, and therefore minimal improvements in production performance were documented. Moderate flow regimes improved feed efficiency but did not influence other feed and growth metrics or spinal health, including spinal anomaly incidences. This study did reveal unfavourable relationships between susceptibility to spinal curvature and enhanced growth performance, suggesting a potential consequence of selecting for enhanced growth. Improving skeletal health broadly remains a key goal for some farms in NZ, and more attention needs to be paid to assessing ways to improve spinal health specifically and the relationship with production performance especially as the industry moves towards integrating flow regimes in hatchery production, and rearing fish in high-energy, high-flow offshore environments.

Chapter Four Genetic parameters and genotype-by-environment interaction estimates for growth and feed efficiency related traits in Chinook salmon, *Oncorhynchus tshawytscha*, reared under low and moderate flow regimes

All the research contained within this chapter has been accepted as Prescott, L.A., Scholtens, M.R., Walker, S.P., Clarke, S. M., Dodds, K.G., Miller, M.R., Semmens, J.M., Carter, C.G., Symonds, J.E., 2024. Genetic parameters and genotype-by-environment interaction estimates for growth and feed efficiency related traits in Chinook salmon, *Oncorhynchus tshawytscha*, reared under low and moderate flow regimes. Genetics Selection Evolution.

4.1 Abstract

A genotype-by-environment (G×E) interaction is defined as genotypes responding differently to different environments. In salmonids, G×E interactions can occur in different rearing conditions, including environmental variations such as salinity or temperature. However, water flow, an important variable that can influence metabolism (i.e., higher swimming speeds), has yet to be considered for a potential G×E interaction, even though water flows differ across production stages. The salmonid industry is now manipulating flow in tanks to improve welfare and production performance, rearing juveniles for longer on land, and expanding sea pen farming offshore, where flow dynamics are substantially greater. Therefore, there is a need to test whether G×E interactions occur under low and higher flow regimes to determine if it is necessary for industry to modify their performance evaluation and selection criteria to account for different flow environments. Here a genotype-by-sequencing was used to create a genomic-relatedness matrix of 37 Chinook salmon, *Oncorhynchus tshawytscha*, families to assess possible G×E interactions for production performance under two flow environments: a low flow regime (0.3 body lengths per second; bl s^{-1}) and a moderate flow regime (0.8 bl s^{-1}). Genetic correlations between the same production performance traits suggest there is minimal evidence of a G×E interaction for production performance between the low and moderate flow regimes tested in this study, for Chinook salmon reared from 82.9 ± 16.8 g ($\overline{x}$ ± s.d.) to 583.2 ± 117.1 g ($\overline{x}$ ± s.d.). Genetic and phenotypic correlations between traits, irrespective of the flow regime, did not reveal any unfavorable trait correlations for size- (weight and condition factor) and growth-related traits, but did suggest measuring feed intake would be the preferred approach for improving feed efficiency because of stronger correlations, consistent with previous studies. This new information suggests that Chinook salmon families do not need to be selected separately for performance across different flow regimes; however, further studies are needed to confirm this across a wider fish size- and flow range. This information is key for salmon breeding programs

to determine if separate evaluation groups are required depending on the flow regimes being used for production (e.g., hatchery, post smolt recirculating aquaculture system, or offshore).

4.2 Introduction

Selective breeding programs have revolutionised production efficiency in animal farming by selecting broodstock that exhibits desirable traits (Zuidhof et al., 2014). Selective breeding to genetically improve production performance in salmon farming began in the 1970s (Gjedrem, 1985) and since then, selecting for fast growth has achieved significant genetic gains for the industry (Kause et al., 2006; Gjedrem et al., 2012; Walker et al., 2012; de Verdal et al., 2018b; Symonds et al., 2019). Ideally, a successful breeding program generates populations that can perform equally across multiple production systems. There are several factors that can limit the success of breeding programs, one of which is environmental variation influencing genotype differences in performance. This is termed a genotype-by-environment (G×E) interaction (Falconer, 1952). If G×E interactions exist, genetic breeding programs can be adjusted by widening the selection criteria to include different environments to achieve a balanced genotype performance across multiple environments (Cooper et al., 2023).

In salmon farming, determining if G×E interactions exist is important because of the large environmental range that occurs across a production cycle. As salmonids are anadromous species (migrating from seawater to freshwater spawning grounds), commercial production begins in freshwater and ends (typically) in seawater; two environments that require opposing osmo-regulatory mechanisms (Groot, 1991; Kirschner, 1995; Evans, 1998). Production stages also vary from controlled hatchery facilities (e.g., recirculating aquaculture systems; RAS, flow-through raceways) to uncontrolled sea pens. Salinity, temperature, dissolved oxygen, and water movement are some of the abiotic factors that can vary across the entire salmonid production cycle and alter fish metabolism and activity (Kirschner, 1995; Angilletta Jr et al., 2002; Johansson et al., 2007). Metabolism and activity can dictate growth and feed efficiency, which are key traits in selective breeding criteria, and therefore could be potential mechanisms for G×E interactions to occur.

Several G×E interactions have been identified for salmonid species and other finfish species. These include performance interactions between freshwater and seawater (Winkelman and Peterson, 1994; Chiasson et al., 2014; Correa et al., 2018; Gonzalez et al., 2022), low and elevated temperatures (Heath et al., 1993), as well as rearing environments (e.g., pen vs. pond, tanks vs. streams, breeding nucleus vs. test stations or commercial farms) (Kause et al., 2003; Sundström et al., 2007; Mas-Muñoz et al., 2013; Sae-Lim et al., 2013; Sundström et al., 2016). See Sae-Lim et al. (2016) for a review of G×E interactions in aquaculture. An environmental

factor that has yet to be considered in isolation or under controlled environmental conditions but could be linked to G×E interactions detected across different rearing environments, is water flow.

Flow regimes are likely to vary across the salmonid production cycle. In juvenile salmon production, pre- and post-smolts are being reared for longer in controlled tank-based RAS (Timmerhaus et al., 2021; Prescott et al., 2023) with optimal flow regimes to moderately exercise and improve production and animal welfare (Davison, 1997; McKenzie et al., 2020). In later production stages, salmonids are farmed to harvest-size in nearshore protected sites, but the industry has plans to expand into offshore high energy environments (Buck and Langan, 2017; NZKS, 2020). This transition means salmon will be reared in strong environmental currents requiring increased and sustained swimming speeds (Buck and Langan, 2017; Campos et al., 2019; Newcombe et al., 2019; NZKS, 2020). Investigating if G×E interactions exist between different levels of flow is critical for the salmonid industry as they need to determine whether flow regimes are to be considered within their breeding program to improve performance across existing and future production environments.

The aims of this study were to 1) determine the phenotypic responses and genetic parameters for key performance traits when commercial Chinook salmon families are reared under two flow regimes, 2) determine if the different flow regimes result in a significant G×E interaction, and 3) assess the genetic and phenotypic correlations among traits. This information is important when determining how families are evaluated, if performance at different flows need to be considered in the industry's breeding programs, and to better improve genetic selection.

Two flow environments were chosen to reflect regimes that can be adopted in future RAS by the New Zealand (NZ) Chinook salmon (*Oncorhynchus tshawytscha*) industry and were based on available information, such as publications identifying flow regimes that enhance production traits in salmonids (Davison and Herbert, 2013), previous flow regimes used with Chinook salmon as a subject species (Thorarensen et al., 1993; Kiessling et al., 1994; Gallaugher et al., 2001; Kiessling et al., 2005), and comparisons of swimming performance between Chinook salmon and Atlantic salmon *Salmo salar* (Gallaugher et al., 2001; Remen et al., 2016; Hvas et al., 2017; Hvas and Oppedal, 2019; Prescott et al., 2023).

4.3 Methods and Materials

4.3.1 Genotyping-by-sequencing

All-female pedigree Chinook salmon smolts (2020-year class) from 37 selectively bred families were sourced from Sanford's Kaitangata commercial salmon hatchery, where the fish (age at tagging = 162 days old – 183 days old) were tagged with passive integrated transponder

tags (HIDGlobal, EM4305, 12 mm long and 2 mm diameter glass tags), fin-clipped for genotyping, and transferred to the Finfish Research Centre at Cawthron Aquaculture Park, Glenduan Nelson, New Zealand on 7th December 2020. Full and half-sib families were generated from 21 sex-reversed XX sires and 32 dams from 6th of May 2020 to 25th of May 2020, where sires and dams were crossed up to four and two times, respectively. The families were pooled at the eyed egg stage. A total of 3600 individually PIT tagged fish ($\bar{x}$ weight = 11.86 ± 0.04 g) were genotyped using restriction enzyme based Genotyping-by-Sequencing (GBS; PstI/MspI double digest) following the methods outlined in Dodds et al. (2015), with the modifications described in Scholtens et al. (2022). TagDigger (Clark and Sacks, 2016) was used to count the reference and alternate alleles for each variant of a previously developed catalogue of 42,839 single nucleotide polymorphisms (SNPs) (Scholtens et al., 2023). Single nucleotide polymorphisms that were monomorphic (969 SNPs) or with no reads (11 SNPs) were removed. Fish with mean read depth < 0.3 (6 fish) were removed. Further quality control removed SNPs if minor allele frequency < 0.01, Hardy-Weinberg (HW) disequilibrium < -0.05 or with a depth-adjusted HW test (Dodds et al., 2018) *P*-value $<10^{-100}$. After filtering, 34,557 SNPs remained with a callrate of 0.46 and mean read depth of 1.31. From the 3,594 genotyped fish on the farm, 3,438 were successfully assigned to only one family of the 37 possible families, 3,191 were transferred to the finfish research facility, and 3,174 fish ($\bar{x}$ = 86 offsprings per family, min = 44 offspring per family, and max = 113 offspring per family) were used in the study.

4.3.2 Fish husbandry and experimental conditions

The fish were transferred into 8,000 L tanks containing water with a salinity of 14-15 ppt at 13 ± 0.2 °C on arrival. Fish were acclimatised to full seawater (35 ppt) and a rearing temperature of 17 °C (maintained within 0.2 °C) over seventeen days. Fish were then continuously supplied with filtered recirculating seawater (35 ppt, 17 °C and maintained within 0.2 °C, 24 h light photoperiod). From the 29th to 31st December 2020, fish were sorted into twelve treatment tanks (8,000 L) with approximately 260 fish per tank, ensuring families were evenly represented across all treatment tanks, and tank velocities were set to 4.93 ± 0.08 cm s^{-1} for ~3 weeks. All fish were weighed (WT) and measured for fork length (FL) prior to the tank flow changes (length = 174.6 ± 1.7 mm; weight = 82.90 ± 0.30 g; mean ± SE). Tank flow regimes were then increased by 1.5 cm s^{-1} day^{-1} across three to seven days until the target speed was achieved. Tank flow regimes were maintained by directing the incoming water in a clockwise direction at either low flow regime (LFR; 0.3 bl s^{-1}) or moderate flow regime (MFR; 0.8 bl s^{-1}; six tanks per treatment). Exchange rates were maintained at 224 ± 0.07 L min^{-1} (mean ± S.E.M.). Tank flow regimes were measured daily and adjusted monthly to account for fish growth and to maintain the 0.3 bl s^{-1} and 0.8 bl s^{-1} flow regimes. Tank flow regimes were based on growth data

obtained in previous experiments (Elvy et al., 2022b) and readjusted to match FL data during routine growth assessments.

Fish were hand fed (crude protein 37.5 g $100g^{-1}$, total fat 24.2 g $100g^{-1}$, energy 1705 kJ $100g^{-1}$) with a commercial feed to satiation daily and pellet size was increased with fish growth, as per manufacturer's recommendation. Fish were first fed five times per day until one week prior to flow regimes being set. The frequency of feeding per day was reduced slowly, where the fish were fed three times per day for the following two weeks, then reduced to two feeds per day for the following four weeks, and then one feed per day for the remainder of the trial. Fish were fed once per day when feed intake rates were measured using the ballotini X-ray method described below. Tank daily feed intake (tank DFI) was measured by subtracting final feed bucket weight including uneaten pellets (retrieved by swirl separator) from the initial feed bucket weight. Uneaten pellets were counted using an automated counter (Contardor2, PFEUFFER GMBH, Kitzingen, Germany) and multiplied by the average pellet weight.

4.3.3 Trait assessments

At 4-week intervals (4 wks = 273 – 301 days old, 8 wks = 301 – 329 days old, and 12 wks = 329 – 357 days old), all fish were anaesthetised using tricane methanesulfonate (65 ppm; Syndel, Canada), and over two consecutive weeks (two tanks per day) their WT and FL were measured. Condition factor (K) and daily weight gain (DWG; two time periods: 4 - 8 and 8 - 12) were later calculated as previously described in Prescott et al. (2024). Condition factor (K) was calculated as:

$$\mathrm{K} = 100000 \times \frac{WT}{FL^3}, \quad (1)$$

where WT is the weight of the fish (g) and FL is the fork length (mm). Daily weight gain (DWG; g day^{-1}) was calculated as:

$$\mathrm{DWG} = \frac{WT_f - WT_i}{days}, \quad (2)$$

where WT_f is the final weight (g), WT_i is the initial weight (g), and days is the number of days between measurements.

At eight and twelve weeks, all fish prior to the assessment were fed pellets (of equal composition to the feed fed daily, composition described above) containing X-ray opaque ballotini beads (~1 mm; fish received ballotini feed for: mean = 20 min 38, s.d. = 3 min 29 s) and immediately after were anaesthetised (tricane methanesulfonate, Syndel, Canada; 65 ppm) for size measurements (as described above) and laterally radiographed (Difford et al., 2023) at 60kV and 0.1 mAs^{-1} 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; image area = 430 × 420 mm, resolution = 3408 x 3320 pixels, pixel pitch = 125 μm) set at 50 cm distance. Daily feed intake (DFI) was estimated by counting the number of beads present in the X-ray (semi-automated using "Bead Counter" software, AgResearch, NZ) and using a standard curve to convert the bead count into grams of food ingested (McCarthy et al., 1992; McCarthy et al., 1993; Walker et al., 2012). Subsequently, feed conversion ratio (FCR) was calculated (Walker et al., 2012; Elvy et al., 2022b). Feed conversion ratio (FCR) was calculated as:

$$FCR = \frac{TFI}{MG} \ , \tag{3}$$

where *TFI* is the mean share of the meal ($\overline{SOM}$) multiplied by the total tank feed intake between the two assessments, and MG is the fish's mass gained between the two sampling assessments.

Share of the meal was calculated following McCarthy et al (McCarthy et al., 1992):

$$SOM = \frac{DFI}{tank\ DFI} \ . \tag{4}$$

Figure 4.1 presents a schematic illustration showing the sequence of sampling timepoints throughout the experiment.

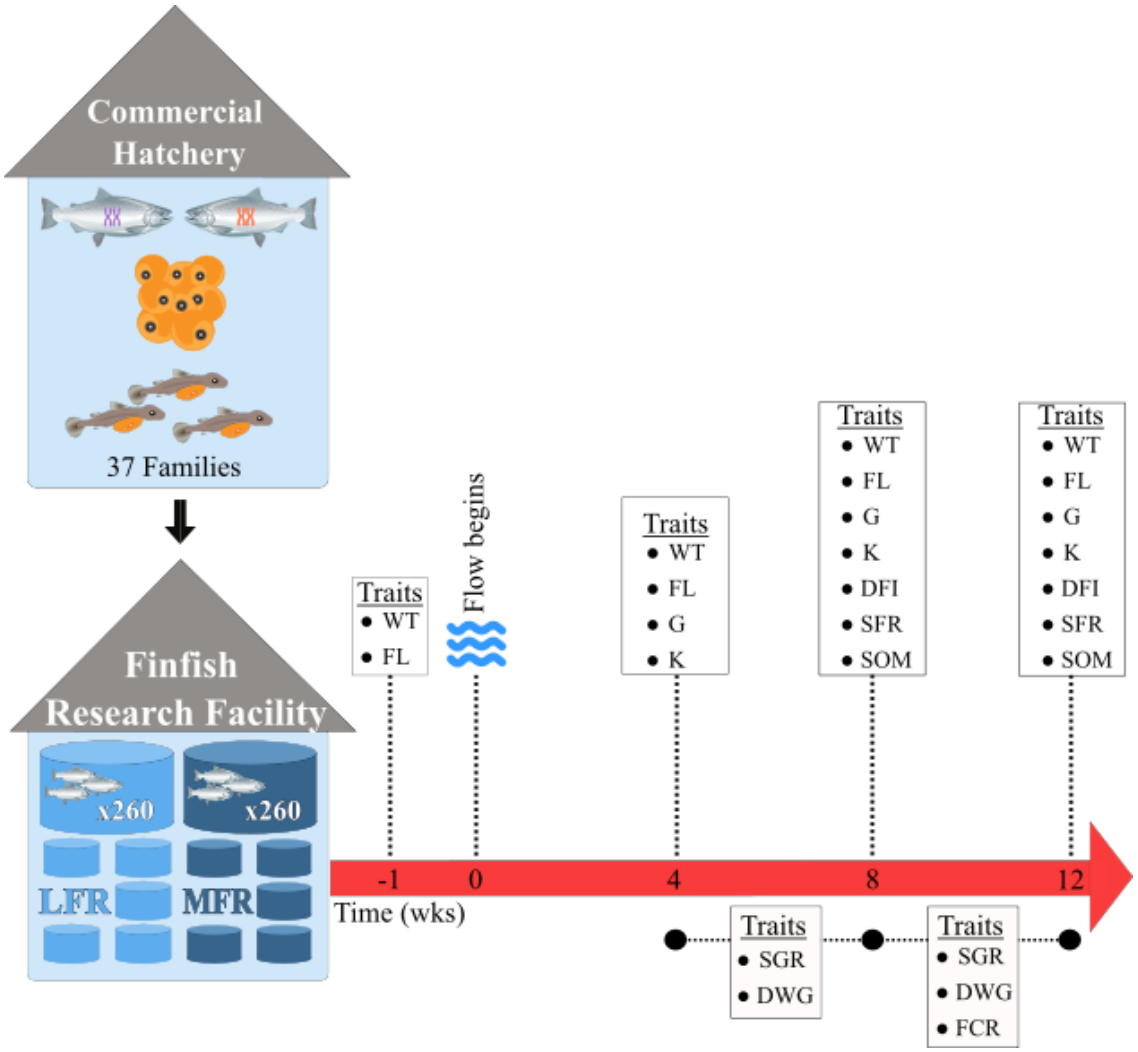


Figure 4.1 A schematic illustration depicting the experimental timeline, from the first sampling timepoint to the final sampling timepoint and the respective traits measured. Vertical dashed lines represent timing of traits measured, while horizontal dashed lines represent the period that traits were calculated across. LFR, low flow regime; MFR, moderate flow regime; WT, weight; FL, fork length; K, condition factor; DWG, daily weight gain; DFI, daily feed intake; FCR, feed conversion ratio.

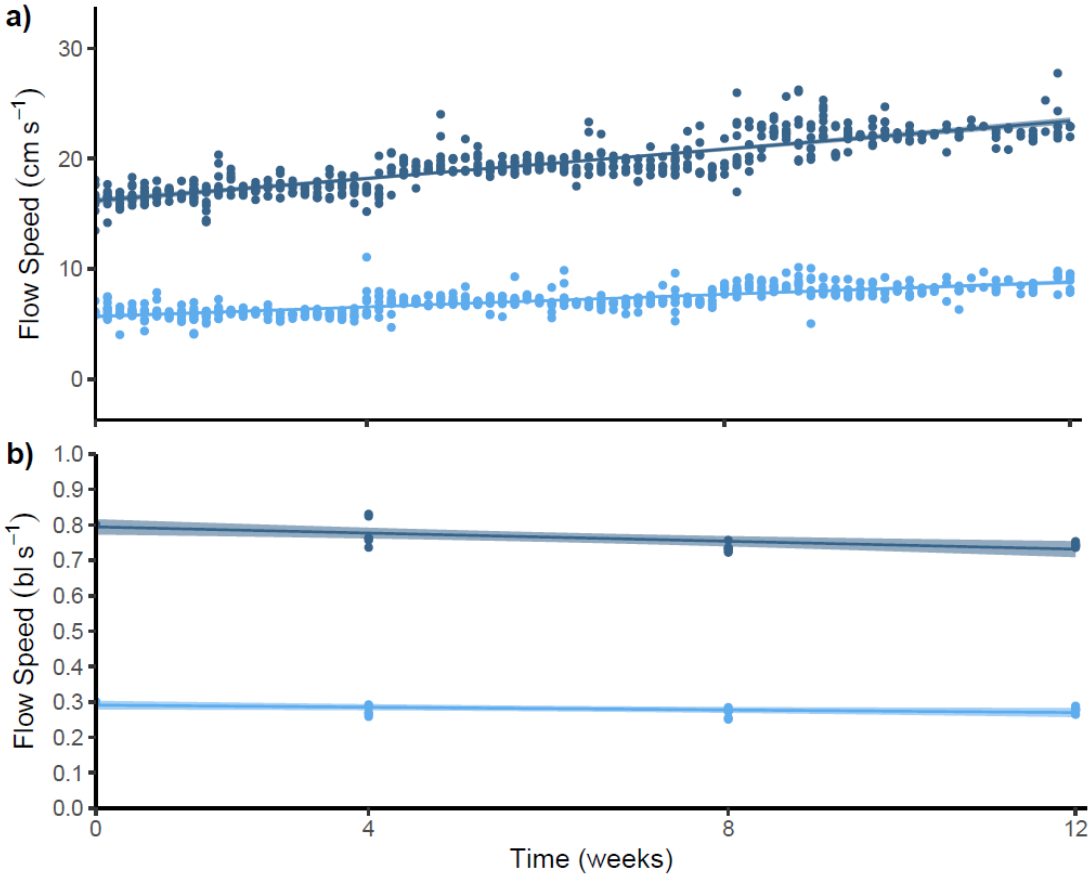


Figure 4.2 Absolute (cm s^{-1}) a) and relative (body lengths; bl s^{-1}) b) flow speeds measured from Chinook salmon tank setups with low (light blue) and moderate (dark blue) flow regimes during the experimental duration (time in weeks). Tanks are represented by individual points, solid lines represent linear relationships between flow speed (in a) cm s^{-1}, in b) bl s^{-1}) and time (weeks), and in b) shading represents the 99% confidence interval.

4.3.4 Genetic parameters and genotype-by-environment analysis

All fish that were used in the study were included in the statistical analysis (assessment data for individuals were included unless deceased), refer to Table 4.1 for sample size. Estimates of variance and covariance components were obtained using the Restricted Maximum Likelihood procedure in ASReml version 3 (Gilmour et al., 2009) fitting a univariate animal model. The model included the fixed effect of tank history and the random effect of animal. Heritabilities and genomic estimated breeding values (GEBV) were obtained for each trait in each environment at each timepoint. Heritability (h^2) was calculated as the proportion of additive genetic variance with respect to the phenotypic variance.

A bivariate model was used to estimate the genetic correlations (r_g) when treating the traits recorded in different flow regimes as separate traits, as an indicator for G×E interactions. Subsequently, due to these r_g between flow regimes being high, indicating they were similar traits, bivariate models were fitted with traits in both environments (i.e., LFR and MFR) being treated as the same trait. These models were to estimate the r_g and phenotypic correlations (r_p) for traits at the same timepoint, and for the same trait at the week eight and twelve timepoints. The rearing environment was not included in the model because it did not have a significant effect and there was minimal evidence for G×E interaction between traits. Therefore traits (e.g., WT) measured from individuals reared under LFR and MFR were considered the same when estimating r_g and r_p between traits at the same timepoint and for the same trait at the week eight and twelve timepoints. Age was not included in the models, as age was not found to have a significant main effect when examining all effects simultaneously. The bivariate animal model is represented as:

$$\begin{bmatrix}\mathbf{y}_i\\ \mathbf{y}_j\end{bmatrix}=\begin{bmatrix}\mathbf{X_i} & 0\\ 0 & \mathbf{X_j}\end{bmatrix}\begin{bmatrix}\mathbf{b}_i\\ \mathbf{b}_j\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_i & 0\\ 0 & \mathbf{Z}_j\end{bmatrix}\begin{bmatrix}\mathbf{u}_i\\ \mathbf{u}_j\end{bmatrix}+\begin{bmatrix}\mathbf{e}_i\\ \mathbf{e}_j\end{bmatrix}, \quad (5)$$

where, for i and j, $\begin{bmatrix}\mathbf{y}_i\\ \mathbf{y}_j\end{bmatrix}$ is a vector of phenotypes (for the G×E model i and j represent the different environments, i.e., LFR and MFR, and for the between trait analysis i and j represent different traits or timepoints, e.g., weight vs condition factor), $\begin{bmatrix}\mathbf{b}_i\\ \mathbf{b}_j\end{bmatrix}$ is a vector of fixed effect of contemporary group of tank history, $\begin{bmatrix}\mathbf{u}_i\\ \mathbf{u}_j\end{bmatrix}$ is a vector of random animal genetic effects, $\begin{bmatrix}\mathbf{e}_i\\ \mathbf{e}_j\end{bmatrix}$ is a vector of random residuals, **X** and **Z** are design matrices for the corresponding fixed and random effects for traits i and j. It is assumed that $\begin{bmatrix}\mathbf{u}_i\\ \mathbf{u}_j\end{bmatrix}\sim N\left(\begin{bmatrix}0\\ 0\end{bmatrix},\begin{bmatrix}\sigma^2_{a_i} & \sigma_{a_{ij}}\\ \sigma_{a_{ji}} & \sigma^2_{a_j}\end{bmatrix}\otimes G\right)$, where $\begin{bmatrix}\sigma^2_{a_i} & \sigma_{a_{ij}}\\ \sigma_{a_{ji}} & \sigma^2_{a_j}\end{bmatrix}$ is the additive genetic variance and covariance structure, and G is the genomic relationship matrix, calculated using the GBS data while taking read depth into account (following the KGD method)

(Dodds et al., 2015); and $\begin{bmatrix}\mathbf{e}_i\\ \mathbf{e}_j\end{bmatrix} \sim N\left(\begin{bmatrix}0\\0\end{bmatrix}, \begin{bmatrix}\sigma^2_{e_i} & \sigma_{e_{ij}}\\ \sigma_{e_{ji}} & \sigma^2_{e_j}\end{bmatrix} \otimes I\right)$, where $\begin{bmatrix}\sigma^2_{e_i} & \sigma_{e_{ij}}\\ \sigma_{e_{ji}} & \sigma^2_{e_j}\end{bmatrix}$ is the residual variance and covariance structure and I is an identity matrix.

For genetic correlation estimates that were less than 0.95, a likelihood ratio test was undertaken to test if they were significantly less than 1, where the null likelihood was found from an analysis with the correlation fixed at 1. Minus twice the difference in log likelihoods was compared to a mixture of $½\chi_1^2$ and ½ distribution with all its mass at 0 (Self and Liang, 1987; Butler et al., 2017).

4.4 Results

4.4.1 Environmental conditions and descriptive statistics

The absolute flow speeds increased through time and the relative flow speed was maintained at ~0.8 and ~0.3 bl s^{-1} across the experimental duration (Figure 4.2). On average, WT, K, DWG, and DFI increased over time (Table 4.1). Coefficient of variation was the highest for DFI and lowest for K (Table 4.1).

Table 4.1 Descriptive statistics[1] of production performance traits[2] in Chinook salmon under low and moderate flow regimes.

Trait	Timepoint (wks)	Flow regime	n	$\bar{X}$	σ	CV
WT (g)	4	Low	1583	229.8	44.3	19.3
		Moderate	1589	226.6	43.5	19.2
	8	Low	1579	397.2	76. 9	19.4
		Moderate	1574	383.9	75.46	19.6
	12	Low	1491	591.5	118.7	20.1
		Moderate	1491	575.0	115.0	20.0
FL (mm)	4	Low	1583	234.5	12.6	5.4
		Moderate	1589	233.6	12.7	5.4
	8	Low	1579	271.4	14.6	5.4
		Moderate	1574	269.5	14.6	5.4
	12	Low	1491	305.6	16.9	5.5
		Moderate	1491	303.1	16.6	5.5
K	4	Low	1583	1.8	0.1	6.6
		Moderate	1589	1.8	0.1	6.5
	8	Low	1579	2.0	0.1	7.3
		Moderate	1574	1.9	0.1	7.2
	12	Low	1491	2.1	0.2	7.8
		Moderate	1491	2.0	0.2	7.8
DWG (g)	4 - 8	Low	1579	167.5	36.7	21.9
		Moderate	1574	157.5	36.0	22.9
	8 - 12	Low	1491	194.3	48.2	24.8
		Moderate	1491	189.0	46.4	24.6
DFI (g)	8	Low	1579	8.6	3.4	39.1
		Moderate	1573	8.3	3.2	37.8
	12	Low	1491	9.6	4.3	45.3
		Moderate	1491	9.2	4.1	44.5
FCR	8 - 12	Low	1472	1.2	0.3	26.9
		Moderate	1474	1.2	0.3	27.3

[1] Descriptive statistics: $\bar{x}$ = Mean; σ = standard deviation; CV = coefficient of variation.
[2] Traits: WT = weight; FL = fork length; K = condition factor; DWG = daily weight gain; DFI = daily feed intake; FCR = feed conversion ratio.

4.4.2 Estimates of heritability within flow regimes

Table 4.2 presents the additive genetic variance, residual variance, and heritability of traits within each environment throughout the experiment and r_g of traits between environments for a given timepoint. Additive genetic- and residual variances for traits relating to size (i.e., WT and K) increased with time, while heritability remained similar with time and within environments. Additive genetic- and residual variance as well as heritability for traits relating to growth (i.e., DWG), feeding (i.e., DFI), and their relationship (i.e., FCR) remained similar with time and within environments.

Heritability estimates for production performance traits across each experimental timepoint were similar under LFR and MFR with small standard errors. Heritability estimates for WT and K were the highest amongst the traits evaluated (i.e., > 0.4). For DWG and DFI, the heritability estimates were slightly lower but within a moderate to high range (i.e., 0.2 – 0.45) (Bennett et al., 2014). The heritability for FCR was estimated to be the lowest (i.e., 0.15 – 0.22) amongst the production performance traits.

4.4.3 Genotype by flow regime interaction

Genetic correlations for most traits between the two flow regimes were high (> 0.85), with low standard error (< 0.11). A re-ranking plot of family level GEBVs for FCR showed that most family's FCR GEBVs were similar under LFR and MFR, but some families appeared to re-rank across the environments, suggesting that these families may perform better or worse in different flow environments (Figure 4.3).

4.4.4 Genetic and phenotypic correlations

Outlined in Table 4.3 are r_g and r_p among the traits (regardless of the flow regime) measured at twelve weeks. The LFR and MFR traits were treated as the same traits due to the high r_g values presented in Table 4.2. Genetic and phenotypic correlations remained similar at each experimental timepoint (results not shown), therefore only r_g and r_p at twelve weeks are reported.

Directly measured traits, such as size (i.e., WT and K), DWG, and DFI typically showed the highest r_g (> 0.5). For FCR, r_g with other traits tended to be lower (< 0.5), with some exceptions; FCR against DFI > 0.6. Phenotypic correlations were typically lower than the respective r_g but showed similar patterns. Size traits (i.e., WT and K) had strong correlations amongst themselves and with DWG. DWG showed strong r_p with DFI (> 0.5), but not against FCR. DFI and FCR tended to have the lowest r_p with size (i.e., WT and K). FCR only present strong r_p with DFI.

All r_g estimated for traits (i.e., WT, K, DFI, and DWG) measured at week eight and week twelve were high (0.90 – 0.98) with small standard error values (0.00 – 0.03). The r_g for DFI between week eight and week twelve timepoints was estimated to be the weakest correlation (0.90 ± 0.03), whereas WT was estimated to have the strongest correlation (0.98 ± 0.00).

Table 4.2 Genetic parameters for production performance traits[1] of Chinook salmon under low and moderate flow regimes.

Trait	Timepoint (wks)	Flow regime	Additive genetic variance	Residual variance	Heritability	Genetic correlation
WT	4	Low	751.090 ± 66.456	842.478 ± 34.333	0.471 ± 0.025	0.978 ± 0.25
		Moderate	692 ± 64.896	882.819 ± 35.907	0.440 ± 0.026	
	8	Low	2556.734 ± 229.795	2876.105 ± 117.871	0.471 ± 0.025	0.995 ± 0.02
		Moderate	2297.722 ± 222.214	2970.785 ± 122.380	0.436 ± 0.027	
	12	Low	5979.066 ± 577.588	7067.323 ± 300.579	0.458 ± 0.028	0.994 ± 0.02
		Moderate	5262.617 ± 535.389	7196.552 ± 304.669	0.422 ± 0.028	
K	4	Low	0.0053 ± 0.0005	0.0065 ± 0.0003	0.452 ± 0.026	0.989 ± 0.07
		Moderate	0.0053 ± 0.0004	0.0058 ± 0.0002	0.476 ± 0.024	
	8	Low	0.0080 ± 0.0008	0.0102 ± 0.0004	0.441 ± 0.026	0.977 ± 0.02
		Moderate	0.0085 ± 0.0007	0.0088 ± 0.0004	0.491 ± 0.024	
	12	Low	0.0094 ± 0.0009	0.0122 ± 0.0005	0.435 ± 0.028	0.998 ± NA
		Moderate	0.0103 ± 0.0009	0.0113 ± 0.0005	0.476 ± 0.025	
DWG	4 - 8	Low	613.117 ± 58.892	750.192 ± 31.119	0.450 ± 0.027	0.995 ± 0.02
		Moderate	535.332 ± 56.770	785.792 ± 32.804	0.405 ± 0.029	
	8 - 12	Low	796.345 ± 98.107	1378.404 ± 60.245	0.366 ± 0.033	0.996 ± 0.02
		Moderate	750.813 ± 89.436	1337.344 ± 57.558	0.360 ± 0.031	
DFI	8	Low	1.203 ± 0.165	2.713 ± 0.115	0.307 ± 0.033	0.999 ± 0.03
		Moderate	1.066 ± 0.149	2.526 ± 0.107	0.297 ± 0.033	
	12	Low	1.346 ± 0.249	5.224 ± 0.223	0.205 ± 0.033	0.939 ± 0.06
		Moderate	0.867 ± 0.154	3.036 ± 0.131	0.222 ± 0.034	
FCR	8 - 12	Low	0.022 ± 0.004	0.078 ± 0.003	0.217 ± 0.033	0.967 ± 0.07
		Moderate	0.015 ± 0.003	0.082 ± 0.003	0.159 ± 0.031	

Estimates ± S.E.M.

[1] Traits: WT = weight; K = condition factor; DWG = daily weight gain; DFI = daily feed intake; FCR = feed conversion ratio.

NA: Standard error was not reported due to the estimate being at its upper bound.

4.5 Discussion

The current experiment investigated if a G×E interaction exists between LFR and MFR for production performance traits in NZ-farmed Chinook salmon families. Based on high r_g estimates, there is minimal indication for genotype re-ranking across the two flow regimes for all traits measured. Heritabilities were similar in both flow environments. The family mean of GEBV for FCR re-ranking plot shows most families had similar performance in the two environments supporting that there is no indication of G×E interaction, although family re-ranking occurred for some families. There was no evidence to suggest that families need to be selected separately for performance in different tank-based flow regimes of 0.3 to 0.8 bl s^{-1} up to 600 g.

4.5.1 Genotype by flow regime interaction

Genetic correlations between the same traits under LFR and MFR were high (i.e., > 0.8) and remained constant through time. A r_g of 0.8 or higher is often taken as an indication of a minimal G×E interaction for the measured trait so then traits can be considered as the same trait in a breeding program (Robertson, 1959; Gjedrem and Baranski, 2010; Sae-Lim et al., 2016). However, it is important to determine if G×E interactions are significant both biologically and economically, which can be achieved by simulating multiple breeding programs and conducting cost-benefit analyses (Sae-Lim et al., 2016). In rainbow trout, the break-even correlation is suggested to be 0.7 (Sae-Lim et al., 2013; Sae-Lim et al., 2016), further supporting the findings of minimal G×E interactions. Re-ranking plots for feed efficiency showed most families have similar performance under LFR and MFR, although some families did perform differently under the two flow regimes. In other contrasting environments, G×E interactions have previously been documented, for example growth and feed performance responses differ between freshwater and seawater (Winkelman and Peterson, 1994; Chiasson et al., 2014; Correa et al., 2018; Gonzalez et al., 2022), low and elevated temperatures (Heath et al., 1993), as well as tank and stream environments (Sae-Lim et al., 2016; Sundström et al., 2016).

Weak re-ranking means that the best families in LFR were also likely to be the best families in MFR, and likewise for poorer performing families. A possible reason that flow regimes did not cause a G×E interaction could be because this study assessed traits during the peak growth period (< 1 kg) and before the critical size when Chinook are believed to become more sensitive to environmental factors. Another reason could be associated with Chinook salmon swimming at similar speeds in both environments, and that energy expenditure was equivalent. In Prescott et al. (2024), measured swimming speeds of Chinook salmon under LFR and MFR were not different. This study only included 37 families from one breeding program in New Zealand, and therefore, evaluating more families from multiple breeding programs would confirm if these results hold more generally.

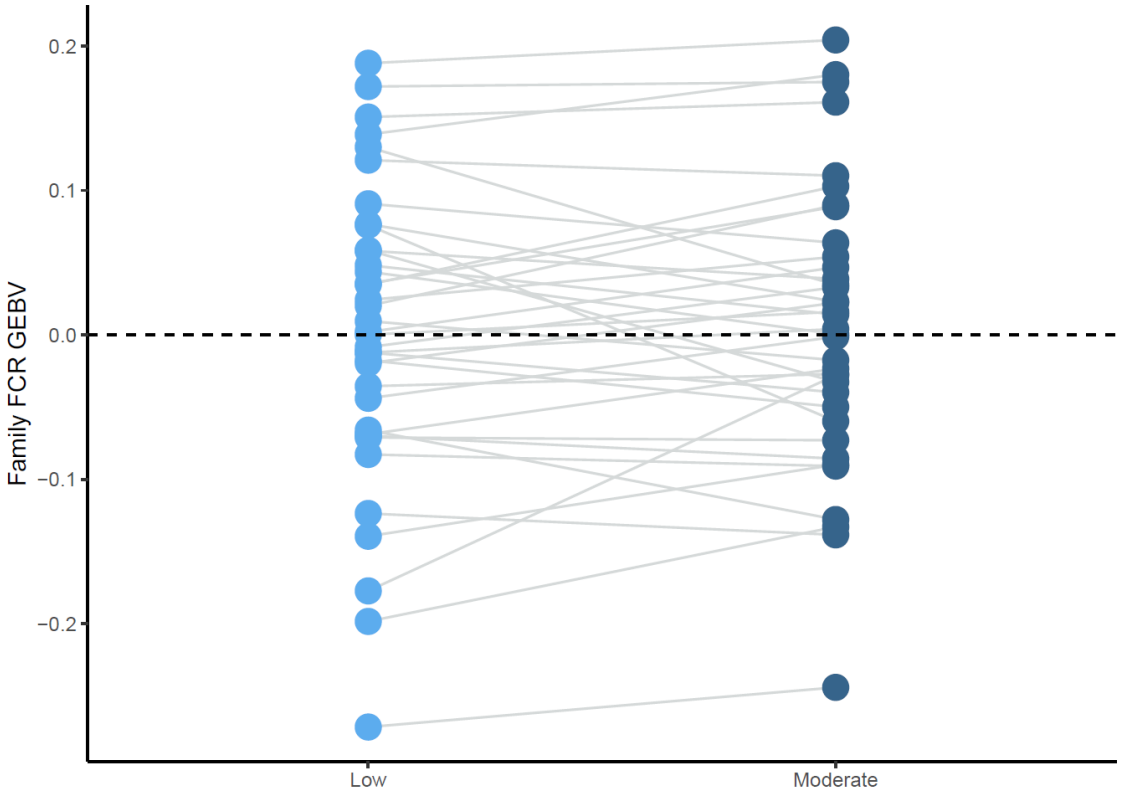


Figure 4.3 Re-ranking of family genomic estimated breeding values (GEBV) for feed conversion ratio (FCR) between low (light blue) and moderate (dark blue) flow regimes.

4.5.2 Genetic parameters for production performance

Heritability estimates for each trait remained comparable throughout the experiment. The sampling timepoints coincided with when post-smolt salmon undergo rapid and peak growth, 2.4% WT day^{-1} to 1.4% WT day^{-1} (unpublished growth data on Chinook salmon in seawater) (Handeland et al., 2008). The size ranges at each sampling timepoint did overlap, which may provide some explanation for these consistent heritabilities. In Scholtens et al. (2023), heritabilities estimated for similar traits, but in larger Chinook salmon (~0.9, ~1.5, ~1.9, and ~2.1 kg), were also comparable through time. In other salmonid species (i.e., Atlantic and coho salmon *Oncorhynchus kisutch*), consistent heritabilities were also observed for production-related traits over time (Myers et al., 2001; Thorland et al., 2020).

Trait heritabilities measured in the current experiment are comparable with other published Chinook salmon studies (Walker et al., 2012; Symonds et al., 2019; Scholtens et al., 2022; Scholtens et al., 2023). These other studies sourced families from two breeding programs that were evaluated in a range of environments from tanks to sea pens and at different times during the production cycle. In Scholtens et al. (2022), r_g between traits in tanks versus sea pen environments ranged between 0.46 to 0.78, and heritability estimates were similar across the two environments. The combined results suggest that heritabilities are consistent throughout the production cycle and that tank-based family evaluation can be used to inform the industry's selective breeding programs, but information from all rearing environments would provide the most benefit to selection (Scholtens et al., 2022).

Similarly, heritability estimates for size (i.e., WT and K) and growth (i.e., DWG) traits were comparable to other salmonids (i.e., Atlantic and coho salmon, and rainbow trout) (Carlson and Seamons, 2008; Gonzalez et al., 2022), and non-salmonid fish species (i.e., Indonesian hybrid tilapia and silver trevally *Pseudocaranx georgianus*) (Setyawan et al., 2022; Valenza-Troubat et al., 2022). The heritabilities of the feed related traits (i.e., DFI, SFR, and SOM) were lower (Silverstein et al., 2001; Quinton et al., 2007; de Verdal et al., 2018a) or similar to other published fish studies (Besson et al., 2022). Heritabilities for FCR were similar to Nile tilapia (*Oreochromis niloticus*) (de Verdal et al., 2018a; de Verdal et al., 2022) and sea bass (*Sparus aurata*) (Besson et al., 2019) but higher than other salmonids (i.e., rainbow trout and European whitefish *Coregonus lavaretus* L.) (Kause et al., 2006; Quinton et al., 2007; Kause et al., 2016). The moderate to high heritability estimates for desired traits, such as growth, which can be easily measured in commercial settings, provides significant scope for genetic gains to be achieved through breeding programs, which can generate significant economic gains for the NZ Chinook salmon aquaculture industry.

Genetic correlations for the same traits between the eight and twelve week timepoints showed strong correlations, suggesting these traits remained stable through time. These results could be contributed to the measurements reoccurring only four weeks apart and during the peak growth period for NZ farmed Chinook salmon (unpublished growth data on Chinook salmon in seawater). In Scholtens et al. (2023), larger (> 985 g) NZ-farmed Chinook salmon showed moderate r_g between growth rate and DFI at consecutive timepoints (i.e., time 1 compared to 2, ~six - eight weeks apart) and weaker r_g when comparing non-consecutive (i.e., time 1 compared to 3) timepoints. However, WT and K had high r_g across all timepoints, similar to r_g estimated in the current experiment. In Thorland et al. (2020), r_g for thermal growth coefficients in farmed Atlantic salmon were low across the production cycle, even though r_p were significant. Together, these results suggest that other factors could be influencing traits differently across an entire production cycle, and that they may not be considered the same trait at the beginning and end of the production cycle. Farmers need to consider this when using these traits in their selection criteria, as the timing is important for when phenotypes are measured to generate genetic parameters.

Genetic correlations did not reveal any unfavourable correlations among the size (i.e., WT and K) and growth traits (i.e., DWG) and were comparable to previous Chinook salmon genetic evaluations of similar traits (Scholtens et al., 2023) and those for other salmonid species (Myers et al., 2001; Garber et al., 2019). Harvest weight is a priority breeding objective used in commercial breeding strategies in NZ (Symonds et al., 2019; Scholtens et al., 2023), and in this study r_g and r_p for WT and DWG against FCR were low. For other commercial species, such as Nile tilapia, gilthead sea bream (*Sparus aurata*) and Atlantic salmon, r_p between growth traits (e.g., WT, DWG or thermal growth coefficient) against FCR are typically high (> 0.4) (Thodesen et al., 1999; Thodesen et al., 2001; de Verdal et al., 2018a; Besson et al., 2022; de Verdal et al., 2022) and higher than those found in this study; however, in Nile tilapia r_g between body weight gain and FCR was found to be low (−0.07) (de Verdal et al., 2018a). For Chinook salmon's commercial breeding programs in NZ, these results suggest that when selecting for families that have the largest harvest weight, selection for fish with poorer FCR could occur. Genetic correlations show unfavourable correlations between FCR and feed-related traits (i.e., DFI) as well as DFI against WT, suggesting that families the best FCR would have lower DFI and DWG. Based on these r_g, better genetic gains for FCR can be achieved by selecting for feed intake traits rather than harvest weight or growth. However, difficulties in accurately measuring feed intake in commercial settings, alongside the unfavourable r_g estimated in this study, makes selection for feed-efficient salmon difficult and is reflected by slow FCR improvements in the industry (Walker et al., 2012; Scholtens et al., 2023).

Table 4.3 Genetic and phenotypic correlations for production performance traits[1] of Chinook salmon after twelve weeks.

	WT	K	DWG	DFI	FCR
WT		0.719 ± 0.012	0.926 ± 0.003	0.468 ± 0.018	0.041 ± 0.023
K	0.661 ± 0.029		0.719 ± 0.012	0.401 ± 0.020	0.066 ± 0.023
DWG	0.947 ± 0.007	0.647 ± 0.032		0.502 ± 0.017	-0.065 ± 0.023
DFI	0.784 ± 0.039	0.560 ± 0.051	0.811 ± 0.037		0.634 ± 0.013
FCR	0.224 ± 0.077	0.215 ± 0.073	0.126 ± 0.083	0.609 ± 0.063	

Estimates ± S.E.M.
Genetic correlations = above diagonal; Phenotypic correlations = below diagonal.
Note: Traits under both environments were treated as the same trait.
[1] Traits: WT = weight; K = condition factor; DWG = daily weight gain; DFI = daily feed intake; FCR = feed conversion ratio.

4.5.3 Implications for future breeding programmes in the context of variable flow

Stronger G×E interactions may exist in Chinook salmon if reared under different flow regimes than tested in this study, as several other aspects have yet to be determined:

1. The optimal flow regime for rearing pre- and post-smolt Chinook salmon to achieve exercise-enhanced growth has yet to be identified, where higher flow regimes may be required to achieve this (Thorarensen et al., 1993; Gallaugher et al., 2001; Prescott et al., 2024). In that case, G×E interactions may need to be re-evaluated under these flow regimes.

2. Production performance in harvest-size Chinook salmon reared under different flow regimes and/or in low and high energy farms may respond differently to that for smaller fish (as measured in this study), indicating a longer-term study with flow regimes more representative of offshore environments (e.g., faster and oscillating speeds) is needed to determine if G×E interactions exist when farming to harvest-size.

3. Performance may respond differently when fish are reared under different flow regimes in freshwater versus seawater (i.e., pre- versus -post smolt), therefore future studies should consider G×E interactions based on both flow and salinity.

4.5.4 Conclusion

Additive genetic variation is a significant component in salmon size, growth, and feed related traits, including feed intake and FCR. In the current experiment, there was minimal evidence to suggest that a G×E interaction exists for production performance between LFR and MFR for Chinook salmon between 82.9 ± 0.3 g and 583.2 ± 2.1 g. This demonstrates that family genetic merit is relatively consistent when individuals are reared under different flow regimes. This study provided important information for industry to consider when they integrate different tank-based environments and offshore high energy sites into their farming strategy.

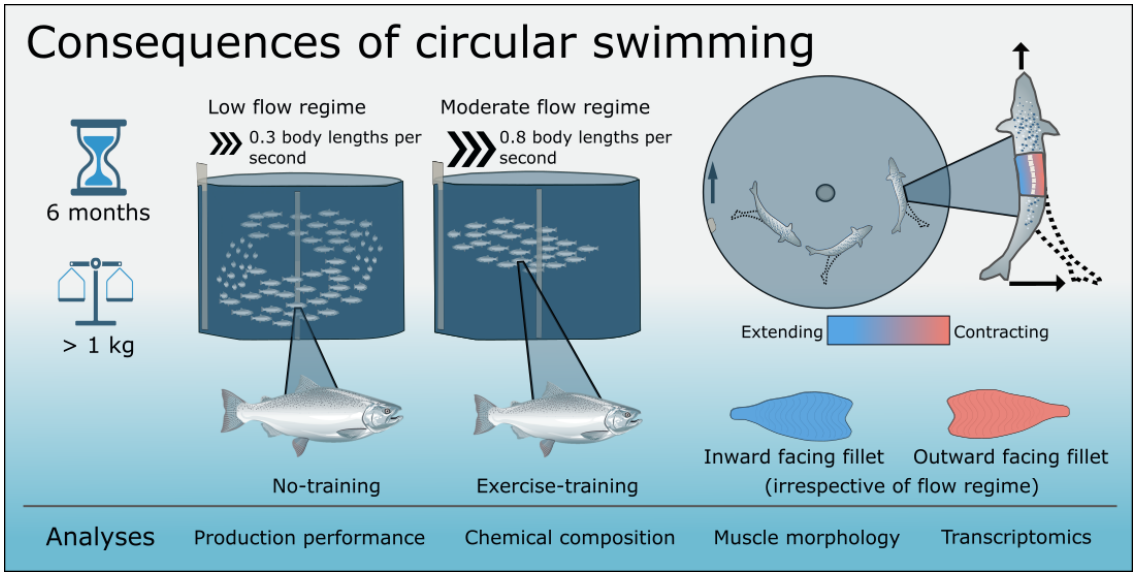
Chapter Five Consequences of long-term circular swimming on the lateral aspects of white muscle physiology and symmetry

Part of the research contained within this chapter is in preparation as Prescott, L.A., Canepa, M.M., Symonds, J.E., Walker, S.P., Miller, M.R., Semmens, J.M., Carter, C.G., 2023. The side effects of swimming: white muscle responses in long-term exercise-trained Chinook salmon reveal consequences for muscle physiology and symmetry.

5.1 Abstract

Using exercise to optimise salmonid production performance has been extensively studied; however, most of these studies use short exercise-training periods (1 to 3 months) with small size classes (< 500 g). There is urgency to understand the influence of sustained swimming on larger individuals and how to adequately scale exercise regimes for the larger smolt sizes that are now being transferred to sea. There is also a considerable knowledge gap in understanding the use of circular tanks to exercise salmonids and the impact that continuous circular swimming may have on muscle physiology and symmetry. This study aims to determine if exercise regimes optimised for small post-smolt salmonids can enhance production performance in larger post-smolts and to identify any muscle asymmetry resulting from exercise-training salmon in circular tanks. Post-smolt Chinook salmon were reared under low (LFR) and moderate flow regimes (MFR) for six months, to a larger size (1056 ± 11 g and 374 ± 1 mm) than typically studied. Several aspects of whole-animal performance were measured and matched with physical and biochemical responses on lower biological levels (i.e., tissue, cellular and molecular) in the white muscle from both sides of the fish. Production performance (individual growth, tank level feed intake, growth and efficiency) was significantly improved in Chinook salmon reared under LFR compared to MFR. Chemical composition (lipid and protein content) and muscle morphometrics did not differ between the flow regimes, even though genes involved in lipid metabolism, muscle development and contraction, and muscle repair and maintenance were upregulated in Chinook salmon reared under MFR. Lateral investigations of white muscle showed the right fillet (outward facing fillet) to have higher lipid content in the lateral and visceral regions, lower protein content across the entire fillet, and denser white muscle fibres. Genes involved in lipid metabolism and muscle development and contraction were also upregulate in the white muscle in the right fillet. This study shows that optimal exercise regimes for small post-smolt salmon is not always suited to larger post-smolt salmon, and that exercise regimes for larger post-smolt salmonids may need to be optimised separately. Additionally, the results from this study suggest that continuous

circular swimming may stimulate the inward and outward facing muscle blocks unevenly leading to muscle asymmetry and reveals a new angle of questions associated with exercising salmonids in circular tanks. Further exploration is warranted to determine the relevance for commercial settings when larger rearing tanks are used.



Graphical Abstract 1 Consequences of circular swimming.

5.2 Introduction

Using flow regimes to encourage swimming in commercial aquaculture and hatchery settings is becoming increasingly important. Flow regimes provide a means of natural enrichment and can improve fish robustness and resilience (Palstra and Planas, 2011; Davison and Herbert, 2013; McKenzie et al., 2020). Across multiple fish species, particularly in salmonids, exercise training improves production-biology (e.g., growth and feed efficiency), product quality, swimming performance, health, and disease resilience (refer to reviews by Palstra and Planas, 2011; Davison and Herbert, 2013; McKenzie et al., 2020; Huang et al., 2021; Rodgers and Gomez Isaza, 2023). Enhancing these traits through exercise training can lead to large industry advancements, as it reduces pressure on selective breeding programs and vaccine development, and accelerates the production of larger, high-quality fish that can be reared across multiple environments (e.g., recirculating aquaculture systems (RAS), nearshore, offshore). However, limited information is available on the optimal exercise regime needed to promote exercise-enhanced traits across different sizes in the production cycle.

Most exercise studies to date, using salmonid species, have focused on early production stages, with small salmonids (final size < 500 g) and training regimes typically lasting for one to three months, and up to five months. Some studies have used training regimes longer than five months, but the final fish size often remains < 500 g. As such, there is a considerable knowledge gap regarding the effects of long-term exercise on larger fish and whether the optimal exercise regimes for small post-smolts are suitable for larger individuals (reviewed by Rodgers and Gomez Isaza, 2023). This is problematic, as some fish aquaculture industries are rearing or considering rearing stocks to a larger size (e.g., 1000-1500 g in salmonids) before sea transfer (Timmerhaus et al., 2021). Only a few studies have investigated the responses of salmonids to exercise lasting longer than five months and involving a size larger than 500 g (Totland et al., 1987; Nilsen et al., 2019; Prescott et al., 2023). In Totland et al. (1987) and Nilsen et al. (2019), Atlantic salmon (*Salmo salar*) were exercised for eight (initial size: ~2000 g; final size: ~3000 g) and six months (initial size: ~800 g; final size: ~3000), respectively, and showed better growth and enlarged muscle fibres. These studies suggest there is scope for defining optimal exercise regimes for larger individuals and possibly across the entire production cycle through to harvest. With this information, the industry could then scale flow regimes to enhance production performance in their stocks prior to sea transfer.

To accurately inform the industry about opportunities to enhance production in larger post-smolt salmon, a better understanding of the underlying mechanisms behind exercise-enhanced growth is needed (Koganti et al., 2020). It is well-documented that exercise-enhanced growth is predominantly driven by increases in muscle fibre size i.e., hypertrophy (Davie et al.,

1986; Rasmussen et al., 2011; Timmerhaus et al., 2021) and sometimes protein content (reduced total lipid content: Lauff and Wood, 1997; Huang et al., 2021), but the underlying molecular changes are still being explored. In rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon, exercise-enhanced growth is linked to the upregulation of genes involved in myogenesis (e.g., insulin growth factor hormone) and aerobic energy use and generation (Magnoni et al., 2013; Pengam et al., 2021; Timmerhaus et al., 2021). Similarly, in mammals, insulin-like growth factors (IGFs) are responsible for muscle regeneration and hypertrophy (Zanou and Gailly, 2013). In non-salmonid species such as zebrafish, *Danio rerio* (Palstra et al., 2010; Palstra et al., 2014) and gilthead seabream, *Sparus aurata* (Sánchez-Gurmaches et al., 2013), the insulin growth factor hormone is also described as a key gene involved in muscle growth. However, this was not consistent in a follow up study on gilthead seabream (Palstra et al., 2020). Other studies assessing changes in biochemical pathways show exercise training to increase activity levels of key oxidative- (e.g., succinate dehydrogenase, citrate synthase; Johnston and Moon, 1980; Anttila et al., 2006; Anttila et al., 2011; Zhang et al., 2016) and muscle excitation-contraction coupling enzymes (e.g., ryanodine and dihydropyridine receptors; Anttila et al., 2006; Anttila et al., 2008) in both the red and white muscle, which could also be playing important roles in exercise-enhanced growth.

Furthermore, some studies have also documented markers of muscle inflammation as exercise regimes become more intense, which was positively correlated with growth (Palstra et al., 2020; Timmerhaus et al., 2021). This could suggest that there is an upper limit for exercise-enhanced growth with respect to animal welfare. In mammalian species, acute muscle inflammation is a key parameter involved in muscle growth, where maintaining a balanced cycle of inflammation and repair is important (Yang and Hu, 2018). This balance is yet to be defined in fishes, and applying this model to studies investigating the influence of exercise in fishes is still in its infancy. Research is needed to identify the key genes involved in muscle growth by exploring how exercise influences gene expression and specific markers (e.g., IGF and inflammatory genes), both when exercise-enhanced growth is achieved and when it is not.

Similarly, minimal research effort has been dedicated to understanding how exercising salmonids in circular tanks may influence the left and right muscle blocks differently. When exercise-training salmonids in circular tanks, the subjected fish are continuously swimming and turning in one direction. The effect this has on muscle physiology and symmetry is unknown. Lateral asymmetry has been alluded to in a previous exercise study with Chinook salmon, where they documented lateral changes in muscle composition and morphology, which was suggested to influence the direction of spinal scoliosis (Prescott et al., 2023). Lateral asymmetry, however could be a natural occurring phenomenon, as larger muscle blocks have been associated with the turning preference in zebrafish (Heuts, 1999) and in largemouth bass, *Micropterus salmoides*

(Nakajima et al., 2007). However, the extent of asymmetry between the left and right muscle blocks and the potential links to reduced spinal health, which could impact product quality of farmed Chinook salmon, demands research attention.

Using transcriptomics and other 'omic approaches (e.g., proteomic, metabolomic) to explore molecular changes are valuable emerging tools (Esmaeili et al., 2021; Anderson et al., 2022; Esmaeili et al., 2022; Young et al., 2023), and these tools could improve understanding of the mechanisms driving muscle growth and possible lateral asymmetry when salmon are exercised in circular tanks. Chinook salmon (*Oncorhynchus tshawytscha*) remain as one of the only studied salmonid species yet to exhibit exercise-enhanced growth (Thorarensen et al., 1993; Gallaugher et al., 2001; Kiessling et al., 2005; Prescott et al., 2024), where improvements have only been documented in their feed efficiency, swimming performance, aerobic capacity, and recovery efficiency (Gallaugher et al., 2001; Prescott et al., 2023; Prescott et al., 2024). It is not yet clear why Chinook salmon do not benefit from exercise in terms of growth, and most studies focusing on exercising Chinook salmon have only measured responses at the whole animal level. Therefore, exploring the molecular changes in exercise-trained Chinook salmon could help identify common molecular markers associated with exercise, and differentiate them from those previously described as key genes in exercise-enhanced growth in other species. This provides an alternative perspective for improving understanding of the molecular drivers behind exercise-enhanced growth.

This study used a low flow regime (LFR) and moderate flow regime (MFR) to exercise-train New Zealand (NZ) farmed Chinook salmon for six months, to determine whether flow regimes optimised for small post-smolt salmonids are suitable for larger individuals and for extended durations. Several physiological measures were used to match changes at whole-animal level through to tissue, cellular, and molecular levels. Specifically, the production performance of the whole-animal and the chemical composition, morphology, and transcriptomics analysis of the white muscle were explored to understand the impact of long-term exercise in larger individuals. Investigations in the white muscle were applied to both sides of the fish to identify possible muscle asymmetry resulting from training salmon in circular tanks. The fish used in this study were sampled after being exercised for six months, while the remaining fish continued to be exercised up to 10-11 months, during which other physiological aspects were investigated (Chapter 6; Prescott et al., 2023).

5.3 Materials and Methods

All protocols performed on animals followed animal ethics protocols through Nelson Marlborough Institute of Technology Animal Ethics Committee (AEC2018 CAW01).

5.3.1 Fish husbandry and experimental conditions

Fish sourcing, husbandry, and experimental conditions followed protocols outlined in Prescott et al. (2023) and Prescott et al. (2024). Fish used in this study were supplied from Sanford's Kaitangata commercial salmon hatchery, which consisted of all-female Chinook salmon smolts. Fish were tagged with passive integrated transponder tags (HIDGlobal, EM4305, 12 mm long and 2 mm diameter glass tags) at the hatchery and transferred to the Fisheries Research Centre at Cawthron Aquaculture Park, Glenduan Nelson, NZ on 7th December 2020. The fish were held in 8,000 L tanks with a salinity of 14 – 15 ppt at 13 °C (maintained within 0.2 °C) on arrival. Exchange rates were set to 224 ± 0.07 L min^{-1} (mean ± S.E.M.) to ensure high water quality was maintained. Over seventeen days, fish were acclimatised to full seawater (35 ppt) and a rearing temperature of 17 °C (maintained within 0.2 °C), and continuously supplied with filtered recirculating seawater (35 ppt, 17 °C maintained within 0.2 °C, 24 h light photoperiod). Fish were sorted (29th-31st December 2020) across twelve 8,000 L treatment tanks with approximately 260 fish per tank, and tank velocities were set to 4.93 ± 0.08 cm s^{-1} for ~3 weeks. All fish underwent a routine assessment, where mass and fork length (fork length = 174.6 ± 0.2 mm; weight = 82.9 ± 0.3 g; mean ± SE) were measured allowing the initial tank velocities to be set (i.e., beginning of flow regime treatments). Tank velocities were slowly increased (by 1.5 cm s^{-1} day^{-1}) to either LFR (0.3 bl s^{-1}) or MFR (0.8 bl s^{-1}; six tanks per treatment) across seven days or until the target speed was achieved. Tank velocities were measured daily and adjusted monthly to account for fish growth. Fish growth was estimated based on data generated in previous experiments (Elvy et al., 2022) and adjusted following routine growth assessments (Figure 5.1). After 79 days under treatment, 269 fish (fork length 304.4 ± 0.6 mm; mass = 577.6 ± 3.7 g) were transferred into smaller experimental tanks (3,200 L) to continue rearing the fish under LFR and MFR. Flow regimes were measured 2–3 times per week and adjusted monthly to account for fish growth.

5.3.2 Tail beat frequency

GoPro Hero 7 cameras (frame rate: 60 Hz) were placed inside the 3,200 L tanks and were pre-set to record for ten-minute recordings every hour, allowing for four videos to be obtained before the battery needed replacement. The video captured a side-angle of the fish occupying approximately 50% of the tank. Video recordings were obtained weekly from 105 days to 161 days under treatment. From these videos, three randomly selected minutes (excluding first video as this capture fish being disturbed) were used to measure the time taken for five consecutive tail beats (complete caudal fin oscillation) from five fish to be complete and expressed as tail beat frequency (TBF) in hertz (Hz). Each fish assessed for TBF were selected based on their

positioning in the tank (i.e., top, bottom, middle, left, and right) to avoid pseudo replication and to account for flow variation across the tank profile.

5.3.3 Feed and growth assessments

Fish were hand fed a commercial feed (Orient A 2000, Skretting, Australia: crude protein 37.5 g $100g^{-1}$, total fat 24.2 g $100g^{-1}$, energy 1705 kJ $100g^{-1}$ measured by the Cawthron commercial food testing laboratory) to satiation daily (AM) and pellet size was increased with fish growth, as per manufacturer's recommendation. Tank daily feed intake (tank DFI) was measured by calculating the difference between the final feed bucket weight including uneaten pellets (retrieved by swirl separator) and the initial feed bucket weight. Uneaten pellets were dried and counted using an automated counter (Contardor2, PFEUFFER GMBH, Kitzingen, Germany) and multiplied by the average pellet weight.

After 22, 51, 79, 132, and 167 days, under treatment, on average, all fish were anaesthetised using tricane methanesulfonate (65 ppm; Syndel, Canada), and their mass (M), fork length (FL), and girth (G; not measured at 22 days) were measured. Condition factor (K) was later calculated as:

$$\mathrm{K} = 100000 \times \frac{M}{FL^3}, \quad (1)$$

where M is the mass of fish (g) and FL is the fork length (mm). Specific growth rate (SGR; (% day^{-1}) was calculated as:

$$SGR = \frac{\ln(M_f) - \ln(M_i)}{days} \times 100, \quad (2)$$

where M_f is the final mass (g), M_i is the initial mass (g), and days is the number between measurements.

Tank specific feed rate (SFR_t; % g^{-1}) was calculated as:

$$SFR_t = \frac{tank\ DFI}{M_t} \times 100\ , \quad (3)$$

where M_t is the total mass of the tank.

Tank feed conversion ratio (FCR_t) was calculated as:

$$FCR_t = \frac{TFI}{MG}\ , \quad (4)$$

where *TFI* is the total tank feed intake between the two assessments, and MG is the total mass gained by this fish between the two sampling assessments.

5.3.4 Sampling assessments

After 167 days under treatment, six fish per tank were euthanised with a lethal dose of AQUI-S (100 ppm). Blood was drawn from the caudal blood vessel and centrifuged in a HemoCue Microhematocrit tube at 9000 rpm for seven minutes to measure haematocrit (Hct). White muscle tissue samples were excised from both sides (back of dorsal) of the fish with clean forceps and a scalpel, flash frozen in liquid nitrogen, and stored at -80°C for later transcriptomic analysis. Gill (second arch, left side), skin (below dorsal fin, left side), and additional white muscle samples (back of dorsal, left and right side) were removed and fixed in 10% neutral-buffered formalin for histological processing. Whole viscera, heart, liver, and gonads were weighed, and belly-flap thickness at three locations were measured: in line with pectoral, pelvic, and anal fins. Remaining viscera, liver, fillets, and carcass were stored frozen for compositional analysis.

5.3.5 FT-NIR approximate composition

Chemical composition was measured on the remaining viscera, liver, fillets, and fish carcass using Fourier transform – near infrared (FT-NIR) spectroscopy. Following protocols from Miller et al. (2019), FT-NIR was used to estimate total lipid in whole-body, fillet, liver and viscera, and total protein, ash, and moisture in whole-body and fillet only. The fillet was divided into four regions (dorsal, lateral, visceral, and posterior dorsal) as outlined in Prescott et al. (2023). The viscera and liver were homogenised using an IKA T18 ULTRA TURRAX for 30-60s, whereas a food processer was used to homogenise the fillet. Uniform samples from each prepared tissue were placed in a 50 mm rotating cup and scanned in reflectance mode using a Bruker MPA FT-NIRs (Bruker, Ettlingen, Germany). The remaining carcass and already homogenised fillet, viscera, and liver were broken down and mixed using a commercial meat mincer to obtain a whole-body homogenate. All samples were scanned using models developed and validated by Miller et al. (2019), and further checked by assessing the proximate composition using wet chemistry through a commercial testing laboratory (Food Testing Laboratory of Cawthron Analytical Services; Nelson, NZ) from one fish per treatment. Samples of the whole-body, fillet, liver, and viscera samples were collected from two fish (one per treatment) during NIR spectroscopy and assessed following the methods from Association of Official Analytical Chemists (AOAC) for crude protein (AOAC 981.10), total lipid (AOAC 948.15), moisture at 105˚C (AOAC 950.46), and ash (AOAC 920.153). FT-NIR lipid and protein values were < 10% (R^2 = 0.96) and <7% (R^2 = 0.99) different from values obtained from wet chemistry, respectively.

5.3.6 Histology and morphometrics

Histological techniques were performed using the formalin fixed samples taken from the sampled fish (six fish per tank). Muscle samples were processed by the histology department at

the University of Otagao, NZ. Muscle samples from both sides of the fish were decalcified in 5% formic acid for five days. The samples then underwent a series of dehydration and wax infiltrations (Thermo Excelsior ES processor), before being cut to 5 µm using a microtome (Leica RM2235). Muscle sections were automatically stained (Thermo Genini Autostainer) with haematoxylin and eosin (H&E) and automatically scanned using a Leica Aperiod Scansope at 20x magnification. Following Prescott et al. (2023), three randomly selected 500,000 μm^2 regions were used to manually measure muscle fibre size and perimeter using QuPath (version 0.3.2; Bankhead et al., 2017). From these measurements, the average muscle fibre diameter was estimated as:

$$\emptyset = 2\sqrt{A * \pi^{-1}}, \quad (5)$$

where, A is the area (μm^2). Muscle fibre density was calculated as:

$$d = \frac{n}{A}, \quad (6)$$

where, n is the count of fibres and A is the area of analysed area (500,000 μm^2).

Gill and skin samples were processed and stained with H&E by Gribbles Veterinary, Christchurch, NZ. The slides were visualised using an Olympus BX53 upright light microscope and micrographed with an Olympus DP27 camera attached to the microscope at 200x magnification. Five micrographs per slide (containing three histological sections) were taken for each tissue. Gill micrographs contained lamellae from the lower (x2), upper (x2), and middle (x1) regions of the gill filaments. Gill and skin morphometrics were obtained using ImageJ (v. 1.53e, National Institutes of Health, Rockville, MD, USA). For each gill micrograph, three randomly selected lamellae were measured for lamellae length, filament thickness, lamellae density, and thickness of the lamellae epithelium (proxy for oxygen diffusion distance) on each micrograph, meaning fifteen lamellae per fish were analysed, following Hess et al. (2015); (2017) and Prescott et al. (2023). For each skin micrograph, epidermis and dermis thickness were measured on fifteen randomly selected locations, following methods from Timmerhaus et al. (2021) and Prescott et al. (2023).

5.3.7 Transcriptomics

RNA extraction was performed by staff from Cawthron's molecular biology laboratory, Nelson, NZ. Total RNA was isolated from each white muscle (left and right side) sample using the trizol extraction method and eluted in 35 µl RNase-Free water, treated turbo DNase, and stored at -80°C.

A sample of RNA from all thirty-six samples (9 biological replicates × 2 biological locations, i.e., left and right fillet × 2 treatment groups, i.e., LFR and MFR) were sent to

AgResearch, NZ, for quality checking (RNA integrity number (RIN) values were > 9.2) on the Agilent 4150 TapeStation system. All RNA-Seq libraries were prepared using the Illumina Stranded mRNA Prep KIT, where messenger RNA (mRNA) was isolated from 200 ng total RNA. Libraries were sequenced on the Illumina NoveSeq6000, on one lane of a S4 flowcell and produced an average of 22 million individual 150-bp paired-end reads per sample.

All high-performance computing was performed on an Ubuntu 22.04.3 LTS platform in a bioconda environment. The quality of Illumina raw reads was assessed using FastQC 0.12.1 (Babraham Institute; www.bioinformatics.babraham.ac.uk) and then the wrapper Trim Galore! 0.6.4 (Babraham Institute) was used in conjunction with Cutadapt 2.4 (https://github.com/marcelm/cutadapt) to remove any remaining adaptors, trim bases with a Phred value of < 25, and exclude reads with a length of < 40 bp. Kallisto (Bray et al., 2016) was used to assemble the transcriptome *de novo* using reads from each sample. High quality reads (Q>30) were mapped to Chinook salmon genome Otsh_v2.0 (version Otsh_v2.0, INSDC Assembly GCA_018296145.1) using Kallisto with default parameters. Raw reads with less than 0.5 counts per million in at least nine samples were removed and the remaining reads were normalised using the edgeR version 4.2.0 (Robinson et al., 2010) in the R statistical computing environment. As library sizes were quite variable between samples, the voom transformation was applied to the normalised and filtered DGEList object generated from EdgeR. The voom method (Limma, version 3.60.2) was used to estimate the mean-variance relationship of the log2 counts. This method provides a precision weighting for each observation and incorporates them into the limma empirical Bayes analysis (Law et al., 2014; Ritchie et al., 2015). To identify differential expression between samples and tissues, a fold-change and false discovery rate (FDR) thresholds were applied. Specifically, the FDR was set to ≤ 0.05 and log2 (fold change) ≥ 1 to declare significance in line with previous recommendations (Haas et al., 2013; Schurch et al., 2016). It is important to note a log2 (fold change) >1 equates with a minimum 2-fold change.

Gene Ontology (GO) Enrichment Analysis of the Tissue-Specific Gene Clusters were performed using g:Profiler (https://biit.cs.ut.ee/gprofiler/gost). The orthololegous ensemble_gene_id for rainbow trout of the gene clusters from Chinook salmon was used as identifier and rainbow trout was selected as the background dataset for the enrichment analysis. The g:Profiler uses Fisher's exact test to ascertain statistically significant enrichment of pathways amongst differentially expressed genes (DEGs) relative to the background transcriptome. For the purpose of the enrichment analysis, GO categories with a g:SCS threshold $P \leq 0.05$ were considered significant (Huang et al., 2009). Due to the lack of enriched GO pathways, DEGs were manually assigned a functional group based on the available function characterisation for further exploration.

5.3.8 Critical swimming speed

After 228 days under treatment, a total of 25 fish (target size = 1,500 g) from LFR and MFR were individually transferred to a swim tunnel and underwent a standard stepwise critical swimming protocol (Brett, 1964; Steffensen et al., 1984). Critical swimming speed (U_{crit}) was measured on 17 fish (fork length = 409.4 ± 3.4 mm; weight = 1460.8 ± 24.2 g; MFR=8; LFR=9) as some fish died during the habituation period (n = 3) or failed to swim (n = 5). The number of fish assessed across each tank was not equal and one tank from LFR was not represented. The critical swim test took place in one of two identical 180 L swim tunnel with a working section of 72 × 27 × 30 cm (length × width × depth). The flow was calibrated using a Hontzsch flow meter with a vane wheel (Waiblingen, Germany) in the working section of the tunnel to obtain a ten-point calibration ($r^2 = 0.99$). Solid blocking effects of the fish were corrected following Bell and Terhune (1970):

$$U_f = U_t(1 + \varepsilon_S), \quad (7)$$

where U_f is the corrected flow speed, U_t is the uncorrected flow speed and ε_s is the solid blocking fraction error. The solid blocking fraction error was calculated as:

$$\varepsilon_S = 0.8\lambda(A_O / A_T)^{0.5}, \quad (8)$$

where λ is the constant obtained for the animal shape (0.5*FL/body thickness), A_O is maximum cross section of animal, and A_T is the cross section of the swim chamber. Fish were fasted for 48 h before being transferred to the tunnel. Fish were allowed to acclimate overnight at 0.5 bl s^{-1} before commencing a U_{crit} test the following morning. Swimming speeds were increased incrementally by 0.25 bl s^{-1} every 30 minutes, until the fish reached fatigue. Fish were considered fatigued when they could no longer swim against the current and rested on the back gate for >5s. The speed and time at which the fish reached fatigue was recorded and U_{crit} was later calculated as:

$$U_{crit} = U_f + U_i T_f T_i^{-1} \quad (9)$$

where U_f is the highest speed maintained for the entire swimming period, U_i is the speed increment (cm s^{-1}), T_f is the time elapsed at fatigue speed, and T_i is the set interval time (min).

5.3.9 Statistical Analysis

Statistical analysis, data handling, and figures were produced using the R statistical language and lme4, MuMin, nnet, MASS, tidyverse, and ggplot2 packages. Models were assessed for normal distribution and equal variances through Q-Q plot and residual versus fitted plot. Data that failed to meet the model parameters were log-transformed. Comparisons between exercise treatments, through time, and/or between sides of the fish were evaluated using general

linear mixed effects model analysis. Models were selected using Akaike's information criterion (AIC) for the inclusion of predicator and fixed variables, where appropriate (Richards, 2005). Interactions between predicator and fixed variables were assessed and removed if not significant, in that case main effects in the model were assessed. Tank, fish identification and tissue regions were included as repeated or random effects, where appropriate, and removed if not significant. A $P < 0.05$ was used as the significance level for all statistical tests.

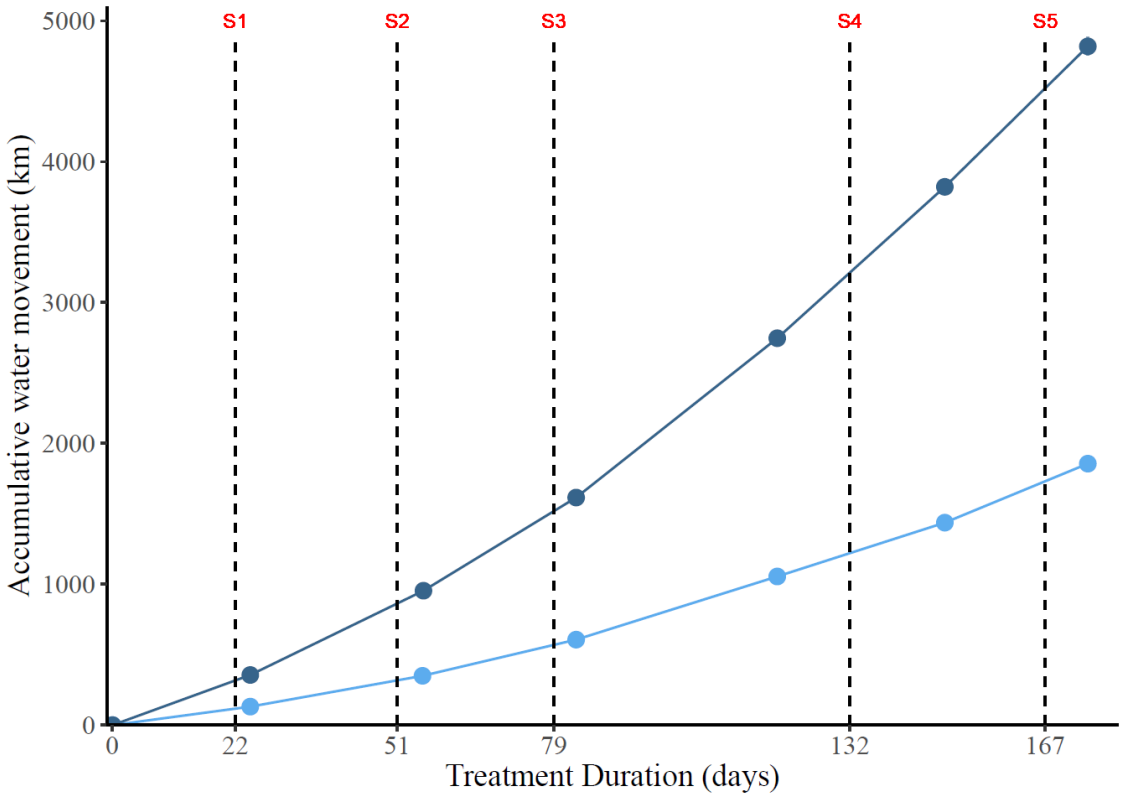


Figure 5.1 Accumulated water movement in the low (light blue) and moderate (dark blue) flow regimes throughout the experiment. Points represent the average accumulated water movement at the end of each month, as flow regimes were adjusted monthly. The sampling timepoints are depicted by vertical dashed lines and labelled from "S1" through to "S5".

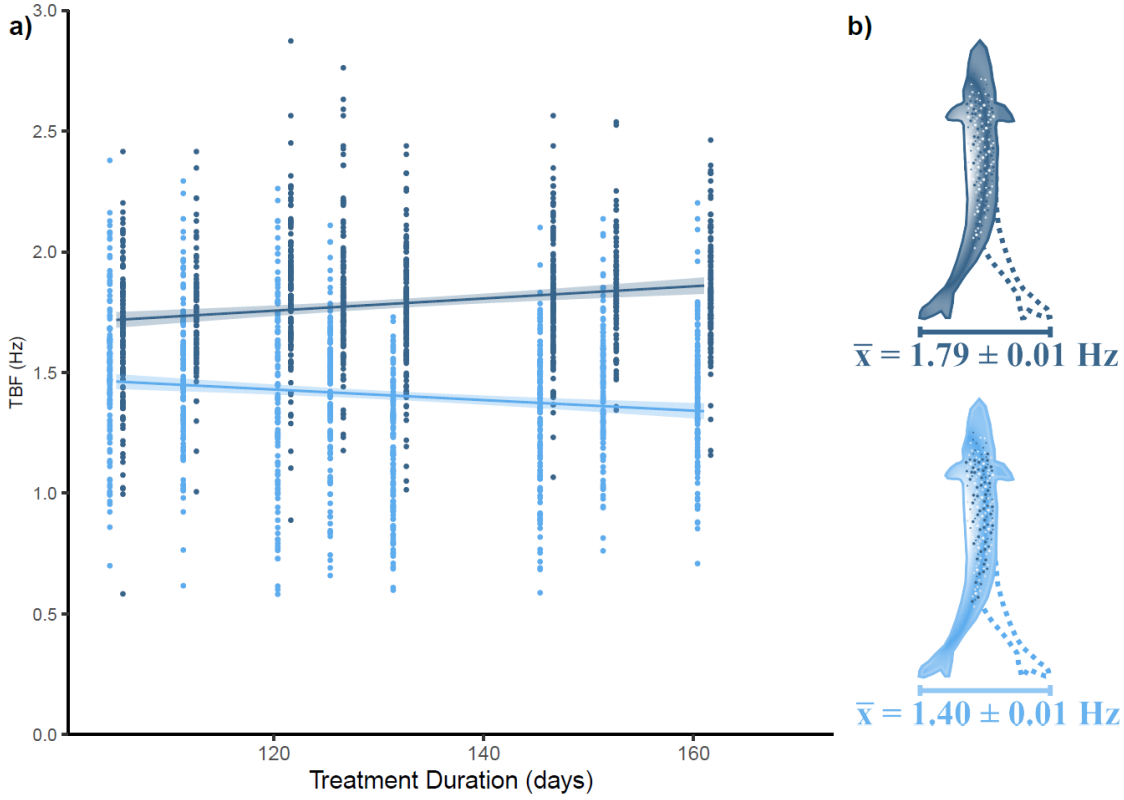


Figure 5.2 Tail beat frequency (TBF) a) as a function of treatment duration in Chinook salmon reared under low (light blue) and moderate (dark blue) flow regimes. Figure b) presents mean TBF of Chinook salmon reared under low (light blue) and moderate (dark blue) flow regimes. Points represent individual measurements, and the solid lines represent the linear relationship between TBF and treatment duration. $P < 0.05$ represents significantly different linear regression (TBF as a function of treatment duration) between flow regimes.

5.4 Results

5.4.1 Swimming effort

From 105 to 161 days, TBF was significantly higher in individuals reared under MFR compared to LFR (LMER; $F_{1,2066} = 45.57$, $P < 0.0001$; Figure 5.2) and the coefficient of variation for TBF was significantly lower in individuals reared under MFR compared to LFR (LMER; $F_{1,2060} = 46.82$, $P < 0.0001$).

5.4.2 Fish assessments

The M, FL, G, SGR, and FCR_t were all significantly different between exercise regimes across the experimental period (outlined in Table 5.1). Mass (LMER; $F_{1,6600} = 0.20$, $P < 0.0001$), FL (LMER; $F_{1,6570} = 45.97$, $P <0.0001$), and G (LMER; $F_{1,3464} = 3.89$, $P < 0.05$) were significantly greater in individuals reared under LFR compared to MFR as a function of time. This was most evident at the final assessment period (i.e., 167 days; Table 5.1). Condition factor did not differ between treatments as a function of time (LMER; $F_{1,6685} = 5.84$, $P < 0.05$; Table 5.1). Specific growth rate was significantly faster in individuals reared under LFR (LMER; $F_{1,6678} = 7.98$, $P < 0.01$; Table 5.1) as a function of time, but SFR_t was not different between exercise regimes (LMER; $F_{1,11} = 0.29$, $P > 0.05$) and significantly decreased with increasing time (LMER; $F_{1,313} = 449.5$, $P <0.0001$; Table 5.1). The tank based FCR was significantly greater after 167 days in individuals reared under MFR (LMER; $F_{5,48} = 2.54$, $P < 0.05$; Table 5.1).

Relative tissue weights and somatic indices for viscera, heart, liver, and gonad did not differ between exercise regimes ($P > 0.05$; metrics are presented in Table A.6). Belly-flap thickness at the pectoral, pelvic, and anal locations were not different between exercise regimes ($P > 0.05$; Table A.6). Hematocrit was also not different between exercise regimes ($P > 0.05$; Table A.6).

Table 5.1 Long-term tank performance[1] of Chinook salmon under low and moderate flow regimes.

Treatment	n	M (g)	FL (mm)	G (mm)	K	SGR (% day^{-1})	SFR_t (% day^{-1})	FCR_t
Low Flow Regime								
Pre-trial	1587	83.02 ± 0.43	174.47 ± 0.24		1.54 ± 0.00			
22 days	1586	229.71 ± 1.11	234.49 ± 0.32		1.76 ± 0.00	2.42 ± 0.01	2.37 ± 0.03	1.00 ± 0.01
51 days	1582	397.12 ± 1.93	271.42 ± 0.37	199.87 ± 0.40	1.96 ± 0.00	1.96 ± 0.01	2.10 ± 0.03	1.02 ± 0.01
79 days	1494	591.52 ± 3.07	305.57 ± 0.44	229.72 ± 0.50	2.05 ± 0.00	1.42 ± 0.00	1.72 ± 0.03	1.22 ± 0.01
132 days	132	940.87 ± 10.79	356.66 ± 1.27	264.49 ± 1.21	2.07 ± 0.01	0.86 ± 0.01	1.15 ± 0.03	1.34 ± 0.02
167 days	130	1112.42 ± 14.73	378.49 ± 1.49	282.99 ± 1.44	2.04 ± 0.01	0.47 ± 0.02	0.95 ± 0.03	1.64 ± 0.06[a]
Moderate Flow Regime								
Pre-trial	1597	82.72 ± 0.41	174.66 ± 0.23		1.53 ± 0.00			
22 days	1590	226.45 ± 1.09	233.58 ± 0.32		1.76 ± 0.00	2.40 ± 0.0 1	2.36 ± 0.02	1.00 ± 0.01
51 days	1575	383.89 ± 1.89	269.44 ± 0.37	197.70 ± 0.39	1.94 ± 0.00	1.88 ± 0.01	2.08 ± 0.03	1.05 ± 0.01
79 days	1492	574.95 ± 2.98	303.06 ± 0.43	227.50 ± 0.48	2.04 ± 0.00	1.42 ± 0.00	1.68 ± 0.03	1.20 ± 0.01
132 days	134	872.81 ± 11.61	348.57 ± 1.21	258.92 ± 1.55	2.05 ± 0.01	0.79 ± 0.02	1.11 ± 0.03	1.36 ± 0.02
167 days	131	999.97 ± 15.30	369.68 ± 1.40	272.90 ± 1.85	1.96 ± 0.01	0.34 ± 0.02	0.81 ± 0.03	1.91 ± 0.13[b]
P-value		***P < 0.01***	***P < 0.0001***	***P < 0.05***	*P > 0.05*	***P < 0.01***	*P > 0.05*	*P < 0.05*

Values are means ± S.E.M.

Significance level = $P < 0.05$; bolded *P*-values indicate significant linear regression.
Letters indicate significant differences between experimental treatments and within sampling events.
[1] Performance: n = sample size; M = mass; FL = fork length; G = girth; K = condition factor; SGR = specific growth rate; SFR_t = tank specific feed rate; FCR_t = tank feed conversion ratio.

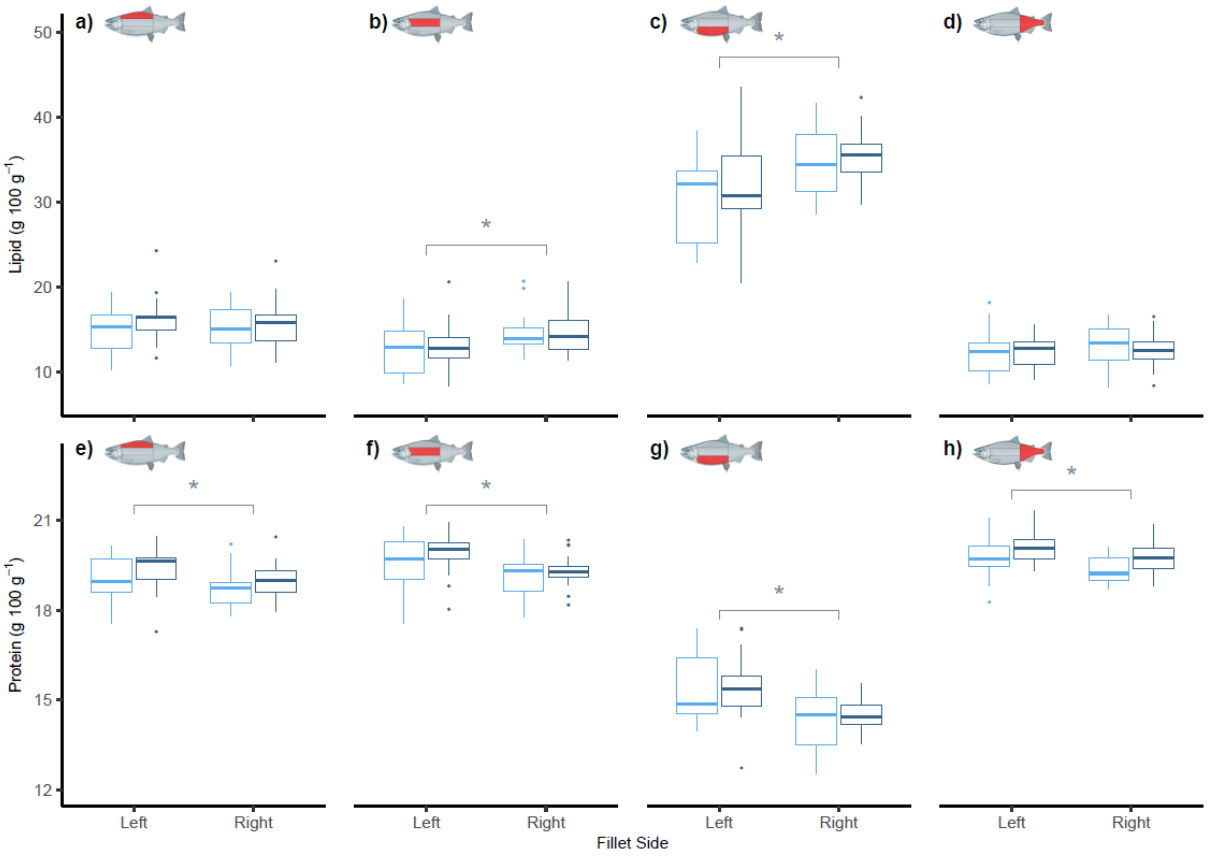


Figure 5.3 Lipid and protein composition across four regions of the left and right fillet in Chinook salmon reared under low (light blue) and moderate (dark blue) flow regimes for 167 days. Lipid composition in the dorsal a), lateral b), visceral c), and posterior dorsal d) regions of the left and right fillet. Protein composition in the dorsal e), lateral f), visceral g), and posterior dorsal h) regions of the left and right fillet. Red shading on the insert figure of Chinook salmon depicts location of nutrient composition assessed. Boxplots present the median (middle bar), first and third quartiles (upper and lower bars), the largest and smallest value within 1.5 interquartile range (IQR; vertical bars) and outliers (i.e., > third quartile + 1.5*IQR, < first quartile + 1.5* IQR; points). Brackets and asterisks represent significant differences ($P < 0.05$) between left and right fillets.*

5.4.3 Chemical composition

The chemical composition of fish reared under LFR and MFR are outlined in Table A.7. Lipid composition did not differ between exercise regimes in the liver (LM; $F_{1,0.11} = 2.21$, $P > 0.05$), viscera (LM; $F_{1,20.83} = 0.24$, $P > 0.05$), fillet (LM; $F_{1,159.4} = 2.9$, $P > 0.05$), or across the whole body (LM; $F_{1,0.2345} = 0.08$, $P > 0.05$). In the fillet, lipid composition was not different between the left and right sides of the fish (LM; $F_{1,20.3} = 2.61$, $P > 0.05$), except when comparing lateral and visceral fillet regions (Figure 5.3b,c). Protein composition was not different between exercise regimes in the fillets (LM; $F_{1,0.21} = 0.49$, $P > 0.05$) or across the whole body (LM; $F_{1,0} = 0.0018$, $P > 0.05$), but was significantly greater in the left fillet compared to the right (LM; $F_{1,2.78} = 6.47$, $P < 0.05$; Figure 5.3e-h). Ash and moisture were also not different between exercise regimes in the fillets (LM; $F_{1,0} = 0.61$, $P > 0.05$, LM; $F_{1,8.75} = 2.18$, $P > 0.05$, respectively) or across the whole body (LM; $F_{1,0} = 22.87$, $P > 0.05$, LM; $F_{1,3.47} = 1.57$, $P > 0.05$, respectively).

5.4.4 Muscle, gill, and skin morphometrics

White muscle fibre size did not significantly differ between exercise regimes (LMER; $F_{1,32.6} = 2.48$, $P > 0.05$), but did show a significant interaction between mass and fillet side (LMER; $F_{1,27405} = 11.79$, $P < 0.001$; Figure A.2), where muscle fibres on the left fillet were significantly greater than muscle fibres on the right fillet. Similarly, white muscle fibre density did not significantly differ between exercise regimes (LMER; $F_{1,33} = 0.86$, $P > 0.05$), but was significant greater in the right fillet with respect to mass compared to the left fillet (LMER; $F_{1,178} = 4.70$, $P < 0.05$; Figure A.2).

Gill morphology was unaffected by LFR and MFR. Lamellae length (LMER; $F_{1,34} = 0.0288$, $P = 0.8661$), distance between lamellae (LMER; $F_{1,34} = 0.0271$, $P = 0.8703$), filament thickness (LMER; $F_{1,34} = 0.7588$, $P = 0.3898$) and diffusion distance (LMER; $F_{1,34} = 0.276$, $P = 0.6028$) were not different between exercise regimes. Similarly, skin epidermis (LMER; $F_{1,34} = 1.1943$, $P = 0.2821$) and dermis (LMER; $F_{1,34} = 2.0188$, $P = 0.1645$) did not differ between exercise regimes.

5.4.5 Transcriptomics

A total of 38,160 transcripts were yielded after quality control. Of these, 575 were differentially expressed transcripts (DETs) between Chinook salmon reared under MFR and reared under LFR, 420 were DETs between the right fillet and left fillet in the LFR, and 271 were DETs between the right fillet and left fillet in the MFR. A total of 221 DETs were upregulated and 354 DETs were downregulated in Chinook salmon reared under MFR compared to LFR, a total of 308 DETs were upregulated and 112 DETs were down regulated in the right fillet

compared to the left in the LFR, and a total of 191 DETs were upregulated and 80 DETs were downregulated in the right fillet compared to the left fillet in the MFR.

The number of DEGs potentially involved in different functional groups are presented in Figure 5.4. Chinook salmon reared under MFR had more DEGs upregulated in lipid metabolism, muscle development and contraction, muscle repair and maintenance, and apoptosis than downregulated DEGs in the respective functional group, and more DEGs were downregulated in circulatory system development, collagen, stress response, cell signalling, and cytoskeleton processes then upregulated in the respective function group. In the right fillet, most functional groups had more upregulated DEGs than downregulated, except for DEGs involved in muscle repair and maintenance but only in Chinook salmon reared under MFR.

The log fold change for DEGs involved in lipid metabolism, muscle development and contraction, collagen, and immune system processes for each comparison group (i.e., MFR vs. LFR, LFR: right fillet vs. left fillet, and MFR: right fillet vs. left fillet) is presented in Figure 5.5. Within these functional groups, more DEGs were found when comparing flow regimes compared to lateral comparisons of either flow regime. The DEGs in lipid metabolism and muscle development and contraction typically had higher log fold change and were mostly consistent across three comparison groups. Similarly, DEGs relating to collagen and immune system response had a mix of high and low log fold changes and also responded consistently across the three comparison groups.

Of the DEGs genes, only four were exclusively upregulated and seven were exclusively downregulated in the MFR compared to the LFR irrespective of the lateral aspect, and eight were exclusively downregulated and 52 were exclusively upregulated in the right fillet compared to the left fillet irrespective of the flow regimes (Figure 5.6). The four DEGs that were exclusively upregulated in the MFR compared to the LFR irrespective of the lateral aspect are involved in immune system responses, transcription, cellular processes, and cytoskeleton, while the seven DEGs that were downregulated are involved in immune system responses, stress response, transcription, and cell signalling. The 52 DEGs that were exclusively upregulated in the right fillet compared to the left fillet irrespective of the flow regimes are involved in several functional groups, but most are involved in transcription, cell signalling, and cellular processes. The eight that were exclusively downregulated in the right fillet compared to the left fillet irrespective of flow regime are involved in lipid metabolism, circulatory system development, cell signalling, and cellular processes.

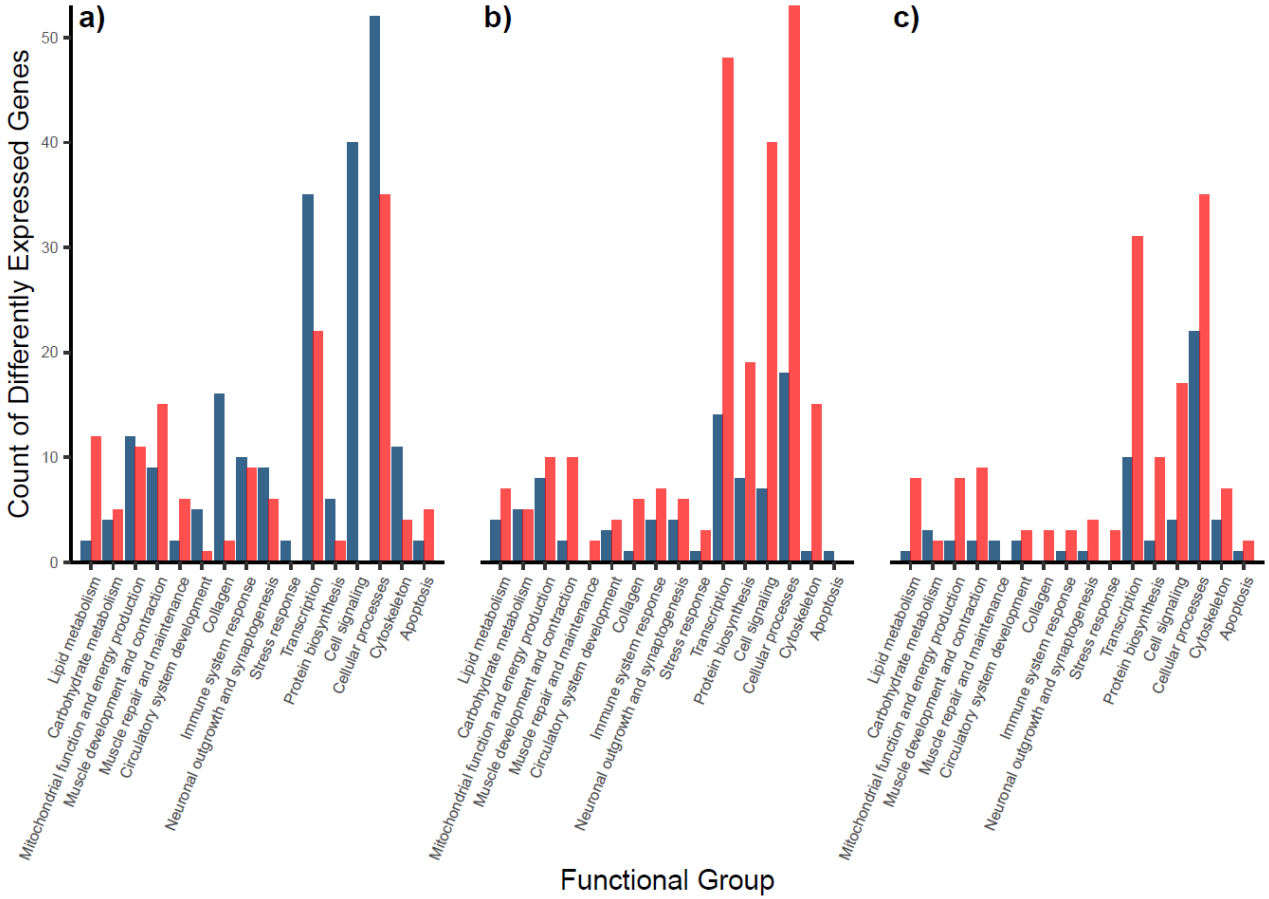


Figure 5.4 Counts of differently expressed genes in functional groups from white muscle tissue of Chinook salmon reared under moderate flow regime (MFR) and low flow regime (LFR). Panel a) presents counts in MFR compared to LFR, panel b) and c) presents counts in the right fillet compared to the left fillet from LFR and MFR, respectively. Blue bars represent the number of downregulated genes and red bars represent the number of upregulated genes.

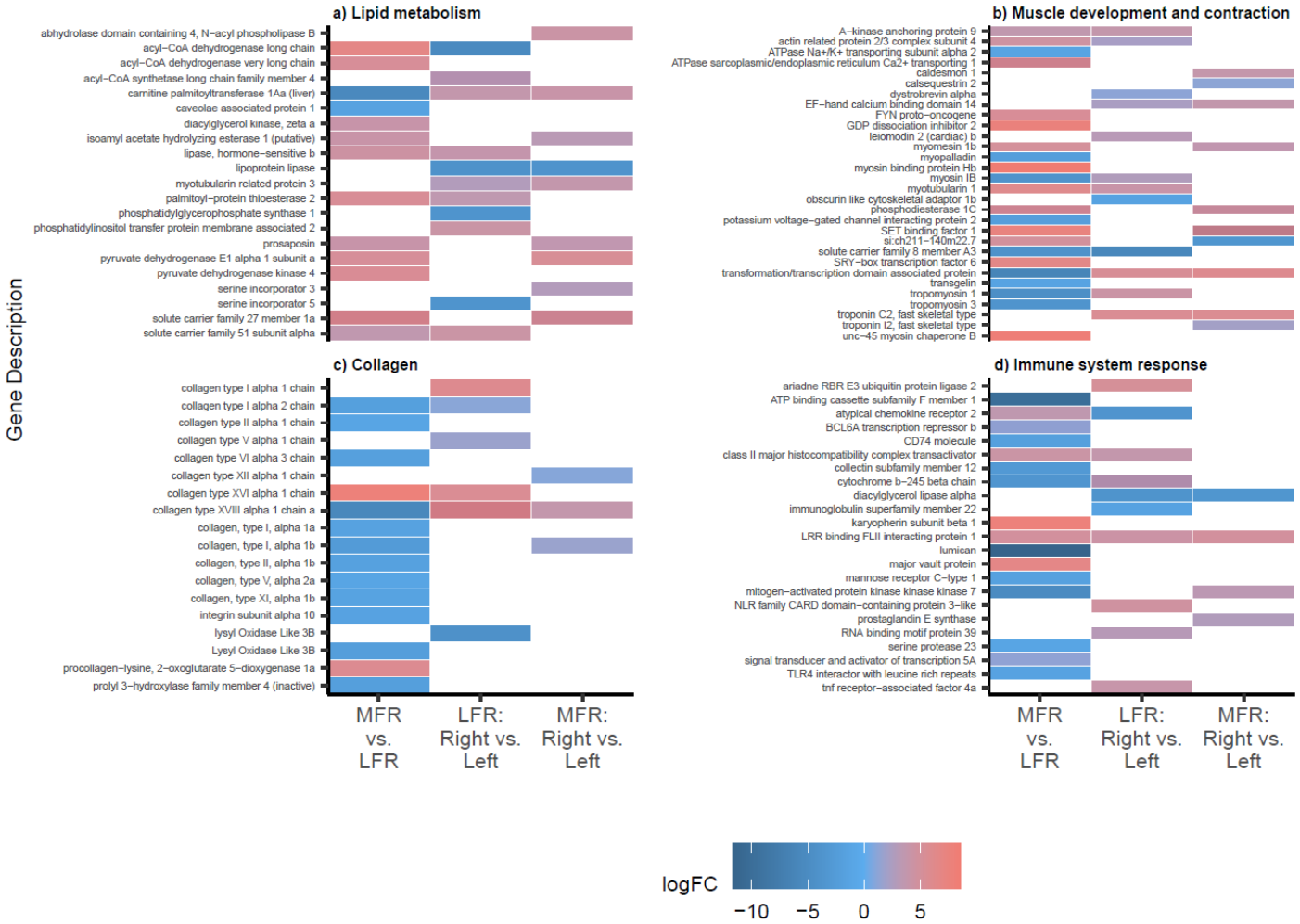


Figure 5.5 Log fold changes of significantly differentially expressed genes from white muscle tissue of Chinook salmon reared under moderate flow regime (MFR) and low flow regime (LFR). Panels present genes involved in selected functional groups: a) lipid metabolism, b) muscle development and contraction, c) collagen, and d) immune system response for each comparison group (MFR vs. LFR, and right fillet vs left fillet for LFR and MFR).

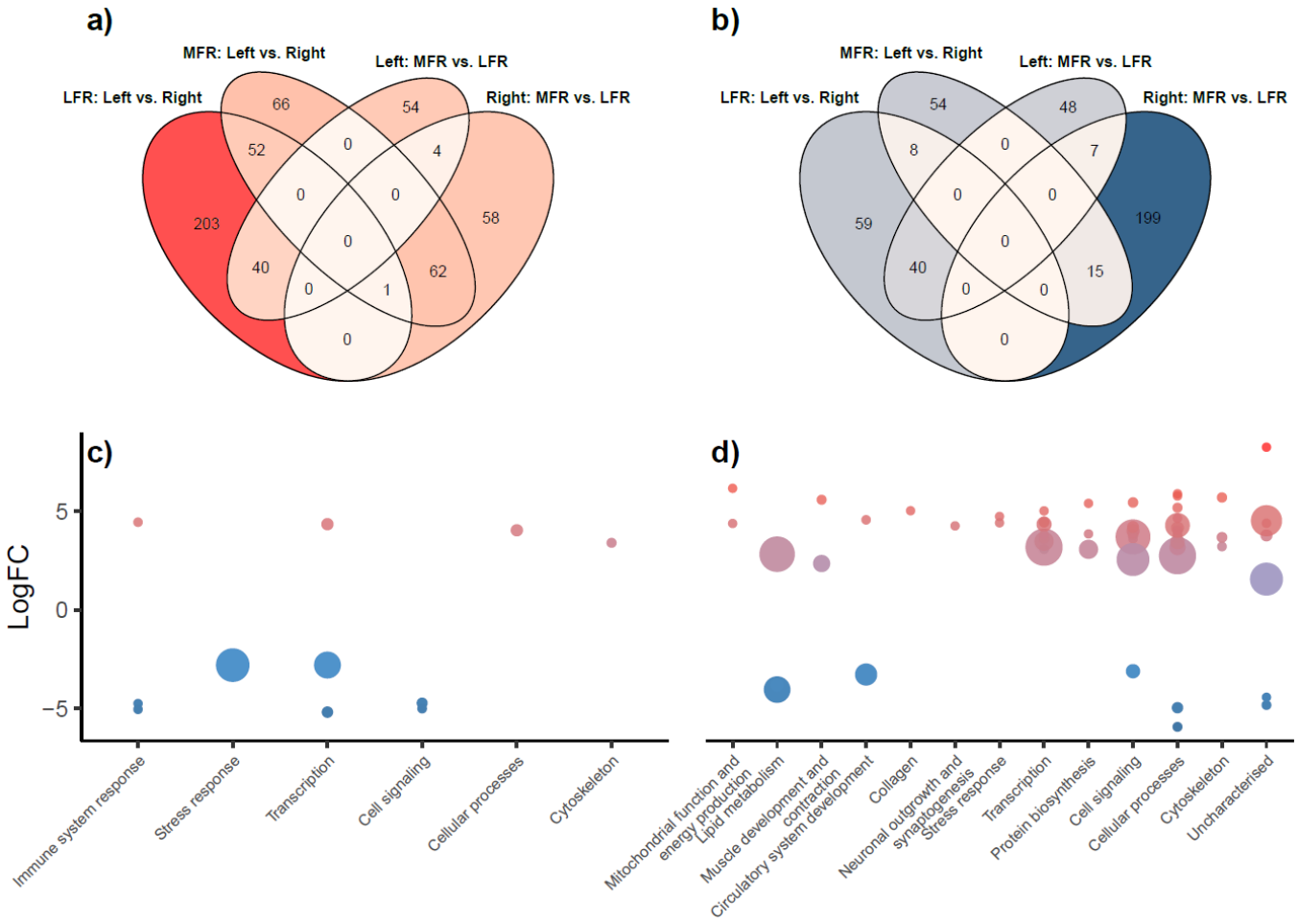


Figure 5.6 Venn diagrams and Manhattan plots of significantly differentially expressed genes (DEGs) from white muscle tissue of Chinook salmon reared under moderate flow regime (MFR) and low flow regime (LFR). Venn diagrams show common and unique a) upregulated and b) downregulated DEGs. The log fold change of unique DEGs when comparing c) MFR with LFR and d) right fillet with left fillet and their assigned functional groups. Red circles present upregulated DEGs and blue circles present downregulated DEGs. The circle size scales with the level of P-value significance (small = 0.01 – large = 0.04).

5.4.6 Swimming performance

The critical swimming speed did not significantly differ between exercise regimes (LM; $F_{1,15} = 2.64$, $P = 0.125$), but was slightly higher in Chinook salmon reared under MFR ($U_{crit} = 2.15 \pm 0.14$ bl s^{-1}) compared to Chinook salmon reared under LFR ($U_{crit} = 1.76 \pm 0.19$ bl s^{-1}).

5.5 Discussion

There is a considerable knowledge gap regarding the effects of long-term exercise regimes on larger individuals for longer durations and how exercise-training salmon in circular tanks can impact their muscle physiology and symmetry. Yet, this information is pivotal for the integration of flow regimes in aquaculture settings to improve production performance in the larger individuals that are now being transferred to sea. This study found production performance to be significantly better in individuals reared under LFR compared to those reared under MFR for six months. These responses were not in accordance with changes in chemical composition or muscle morphology and were not matched with an upregulation in growth markers in the white muscle transcriptome. Interestingly, differences in the chemical composition, muscle morphology, and gene expression were evident in the white muscle between the left and right fillets, suggesting that continuous circular swimming stimulates muscle blocks unevenly, leading to lateral asymmetry.

5.5.1 Long-term animal performance and physical and biochemical properties of white muscle in response to low and moderate flow regimes

There are limited studies investigating how exercise training influences production performance in larger fish (final size < 500 g) for longer durations (< five months), and whether there is a size limitation for exercise-enhanced growth (Rodgers and Gomez Isaza, 2023). Flow regimes used within this experiment were matched to the recommended exercise levels to promote production-related traits in salmonid species (Davison, 1997; Palstra and Planas, 2011; Davison and Herbert, 2013), but were maintained for longer and with larger fish than typically used (Davison and Herbert, 2013; Rodgers and Gomez Isaza, 2023). This study found that after 167 days under treatment, Chinook salmon reared under LFR were larger and grew faster, which led to improvements in feed efficiency (tank level comparison), providing no evidence of exercise-enhanced production performance in larger salmon. These results disagree with previous exercise studies: Atlantic salmon of similar size (initial sizes: ~800 g and ~2000 g) exercise trained for six and eight months exhibited exercise-enhanced growth (Totland et al., 1987; Nilsen et al., 2019); smaller Chinook salmon (< 600 g) exercise-trained for three months exhibited

improved feed consumption-growth relationships and FCR (Prescott et al., 2024). However, in Totland et al. (1987) and Nilsen et al. (2019), raceways and closed containment systems were used, respectively, both of which were equipped with booster pumps to generate the target flow. These differing setups could be contributing to the inconsistent results found in the current experiment. Nonetheless, these studies suggest that larger Chinook salmon may respond differently to exercise in comparison to other salmonids and that the optimal flow regime for smaller salmon is not easily scaled to larger sizes (1000 g in this study). Exercise-regimes may need to be optimised separately for larger individuals.

Further physical and morphological measurements presented minimal differences between Chinook salmon reared under LFR and MFR: chemical composition (i.e., lipid and protein content) was comparable across several tissues (i.e., liver, viscera, fillet, and whole body); histological assessment of the white muscle morphology showed no responses to different exercise levels. Typically, salmon held under substantial training regimes (moderate to high speeds) lead to decreases in whole-body lipid content (Bugeon et al., 2003; Simpkins et al., 2003a, 2003b; Rasmussen et al., 2011), while protein content remains tightly regulated (Nahhas et al., 1982b; Totland et al., 1987; Kiessling et al., 1994; Rasmussen et al., 2011; Grisdale-Helland et al., 2013). Reduced lipid content was documented in larger Chinook salmon, from the same group of fish used in this study, that were exercise-trained for longer (10-11 months; Prescott et al., 2023), and not in smaller size ranges (< 600 g) (Kiessling et al., 2005; Prescott et al., 2024). Similarly, changes in white muscle morphology through hypertrophic processes is a common response when salmonids are exercise trained (Bugeon et al., 2003; Rasmussen et al., 2011; Palstra et al., 2014; Timmerhaus et al., 2021), which is thought to underpin increases in muscle mass. For Chinook salmon, this change has not been reported, where only increases in muscle fibre density have been observed (hyperplasia), which was linked to superior swimming abilities (Prescott et al., 2023).

The transcriptome showed changes in gene regulation between the two flow regimes, where genes involved in lipid metabolism, muscle development and contraction, muscle repair and maintenance, and apoptosis were upregulated in Chinook salmon reared under MFR. Previous exercise studies with Atlantic salmon, rainbow trout, and zebrafish observed an upregulation in growth and insulin-like growth factor hormones, as well as markers in muscle contraction and development, carbohydrate and lipid metabolism (Palstra et al., 2010; Felip et al., 2012; Magnoni et al., 2013; Huang et al., 2021; Pengam et al., 2021; Timmerhaus et al., 2021). These responses were associated with individuals presenting exercise-enhanced growth and is described as a protein-sparing mechanism leading to increased muscle mass (Palstra et al., 2010; Magnoni et al., 2013; Huang et al., 2021; Timmerhaus et al., 2021). In the current experiment, no growth-related genes were differently expressed in either flow regime, but myosin

and actin genes that are involved in muscle contraction and development were common with genes upregulated with exercise in Magnoni et al. (2013) and Palstra et al. (2020). Markers of carbohydrate metabolism and protein biosynthesis were found to respond similarly between the two flow regimes. In previous studies, genes involved in carbohydrate metabolism and protein biosynthesis have been documented to increase (Magnoni et al., 2013; Planas et al., 2013); however, these responses are more pronounced in the red muscle tissue and to a lesser extent in the white muscle tissue. Red muscle was not assessed in this study. The upregulation of lipid metabolism can be contributed as a direct effect of greater swimming effort depicted by higher TBF. These results alongside those from previous exercise studies presents an interesting comparison, especially since changes in physical parameters were not observed. This information suggests that lipid metabolism may not be directly involved with increased muscle growth as is often observed, but rather provides an energy dense source to sustain muscle function while swimming.

A different group of gene markers that are now being recognised as key players in muscle development are those involved in inflammatory responses. In mammalian species, muscle inflammation is recognised as a key parameter involved in muscle growth (Yang and Hu, 2018), and this model has been described in fish (Johnston et al., 2009), including fish exhibiting exercise-enhanced growth (Magnoni et al., 2013; Palstra et al., 2020; Timmerhaus et al., 2021). In adult zebrafish with different growth trajectories (i.e., recruitment vs. enlarging fibres), genes in immunity and energy metabolism were upregulated in the individuals that were enlarging their muscle fibre size, i.e., hypertrophy (Johnston et al., 2009). In the current study, many markers involved in immune responses, in particular inflammation, were up- and down-regulated, but no distinct pattern could be contributed to either flow regime, or changes in growth. Chemokin genes were found to upregulate in this study and in exercised rainbow trout and sea bream, but major histocompatibility complex genes presented opposite effects (Magnoni et al., 2013; Palstra et al., 2020). Even though Chinook salmon reared under LFR had significantly improved production performance, the numerical differences were small, and considering how similar the whole-body protein content was between these two groups and that muscle morphology was not different, it would be difficult to explain patterns in immune response if found. As such, these results are in support of inflammatory gene markers being involved in muscle growth, and that the presence of inflammatory markers are not a direct indication of compromised health with increased swimming speeds.

The physical and biochemical aspects explored in the current experiment identifies a critical time component when moderately exercising Chinook salmon. Chinook salmon, from the same group of fish used in the current experiment, were exercised longer (10-11 months) and showed significant changes in the physical condition (Prescott et al., 2023). However, the

transcriptome was not investigated in that study (Prescott et al., 2023). The physical changes that occurred with longer exposure to exercise are consistent with some of the transcriptomic changes observed in the current experiment, which may serve as predictors for the longer term changes observed by Prescott et al. (2023). In particular, the current experiment found an upregulation of genes involved in muscle development and contraction in Chinook salmon reared under MFR, similar to findings in previous exercise studies (van der Meulen et al., 2006; Magnoni et al., 2013). This upregulation may indicate improvements in muscle function to support sustained swimming. The U_{crit} in exercise-trained Chinook salmon is faster though not significantly different after six months (in the current study), but is significantly faster after ten months (Prescott et al., 2023). These differences were reflected in their physiology, where they had lower visceral lipid content and denser white muscle fibres: physiological changes that were not described after six months in the current experiment. Together, these studies demonstrate how traits (e.g., production performance, swimming performance) and physical parameters (e.g., chemical composition and muscle morphology) respond differently with increasing exercise duration in larger fish, and that the transcriptome could be a valuable tool to predict the performance and condition of aquaculture fish.

5.5.2 Long-term animal performance and physical and biochemical properties of white muscle in response to continuous circular swimming

White and red muscles support swimming through the shortening and contracting of myotomes (muscle fibres) to oscillate the caudal fin and propel the fish forward (Videler, 1981; Videler, 1993a). Under exercise and higher swimming speeds, this mechanism increases in pace and activates more fibres along the body (Alfonso et al., 2021; Hachim et al., 2021), leading to functional and structural changes of the white muscle (Anttila et al., 2006; Anttila et al., 2008; McKenzie et al., 2012; Prescott et al., 2023). When swimming in circles or maintaining position against a circular flow, muscle blocks on one side of the fish could be activating more than the other (see Graphical Abstract 1). This could explain the lateral differences in the physical properties of white muscles found in this study. The lipid content was significantly higher in the right fillet (outward facing fillet; lateral and visceral fillet regions) and protein content was significantly higher across all regions of the left fillet (inward facing fillet). White muscle fibres were smaller but denser in the right fillet compared to the left fillet. Both responses indicate that the left and right muscle blocks were stimulated unevenly when swimming in circles. When the Chinook salmon in this study were exercised for longer under LFR and MFR (Chapter 6; Prescott et al., 2023) lateral differences in chemical composition and morphology were also observed. In other finfish species, lateral asymmetry in muscle block sizes were reported, which were linked

to the turning preference in zebrafish (Heuts, 1999) and in largemouth bass, *Micropterus salmoides* (Nakajima et al., 2007), suggesting muscle asymmetry could be common across fishes.

In this study, the fish were reared in circular tanks with a clockwise flow regime and were continuously turning towards the left when swimming. This promoted their body to position in a reverse c-shape, therefore only allowing half of a caudal fin oscillation to occur and propel the fish forwards, i.e., through contracting the right (outward facing fillet) muscle blocks. Under these conditions the right muscle blocks are presumed to be working harder than the left muscle blocks to support swimming, with the right muscle blocks being the primary driver for generating thrust. Therefore, based on the hypothesis that continuous circular swimming excites the inward and outward muscle blocks differently, it would be expected that the fillet transcriptomes would mirror these differences and the right fillet would show similar gene expression patterns as to what is shown in individuals reared under MFR. In agreement, most DEGs involved in lipid metabolism and muscle development and contraction were upregulated in the right fillet compared to the left in individuals from both flow regimes, as also found in individuals reared under MFR compared to LFR individuals. The upregulation of lipid and muscle function genes are comparable to the transcriptomic changes shown in individuals reared under MFR that were exhibiting greater swimming effort, further supporting this hypothesis. This explanation further supports the findings of increased muscle fibre density in the right muscle blocks, when these fish were exercised for an additional three months (Prescott et al., 2023). It should be noted that these lateral changes may be an artefact of small tank sizes and may not be representative of commercial production tanks and sea pens, therefore their relevance to industry remains in question.

Genes from other functional groups were upregulated in the right muscle blocks in comparison to the left muscle blocks. For example, irrespective of flow regime, most immune related genes were upregulated in the right fillet, which had lower protein content and hyperplasia growth trajectories. This is opposite to the findings in Johnston et al. (2009), who showed links between immune-related genes and hypertrophy, and previous studies that linked inflammatory markers with exercise-enhanced growth and thus hypertrophy (Magnoni et al., 2013; Palstra et al., 2020; Timmerhaus et al., 2021). Several genes involved in collagen, cellular processes, transcription, and biosynthesis were upregulated in the right fillet compared to the left, and opposite to the responses found in individuals reared under MFR when the fillet results were combined. These genes could also be related to the morphological changes described in the right muscle fibres. As the right muscle increased in protein content probably through increased fibre density, it would be expected that increases in other essential components of the muscle would occur simultaneously, such as collagen and the extracellular matrix (Planas et al., 2013).

Together, these transcriptomic changes provide some insight into the phenotypic responses of exercise-training fish in circular tanks.

5.5.3 Future directions and considerations

Future studies investigating the growth-promoting effects of exercise should continue including 'omic approaches to determine the relationships between muscle physiology and kinematics. To date, transcriptomic studies, including the current one, have provided valuable and detailed information on how exercise-training shapes muscle and fish, which could help advance the industry. In the current experiment, few significant gene ontology pathways were found. This is likely because salmon are evolutionarily exceptional swimmers (Eliason and Farrell, 2016), and the two exercise treatments implemented may not be impactful enough given their innate physiology. Thus, some caution must be taken when interpreting the assigned functional groups for common patterns in DEGs. Furthermore, it is urged that future studies should also consider the impact of circular swimming on muscle symmetry to gauge the extent of this condition. Comparing individuals swimming clockwise, anti-clockwise, and in a straight direction can help determine if lateral asymmetry is linked to programmed lateralisation or the experimental setups used.

5.5.4 Conclusion

Unlocking the potential of using exercise training to enhance farmed fish production, health, and welfare is pivotal for industry growth. Various techniques across several layers of biological organisation were used to better understand the influence of exercise training for six months in larger Chinook salmon. Optimal flow regimes that are recommended for smaller salmon did not scale with larger salmon and further attention is needed to define the optimal flow regime for larger salmon and those nearing harvest size. Investigations into the transcriptome supports the hypothesis that inflammation plays a key role in muscle growth, and that this field requires more attention. This research also revealed lateral asymmetry in muscle composition, morphology, and the transcriptome, which is likely caused by continuous swimming in circular tanks, but the relevance to industry settings remains in question.

Chapter Six Long-term sustained swimming improves swimming performance in Chinook salmon, *Oncorhynchus tshawytscha,* with and without spinal scoliosis

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6.1 Abstract

Exercise training during pre-and post-smolt production is becoming a key component in salmon hatcheries as it is known to enhance several production-related traits in salmonids. Exercise conditions for rearing salmonids are continually being optimised and now that the salmonid industry is developing offshore, training is being considered as a tool to prepare domestic stocks for high energy environments. It is unknown if exercise can enhance traits in other understudied salmonid species and in individuals with spinal curvature (scoliosis), which is a common health concern within some salmon farms. Chinook salmon (initial weight: 82.9 ± 0.30 g) were exposed to low (0.3 bl s^{-1}) and moderate (0.8 bl s^{-1}) flow regimes for ten to eleven months and quantified aerobic capacity and swimming performance in individuals with and without mild scoliosis. Further, compositional changes and morphological responses at cellular and whole-body levels were investigated. Raising Chinook salmon under moderate flow regimes improved critical swimming speed, maximum metabolic rate, and aerobic scope in individuals with and without spinal curvature, but recovery processes (exhaustive exercise post oxygen consumption and time) in individuals with spinal curvature required higher energetic costs (measured immediately after reaching critical swimming speed). Fat content was reduced in fish raised under moderate flow regimes, while protein content was higher in individuals with spinal curvature. Exercise regimes caused morphological changes in muscle fibres, gill, and skin. Together, the results of this study show benefits for integrating exercise training into hatchery settings (i.e., pre- and post-smolts) to prepare stocks for offshore farming and provides evidence that some exercise-enhanced traits can be translated into individuals with spinal curvature, but concerns remain for individuals with more severe spinal curvature. Additionally, this study reveals that exercise regimes influence nutrient utilisation and deposition in Chinook salmon, therefore optimising nutrient profiles for offshore feeds should be considered as nutrient demands

in fish farmed in high energy sites may differ to nutrient demands of fish in lower energy nearshore farms.

6.2 Introduction

Rearing post-smolts in tanks for longer periods and transferring larger salmon to sea pens offshore is becoming an important component of modern salmon aquaculture practice (Buck and Langan, 2017; Gentry et al., 2017; Gorle et al., 2018). This change has partly stemmed from traditional nearshore sites becoming limited in terms of obtaining new farming licenses and space, while offshore open ocean sites provide larger areas, deeper waters, and improved water quality (Di Trapani et al., 2014; Froehlich et al., 2017; Envirostrat, 2020; Morro et al., 2021). Open ocean farming, theoretically, reduces human impact and user competition (Chu et al., 2020; Hvas et al., 2020), meaning farming offshore should improve industry development. Therefore, the integration between on-land tank-based systems and offshore farming provides an opportunity for extensive industry expansion, not only for the salmonid industry but across the breadth of aquaculture (Buck and Langan, 2017). However, offshore environments do present their own array of challenges including the high energy hydrodynamic conditions (high water speeds >1m s^{-1}, wave heights up to 5m), especially when compared to farms in protected fjords and coastal bays (Danielssen et al., 1997; Johansson et al., 2007; Oppedal et al., 2011). Therefore, it is of interest to understand how salmon will respond to these high energy environments.

Through salmon domestication and selective breeding, swimming characteristics (e.g., critical swimming speed, prolonged swimming speeds, and endurance), such that are found in wild salmon strains (i.e., river migrations to and from sea), are thought to be compromised (Zhang et al., 2016). This is of concern for the industry as they move towards farming salmon further offshore in high energy sites. Many studies over the past decades have focused on whether training salmon (predominantly Atlantic salmon, *Salmo salar* and rainbow trout, *Oncorhynchus mykiss*) in tank settings (forced swimming against water currents) can improve swimming characteristics in farmed stocks (Farrell et al., 1990; Holk and Lykkeboe, 1998; McKenzie et al., 2012; Zhang et al., 2016) and improve overall production efficiency and resilience in preparation for offshore farming environments. Exercise training regimes during post-smolt stages have shown significant improvements in several key phenotypes of farmed salmon. For instance, training programs, as short as 6 weeks, including a daily sprint (e.g., 30 s exhaustive chase) or prolonged sustained swimming protocols, increase preferred-, optimal-, and critical (U_{crit}) swimming speeds as well as stamina and recovery (Brett et al., 1958; Gamperl et al., 1991; McKenzie et al., 2012). Production-related traits such as, growth rates, feed efficiency, and fillet quality all improved under low to moderate training levels (Palstra and Planas, 2011; Davison and Herbert, 2013; Timmerhaus et al., 2021), as well as fish welfare through increased stress

tolerance and disease resilience (Woodward and Smith, 1984; Castro et al., 2011; Ytrestoyl et al., 2020).

Mechanisms underpinning these training-improved phenotypes occur at tissue and cellular levels within the respiratory, circulatory, and cardiac systems. Adjustments include, increased heart size, shape, and function, increased blood-oxygen uptake, transport, and delivery properties, enhanced gene regulation, elevated expression in oxidative enzymes, as well as increased muscle capillarization and enlarged fibre morphometrics (Zbanyszek and Smith, 1984; Davie et al., 1986; Gallaugher et al., 2001; Timmerhaus et al., 2021). Additionally, changes in chemical composition (e.g., increased protein and reduced fat content) through increased lipid usage and more drag efficient body shapes are a result of aerobic exercise training, which simultaneously enhances swimming performance and growth efficiency (Pakkasmaa and Piironen, 2000; Rasmussen et al., 2011; Yu et al., 2022). It is evident that salmon have remarkable phenotypic plasticity (i.e., blood-oxygen transport properties, tissue remodelling capabilities) in response to varying environmental cues, but understanding as to how these responses will apply to other commercially important salmonid species and scale across domesticated populations are limited.

Furthermore, previous studies investigating exercise-enhanced traits focused on populations of healthy non-deformed individuals, either Atlantic salmon or rainbow trout, as they represent the majority of the salmonid aquaculture industry (FAO, 2022). Therefore, how exercise affects individuals with skeletal deformities remains to be studied. Skeletal deformities (e.g., lower jaw deformity, shortened opercula, spinal curvature, etc.) in salmonid and other finfish aquaculture can be prevalent and severe (Sadler et al., 2001; Lijalad and Powell, 2009; Powell et al., 2009; Perrott et al., 2018). For example, spinal curvature is a late onset deformity (occurring six to twelve months prior to harvest) that can occur in up to 40% of harvested Chinook salmon (*Oncorhynchus tshawytscha)* stocks within some farms in the Marlborough Sounds, New Zealand (NZ; Davie et al., 2018; Perrott et al., 2018; Lovett et al., 2020). A rate of less than 5% is more typical across the industry. Spinally curved fish incur reduced market price and increased processing costs (Fjelldal et al., 2012; Lovett et al., 2018). The underlying causes of spinal deformities in NZ farmed Chinook salmon are not fully understood; however research into the drivers behind spinal deformities and ways to mitigate severity and prevalence is ongoing (Lovett et al., 2018; Perrott et al., 2018; Davie et al., 2019; Lovett et al., 2020; Perrott et al., 2020; Araújo et al., 2022a; Scholtens et al., 2023). Spinal curvature can negatively impact swimming performance by requiring large energetic costs (Powell et al., 2009), potentially leading to higher feed intake and poorer feed efficiency. This could be further exacerbated in offshore high energy farming sites, where sustained swimming at medium to fast speeds is required. Among the literature investigating the influence of exercise training on salmon production biology, no study

(to my knowledge) has focused on salmon with spinal curvature and there is a paucity of information about the influence of sustained swimming on bone structure or mineral content in deformed individuals. It has been shown that swim training can improve bone mineral content (Deschamps et al., 2009; Totland et al., 2011; Ytteborg et al., 2013; Solstorm et al., 2016a); however, no studies to date show that exercise training limits the development of spinal curvature.

Chinook salmon is an understudied, commercially important salmonid species and spinal curvature in some farms in NZ is a large welfare concern (Davie et al., 2018; Perrott et al., 2018; Lovett et al., 2020). The NZ Chinook salmon aquaculture sector is proposing to expand grow-out practices further offshore and adopt an integrated on-land tank-based system, with nearshore sheltered and offshore high energy, grow-out farms (NZKS, 2020; Mutter, 2022). This means that Chinook salmon stocks, including those with spinal curvature, that are currently farmed in low energy nearshore sites in the Marlborough Sounds, with environmental currents ranging between 0.1-0.3 m s^{-1} will be placed in high energy locations that experience environmental currents between 0.40-1.24 m s^{-1} (Gillespie, 2011; Campos et al., 2019; Newcombe et al., 2019). Therefore, to assist the NZ industry in expanding farming practices offshore, it is pivotal to understand the impact of sustained swimming on the form and function of existing NZ Chinook salmon stocks. Information transfer from Atlantic salmon and rainbow trout is limited, as comparisons between these species and Chinook salmon have revealed large species-specific differences (e.g., feed conversion efficiency, intermuscular and whole-body fat, and body shape; Petrell and Jones, 2000; Johnsen et al., 2011; Araújo et al., 2021; Araújo et al., 2022b; Elvy et al., 2022b). Information needed for the NZ salmon industry includes estimating the swimming performance and energy requirements of existing stocks, determining if exercise training can be used in hatchery settings to improve swimming fitness and efficiency in individuals with and without spinal curvature, and how long-term exercise may alter physiological processes and tissue morphology. It is hypothesized that production-related traits will improve under low to moderate exercise regimes, as shown in other salmonid species.

This study aimed to determine the effects of long-term sustained swimming on the form and function of Chinook salmon with and without spinal curvature. Specifically, this study quantified aerobic capacity, swimming performance, and investigated morphological changes at cellular and whole-body levels in response to exercise training. To achieve this, Chinook salmon, including individuals that developed mild scoliosis, were exposed to low (LFR) and moderate flow regimes (MFR) continuously for ten to eleven months. Swimming performance metrics were estimated using a swim tunnel, where individuals underwent a stepwise swim test to determine U_{crit} and concomitantly measure oxygen consumption rates ($\tilde{M}O_2$). The effect of sustained swimming on whole-body shape and composition, and specific tissue morphometrics (gill, skin, and white muscle) were also investigated. The current experiment created and used a

large dataset across a wide range of variables that are potentially influenced by swimming and mild scoliosis to identify which factors are likely to be more important in relation to understanding swimming in Chinook salmon. This dataset provides some of the pivotal information needed to assist the NZ salmon industry as they consider expanding farming practices offshore into high energy sites, as well as contributing to the broader field of fish swimming physiology.

6.3 Materials and Methods

All protocols performed on animals followed animal ethics protocols approved by the Nelson Marlborough Institute of Technology Animal Ethics Committee (AEC2018 CAW01).

6.3.1 Animal husbandry and experimental setup

All-female Chinook salmon smolts were sourced from Sanford's Kaitangata commercial salmon hatchery, where they were tagged with passive integrated transponder tags (HIDGlobal, EM4305, 12 mm long and 2 mm diameter glass tags) and transferred to the Finfish Research Centre at Cawthron Aquaculture Park, Nelson, NZ on 7th December 2020. The fish were transferred into 8,000 L tanks containing water with a salinity of 14-15 ppt at 13 ± 0.2 °C on arrival. Fish were acclimatised to full seawater (35 ppt) and a rearing temperature of 17 ± 0.2 °C over seventeen days. Fish were then continuously supplied with filtered recirculating seawater (35 ppt, 17 ± 0.2 °C, 24 h light photoperiod). From the 29th-31st December 2020, fish were sorted into twelve treatment tanks with approximately 260 fish per tank, and tank flow was set to 4.93 ± 0.08 cm s^{-1} for ~3 weeks. Fish were hand fed (P 37.5 g 100 g^{-1}, F 24.2 g 100 g^{-1}, energy 1705 kJ 100 g^{-1}) with a commercial feed to satiation daily (AM) and pellet size was increased with fish growth, as per manufacturer's recommendation.

All fish (n = 3119) were measured for mass and fork length prior to the start of the experiment (fork length = 174.6 ± 0.17 mm; mass = 82.9 ± 0.30 g; mean ± SE). Flow regimes were then increased by 1.5 cm s^{-1} day^{-1} (<0.1 bl s^{-1} day^{-1}) across seven days or until the target speed was achieved. Flow regimes were maintained by directing the incoming water in a clockwise direction at either low (LFR; 0.3 body lengths bl s^{-1}) or moderate (MFR; 0.8 bl s^{-1}) flow. Exchange rates were maintained at 224 ± 0.07 L min^{-1} (mean ± S.E.M.). After three-months under treatment, a subsample of 269 fish (without any skeletal deformity; fork length 304.4 mm ± 0.61; mass = 577.6 g ± 3.73; mean ± SE) were transitioned into 3,200 L tanks to continue the LFR and MFR. Flow regimes were measured 2-3 times per week and adjusted monthly to account for fish growth. Predicted flow regimes were based on growth data obtained in previous

experiments (Elvy et al., 2022) and readjusted to match fork length data during routine sampling. Exchange rates were maintained at 138 ± 0.13 L min^{-1} (mean ± S.E.M.) for LFR and throughout the remaining duration, whereas MFR required greater exchange rates (ranging from 106 ± 0.06 L min^{-1} to 188 ± 0. 34 L min^{-1}) to achieve monthly increases in flow regimes.

6.3.2 Routine assessment

After nine months under training, all fish (151) were anaesthetised using tricane methanesulfonate (65 ppm; Syndel, Canada) and over three sampling days (two tanks per day) mass, fork length, girth, and condition factor were measured, and external damage and scoliosis were visually assessed (summarised data presented in Table A.8). Scoliosis was visually graded based on the degree of deviation the fish's posterior curved: mild (deviation <20˚), moderate (deviation 20-40˚), and severe (deviation >40˚; Perrott et al., 2018). All fish were allowed to recover for a minimum of two weeks after routine spinal assessment, after which, in preparation for the swimming performance test, individual fish were removed from their source tank, anesthetised using AQUI-S (15-20 mg L-1) and mass, fork length, girth, height, and width were measured. Condition factor (K) was calculated as:

$$\mathrm{K} = 100000 \times \frac{M}{FL^3}, \quad (1)$$

where M is the mass of fish (g) and FL is the fork length (mm). Whole-body photographs were taken in a custom-built photo box (internal LED illumination, camera position ~55 cm from tray, Canon G7X 24-100 mm lens, set to factory settings) for body shape morphometrics. Fish that were deemed suitable for swim performance testing (i.e., no spinal curvature or mild scoliosis) were placed into a fasting tank for 48 h prior to the test.

6.3.3 Swimming respirometry

A total of 40 fish across LFR and MFR with and without mild scoliosis (<20˚deviation) underwent a swimming performance test following a standard stepwise critical swimming protocol with intermittent respirometry (Brett, 1964; Steffensen et al., 1984). Swimming performance testing took place over a four-week duration, meaning training duration for these individuals ranged between ten and eleven months. Swimming performance data were collected on 29 of the 40 fish, because some fish died during the acclimation (n=3; suspected to be linked to stress) or did not swim according to the protocol (n=8). No fish died during the swimming performance test. Data were collected from eight fish per treatment, except for fish with mild scoliosis raised under LFR, where data were collected from only five fish. Swimming respirometry experiments were conducted in two 180 L swim tunnel respirometers with a working section of 72 × 27 × 30 cm (length × width × depth). Swim tunnels were placed in a water bath that was continuously supplied with filtered recirculating seawater. Temperature was

continuously monitored within the swim tunnel and controlled using a temperature controller (17 °C; hysteresis 0.1 °C) connected to a coil that was placed inside the tunnel. The coil was setup on an independent system that was supplied with cold water (4-8 °C) from an external water bath and chiller (HAILEA HC 2200BH). Seawater within the swim tunnel was maintained between 17 ± 0.1 °C. Oxygen levels within the respirometers were recorded using a Firesting Optical Oxygen Meter (Pyro Science e. K., Aschen, Germany) every two seconds, which was then automatically calculated into a mass-specific $\tilde{M}O_2$ ($mgO_2\ kg^{-1}\ hr^{-1}$) using AquaResp software (Svendsen, 2019) as:

$$\dot{M}O_2 = V\ (\Delta PO_2/t)\ \alpha\ M, \quad (2)$$

where V is the volume of the respirometry chamber in litres corrected for by the size of the fish, $\Delta PO_2/t$ is the change in oxygen partial pressure (kPa) per unit of time (hr), α is the oxygen solubility coefficient (salinity 34ppt; 17˚C) in $mgO_2\ kPa^{-1}$, and M is mass of the fish (kg). The flow was calibrated using a Hontzsch flow meter with vane wheel in the working section of the respirometer to obtain a ten-point calibration ($r^2 = 0.99$). Solid blocking effects of the fish were corrected following Bell and Terhune (1970):

$$U_f = U_t(1 + \varepsilon_s), \quad (3)$$

where U_f is the corrected flow speed, U_t is the uncorrected flow speed and ε_s is the solid blocking fraction error. The solid blocking fraction error was calculated as:

$$\varepsilon_s = 0.8\lambda(A_O / A_T)^{0.5}, \quad (3)$$

where λ is the constant obtained for the animal shape (0.5*FL/body thickness), A_O is maximum cross section of animal, and A_T is the cross section of the swim chamber.

Between each fish, the respirometers were cleaned with bleach (and thoroughly rinsed with seawater) to ensure that bacterial respiration rates remained below 10% of the routine metabolic rate at 0.5 bl s^{-1}. Bacterial respiration was measured across two 7.5 min cycles, before and after each testing and the average was then subtracted from each $\dot{M}O_2$ obtained (Johansen and Esbaugh, 2017). Two GoPro Hero 7 cameras (frame rate: 60 Hz) were positioned 50 cm directly above the working section of the swim tunnel and adjacent to the working section, allowing capture of both aerial and side views of the fish. Video recordings were taken for five minutes during the first measurement cycle of each swimming speed. This allowed tail beat frequency to be later calculated.

Individual fish that were selected for swimming respirometry and had been fasted for 48 h, were acclimated to the swim tunnel for 12-24 h at 0.5 bl s^{-1} until $\dot{M}O_2$ stabilised. Once acclimated, the fish underwent a stepwise protocol, where oxygen consumption was measured

for two cycles (Flush: 420s, wait: 30s, measure: 450s) and swim speeds increased incrementally by 0.25 bl s^{-1}. Swimming speed continued to increase until the fish was unable to swim against the current and rested on the back grate for > 5 s. The swimming speed was reduced back to 0.5 bl s^{-1} and the fish was allowed to recover ($\overline{x}$ = 3.37 ± 0.27 h). $\dot{M}O_2$ continued to be measured to estimate excess post-exercise oxygen consumption (EPOC) and recovery time. $\dot{M}O_2$ values were only included in the analysis if the correlation coefficient (R^2) of each slope was greater than 0.95.

U_{crit} was determined as:

$$U_{crit} = U_f + U_i\, T_f\, T_i^{-1} \quad (4)$$

where U_f is the highest speed maintained for the entire swimming period, U_i is the speed increment (cm s^{-1}), T_f is the time elapsed at fatigue speed, and T_i is the set interval time (min).

Maximum metabolic rate (MMR) was defined by the largest $\dot{M}O_2$ measured, typically occurring at U_{crit} (Johansen and Esbaugh, 2017). Standard metabolic rate (SMR) was unable to be estimated, as the relationship between oxygen consumption and swimming performance could not be expressed as:

$$\dot{M}O_2 = a + b\, U^{(c)} \quad (5)$$

This was due to 'skittish' behaviours, resembling an escape response (see Elvy et al., 2022b) being exhibited throughout the swimming performance test. Therefore, routine metabolic rate at 0.5 bl s^{-1} ($RMR_{0.5}$) was used instead of SMR and calculated as the 0·15 quantile (q0.15) of $\dot{M}O_2$ at 0.5 bl s^{-1}, following methods from Chabot et al. (2016b). Subsequently, an active aerobic scope (AS) was calculated for each fish by subtracting $RMR_{0.5}$ from MMR.

Excess post-exercise oxygen consumption and recovery time was estimated on $\dot{M}O_2$ following the U_{crit} test by fitting a decay function to the $\dot{M}O_2$ values:

$$\dot{M}O_2 = a^{(bt)} \quad (6)$$

where a and b are coefficients, determined by the nonlinear least squares function (Rstudios, package: stats) and t is time (h). Recovery time was calculated by determining when the decay function intercepted $RMR_{0.5}$ + 10%. EPOC was calculated by integrating the area under the curve (decay function), above $RMR_{0.5}$ + 10% value and between the initial measurement time and recovery time (Lee et al., 2003; Svendsen et al., 2010; Bouyoucos et al., 2017). Four fish (1x low, 1x low with mild scoliosis, 2x moderate with mild scoliosis) were excluded from estimating EPOC and recovery time, as $\dot{M}O_2$ failed to follow an exponential decay function and $\dot{M}O_2$ did not reduce back to $RMR_{0.5}$ + 10%.

6.3.4 Video analysis

Caudal fin oscillation (tail beat frequency, TBF; top view) were averaged from three randomly selected one-minute periods within the five-minute videos for each fish across each speed using VLC player (Version 3.0.16 Vetinari). A tail beat was defined as one complete oscillation of the caudal fin.

6.3.5 Body shape analysis

Seven body metrics, as proxies for body shape, were measured on images of salmon that were previously taken in a photo box prior to undergoing the U_{crit} test. Following Pakkasmaa and Piironen (2000), body metrics were measured using ImageJ (v. 1.53e, National Institutes of Health, Rockville, MD, USA), where head length (HL), head height (HH), antero to dorsal length (AD), antero to anal length (AA), dorsal fin base (DF), pectoral fin base (PF), dorsal body height, (DH), anal body height (AH), peduncle body height (PH), caudal fin height (CF), and caudal fin area (CA) were measured (refer to Figure A.3a)

6.3.6 Sampling

Upon completion of the swimming performance test, fish were euthanised with a lethal dose of AQUI-S (100 ppm), blood and specific tissues were sampled and measured. Blood was drawn from the caudal blood vessel and haematocrit (Hct) was measured by centrifuging blood in a HemoCue Microhematocrit tube at 9000 rpm for seven minutes. Gill (second arch, left side), skin (below dorsal fin, left side), and muscle samples (back of dorsal, left side) were removed and fixed in 10% neutral-buffered formalin (NBF). Belly-flap thickness at three locations (pectoral, pelvic and anal) was measured, and whole viscera, heart, liver, and gonads were weighed. Remaining fish carcass, liver, and fat were stored frozen for later Fourier transform – near infrared reflect (FT-NIR) analysis, bone mineral analysis, and fillet colour measurements.

6.3.7 Fillet colour

Fillet colour analysis was measured instrumentally using a CR-410 chroma meter (measurement diameter 50 mm) and characterised by three colour parameters defined under the International Commission on Illumination (CIE): a* (red chromaticity), b* (yellow chromaticity), and L* (lightness; Crouse et al., 2018). Frozen fish were thawed overnight, and fillet colour was measured across three fillet regions on both the left and right side of the fish (Figure A.3b). Prior to fillet measurements, the instrument was calibrated on a white calibration plate following manufacturers guidelines.

6.3.8 Bone mineral content

Vertebrae 30 and 31 were dissected from remaining fish carcasses and stored frozen (-20℃). Vertebrae 30 and 31 were microwaved for ~30 sec to allow for easy removal of flesh and then further washed in distilled water, where any remaining flesh was removed before drying overnight in an oven at 105℃. Vertebrae were then crushed into a fine powder using a mixer mill (Retsch Oscillating Mill MM400) for 30 sec at 30 Hz. Bone mineral content (BMC) was assessed through a commercial testing laboratory (Food Testing Laboratory of Cawthron Analytical Services; Nelson, NZ) using an in-house digestion and inductively coupled plasma mass spectrometry (IPC-MS; APHA 3125B) protocol.

6.3.9 FT-NIR composition

Thawed fish carcass (post fillet colour assessments and vertebrae removal), viscera, and liver were used to approximate fat, protein, ash, and moisture composition using FT-NIR spectroscopy, following protocols from Miller et al. (2019). FT-NIR was used to estimate total fat in whole-body, fillet (including the 4 regions described below), liver and viscera. Whole-body and fillet (including the 4 regions) were also assessed for total protein, ash, and moisture. All fish carcasses were filleted, and both (left and right) fillets were divided into four regions (i.e., 8 sections total). Fillet regions consisted of dorsal-, lateral line-, visceral-, and posterior dorsal region (Figure A.3c) (modified from Miller et al., 2019). Each fillet section was homogenised using a food processer for 30-60s, whereas the viscera and liver were homogenised using an IKA T18 ULTRA TURRAX for 30-60s. Uniform samples for testing were placed in a 50 mm rotating cup and scanned in reflectance mode using a Bruker MPA FT-NIRs (Bruker, Ettlingen, Germany). All fillet regions (i.e., left and right) were then recombined and homogenised to obtain an overall fillet reading. The remaining carcass was broken down using a meat processer, before being placed into the food processer, where it was further homogenised along with the fillet regions, viscera, and liver to obtain a whole-body reading.

All samples were scanned using models developed and validated by Miller et al. (2019). The model was validated against proximate composition values assessed through a commercial testing laboratory (Food Testing Laboratory of Cawthron Analytical Services; Nelson, NZ). Whole-body, fillet, liver, and viscera samples were collected from one fish per treatment (i.e., 4 fish) and assessed following the methods from Association of Official Analytical Chemists (AOAC) for crude protein (AOAC 981.10), total fat (AOAC 948.15), moisture at 105˚C (AOAC 950.46), and ash (AOAC 920.153). FT-NIR fat and protein values were <20% (R^2 = 0.93) and <4% (R^2 = 0.79) different from values obtained from wet chemistry, respectively.

6.3.10 Histological techniques and morphological assessment

Muscle samples were decalcified in 5% formic acid for five days before undergoing a series of dehydration and wax infiltrations (Thermo Excelsior ES processor) overnight. Muscle samples were cut to 5 µm using a microtome (Leica RM2235) and stained automatically (Thermo Gemini Autostainer) with H&E. Slides were scanned at 20x magnification using a Leica Aperio Scanscope. Muscle fibre size, diameter, and density were estimated using QuPath (version 0.2.3; Bankhead et al., 2017). Three randomly selected 500,000 µm^2 regions (2 x 500 µm x 500 µm grid) were used to manually measure muscle fibre size and perimeter, and subsequently, the average muscle fibre diameter and density were calculated. Muscle fibre diameter was calculated as:

$$\emptyset = 2\sqrt{A * \pi^{-1}} \quad (7)$$

where, A is the area (µm^2), and muscle fibre density was calculated as:

$$d = \frac{n}{\Sigma A} \quad (8)$$

where, n is the count of fibres and ΣA is the sum of fibre area (µm2; Bugeon et al., 2003; Martin and Johnston, 2005; Rasmussen et al., 2011; Timmerhaus et al., 2021).

Gill and skin samples were processed and stained with haematoxylin and eosin (H&E) and periodic acid-schiff and alcian blue (PAS-AB) by Gribbles Veterinary, Christchurch, NZ. The slides were visualised using an Olympus BX53 upright light microscope and micrographed with an Olympus DP27 camera attached to the microscope. Gill and skin were micrographed at 200x and 100x, respectively, and five micrographs were taken for each stain. For the gills, random filaments (sectioned at appropriate angle) were selected, and micrographs were taken at the lower (x2), upper (x2) and middle (x1) region of the filament that included >10 lamellae. Gill morphometrics were measured on fifteen randomly selected lamellae (three per image) from H&E-stained micrographs in ImageJ (v. 1.53e, National Institutes of Health, Rockville, MD, USA) following Hess et al. (2015); (2017). Measured morphological features included lamellae length, filament thickness, lamellae density, and lamellae epithelium thickness (proxy for oxygen diffusion distance; refer to Figure 6.4e). Lamellae epithelium was estimated by subtracting the area of the lamellae blood-pillar system from the total area of lamellae (i.e., area above filament epithelium) and dividing by twice the lamellae length (refer to figure 1c in Hess et al., 2015). Pathological features were analysed on 100 lamellae per fish, following Timmerhaus et al. (2021). Pathological features include lamellae fusion, aneurysm, epithelial lifting, clubbing, hypertrophy, hyperplasia, and necrosis, described by Mallatt (1985). Goblet cell counts were taken from ten consecutive interlamellar areas, where the area between the blood-pillar system of both lamellae and to the central-venous system were measured and expressed as cells 100 µm-

^{2}. Goblet cell size was measured from one randomly chosen cell in each area. Therefore, fifty interlamellar regions were assessed for goblet cell density and size for each fish.

For skin morphometrics, epidermis and dermis thickness were measured on fifteen randomly selected locations, following methods from Timmerhaus et al. (2021). Goblet cell counts and size were measured across 100 µm, which was randomly chosen from a 10,000 µm^2 grid that was overlayed on the skin micrographs, following methods modified from Fast et al. (2002). Three regions were randomly chosen for goblet cell counts, where the area (i.e., 100 µm^2 by the height of epidermis) was measured and expressed as cells 100 µm^{-2} (fifteen counts for each fish). Goblet cell size was measured from one randomly chosen cell in each area (fifteen counts for each fish).

6.3.11 Statistical Analysis

All statistical analyses were performed using the R statistical language and lme4 and MuMin packages. Data handling and figures were produced using tidyverse and ggplot2 packages. Model selection was assessed using Akaike's information criterion (AIC), following selection criteria from Richards (2005). Model parameters (normal distribution and equal variances) were assessed visually through Q-Q plot and residual versus fitted plot. Data that failed to meet model parameters were log-transformed.

For $\dot{M}O_2$ and TBF as a function of swimming speed, general linear mixed effects model analysis were performed, where interactions between predicator and fixed variables were investigated and repeated measures were included as a random effect nested within fish identification to account for independent responses. Interactions were removed if not significant and the model was assessed for main effects.

For metrics of swimming performance (i.e., $RMR_{0.5}$, MMR, AS, U_{crit}, EPOC, and recovery time), fish size (i.e., mass, fork length, girth, height, width, and condition factor) and tissues (belly flap, viscera, liver, heart, gonad, Hct, gill and skin morphometrics), general linear model analysis were performed, where interactions between fixed variables were investigated and removed if not significant. Mass was included as covariate for models investigating fork length, girth, height, width, condition factor, viscera, heart, liver, and gonad. Tank effect as a random effect was investigated and removed if not significant. Gill pathology was analysed using generalised liner mixed effects models.

FT-NIR composition, BMC, fillet colour, and muscle morphometrics were also assessed using general linear models and general linear mixed effect models, where repeated measures were included as a random effect nested within fish identification to account for independent responses. For models investigating FT-NIR composition and fillet colour, region and side of the

fish were included as fixed effects. Mass was included as a covariate in some models investigating FT-NIR composition when found to be a significant explanatory variable. Tank effect as a random effect was investigated and removed if not significant.

Table 6.1 Size and tissue measurements of Chinook salmon with and without mild scoliosis raised under low and moderate flow regimes.

Response variable	Low flow regime		Moderate flow regimes		*P*-values	
	Without scoliosis	With scoliosis	Without scoliosis	With scoliosis	Exercise treatment	Spinal scoliosis
Sample size	8	5	8	8		
Mass (kg)	1.95 ± 0.12	1.94 ± 0.17	1.74 ± 0.07	1.89 ± 0.13	0.26	0.50
Fork length (mm)	449.8 ± 7.6	440.6 ± 10.9	430.1 ± 4.8	437.5 ± 7.1	0.15	0.16
Girth (mm)	341.0 ± 8.6[a]	339.8 ± 13.0[a]	333.0 ± 6.0[b]	341.0 ± 9.4[b]	**<0.01**	0.49
Height (mm)	131.8 ± 3.7[a]	132.2 ± 5.4[a]	129.3 ± 2.5[b]	132.9 ± 3.4[b]	**<0.05**	0.97
Width (mm)	65.4 ± 1.1	67 ± 2.4	62.6 ± 1.9	66.4 ± 2.3	0.80	0.20
Condition factor	2.12 ± 0.05	2.24 ± 0.08	2.18 ± 0.06	2.21 ± 0.06	0.14	0.40
Fineness ratio	6.89 ± 0.13	6.59 ± 0.14	6.91 ± 0.22	6.63 ± 0.21	0.88	0.14
Belly-flap thickness:						
Pectoral (mm)	8.62 ± 0.49	9.50 ± 1.02	9.83 ± 0.43	9.78 ± 0.60	0.19	0.55
Pelvic (mm)	8.04 ± 0.88	8.82 ± 1.00	9.63 ± 0.39	8.60 ± 0.99	0.19	0.55
Anal (mm)	3.02 ± 0.28	3.33 ± 0.44	3.39 ± 0.25	2.94 ± 0.25	0.90	0.70
Viscera (g)	192.3 ± 19.1[a]	212.2 ± 30.0[a]	153.4 ± 7.5[b]	176.1 ± 16.3[b]	**<0.05**	0.10
Heart (g)	2.08 ± 0.14	1.98 ± 0.23	1.91 ± 0.09	1.95 ± 0.15	0.99	0.39
Liver (g)	19.8 ± 2.3	16.7 ± 4.1	16.8 ± 2.1	16.3 ± 2.3	0.83	0.07
Gonad (g)	1.98 ± 0.16	2.34 ± 0.56	2.28 ± 0.39	2.0 ± 0.36	0.58	0.98
Hct (%)	37.2 ± 1.0	39.4 ± 1.4	38.3 ± 1.2	38.0 ± 1.3	0.90	0.46

Values are means ± S.E.M.
Boldness indicates indicate significant *P*-values. Letters indicate significant differences between experimental treatments.

6.4 Results

6.4.1 Fish size, body shape, and tissue metrics

Fish included within the swim performance test were of a similar size range and were not statistically different between exercise treatments or with and without mild scoliosis (Table 6.1). Irrespective of having similar mass, exercise treatment had a significant effect on fish girth (LM; $F_{1,25} = 9.21$, $P < 0.01$; Table 6.1) and height (LM; $F_{1,25} = 4.53$, $P < 0.05$; Table 6.1), where fish under MFR presented larger (~6.4 mm) girth and developed a taller (~2.7 mm) body shape. Condition factor was not different among treatments (Table 6.1). Among the body shape morphometrics (image analysis), caudal fin height was significantly different between exercise treatments (LM; $F_{1,26} = 4.5563$, $P < 0.05$; Table A.9), where fish raised under LFR had ~5.02 mm larger caudal fin height than fish raised under MFR (Table A.9). Viscera mass was significantly smaller (~21.2 g) in fish raised under MFR (LM; $F_{1,25} = 6.69$, $P < 0.05$; Table 6.1). Other metrics measured were not statistically different between exercise treatments or mild spinal curvature (Table 6.1).

6.4.2 Swimming performance

Fish raised under MFR had a significantly greater $U_{crit,}$ of 2.4 bl s^{-1} (103 cm s^{-1}; 95% C.I. 1.78, 2.99 bl s^{-1}) compared to 1.8 bl s^{-1} (81 cm s^{-1}; 95% C.I. 1.57, 2.12 bl s^{-1}) for fish raised under LFR (LM; $F_{1,26} = 11.19$, $P < 0.01$; Figure 6.1a). Mild spinal curvature did not significantly influence swimming performance (LM; $F_{1,26} = 2.72$, $P = 0.11$). Oxygen uptake rates significantly increased with increasing swimming speed (LMER; $F_{1,35} = 690.47$, $P < 0.001$), but did not differ between flow regimes or between fish with or without mild scoliosis (LMER; $F_{1,58} = 2.07$, $P = 0.16$; LMER; $F_{1,56} = 0.53$, $P = 0.47$). The $RMR_{0.5}$ was not significantly different between flow regimes or between fish with or without mild scoliosis (Figure 6.1b). Significant differences between flow regimes occurred for MMR and AS, where the MMR of fish raised under MFR was ~63 mgO_2 kg^{-1} hr^{-1} (95% C.I. 29.39, 96.17 mgO_2 kg^{-1} hr^{-1}; LM; $F_{1,25} = 15.08$, $P < 0.001$; Figure 6.1b) greater than the MMR of fish raised under LFR and the AS was ~67 mgO_2 kg^{-1} hr^{-1} (95% C.I. 34.86, 98.17 mgO_2 kg^{-1} hr^{-1}; LM; $F_{1,25} = 18.83$, $P < 0.001$; Figure 6.1b) larger than the AS of fish raised under LFR.

Tail beat frequency had a significant interaction between swimming speed where fish with mild scoliosis had a significantly higher TBF as a function of swimming speed (TBF ~ 0.759*SS + 1.5) than fish without scoliosis (TBF ~ 0.714*SS + 1.5; LMER: $F_{1,598.67} = 4.87$, $P < 0.05$; Figure 6.2).

Recovery from exhaustive exercise (i.e., U_{crit} test) was significantly different between fish with and without mild scoliosis. Excess post-exercise oxygen consumption (LM; $F_{1,22} = 4.8$, $P < 0.05$, Figure 6.1c) and recovery time (LM; $F_{1,22} = 4.86$, $P < 0.05$; Figure 6.1d) were significantly higher in fish with mild scoliosis when compared against fish without scoliosis. Fish with mild scoliosis required ~322.7 mgO_2 kg^{-1} and 2.22 h more than fish without scoliosis to recover from exhaustive exercise.

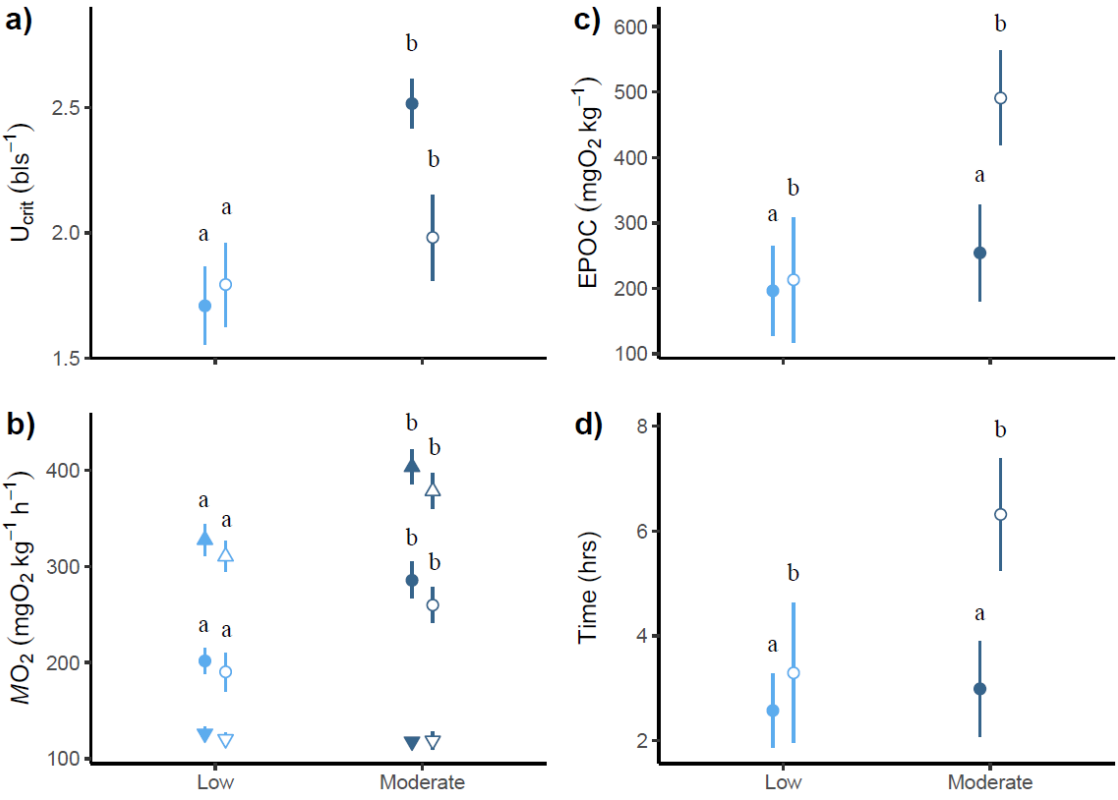


Figure 6.1 Critical swimming speed a), metabolic rates b), excess post-exercise oxygen consumption (EPOC) c), and recovery time d) in Chinook salmon without (filled) and with mild scoliosis (no fill) raised under low (light blue) and moderate (dark blue) flow regimes. Metabolic components b) include maximum metabolic rate (▲), routine metabolic rate at 0.5 BL s-1 (▼), and active aerobic scope (●). Points and vertical bars represent mean ± S.E.M.; significant differences ($P < 0.05$) are indicated by lowercase letters.

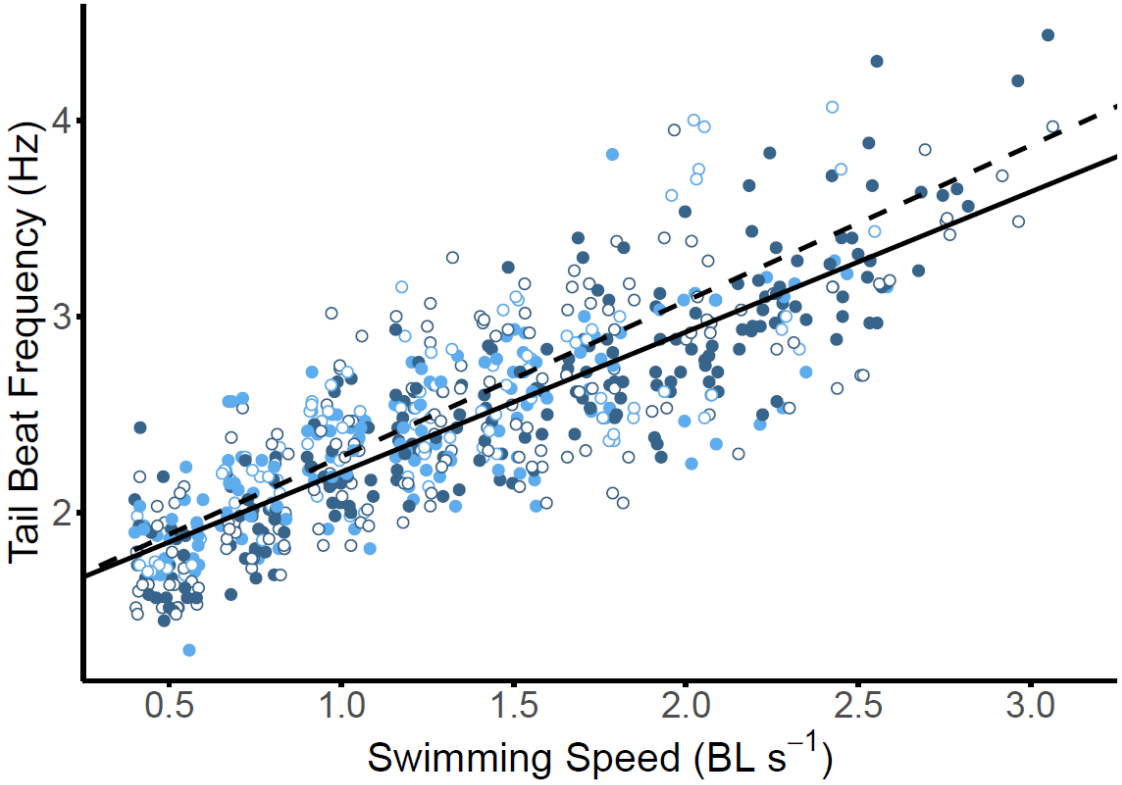


Figure 6.2 Relationship between tail beat frequency (Hz) and swimming speed (BL s-1) in Chinook salmon without (filled) and with mild scoliosis (no fill) raised under low (light blue) and moderate (dark blue) flow regimes. Points are randomly located within each swimming speed to avoid overlap. Solid (without scoliosis) and dashed (with mild scoliosis) lines represent linear relationship between tail beat frequency and interaction between swimming speed and mild scoliosis.

6.4.3 Fillet colour

The posterior fillet region on both the left and right sides of the fish were significantly less red chromaticity in individuals raised under MFR (LRT; $G_1 = 29.68$, $P < 0.001$; Table 6.2). Fillet yellow chromaticity was significantly lower in fish raised under MFR than in fish raised under LFR within the posterior fillet region on the left side of the fish as well as in the anterior fillet region on the right side of the fish (LMER; $F_{1,145} = 3.91$, $P < 0.001$; Table 6.2). Fillet red chromaticity (LRT; $G_1 = 0.02$, $P = 0.90$) and fillet yellow chromaticity (LMER; $F_{1,28} = 2.0$, $P = 0.17$) did not have a relationship with mild scoliosis. Fillet lightness (L*) increased from the anterior fillet location to the posterior fillet location (LMER; $F_{2,152} = 438.47$, $P < 0.0001$) and was greater on the right side of the fish (LMER; $F_{1,152} = 33.67$, $P < 0.0001$), and in fish without scoliosis (LMER; $F_{1,28} = 4.25$, $P < 0.05$; Table 6.2). Fillet lightness did not have a relationship with flow regimes (LMER; $F_{1,28} = 0.79$, $P = 0.38$).

6.4.4 Bone mineral content

Bone mineral content, calcium ($\overline{x}$ =14.93 ± 0.17 g $100g^{-1}$) and phosphorous ($\overline{x}$ = 7.86 ± 0.09 g $100g^{-1}$) were not statistically different among flow regimes (LM; $F_{1,26} = 0.45$, $P = 0.51$, respectively) or fish with and without mild scoliosis (LM; $F_{1,25} = 0.71$, $P = 0.41$; LM; $F_{1,26} = 0.07$, $P = 0.80$, respectively).

Table 6.2 Fillet colour values in Chinook salmon with and without mild scoliosis raised under low and moderate flow regimes.

Response Variable			Low flow regime		Moderate flow regime		*P*-values			
			Without scoliosis	With scoliosis	Without scoliosis	With scoliosis	Region	Side	Exercise treatment	Spinal scoliosis
Red chromaticity (a*)	Fillet regions	L1	28.96 ± 0.89	27.97 ± 1.11	27.57 ± 0.73	27.46 ± 0.98	**Significant interaction <0.001**			0.90
		L2	25.7 ± 0.68	24.55 ± 1.11	23.59 ± 0.83	23.56 ± 0.98				
		L3	16.2 ± 0.67[a]	16.13 ± 1.07[a]	13.58 ± 0.57[b]	14.03 ± 0.63[b]				
		R1	27.61 ± 1.32	27.70 ± 0.69	25.18 ± 1.0	24.90 ± 1.0				
		R2	23.18 ± 0.93	23.09 ± 0.81	21.84 ± 0.80	22.28 ± 0.93				
		R3	15.49 ± 0.70[a]	16.0 ± 0.86[a]	14.54 ± 0.57[b]	14.24 ± 0.67[b]				
Yellow chromaticity (b*)	Fillet regions	L1	24.17 ± 0.96	21.88 ± 0.54	23.25 ± 0.86	22.45 ± 1.09	**Significant interaction <0.001**			0.17
		L2	22.84 ± 0.69	21.39 ± 0.79	21.43 ± 0.75	20.0 ± 0.98				
		L3	16.18 ± 0.62[a]	15.36 ± 0.94[a]	13.82 ± 0.53[b]	13.67 ± 0.45[b]				
		R1	23.83 ± 1.22[a]	22.21 ± 1.30[a]	21.0 ± 0.79[b]	20.65 ± 0.62[b]				
		R2	21.51 ± 0.81	20.43 ± 0.97	20.03 ± 0.77	20.03 ± 0.73				
		R3	16.27 ± 0.50	15.92 ± 0.82	15.45 ± 0.44	14.80 ± 0.47				

Lightness (L*)	Fillet regions	L1	48.96 ± 0.25[a]	47.28 ± 1.39[b]	48.93 ± 0.68[a]	48.35 ± 1.06[b]	**<0.0001**	**<0.0001**	0.38	**<0.05**
		L2	51.45 ± 0.40[a]	50.83 ± 1.15[b]	52.04 ± 0.48[a]	50.46 ± 0.75[b]				
		L3	56.81 ± 0.73[a]	54.81 ± 0.91[b]	58.14 ± 0.58[a]	56.62 ± 0.96[b]				
		R1	49.51 ± 1.36[a]	48.65 ± 0.74[b]	50.65 ± 0.59[a]	49.92 ± 1.03[b]				
		R2	54.23 ± 0.56[a]	52.19 ± 1.03[b]	53.74 ± 0.63[a]	52.0 ± 0.90[b]				
		R3	58.19 ± 0.61[a]	55.84 ± 1.06[b]	57.96 ± 0.58[a]	57.13 ± 0.77[b]				

Values are means ± S.E.M.

Boldness indicates indicate significant *P*-values. Letters indicate significant differences between experimental treatments.

6.4.5 FT-NIR approximate composition

Total fat content within the viscera was significantly higher in fish raised under LFR than in fish raised under MFR (LM; $F_{1,26} = 5.20$, $P < 0.05$; Table 6.3). Fat content was the highest in the visceral fillet region (LMER; $F_{3,199} = 507.49$, $P < 0.0001$) and on the right side of the fish (LMER; $F_{1,199} = 24.87$, $P < 0.0001$; Table 6.3). The visceral fillet region had significantly lower protein content than all other regions and was lowest on the right side of the fish (LMER; $F_{1,196} = 3.79$, $P < 0.05$; Table 6.3). Fish with mild scoliosis had greater ash content than fish without scoliosis (LMER; $F_{1,25} = 5.54$, $P < 0.05$; Table 6.3). Fish raised under LFR had lower moisture content than fish raised under MFR (LMER; $F_{1,26} = 6.11$, $P < 0.05$; Table 6.3).

Whole-fillet fat and moisture content did not statistically differ among flow regimes (LM; $F_{1,25} = 0.72$, $P = 0.40$; LM; $F_{1,24} = 1.79$, $P = 0.19$, respectively) or for fish with or without mild scoliosis (LM; $F_{1,25} = 1.01$, $P = 0.33$; LM; $F_{1,24} = 1.16$, $P = 0.29$, respectively; Table 6.3). In fish with mild scoliosis, protein (LM; $F_{1,24} = 6.99$, $P < 0.05$) was greater and ash (LM; $F_{1,24} = 5.26$, $P < 0.05$) was greater than fish without scoliosis (Table 6.3). Whole-body fat (LM; $F_{1,26} = 18.42$, $P < 0.001$), and ash (LM; $F_{1,26} = 17.45$, $P < 0.001$) was greater than fish raised under LFR (Table 6.3). Whole-body moisture (LM; $F_{1,25} = 30.38$, $P < 0.0001$) was lower in fish raised under LFR than in fish raised under MFR (Table 6.3). Whole-body protein content did not statistically differ among flow regimes (LM; $F_{1,25} = 0.49$, $P = 0.49$) or for fish with or without mild scoliosis (LM; $F_{1,25} = 3.27$, $P = 0.08$; Table 6.3).

Table 6.3 FT-NIR fat and protein composition in Chinook salmon with and without mild scoliosis raised under low and moderate flow regimes.

Response Variable			Low flow regime		Moderate flow regime		*P*-values			
			Without scoliosis	With scoliosis	Without scoliosis	With scoliosis	Region	Side	Exercise treatment	Spinal scoliosis
Fat	Liver		3.13 ± 0.24	3.12 ± 0.28	3.17 ± 0.23	4.15 ± 0.80			0.36	0.28
(g $100g^{-1}$)	Viscera		63.40 ± 3.61[a]	72.30 ±4.24[a]	57.50 ± 30.34[b]	57.46 ± 5.25[b]			**<0.05**	0.38
	Fillet regions						**<0.0001**	**<0.0001**	0.09	0.37
		L1	18.82 ± 1.66	16.03 ± 2.03	15.64 ± 1.28	15.27 ± 1.85				
		L2	17.02 ± 1.73	15.23 ± 2.43	14.08 ± 1.24	13.45 ± 1.32				
		L3	40.67 ± 1.36	37.27 ± 2.46	33.74 ± 1.82	33.96 ± 1.76				
		L4	18.27 ± 0.87	16.16 ± 1.71	14.98 ± 1.94	15.24 ± 1.56				
		R1	21.13 ± 1.39	17.52 ± 3.29	17.05 ± 1.05	17.09 ± 2.05				
		R2	18.01 ± 0.89	15.36 ± 1.90	16.36 ± 1.84	15.14 ± 1.69				
		R3	43.50 ± 2.21	39.38 ± 1.79	38.13 ± 1.62	38.64 ± 2.33				
		R4	18.84 ± 1.21	18.44 ± 1.94	15.88 ± 1.05	17.35 ± 1.69				
	Whole-fillet		23.21 ± 1.59	19.10 ± 2.03	19.88 ± 1.64	19.87 ± 1.62			0.40	0.33
	Whole-body		25.06 ± 1.03[a]	25.17 ± 1.00[a]	21.39 ± 0.91[b]	20.94 ± 0.68[b]			**<0.001**	0.83
Protein	Fillet regions						**<0.0001**	**<0.001**	0.54	0.26
(g $100g^{-1}$)		L1	19.10 ± 0.41	19.49 ± 0.68	18.88 ± 0.23	16.13 ± 0.13				
		L2	19.53 ± 0.40	19.66 ± 0.79	19.21 ± 0.35	18.03 ± 0.19				
		L3	14.03 ± 0.26	14.22 ± 0.39	14.55 ± 0.24	14.94 ± 0.45				
		L4	18.68 ± 0.29	19.30 ± 0.42	18.33 ± 0.48	18.72 ± 0.37				
		R1	18.49 ± 0.33	19.34 ± 0.86	18.26 ± 0.30	18.73 ± 0.32				
		R2	19.36 ± 0.27	19.80 ± 0.33	19.21 ± 0.39	19.69 ± 0.37				
		R3	13.49 ± 0.34	14.63 ± 0.40	13.63 ± 0.36	13.97 ± 0.38				
		R4	18.69 ± 0.36	18.90 ± 0.54	18.28 ± 0.33	18.47 ± 0.35				
	Whole-fillet		17.74 ± 0.23[a]	18.82 ± 0.54[b]	17.55 ± 0.34[a]	18.03 ± 0.19[b]			0.09	**<0.05**

	Whole-body		15.76 ± 0.17	15.87 ± 0.13	15.85 ± 0.13	16.13 ± 0.13			0.49	0.08
Ash	Fillet regions						**<0.0001**	0.42	0.59	**<0.05**
(g $100g^{-1}$)		L1	1.12 ± 0.01^{a}	1.11 ± 0.05^{b}	1.11 ± 0.02^{a}	1.43 ± 0.01^{b}				
		L2	1.13 ± 0.02^{a}	1.15 ± 0.01^{b}	1.14 ± 0.01^{a}	1.15 ± 0.01^{b}				
		L3	0.93 ± 0.02^{a}	0.93 ± 0.04^{b}	1.01 ± 0.02^{a}	1.01 ± 0.02^{b}				
		L4	1.12 ± 0.01^{a}	1.14 ± 0.02^{b}	1.07 ± 0.02^{a}	1.13 ± 0.01^{b}				
		R1	1.13 ± 0.01^{a}	1.12 ± 0.04^{b}	1.12 ± 0.02^{a}	1.15 ± 0.01^{b}				
		R2	1.15 ± 0.02^{a}	1.17 ± 0.01^{b}	1.14 ± 0.01^{a}	1.17 ± 0.01^{b}				
		R3	0.90 ± 0.03^{a}	0.96 ± 0.02^{b}	0.96 ± 0.02^{a}	0.96 ± 0.03^{b}				
		R4	1.11 ± 0.01^{a}	1.13 ± 0.01^{b}	1.09 ± 0.02^{a}	1.13 ± 0.01^{b}				
	Whole-fillet		1.12 ± 0.02^{a}	1.15 ± 0.03^{b}	1.12 ± 0.01^{a}	1.15 ± 0.01^{b}			0.78	**<0.05**
	Whole-body		1.47 ± 0.02^{a}	1.51 ± 0.02^{a}	1.42 ± 0.01^{b}	1.43 ± 0.01^{b}			**<0.001**	0.20
Moisture	Fillet regions						**<0.0001**	**<0.0001**	**<0.05**	0.21
(g $100g^{-1}$)		L1	60.32 ± 0.01^{a}	62.49 ± 1.31^{a}	63.05 ± 0.67^{b}	59.81 ± 0.60^{b}				
		L2	61.09 ± 1.10^{a}	62.68 ± 1.57^{a}	63.94 ± 1.04^{b}	60.37 ± 1.23^{b}				
		L3	45.79 ± 1.20^{a}	48.21 ± 1.19^{a}	50.8 ± 1.3^{b}	51.91 ± 1.14^{b}				
		L4	61.07 ± 0.56^{a}	62.32 ± 0.74^{a}	63.97 ± 1.53^{b}	64.24 ± 1.01^{b}				
		R1	58.39 ± 1.01^{a}	61.23 ± 1.82^{a}	62.12 ± 0.98^{b}	62.58 ± 1.34^{b}				
		R2	60.16 ± 0.78^{a}	62.53 ± 1.07^{a}	62.74 ± 1.30^{b}	63.95 ± 1.12^{b}				
		R3	44.16 ± 1.57^{a}	47.65 ± 1.05^{a}	48.35 ± 1.18^{b}	49.35 ± 1.45^{b}				
		R4	60.84 ± 0.90^{a}	61.54 ± 1.01^{a}	62.63 ± 1.05^{b}	61.86 ± 1.32^{b}				
	Whole-fillet		57.62 ± 1.21	60.10 ± 1.37	60.78 ± 1.03	60.37 ± 1.23			0.24	0.29
	Whole-body		54.035 ± 0.95^{a}	54.23 ± 1.28^{a}	58.44 ± 0.86^{b}	59.81 ± 0.60^{b}			**<0.0001**	0.20

Values are means ± S.E.M.
Boldness indicates indicate significant *P*-values. Letters indicate significant differences between experimental treatments.

6.4.6 Histological morphometrics: muscle, gill, and skin

White muscle fibre size and diameter on the left fillet were significantly larger than muscle fibres on the right fillet in all treatments (LMER; $F_{1,20419} = 6.14$, $P < 0.05$; LMER; $F_{1,20418} = 7.74$, $P < 0.01$, respectively; Figure 6.3a). Standard deviation of white muscle fibre area was significantly larger in the right fillet (LMER; $F_{1,144} = 7.87$, $P < 0.01$) and in fish raised under LFR (LMER; $F_{1,26} = 11.76$, $P < 0.01$; Figure 6.3b). Muscle fibre density was greater in the right fillet (LMER; $F_{1,144} = 20.72$, $P < 0.001$) and in fish raised under MFR (LMER; $F_{1,26} = 7.28$, $P < 0.05$; Figure 6.3c,d,e).

Lamellae length was significantly taller in fish without scoliosis raised under LFR than in fish with mild scoliosis raised under LFR or in fish with and without mild scoliosis raised under MFR (LMER; $F_{1,141} = 3.97$, $P < 0.05$; Figure 6.4a). Lamellae length was not different between fish with and without mild scoliosis raised under MFR. Distance between lamellae (proxy for density) was significantly shorter in fish without scoliosis raised under MFR than in fish raised under LFR, regardless of having mild scoliosis (LMER; $F_{1,141} = 4.65$, $P < 0.05$; Figure 6.4b). Diffusion distance and filament thickness did not differ among flow regimes (LMER; $F_{1,142} = 0.31$, $P = 0.57$; LMER; $F_{1,142} = 0.35$, $P = 0.57$, respectively) or for fish with and without scoliosis (LMER; $F_{1,142} = 0.30$, $P = 0.58$; LMER; $F_{1,142} = 1.38$, $P = 0.24$, respectively). Gill health, assessed by the presence and absence of pathological features, did not differ among flow regimes (LRT; $G_1 = 0.40$, $P = 0.53$) or for fish with or without mild scoliosis (LRT; $G_1 = 1.71$, $P = 0.19$).

Branchial goblet cell density was significantly lower in fish without scoliosis raised under LFR than in fish with scoliosis raised under LFR and fish raised under MFR, regardless of having mild scoliosis (LMER; $F_{1,141} = 4.65$, $P < 0.05$; Figure 6.4c). Branchial goblet cell size was significantly larger in fish without scoliosis than in fish with mild scoliosis (LMER; $F_{1,142} = 6.65$, $P < 0.05$; Figure 6.4d).

Skin epidermis thickness was thinner in fish raised under MFR than in fish raised under LFR (LMER; $F_{1,142} = 14.93$, $P < 0.001$; Figure 6.5a). Dermis thickness did not differ among flow regimes (LMER; $F_{1,142} = 0.25$, $P = 0.62$) or fish with and without mild scoliosis (LMER; $F_{1,142} = 0.31$, $P = 0.58$).

Skin goblet cell density was significantly lower in fish with mild scoliosis raised under MFR than all other groups, and skin goblet cell density in fish with mild scoliosis raised under LFR was significantly higher than fish raised under MFR but not higher than fish without scoliosis raised under LFR (LMER; $F_{1,141} = 16.801$, $P < 0.0001$; Figure 6.5b). Skin goblet cells were larger in fish raised under LFR than in fish raised under MFR (LMER; $F_{1,142} = 14.26$, $P < 0.001$), while fish with mild scoliosis had significantly smaller skin goblet cells than their non-deformed conspecifics within the same flow regime (LMER; $F_{1,142} = 10.70$, $P < 0.01$; Figure 6.5c).

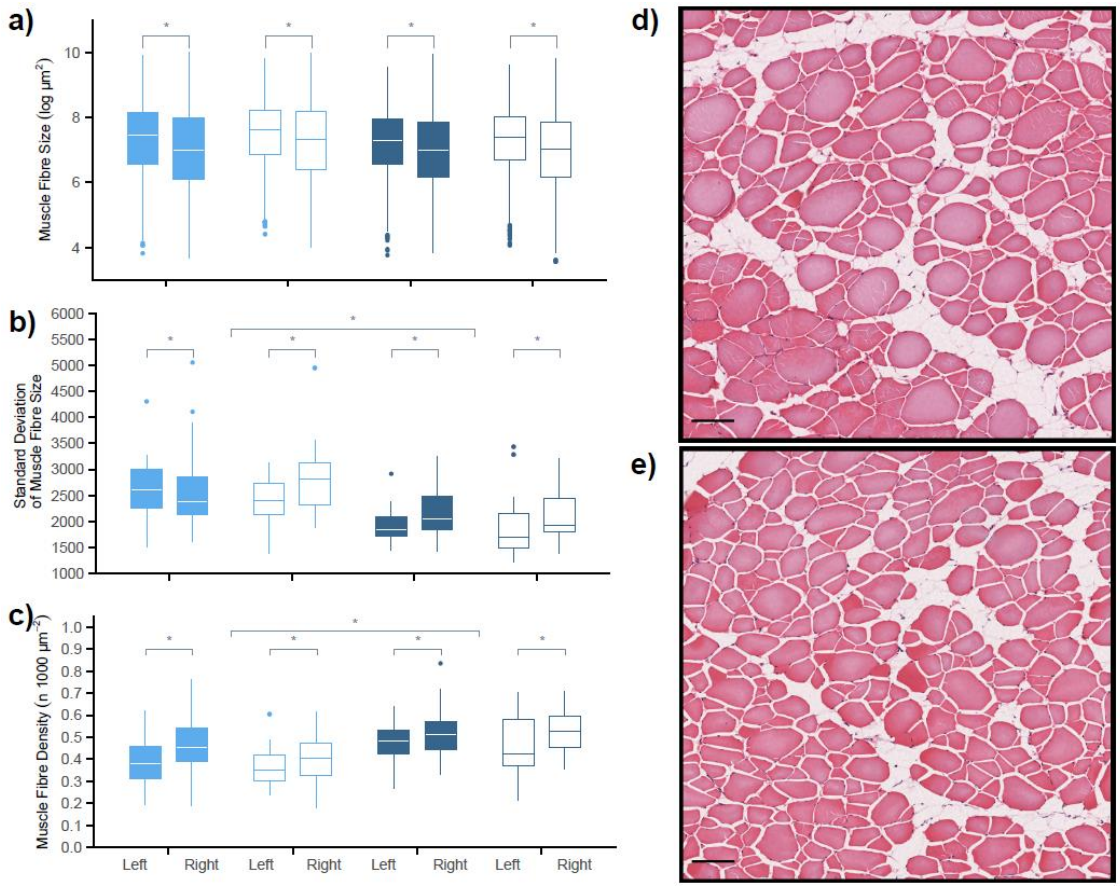


Figure 6.3 Histological analysis of white muscle fibres. Panel a) muscle fibre size, b), standard deviation of muscle fibre size, and c), muscle fibre density shows changes to muscle fibre morphometrics from left and right fillets in Chinook salmon without (filled) and with mild scoliosis (no fill) raised under low (light blue) and moderate (dark blue) flow regimes. Boxplots present the median (middle bar), first and third quartiles (upper and lower bars), and the largest and smallest value within 1.5 interquartile range (IQR; vertical bars). Points represent outliers determined as values beyond the vertical bars (i.e., > third quartile + 1.5*IQR, < first quartile + 1.5* IQR). Brackets and asterisks represent significant differences (P < 0.05) between left and right fillets and exercise treatments. Panel d) and e) present histological micrographs of muscle fibres from left fillet, Chinook salmon without scoliosis raised under low and moderate velocities, respectively. Black scale bars on micrographs indicate 100 µm.*

6.5 Discussion

6.5.1 Swimming performance, aerobic capacity, and recovery

Our results show that long-term training under MFR in Chinook salmon can be used as a tool to improve the overall swimming performance and aerobic capacity in Chinook salmon, including individuals presenting mild spinal curvature. Training under MFR improved U_{crit} by 25% in comparison to individuals from the LFR. In previous exercise studies with Chinook salmon, U_{crit} was recorded to be greater in trained fish than control; however, these results were not significantly different (Thorarensen et al., 1993; Gallaugher et al., 2001). In both these studies, trained Chinook salmon were considerably smaller than those included in the current experiment (< 340 g vs 1,870 g), which may provide some explanation for the opposing results (Thorarensen et al., 1993; Gallaugher et al., 2001). Improving performance through training regimes has been demonstrated in several salmonid studies (in fish < 300 g), including improved 'preferred' swimming speed (Brett et al., 1958), stamina (Nahhas et al., 1982b; Leon, 1986; Houlihan and Laurent, 1987), and U_{crit} (Besner and Smith, 1983; Farrell et al., 1990; McKenzie et al., 2012). Enhanced swimming performance is often linked to improved cardiorespiratory properties, e.g., increased MMR and AS (Nahhas et al., 1982b; Gallaugher et al., 2001; Larsen et al., 2012), as well as, increased blood-oxygen transport properties, haemoglobin (Hb) and Hct (Hochachka, 1961; Zbanyszek and Smith, 1984; Thorarensen et al., 1993; Holk and Lykkeboe, 1998; Gallaugher et al., 2001; Parker and Barnes, 2015). In the current experiment, MMR and AAS improved in fish raised under MFR, but Hct remained similar between the two treatments. Gallaugher et al. (2001) also found MMR to increase in exercised Chinook salmon, contrary to Thorarensen et al. (1993), while both studies found Hct to increase. Improvements in MMR and AAS could be linked to enhanced heart function and efficiency (as shown in Davison, 1989; Farrell et al., 1991; Gallaugher et al., 2001; Nilsen et al., 2019).

Individuals with spinal curvature required higher swimming effort than individuals without spinal curvature, by exhibiting higher TBF for any given swimming speed. Some reasons for an increased TBF could be related to added drag created from having a bent body shape or a compromised musculoskeletal system (e.g., inefficient muscle to spine synchronisation). Consequently, it was expected that individuals with spinal curvature would have reduced swimming performance compared to individuals without spinal curvature. Spinal curvature did not significantly influence U_{crit} (P-value = 0.11), although, in individuals raised under moderate currents, U_{crit} was lower in individuals with mild spinal curvature. Similarly, the relationship between oxygen consumption and swimming speed did not differ between fish with and without spinal curvature. Lack of significant difference in U_{crit} and $\dot{M}O_2$ as a function of swimming speed between fish with and without spinal curvature may have not occurred because of low replication

in fish with spinal curvature raised under LFR and more specifically, the relationships between $\dot{M}O_2$ and swimming speed may have been masked by the skittish behaviour of Chinook salmon within the swim tunnel chamber and using $RMR_{0.5}$ as a proxy for SMR. Despite this, it is suspected that swimming and aerobic performance will be further challenged in individuals that develop more severe spinal curvature than the mild cases that were tested within this study.

Exercise recovery, EPOC and time, were significantly larger and longer in fish with mild spinal curvature than in fish without spinal curvature. Increased recovery in fish with spinal curvature could be associated with them requiring higher TBF for a given swimming speed, potentially leading to earlier activation and dependence of burst swimming and therefore, anaerobic metabolism. Investigation into processes involved in restoring depleted energy stocks (glycogen) and metabolising built-up lactate (Wood, 1991; Kieffer, 2000) between individuals with and without spinal curvature would provide further insight. In addition to these recovery processes, the clearance of stress-related hormones (e.g., cortisol, norepinephrine) could be playing a large role in recovery costs, which is known to occur after exhaustive exercise (Gamperl et al., 1994; Milligan, 1996; Kieffer, 2000). This is particularly interesting, as elevated cortisol levels (without exhaustive exercise) have been shown in fish with spinal deformities (Browning et al., 2012).

6.5.2 Body chemical composition, colour, and shape

Training under MFR altered body structure and composition that could be indirectly supporting swimming performance. Body shape of individuals raised under MFR developed a taller body and larger girth, while the relative visceral mass was significantly lower in fish raised under MFR. Changes in body shape were further reflected in body composition, where lipid composition in the viscera and whole-body were significantly lower in fish raised under MFR. Similarly, developing deeper bodies under higher flows have been shown in Atlantic salmon (Pakkasmaa and Piironen, 2000). Conversely, the Montezuma swordtail, *Xiphophorus montezumae* and a carp species, *Schizothorax wangchiachii*, reduced their body height and increased their length to create a more streamline shape in response to exercise (Alcaraz and Urrutia, 2008; Lu et al., 2020). This suggests that swimming and morphology are closely linked, but responses to exercise are likely to be species-specific. Furthermore, reductions in lipid composition under training programs occurs under moderate to high swimming speeds or under long training durations (Gamperl et al., 1988; Bugeon et al., 2003; Simpkins et al., 2003a, 2003b; Patterson et al., 2004; Kiessling et al., 2005; Rasmussen et al., 2011). Changes to lipid composition and deposition in fish raised under MFR could be caused by increased lipid metabolism to sustain higher exercise levels and therefore, can alter body shape (change in condition factor), indirectly enhancing swimming performance. Inverse relationships between

lipid levels and swimming capacity have been identified, where swimming performance is poorer at higher lipid composition (McDonald et al., 1998). In the current experiment, fish raised under LFR exhibited poorer swimming performance and higher lipid composition, also making this link. Moreover, protein composition in the current experiment did not differ between flow regimes, consistent with other swimming studies involving salmonids (Nahhas et al., 1982b; White and Li, 1985; Totland et al., 1987; Kiessling et al., 1994; Patterson et al., 2004; Castro et al., 2011; Rasmussen et al., 2011; Grisdale-Helland et al., 2013). While protein composition is tightly regulated in fishes and unable to be easily manipulated through exercise, increases in relative fillet size may have occurred under exercise as visceral size and lipid content were reduced under MFR in comparison to fish raised under LFR.

Furthermore, whole-fillet protein was significantly higher in fish with spinal curvature compared to fish without likely linked to the greater swimming effort and increased drag associated with spinal curvature. Similarly, protein composition was significantly higher and lipid composition was significantly lower in the left fillet. The same pattern was reflected in fillet muscle fibres and fillet colour lightness, where muscle fibres were larger and more uniform in the left fillet than fibres in the right fillet and fillet colour lightness was significantly lower in the left fillet as well as in fish with spinal curvature. Positive correlations between protein content and muscle fibre size as well as lipid content and fillet lightness has been reported in Atlantic salmon and other fish species (Christiansen et al., 1995; Rørå et al., 1998; Mørkøre et al., 2001; Huang et al., 2021). In the current experiment, body composition being influenced by spinal curvature and sides of the fish could be explained by the relationship between moving water and swimming in a circular direction. In both treatment groups, water flow moved clockwise, meaning the fish swam in an anti-clockwise direction; however, swimming behaviour differed between the two treatment groups. After ~3-4 months under treatment, fish raised under MFR begun to hold position towards the current, whereas fish under low velocities continued to advance around the tank. The difference in swimming behaviour may have changed myotome activation (e.g., contraction and shortening rates, location along the fillet; Bone and Moore, 2008), and the impact of moving water on the fish causing asymmetrical pressures on muscles, influencing body composition laterally. Additionally, it is worth mentioning that during the final routine spinal assessment, the direction of spinal curvature was recorded and showed that spinal curvature (if present) in fish raised under LFR bent exclusively towards the left, whereas spinal curvature (if present) in fish raised under moderate velocities curved in both directions but predominantly towards the right. Since the direction of spinal curvature was not recorded across all routine assessments, statistical analysis could not be appropriately performed on these data and therefore, interpretation is limited.

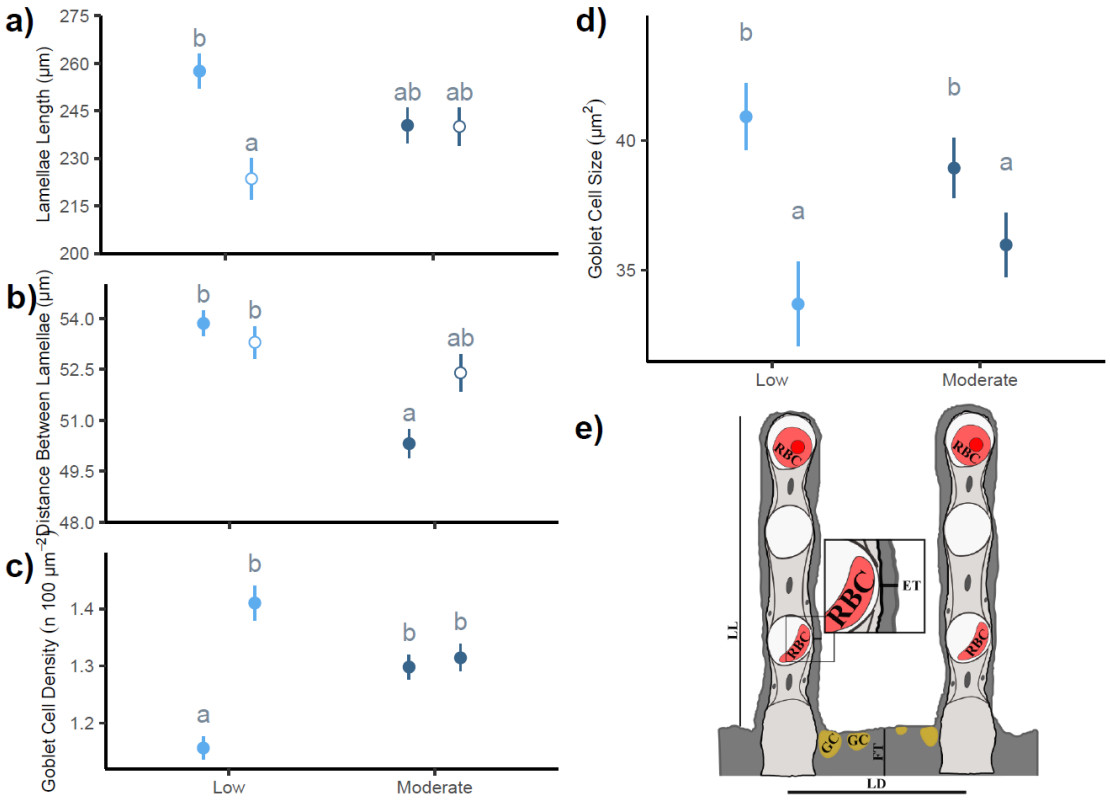


Figure 6.4 Histological morphometrics of the branchial structures and their associated mucous cell communities in Chinook salmon without (filled) and with mild scoliosis (no fill) raised under low (light blue) and moderate (dark blue) flow regimes. Panels a), lamellae length and b), distance between lamellae show morphological changes to branchial structures. Panel c), presents branchial goblet cell density and d), presents branchial goblet cell size. Panel e) presents schematic of two consecutive lamellae depicting morphological features measured. LL, lamellae length; FT, filament thickness; LD, lamellae density or distance between lamellae; ET, lamellae epithelial thickness. Not to scale. Points and vertical bars (panels a:d) represent mean ± S.E.M.; significant differences ($P < 0.05$) are indicated by lowercase letters.

6.5.3 Muscle, gill, and skin histomorphology

In fish, muscle growth is driven by two processes: cell proliferation (hyperplasia) and cell enlargement (hypertrophy). Hyperplasia is more common during earlier life stages when growth rates are high, while hypertrophy continues throughout the fish's life (Higgins and Thorpe, 1990; Zimmerman and Lowery, 1999; Johnston et al., 2011). Thus, competition between these two processes influence fibre size distribution, as hyperplasia would increase the number of smaller fibres and hypertrophy would increase the number of larger fibres. In the current experiment, exercise treatments had a significant influence on the muscle fibre morphometrics, where fish raised under MFR presented a more uniform fibre size and a higher density than fish raised under LFR. These results suggest that exercise may influence the rate of hyperplasia and hypertrophy processes, where it is suspected that hyperplasia may have been more dominant in fish raised under moderate velocities than in fish raised under LFR during early exposure (i.e., beginning of experiment when fish were small), allowing for fibre density to increase. These results are inconsistent with other exercise studies, where hypertrophy is found to be the significant processes influencing muscle fibre morphometrics under exercise (Davie et al., 1986; Postlethwaite and McDonald, 1995; Bugeon et al., 2003; Rasmussen et al., 2011; Palstra et al., 2014; Huang et al., 2021; Timmerhaus et al., 2021). However, in exercised gilthead sea bream (*Sparus aurata*) fingerlings, fibre hyperplasia was the dominant process for muscle growth compared to hypertrophy, likely owing to the young age when investigated (Moya et al., 2019). Documenting changes in muscle growth from early (small size fish) exposure to late (large size fish) exposure will improve understanding of when muscle growth switches between hyperplasia to hypertrophy and its relationship with exercise.

The teleost gill can remodel its structure in response to changing environmental conditions (e.g., temperature, hypoxia, salinity) to enhance oxygen uptake or, conversely, to reduce passive ion and water fluxes (Sollid et al., 2003; Nilsson, 2007; Gilmour and Perry, 2018). It is hypothesised that the teleost gill may make small adjustments to enhance oxygen uptake under long-term sustained swimming, such as increased lamellae length and reduced diffusion distance and distance between lamellae. In the current experiment, diffusion distance did not differ among flow regimes, but lamellae length was significantly shorter in fish raised under LFR with spinal curvature, while all other groups remained similar. Reduced lamellae length in LFR fish with spinal curvature is not consistent with the hypothesis, but similar unexpected alterations in lamellae length have been described in other studies in response to temperature (Bowden et al., 2014; Johansen et al., 2021). These studies suggest that adjustments to lamellae length could be a result of short-term physiological adjustments, e.g., increased perfusion, rather than morphological remodelling or linked to the osmorespiratory compromise, where gills initially respond to enhance oxygen uptake, but later recede to reduce the impact on osmotic homeostasis.

It is unclear as to why lamellae length of fish raised under low velocities with spinal curvature would reduce. Conversely, distance between lamellae, a proxy for density, reduced in individuals raised under MFR, in support of this hypothesis. Lamellae density may have increased to provide larger surface area to enhance oxygen uptake in individuals under higher aerobic pressures.

Branchial goblet cell density was significantly lower in individuals raised under LFR with spinal curvature, while goblet cell size was significantly larger in individuals without spinal curvature, irrespective of the flow regime. In response to chemical and physical irritants, goblet cells respond by proliferating and hypersecreting by more than double compared to control groups (Mallatt, 1985; Hess et al., 2015). This generally results in hyperplasia within the interlamellar space, leading to an increase in filament thickness (Persson et al., 2021). In the current experiment, filament thickness did not differ between flow regimes and considering that the changes in branchial goblet cell density and size were small, it is difficult to infer these results as an indication of declining fish health. Rather, these results suggest that trade-offs exist between goblet cell density and size, where higher densities often result in smaller size and vice versa. Similar patterns were found in skin goblet cell density and size. Skin goblet cell density did not follow a distinct pattern across treatments; rather, they tended to be higher in fish raised under low velocities and skin goblet cell size was significantly larger in fish raised under LFR, likely linked to the epidermis being significantly thicker (i.e., more available space). Available space appears to be the main characteristic driving opposing patterns between goblet cells in the gill and skin, rather than being a physiological response (Elliott, 2011b).

Skin epidermis thickness was significantly reduced in fish raised under MFR, while the dermis thickness did not differ among flow regimes. Similarly, epidermis thickness in Atlantic salmon declined with increasing swimming speeds; however, these reductions were found at speeds above the training regimes used in the current experiment (e.g., 1.8 and 2.5 bl s^{-1}). Reduced epidermis thickness may occur at lower speeds in Chinook salmon under exercise training compared to other salmonid species. However, at similar swimming speeds to the current experiment, Timmerhaus et al. (2021) showed Atlantic salmon trained at 1 bl s^{-1} provided the thickest epidermis. It is suggested that having a thicker epidermis would provide better protection and health to the fish, as the epidermis is a large surface area in direct contact with the external environment and acts as the first defensive barrier to pathogens and parasites (Elliott, 2011a; Elliott, 2011b; Ángeles Esteban, 2012). An opposing view is that epidermal thickening may only occur during periods when protection is needed. In fact, epidermal thickening has been reported in fish infested with skin parasites, exposed to pollutants, or events resulting in increased skin abrasion (e.g., spawning activity; Elliott, 2011a; Hess et al., 2015). Throughout this study, there were no significant signs of poor fish health in either treatment, and mortality rates across the study remained low. Another possible explanation for reduced epidermis thickness in fish raised

under moderate currents could be associated with swimming performance and aerobic efficiency. Although poorly understood, it has been suggested that several properties of fish skin (e.g., shape, formation, and goblet cell distribution) can influence boundary layer properties, functional efficiency as a secondary site for oxygen consumption, as well as reducing friction along the body during oscillatory movements when swimming (Elliott, 2011b). Thus, reducing epidermis thickness in fish raised under moderate velocities could be due to a combination of these reasons.

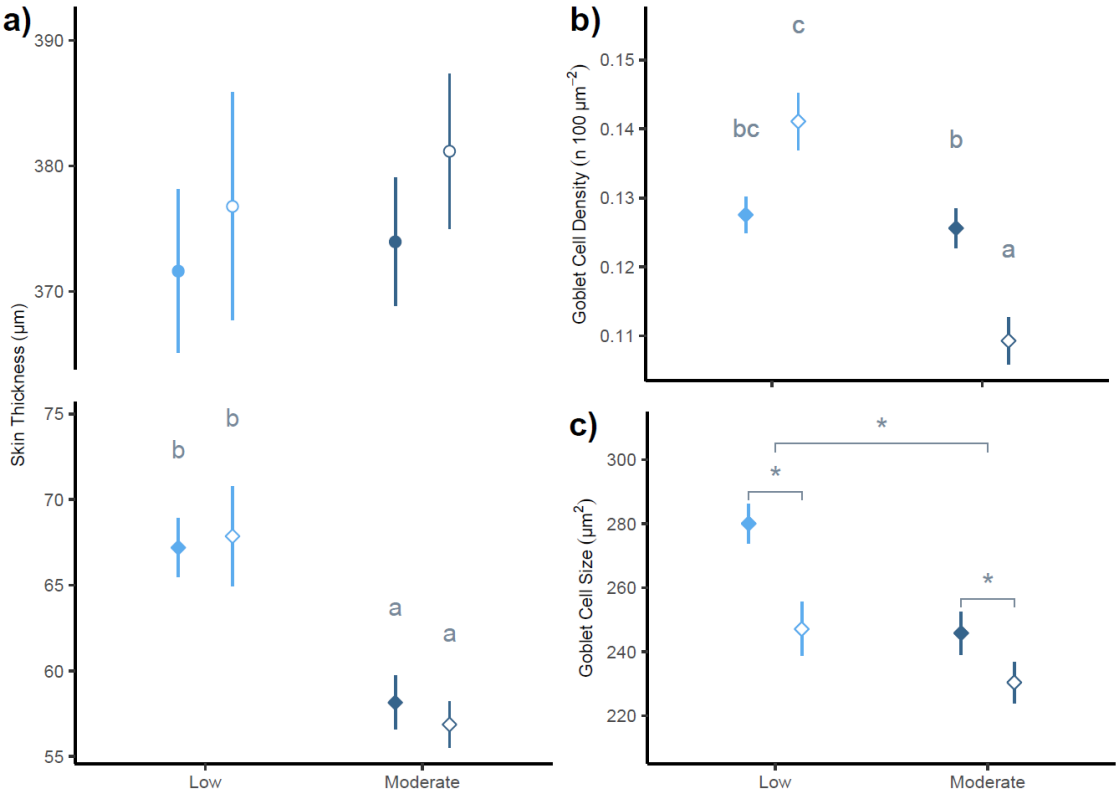


Figure 6.5 Histological morphometrics of the skin structures and their associated mucous cell communities in Chinook salmon without (filled) and with mild scoliosis (no fill) raised under low (light blue) and moderate (dark blue) flow regimes. Panel a), presents skin thickness of the epidermis (♦) and dermis layers (●). Panel b), presents skin goblet cell density and c), presents skin goblet cell size. Points and vertical bars represent mean ± S.E.M.; significant differences (P < 0.05) are indicated by lowercase letters and brackets (depicting comparison) with asterisk in panel c).

6.5.4 General discussion

This study used flow regimes that were considerably slower than environmental currents likely to be encountered in an offshore farming site. These speeds, however, were chosen as they can easily be implemented into an industry's nursery facility without major modifications to the existing infrastructure as well as being aerobically maintained by larger fish. This study did not attempt to implement an exercise regime that mimicked the optimal swimming speed with increasing fish size. It is possible that the speeds at the start of the experiment, when the fish were small, were not aerobically challenging, but with increasing fish size would likely have aligned closely to the optimal swimming speed. Nonetheless, the exercise regimes implemented elicited changes to the U_{crit}. To further assist NZ salmon industry expanding offshore, more research is warranted to understand the dynamics of Chinook salmon swimming performance at higher flow regimes. Future studies may include more sophisticated exercise regimes (e.g., more intense exercise regimes, exercise regimes following optimal swimming speeds) and challenging swimming performance tests at a sea transfer size (e.g., oscillating swimming speeds, change in swimming direction) to better understand if exercise can prepare Chinook salmon for offshore farming, ensure animal welfare, and to inform guidelines for determining site suitability.

This study was initially designed to understand the impact of sustained swimming on form and function in 'healthy' Chinook salmon. However, individuals with mild spinal curvature were included as a predicator variable within this study, as this phenotype was advantageously present within the study population and the influence of exercise on spinal curvature incidence was observed. However, as fewer individuals presented mild spinal curvature in the LFR during the assessment period, this limited the number of fish analysed with spinal curvature in this group. It is possible if more individuals were measured within this group, more significant relationships between fish with and without spinal curvature and interactions with flow regimes may have been detected (e.g., U_{crit}, whole-body protein composition). Nonetheless, this study still provides an insight into the impacts of sustained swimming on individuals with spinal curvature and is relevant to the future expansion of the NZ salmon industry into high energy waters and is worthy of future attention.

Spinal curvature and other spinal deformities are among the main health concerns some farms face within the NZ salmon aquaculture industry. This study is the first to provide insight into how individuals with and without spinal curvature will perform under training programs and if exercise training can be used as a tool to prepare stocks for high energy open ocean farming. Long-term MFR was found to improve swimming performance in domesticated Chinook salmon with and without mild scoliosis; however, the longer recovery process in individuals with spinal curvature is of concern. Individuals with spinal curvature could exhibit poor feed and growth

performance, if they are unable to successfully recover from stressful events, such as handling procedures, predator encounters, or bad weather events. This study also found MFR and spinal curvature to alter lipid levels within visceral regions, while the relationship between spinal curvature and swimming effort may influence protein deposition within the left and right fillets. These results highlight how nutrient utilisation and deposition in Chinook salmon trained in tanks and/or farmed in higher energy sites may differ from those farmed in near shore protected bays, highlighting the need for diet composition to be evaluated and, if required, alterations made to feeds to sustain higher lipid metabolism. Fibre structure within white muscles was also a product of exercise training, but mainly in relation to swimming direction, where fibre morphology differed between the lateral sides of the fish. Gill and skin histology showed only small cellular changes in response to flow regimes and spinal curvature, therefore inferring a physiological response is limited. Nonetheless, improving health and welfare through flow regimes provides an interesting avenue to further explore, especially when considering the rate of emerging parasites and pathogens within finfish farms globally (Murray and Peeler, 2005; Nowak, 2007) and for protection against the already existing local health problems (skin lesions; Johnston et al., 2021; Lane et al., 2022). With further research, e.g., different life stages, higher and varying velocities etc., applying swim training during pre- and post-smolt production within the NZ salmon industry could present a viable option to make a more robust and resilient salmon for offshore farming.

Chapter Seven General Discussion

Part of the research contained within this chapter is in preparation as Prescott, L.A., Symonds, J.E., Walker, S.P., Miller, M.R., Semmens, J.M., Carter, C.G., 2023. Exercise training as an early life shaping tool for enabling high-energy offshore salmonid farming.

This thesis investigated the short- and long-term impacts of sustained swimming on Chinook salmon form and function. The new knowledge gained supports New Zealand's industry advancements of expanding grow-out practices offshore into high-energy environments and transitioning to recirculating aquaculture systems (RAS) with integrated flow regimes to enhance production and fish resilience. These farming developments are representative of the global salmon industry's goals and therefore the thesis provides larger application to the wider salmon aquaculture community. The research set out in this thesis is also of particular interest to fields of fish physiology and bioenergetics, placing a large contribution to fundamental science. This final chapter first highlights the major findings of each chapter and discusses how this new knowledge can contribute to local industry practices of integrating flow regimes during early production stages. Further focus is given to the global offshore farming developments, where New Zealand's (NZ) Blue Endeavour site is used as a case study to demonstrate the benefits of pre-conditioning through a bioenergetics analysis. Lastly this chapter identifies some specific points of interest for future research to better farm robust and resilient salmon for exposed offshore farming.

7.1 Summary of findings

The thesis presented here has created a large dataset to explore how a wide range of key parameters are influenced by sustained swimming and to quantify how they impact Chinook salmon form and function, leading to benefits in fish welfare and production efficiencies (Table 7.1). The primary techniques utilised were tank-based experiments with a low flow regime (0.3 bl s^{-1}; LFR) and moderate flow regime (0.8 bl s^{-1}; MFR) to encourage sustained swimming and analyse a time-series of responses. Several assessments were used throughout the experiment to measure swimming behaviour (**Chapter 3)**, effort (**Chapter 5)**, and performance (**Chapters 5 and 6**), production performance at individual (**Chapter 3**) and tank (**Chapter 5**) level, spinal health (**Chapters 3 and 6**), genetic parameters and environmental interactions (**Chapter 4**), as well as internal form: chemical composition (**Chapters 3, 5, and 6**), morphometrics (**Chapters 5 and 6**), and transcriptome (**Chapter 5**). Overall, increasing exposure to sustained swimming elicited different impacts on production performance, composition, and swimming performance.

7.1.1 Swimming behaviour, effort, and performance

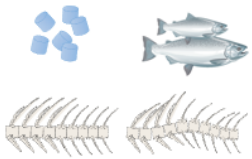
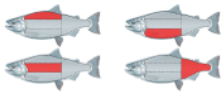
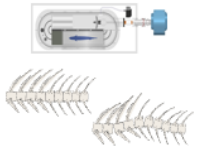
The results of **Chapter 3** indicate that swimming speeds do not differ between low to moderate flow regimes; however, in **Chapter 5** there was a significant difference in swimming effort between the two flow regimes, indicating that MFR became challenging to Chinook salmon when they reached ~600 g and ~300 mm. Measuring indicators of swimming (e.g., speed or effort) provides better information, relative to inferring from the set flow speeds, to understand how salmon respond to sustained swimming, because fish may choose to swim faster than the set flow, as found in **Chapter 3**. Moreover, these measurements provide an indication of how to choose appropriate flow regimes for assessing different training levels with Chinook salmon (e.g., voluntary verse forced swimming).

The timespan of training was critical for the outcome of these experiments, as shown by differences in swimming performance between salmon reared for six and ten months. Rearing Chinook salmon under LFR and MFR for six months did not significantly improve swimming performance, demonstrated by little change in the critical swimming speed (U_{crit}; **Chapter 5**); however, after ten months of training, U_{crit} and aerobic scope were significantly enhanced (**Chapter 6**). These results suggest that as the salmon grew larger, MFR became more challenging than LFR, resulting in fish better conditioned for swimming endurance.

7.1.2 Production performance

Even though exercise training in the first three months was similar under LFR and MFR, feed consumption-growth relationships and feed efficiency (on an individual level) did improve in individuals reared under MFR, which could be related to more uniform swimming speeds and less spontaneous activity (**Chapter 3**). However, after six months under treatment, production performance was superior in individuals reared under LFR, where they grew larger and faster, and with improved feed efficiency (on a tank level; **Chapter 5**). Changes in production performance under LFR and MFR suggest there is an interaction with increasing size, where MFR became challenging when Chinook salmon reached ~600 g. Chinook salmon still remain as one of the only salmonid species (of those investigated) to not exhibit exercise-enhanced growth.

Table 7.1 Physiological assessments and the responses of Chinook salmon reared under low and moderate flow regimes for each chapter of this thesis.

	Chapter 3: Production biology and spinal health		Chapter 4: Genotype by environment interaction		Chapter 5: Metabolic, mechanistic, and locomotory		Chapter 6: Swimming performance and recovery efficiency	
								
Physiological assessments	**Three months**		**Three months**		**Six months**		**Ten months**	
	LFR	**MFR**	**LFR**	**MFR**	**LFR**	**MFR**	**LFR**	**MFR**
Swimming:								
behaviour	N.D.	N.D.						
effort					↓	↑		
performance							↓	↑
Production performance:								
feed intake	N.D.	N.D.			N.D.	N.D.		
growth	N.D.	N.D.			↑	↓		
feed efficiency	↓	↑			↑	↓		
Spinal health:								
frequency	N.D.	N.D.						
severity	N.D.	N.D.						
Genetic parameters:								
heritability			N.D.	N.D.				
genotype by flow regime			N.D.	N.D.				
Internal form:								
chemical composition	N.D.	N.D.			N.D.	N.D.	↓	↑
muscle morphology					N.D.	N.D.	↓	↑
transcriptome					↓	↑		

↑ = improved; ↓ = reduced; N.D. = not significantly different; LFR = low flow regime; MFR = moderate flow regime

7.1.3 Spinal health

Previous studies have revealed NZ farmed Chinook salmon to exhibit unfavourable genetic and phenotypic relationships between higher condition factor and spinal curvature development (Perrott et al., 2018; Scholtens et al., 2023). In agreement with these studies, **Chapter 3** showed individuals that developed spinal curvature were significantly heavier, longer, and had higher condition factor, and the probability of individuals developing spinal curvature increased with higher condition factor. These results suggest that spinal curvature occurrence is driven by myogenic factors, and possibly linked to genetically-selected faster growth. It was initially hypothesised that exercise training would improve spinal health, because previous studies have shown that bone mineral content increases with exercise, leading to better spinal health (Baeverfjord et al., 2019). However, this thesis did not find any evidence to suggest this, and bone mineral content was not significantly different between exercise regimes, supporting the theory that myogenic factors are the underlying driver behind spinal curvature. This information can be used by the NZ Chinook salmon industry for consideration in their selective breeding criteria.

Furthermore, the impact of developing spinal curvature on individual respiratory and locomotory performance was quantified. Using swimming respirometry, swimming performance was estimated in Chinook salmon that were reared under LFR and MFR for ten months and included individuals that presented with mild spinal curvature (**Chapter 6**). The findings show that being reared under MFR improves the U_{crit} and widens aerobic scope, regardless of the spinal condition. Nevertheless, compromised spinal health did lead to greater recovery costs from reaching exhaustion. These findings question the welfare and production performance of individuals with spinal curvature, and possibly other spinal deformities, and suggest that it may be unsuitable and less economical to farm individuals with compromised spinal health offshore in high energy farm sites.

7.1.4 Genetic parameters and environmental interactions

For the first time, this thesis has assessed the potential for genotype-by-environment (G×E) interactions for production performance between two different flow environments (**Chapter 4**). This information is key to the robustness and resilience of selective breeding programs as the industry advances. Here, there was no indication of G×E interactions between any production performance trait and flow environments, suggesting that, for Chinook salmon up to 600 g, the industry's selective breeding program is robust under low to moderate flow regimes. However, the results of **Chapter 3** show that swimming speeds were not different between these two flow environments and that production performance was measured across size classes that typically exhibit peak growth, which may have masked possible interactions.

Different variables (e.g., flow environments) across a large size class need to be investigated, before concluding if G×E interactions exist between flow regimes.

This study also revealed that there were no unfavourable trait correlations for size- and growth-related traits, but using measuring feed intake would provide better genetic gains for feed efficiency. This genetic information is important for the industry's selective breeding programs as one of their priority breeding goals is to improve feed efficiency by selecting faster growth.

7.1.5 Internal form: chemical composition, morphology, and transcriptome

Chemical composition of the liver, viscera, fillet, and whole body was assessed multiple times throughout this study to quantify temporal changes with exercise training. Short (three months) and long (six months) term exposure to LFR and MFR did not influence chemical composition (**Chapter 3 and 5**), likely associated with similar swimming speeds being performed for the first three months under LFR and MFR. However, after ten months of being reared under LFR and MFR, the visceral and whole-body fat contents were significantly lower in Chinook salmon reared under MFR, suggesting that lipid catabolism was upregulated to support the energy requirements of faster swimming speeds. Fat content did not change in the white muscle, which suggests that the diet composition contained enough lipids and energy to support this level of physical exertion and did not compromise product quality.

White muscle morphology was influenced by the two flow regimes differently, but only when individuals were reared for ten months. In **Chapter 5**, white muscle fibre size and density did not differ between individuals reared under LFR and MFR (six months under treatment), but in **Chapter 6**, after ten months under treatment, white muscle fibres in individuals reared under MFR were denser than fibres in individuals reared under LFR. This result was driven by individuals exhibiting a more uniform size range (suggested by differences in standard deviation), and not because of smaller muscle fibres. The morphological changes in response to flow regimes suggest that hyperplasia (cell proliferation) outcompeted hypertrophy (cell enlargement) towards the later stages of training. This differs from other exercise studies with salmonids, where hypertrophy drives morphological changes in the white muscle (Davie et al., 1986; Postlethwaite and McDonald, 1995; Bugeon et al., 2003; Rasmussen et al., 2011; Palstra et al., 2014; Huang et al., 2021; Timmerhaus et al., 2021), while hyperplasia is described to be the predominant process behind muscle growth in early life stages, before reaching 50% of their total length (Higgins and Thorpe, 1990; Zimmerman and Lowery, 1999; Johnston et al., 2011). This indicates that Chinook salmon present unique responses to exercise regimes, inconsistent with closely related species.

In **Chapters 5 and 6**, the right (outward facing) fillet had significantly lower protein and higher lipid content compared to the left (inward facing) fillet. Lateral asymmetry was also

reflected in white muscle morphology, where the white muscle fibre size was significantly more dense in the right fillet compared to the left fillet. These responses match the lateral changes in white muscle composition and suggests that swimming in an anti-clockwise direction may increase white muscle contraction on the right fillet over the left fillet. However, it is unknown if these lateral responses are because of continuous circular swimming or because of the tank size (i.e., 3,200 L experimental tanks vs. 30,000L commercial tanks), thus it questions the relevance of these results to the industry; Is lateral asymmetry a concern during the hatchery phase when circular tanks (e.g., in RAS) are being used and/or during grow out in nearshore sea pens when salmon form schools circling the pen edge? More work is needed to gain a better understanding of the extent of lateral asymmetry by assessing historical harvest data, observing salmon swimming in sea pens, and analysing current speeds within the sea pens.

In **Chapters 5,** transcriptomic approaches were used to assess the molecular changes inflicted by different levels of swimming and how these underlying changes are reflected in the physical condition. Upregulation of genes involved in lipid metabolism, muscle development and contraction, and muscle repair and maintenance were observed in individuals reared under the MFR. These transcriptomic changes agree with previous studies with Atlantic salmon and rainbow trout that exhibited exercise-enhanced growth (Felip et al., 2012; Magnoni et al., 2013; Huang et al., 2021; Pengam et al., 2021; Timmerhaus et al., 2021); however, unlike these studies, changes in production-biology, chemical composition, and muscle morphology did not complement the changes in gene expression. Previous studies exercising salmonids also identify growth and insulin-like growth factor hormones to upregulate and be the key markers driving exercise-enhanced growth (Felip et al., 2012; Magnoni et al., 2013; Huang et al., 2021; Pengam et al., 2021; Timmerhaus et al., 2021). These growth-related genes were not differentially expressed in this evaluation, which is consistent with the lack of evidence supporting exercise-enhanced growth in Chinook salmon. Furthermore, several genes involved in immune responses, such as inflammatory markers, were up-and down-regulated. Immune-related genes are recognised as important markers in mammalian muscle growth, and their role in teleost muscle growth is now being explored (Johnston et al., 2009), including in species exhibiting exercise-enhanced growth (Magnoni et al., 2013; Palstra et al., 2020; Timmerhaus et al., 2021). Overall, the transcriptome showed several changes between individuals reared under LFR and MFR, yet these differences were unable to be linked to modifications on higher biological levels but could be identifying time as a critical component when moderately exercising Chinook salmon. As such, the transcriptomic may act as a precursor for the changes yet to come, such as those documented in **Chapter 6**.

In **Chapter 5,** the physical differences observed between the left and right muscle blocks supports the hypothesis that circular swimming stimulates muscle blocks unevenly. To further

test this hypothesis, transcriptomic approaches were also applied to white muscle sampled from both sides of the fish. The right (outward facing) white muscle from fish reared in both flow regimes upregulated genes involved in lipid metabolism and muscle development and contraction, comparable to individuals reared under MFR that exhibited greater swimming effort (i.e., higher TBF). Other genes which were upregulated in the right muscle blocks are involved in immune responses, collagen, transcription, and protein biosynthesis, which could also be linked to the morphological and compositional changes between the left and right muscles. These asymmetrical findings could be explained by the right muscle blocks being the primary driver to generate thrust. When using circular tanks with a clockwise flow regime to encourage swimming, the fish are continuously turning towards the left promoting their body to position in a reverse c-shape. This only allows the fish to propel forward by completing half of a caudal fin oscillation through contraction of the right muscle blocks. Thus, continuous circular swimming exercises the outward facing muscle blocks more than the inward facing muscle blocks.

7.1.6 Limitations

The experiments conducted throughout this thesis used tank-based RAS to rear Chinook salmon under low or moderate flow regimes for up to ten months. A limitation of this experimental approach is that the tank size (8,000 L and 3,200 L experimental tanks) may not be representative of commercial setups (30,000 L tanks) and the flow regimes used were slow in comparison to environmental conditions, particularly during the beginning of the experiment. However, the flow regimes were chosen based on existing literature (Thorarensen et al., 1993; Kiessling et al., 1994; Kiessling et al., 2005; Hoffnagle et al., 2006) and aligned to speeds that could be achieved in hatchery facilities without major modifications. As quantified in **Chapter 3**, the flow regimes at the start of the experiment, when the fish were small, were not aerobically challenging, suggesting future research should include more sophisticated exercise regimes (e.g., aligning with optimal, oscillating speeds, etc.). Similarly, these experiments were conducted using seawater, even though exercise training may begin during a pre-smolt stage in freshwater. Majority of studies exercising salmonids are conducted in freshwater (refer to Table 2.1 in **Chapter 2**) and as one of the main aims of this thesis was to gain insights into how Chinook salmon may perform in offshore environments that elicit sustained swimming, it was appropriate to assess the Chinook salmon's performance in seawater.

On a more technical level, there are some limitations associated with the number of families used in **Chapter 4** when assessing for potential G×E interactions between flow environments. Initially, this experiment was designed to include 80 families from two separate commercial breeding programs, but unfortunately, offsprings from one commercial breeding program failed to acclimate to Cawthron's finfish research centre (i.e., not feeding), and therefore

could not be used in this study. Similarly, **Chapter 6** evaluated swimming performance on fewer Chinook salmon reared under LFR with mild spinal curvature than the other three groups. More significant relationships in swimming performance may have been detected if more individuals were measured, but unfortunately limited individuals presenting with this case.

7.2 New Zealand salmon aquaculture towards integration of RAS and flow regimes

Integrating flow regimes in RAS to exercise and improve production performance in NZ farmed Chinook salmon remains a challenging task. Even though, in **Chapter 3**, there were some improvements in production performance under MFR during the first three months, exercise-enhanced growth was not documented in this thesis. This thesis supports the theory that Chinook salmon respond differently to flow regimes compared to other salmonids (Davison and Herbert, 2013; Prescott et al., 2024). Future research should focus on higher flow regimes with consideration of the preferred swimming speeds to determine the effects of exercise on Chinook salmon production. In this instance, rather than comparing different exercise levels, comparison between voluntary swimming and forced swimming would be relevant.

In addition to exploring higher flow regimes, investigation into the most appropriate tank size and swimming direction needs to be considered. Throughout this thesis, there were significant lateral responses in chemical composition, white muscle morphology, and gene expression, suggesting that continuous swimming in one direction may exercise the sides of the fish unevenly. There is concern that circular swimming could influence the direction of the spinal curvature and perhaps even increase susceptibility to developing spinal scoliosis. Comparing Chinook salmon swimming straight, clockwise, anti-clockwise, and a combination of both directions would reveal relationships between muscle contraction and extension, and possibly identify a way to manage spinal scoliosis susceptibility.

A further understanding of whether lateral pressures from swimming in circles is only relevant to tanks or is also relevant to salmon in sea pens is essential. Typically, in nearshore sea pens, salmon continuously swim in circles around the pen edge, as the water movement through the pen is considerably slower than their preferred swimming speed (Johansson et al., 2014). As such, swimming behaviour in sea pens could be influencing the direction of the spine and susceptibility to spinal curvature. Spinal curvature is a late-onset spinal deformity in Chinook salmon (Davie et al., 2018; Perrott et al., 2018; Lovett et al., 2020), and there is building evidence suggesting myogenic factors drive spinal curvature (i.e., muscle development outpacing spinal mineralisation causing strain on the spine and therefore bending) (Scholtens et al., 2023; Prescott et al., 2024). Spinal curvature leads to welfare and economic issues, where fish with spinal

curvature require increased processing time and have reduced economic value. If Chinook salmon were predominantly holding position into a current, such as what is assumed to be a main behaviour in offshore farming, the prevalence of spinal curvature may decline. However, further research is needed to confirm this.

Exploring the use of interval training would be extremely valuable for fish production, since recovery periods are essential for healthy muscle growth in mammals (Yang and Hu, 2018). In Atlantic salmon, growth increases with increasing swimming speeds, but muscle inflammation and reduced welfare were also associated with the higher swimming speeds (Timmerhaus et al., 2021), although this correlation was not evident in **Chapter 5**. Recovery periods may be important to alleviate potential negative effects from over stimulation. In this thesis, daily feed intake (on a tank level) was reduced in the MFR after ten months and possibly even after six months (though not significantly different from LFR). Lower feed intake in MFR could indicate the fish lacked sufficient energy or cognitive abilities to collect food and maintain position in the current. As such, having a period of lower flow (i.e., interval training regime or recovery period) could allow for subsequent tissue recovery and ingestion of feeds. An oscillating current regime would also relate closer to environmental current speeds that may be encountered when transferred to sea, and being exposed to these fluctuations during earlier developmental stages could improve plasticity. Interval training has received less attention amongst the salmonids. However, Castro et al. (2011) demonstrated that Atlantic salmon exposed to interval exercise training exhibited the highest survivability during the disease challenge than continuously exercised fish.

A large factor restricting the integration of flow regimes into the aquaculture industry, is being able to appropriately scale flow with commercial set ups, particularly for larger fish (> 500 g), in an economically and efficient way. A cost-analysis showing the benefits of exercise-trained farmed fish may initiate interest for industry to work with engineers and hydro-dynamic physicists to better design commercial setups to integrate flow regimes.

7.3 Offshore high energy finfish farming: a case study of New Zealand's proposed open ocean farming

Challenging the expansion of fish farming offshore are the hydrodynamic conditions that can characterise these locations, i.e., strong current speeds and wave action, and how these factors impact farming infrastructure, operations, and animal welfare. If fish encounter conditions that challenge their swimming abilities, particularly for extended periods, exhaustion can occur, possibly causing fish to collide against each other and the sea pen (Johansson et al., 2014; Hvas et al., 2020). Even if exhaustion is not reached, physiological processes change when fish swim

at higher speeds (fatigue), which could negatively impact farming productivity, and therefore need to be considered.

When fish increase their swimming speed, blood flow is redirected to supply oxygen to, and remove wastes from the muscles and therefore, blood flow is reduced from other tissues and processes, in particular the liver, spleen, and stomach (Randall and Daxboeck, 1982; Thorarensen et al., 1993; Thorarensen and Farrell, 2006). Studies have shown that reduced oxygen supply to the stomach increases digestion time (Thorarensen et al., 1993; Eliason and Farrell, 2014), and that reduced rates of digestion would lead to lower rates of absorption and a reduced rate of metabolic processes that follow absorption. For example, fed fish can only reach 90-95% of their U_{crit} (Farrell et al., 2001; Thorarensen and Farrell, 2006). The effects of blood redistributing between tissues and the impact on their function during periods of increased swimming speeds requires further research attention. For aquaculture especially, understanding the impact of reduced blood flow to the stomach and the impact on digestion efficiency and nutrient retention is critical, as feeds are very expensive and feed efficient and fast-growing fish are highly desired.

As swimming speeds increase, a speed will be reached that initiates a gait transition from steady body-caudal fin propulsion (or from median-paired fin propulsion in other fishes) to burst and coast propulsion (Webb, 1994; Webb, 1998). A fish's gait transition is generally triggered at 70 - 85% of their U_{crit} and indicates a switch from aerobic metabolism (red muscle) to anaerobic metabolism (white muscles; Svendsen et al., 2010; Hvas et al., 2017; Hvas et al., 2021a; Frank et al., 2024). When fish are swimming near their maximum swimming speed reaching fatigue (unable to exercise at maximum capacity) or exhaustion (unable to exercise), energy stores are consumed, metabolic acidosis can occur because of building lactate levels from anaerobic metabolism and increased cortisol levels from stress (Boutilier et al., 1993; Peake and Farrell, 2004). To recover from intensive swimming and to alleviate the subsequent metabolic acidosis, a large amount of the metabolic scope is redirected to the associated tissues to restore depleted myoglobin oxygen, muscle phosphocreatine and ATP, and regain osmotic and ionic balance (Wood, 1991; Milligan, 1996; Kieffer, 2000; Svendsen et al., 2010). The amount of oxygen required to fully recover is termed the excess post-exercise oxygen consumption (EPOC; Zhang et al., 2018b). Full recovery of oxygen consumption rates ($\dot{M}O_2$), heart function, muscle energy stores, muscle pH, and blood properties after reaching exhaustion can require 10 – 16 h, though $\dot{M}O_2$ significantly reduces within the first 1 – 3 h (Zhang et al., 2018b).

By understanding the physiological responses of fish to different levels of swimming, and the consequences associated with reaching fatigue or exhaustion, the aquaculture industry can better manage offshore finfish farms. For example, measuring farmed salmon's swimming speeds and associated physiological responses in laboratory settings, the industry can determine

what current speeds to avoid when selecting offshore fish farms and how to appropriately design farming infrastructure to protect their stocks. Newer information suggests salmon are capable of higher sustained swimming speeds than previously thought, indicating that the industry may be overly cautious regarding the appropriate exercise levels for their stocks. Using these new data in combination with oceanographic data collected from the proposed offshore farm site in NZ (Blue Endeavour), bioenergetic models estimating energy requirements of Chinook salmon farmed offshore, with and without prior training, were produced. The models are preliminary because of the minimal studies conducted on Chinook salmon, beyond the available data collected throughout this PhD. For example, the models contain limited information in size ranges, temperature fluctuations, and the influence of other extrinsic factors that can influence salmon physiology and behaviour (e.g., oxygen, stress, salinity, etc.).

7.3.1 Blue Endeavour's oceanographic conditions

Environmental current data collected in the Blue Endeavour site by the oceanographic team at the Cawthron Institute, were used to show the range and frequency of current speeds salmon may encounter if farmed in Blue Endeavour site (Newcombe et al., 2019). Current speed and depth data were collected using deployed Acoustic Doppler Current Profilers (ADCPs), measured every thirty minutes from October 2018 until January 2019. Current speed data were collected across the entire depth profile (i.e., -90m), but of interest to this thesis and the proposed sea pen being considered (ScaleAQ Subsea System), current speeds to depths of -35m were only included (Figure 7.1).

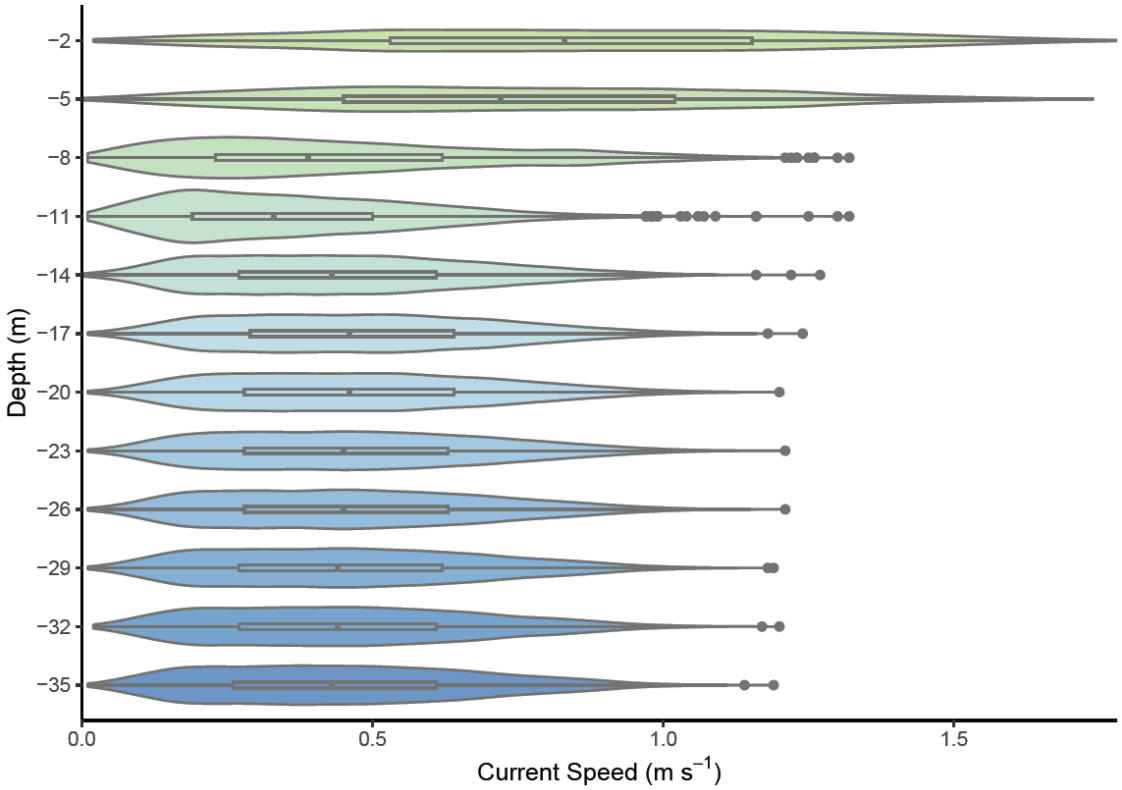


Figure 7.1 Violin and box plots presenting current speeds by depth in the Blue Endeavour proposed offshore salmon farm site. Box plots present the median (middle bar), first and third quartiles (upper and lower bars), and the largest and smallest value within 1.5 × the interquartile range (IQR; vertical bars). Points represent outliers determined as values beyond the vertical bars (i.e., > third quartile + 1.5 × IQR, < first quartile + 1.5 × IQR).

The current speeds measured in the Blue Endeavour site are high relative to other aquaculture sites in NZ (Gillespie, 2011; Campos et al., 2019) and internationally (Johansson et al., 2014; Jónsdóttir et al., 2019; Klebert et al., 2023), reaching up to speeds of ~1.5 m s^{-1}, with an average of 0.49 m s^{-1}. The current speeds in the first ten metres (0.63 ± 0.003 m s^{-1}) are the highest and most variable, but become more consistent with increasing depth, where 11 – 20 m was 0.44 ± 0.002 m s^{-1} and 21 – 35 m was 0.46 ± 0.002 m s^{-1}. According to the Norwegian aquaculture site classes (Standard NS9415; Klebert et al., 2023), the current speeds in the Blue Endeavour site would be classified as medium to high. However, following criteria developed around swimming behaviour and performance of Atlantic salmon in Jónsdóttir et al. (2019), the Blue Endeavour site current speeds would be classified as strong to very strong, and would be predicted to exceed the swimming abilities of Atlantic salmon. Current speeds in some of the exposed Atlantic salmon farm sites reach maximum speeds of 0.7 m s^{-1} (classified medium) in the Faroe Islands, Denmark (Johansson et al., 2014), 0.32 m s^{-1} (classified low to moderate) in Trøndelag, Norway (Klebert et al., 2023), and 1.12 m s^{-1} (classified high) in Frøya archipelago, Norway (Jónsdóttir et al., 2019). Current speeds in the Blue Endeavour site are perhaps some of the most challenging currents in the context of existing and proposed offshore farm sites for salmonids.

The violin and box plots presented in Figure 7.1 show the large variation in current speeds of the Blue Endeavour site. Current variation in the Blue Endeavour site is driven by tides, where across diurnal cycles, current speeds peak during the change of tides, and within a moon cycle, current speeds peak during spring tides (full and new moon; Figure 7.2). To illustrate the variation in current speeds Chinook salmon may be exposed to and how the lunar cycle influences the speeds, the maximum, minimum, and average current speed were extracted for each day of the lunar cycle across three depth groups (i.e., 0 – 10 m, 11 – 20 m, 21 – 35 m; Figure 7.2).

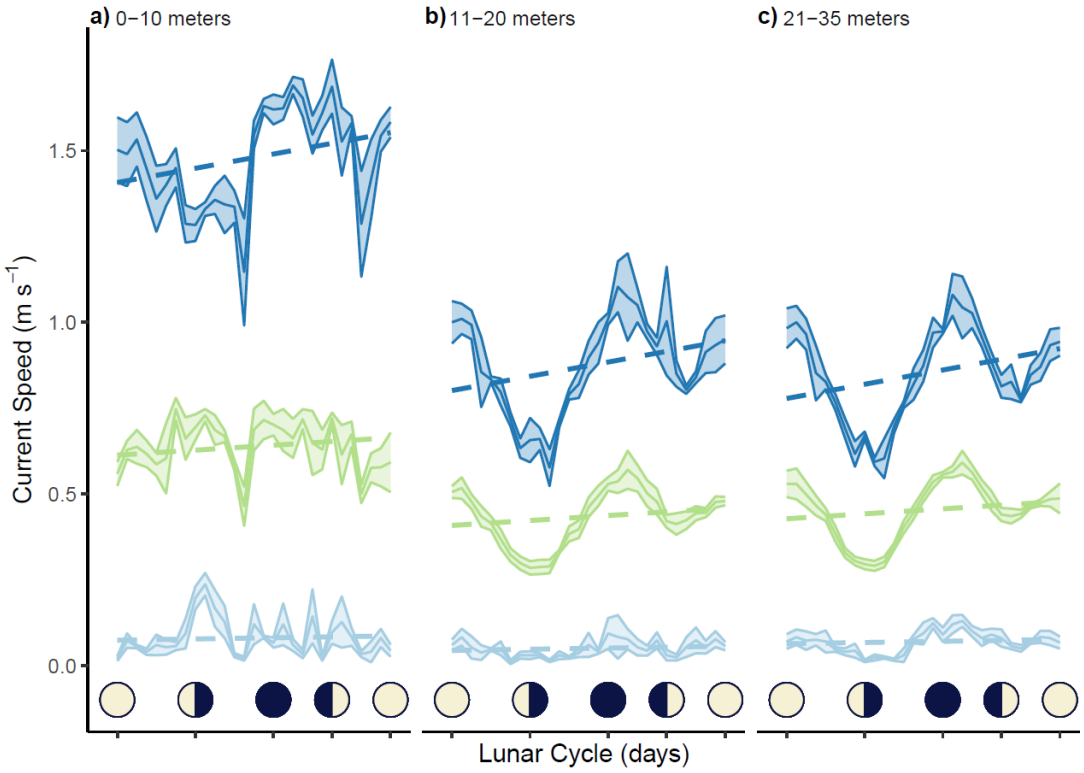


Figure 7.2 Current speeds at a) 0 – 10 m, b) 11 – 20 m, and c) 21 – 35 m depths in the Blue Endeavour site standardised against the lunar cycle (depicted by moon symbols). Dark blue indicates the maximum current speeds, green indicates the average current speeds, and light blue indicates the minimum current speeds. Solid lines and shading represent the averaged speeds and standard error, while the dashed lines represent the linear relationship.

7.3.2 Swimming speeds of salmon in the Blue Endeavour

Figure 7.3 presents the absolute current speeds (m s^{-1}) encountered in the Blue Endeavour site matched to the required swimming speeds (in body lengths per second; bl s^{-1};) for 190 mm (~120 g), 300 mm (500 g), and 500 mm (2.5 kg) fish. The same relationship is provided in the bottom panel but with current speeds reduced by 20% to estimate the protection provided by a sea pen (ScaleAQ pers. comm. 2024; Johansson et al., 2014). The swimming speeds were grouped by incremental increases of 0.25 bl s^{-1} (i.e., 0 – 0.25 bl s^{-1}, 0.25 – 0.5 bl s^{-1}, etc.) to estimate frequency at each swimming speed for each depth grouping (Figure 7.3). The black dashed line represents U_{crit} derived from farmed Atlantic salmon (Hvas et al., 2020), as U_{crit} across multiple size classes is not known for Chinook salmon. Critical swimming speeds increase with body size, but present a negative relationship when expressed in relative terms (i.e., relative to body length) (Videler, 1993a; Videler, 1993b). The critical swimming speed is a widely-used and informative measurement of swimming performance, however this measurement may underestimate the complete swimming speed range fish can achieve, especially when swimming with more available space (sea pen vs. swim tunnel) and in groups.

The current speeds of the Blue Endeavour site could be the most challenging aspect to successfully farming Chinook salmon and it is important to ensure these speeds do not exceed the salmon's swimming performance. Currently, New Zealand King Salmon (NZKS) transfers smolts (~120 g) to nearshore sea pens and harvest them in the 4 - 5 kg range. This thesis recommends salmon in a weight range of 0.5 – 1 kg could be transferred offshore, provided current speeds are reduced by at least 20%. As there is a size limitation to when fish are equipped with swimming abilities able to match current speeds offshore, operational (e.g., nearshore sites acting as intermediate phase) and technical (e.g., sea pen mesh, infrastructure, positioning, skirts etc.) solutions for physical mitigation could allow for smaller fish to be farmed offshore.

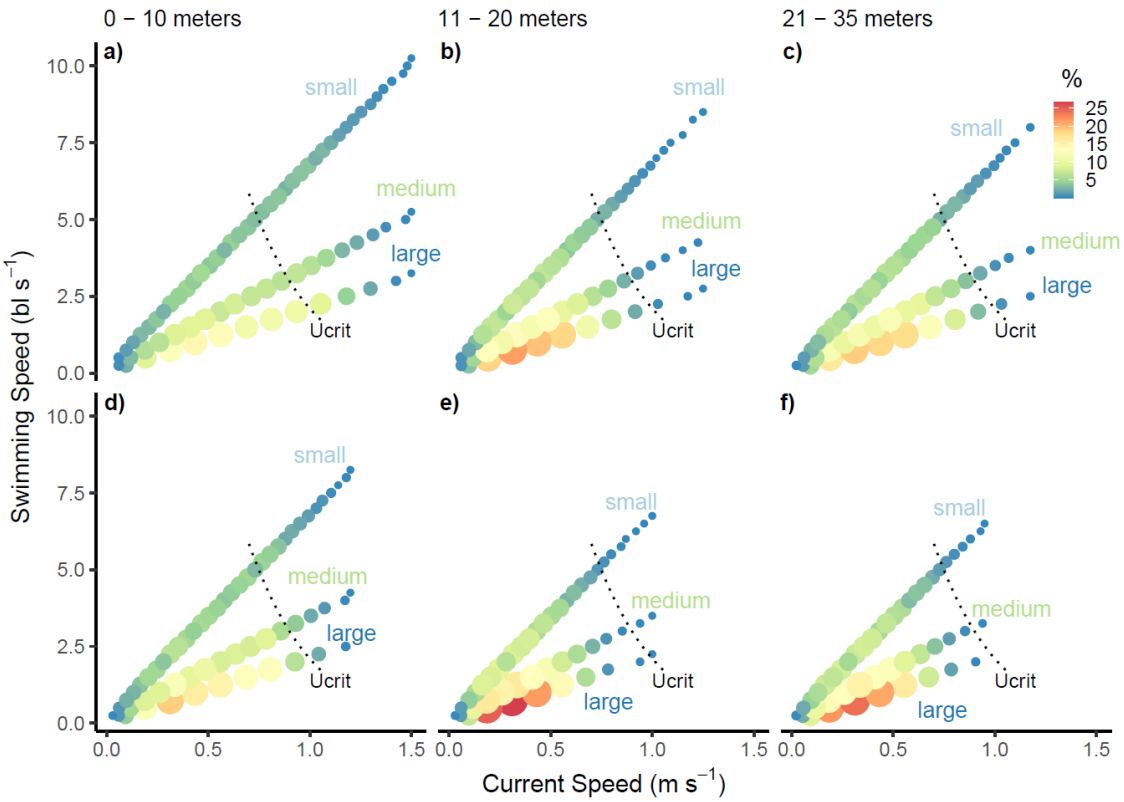


Figure 7.3 Swimming speed as a function of current speed, for small (190 mm, ~120 g), medium (300 mm, ~500 g), and large (500 mm, ~2.5 kg) fish in relation to absolute current speeds measured at a) 0 – 10 m, b) 11 – 20 m, and c) 21 – 35 m depths and reduced by 20% (estimated drag imposed by a clean sea pen) at d) 0 – 10 m, e) 11 – 20 m, and f) 21 – 35 m in the Blue Endeavour site. Each point represents a swimming speed grouping, binned by 0.25 bl s^{-1}. The size and spectral colouring of points represents the percent of time each speed recorded (i.e., blue = low, red = high). Black dotted line represents the critical swimming speed (U_{crit}) based on a U_{crit} by size relationship of farmed Atlantic in Hvas et al. (2020).

To provide an example of the current conditions salmon may experience in 24 h of being farmed in the Blue Endeavour site, the current speed data from 11 – 20 m deep (sea pen depths salmon predominantly occupy; Johannesen et al., 2022) during a full moon lunar cycle were extracted. The current speeds in the following discussion do not consider how farming infrastructure, positioning, or sea pen size can reduce current speeds, and therefore may be an overestimation, albeit a worse-case scenario.

The daily profile of currents speeds in the Blue Endeavour in Figure 7.4 highlights the benefit of pre-conditioning salmon before transferring to offshore farm sites. Depicted by the green oscillating line, current speeds (without any protection) would exceed U_{crit} of the Chinook salmon reared under LFR in **Chapter 6**, whereas current speeds only approach the U_{crit} of Chinook salmon reared under MFR, showing that pre-trained salmon are capable to match the required swimming speeds in the Blue Endeavour. The figure also highlights how tidally influenced the Blue Endeavour site is, and that current speeds will peak four times each day (Figure 7.4). Therefore, salmon farmed in the Blue Endeavour site may be required to switch between swimming gaits up to eight times per day in response to oscillating current speeds. It is expected that proficient cardiorespiratory and locomotory abilities (both of which can be enhanced with exercise training; Farrell et al., 1990; Farrell et al., 1991; Palstra et al., 2015b; Prescott et al., 2023) will be required to cope with varying swimming speeds and to adequately recover from increased energy expenditure. Determining what the maximum swimming speed can be achieved repeatedly with respect to tidal durations (approximately 6 h 12 min) would be a key parameter to inform the industry so they can achieve high welfare standards when farming offshore.

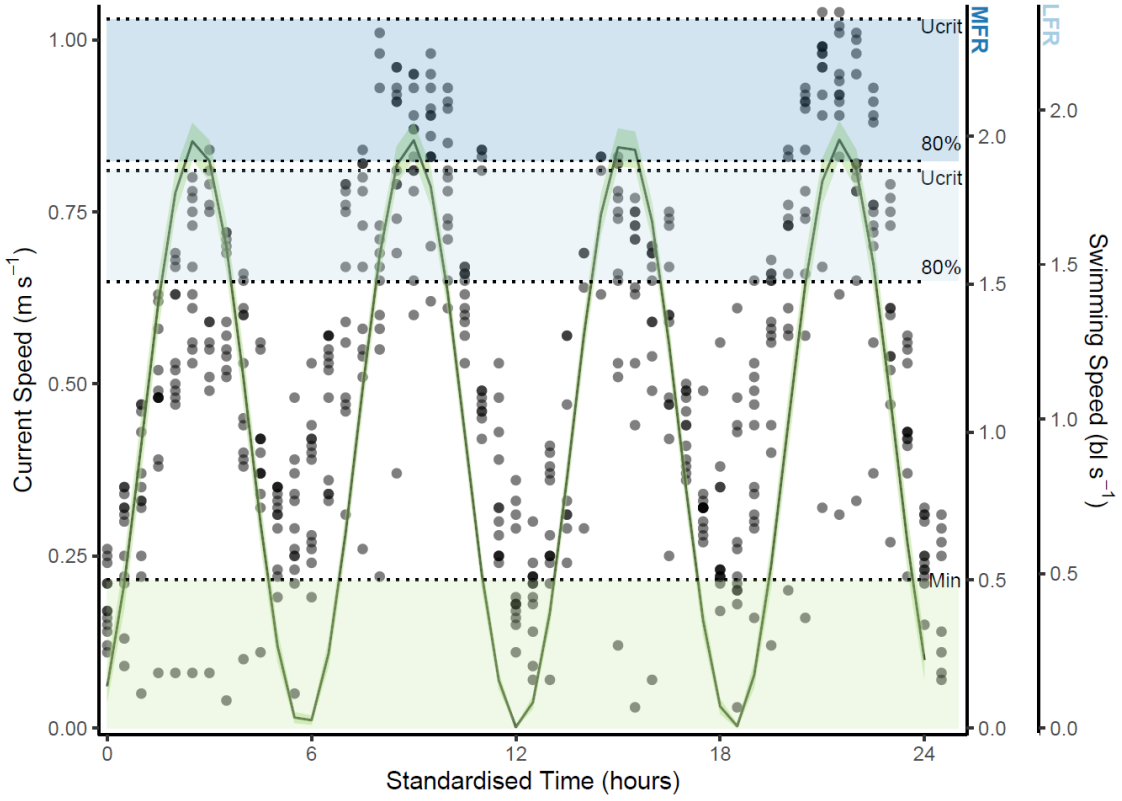


Figure 7.4 Daily profile of the current speeds encountered in the Blue Endeavour offshore farm site at 11-20 m depth during a full moon phase. Time was standardised against the tidal phases, starting at the first high tide of the day. Grey points represent individual data points collected using Acoustic Doppler Current Profilers. Green line represents the derived sin relationship between current speed and time. Second y-axes, labelled with MFR (moderate flow regime) and LFR (low flow regime) represent the swimming speeds in relation to Chinook salmon reared under LFR (1.95 kg mass and 449 mm fork length) and MFR (1.74 kg mass and 430 mm fork length) for ten months in ***Chapter 6****. Dark blue shaded area represents 80% of U_{crit} to absolute U_{crit} measured in Chinook salmon reared under MFR and light blue shading represents the same but for Chinook salmon reared under LFR. Green shading represents the minimum swimming speed (0.5 bl s^{-1}), taken from the preferred swimming speeds encountered in nearshore sites when current speeds are < 20 cm s^{-1}(Johansson et al., 2014).*

Hvas et al. (2020) suggests a conservative approach to inform welfare standards and guidelines for offshore farm locations is to match the maximum current speeds to the fish's maximum sustainable swimming speed. For Atlantic salmon, this equates to 80% of the fish's U_{crit} (Hvas and Oppedal, 2017; Hvas et al., 2021a), which is in agreement with Svendsen et al. (2010), where the striped surf perch (*Embiotoca lateralis*) did not incur an oxygen debt when swum at 80% of U_{crit} for 30 minutes, despite this swimming speed potentially activating white muscle function (Hachim et al., 2021). To further validate if 80% of the fish's U_{crit} is a viable welfare standard, assessing this speed using a domed swimming speed approach would be valuable. The maximum domed swimming speed considers the current speeds fish may encounter with respect to tidally influenced locations, where within half a tidal period (i.e., approximately 3 h) the current speed will increase from zero to the maximum speed (or in this case maximum sustainable speed) and in the following 3 h will reduce back to zero (Gui et al., 2014). Repeating this process (four times to represent 24 h) and assessing whether fish reach fatigue would determine how viable 80% of the fish's U_{crit} is for guiding appropriate currents speeds for offshore farm sites influenced by tides. Exploring the interaction between oscillating current speeds and other abiotic and biotic factors on salmon swimming performance and whether production performance is maintained would be essential to the success of moving salmon farming offshore.

7.3.3 Bioenergetics of salmon in the Blue Endeavour

Using the daily profile of current speeds in the Blue Endeavour site (Figure 7.4) and combining with the swimming respirometry data collected in **Chapter 6**, Figure 7.5 presents the required MO_2 for Chinook salmon reared under LFR and MFR to maintain swimming performance. The analysis shows the required MO_2 to fluctuate through time because of changes in swimming speeds the currents impose, and that some current speeds may lead fish to approach their maximum metabolic rate (MMR; Figure 7.5). These results highlight the possibility of untrained Chinook salmon to be left with minimal aerobic capacity for other physiological processes, such as digestion and growth, when swimming speed is increased. For Chinook salmon reared under MFR, the required MO_2 to maintain swimming performance in the Blue Endeavour site remain well below their aerobic ceiling, suggesting that trained Chinook salmon are better equipped to perform in the Blue Endeavour site, which allows more available oxygen for other physiological processes. This underscores the importance of conditioning salmon during early production stages before transfer to a high flow site.

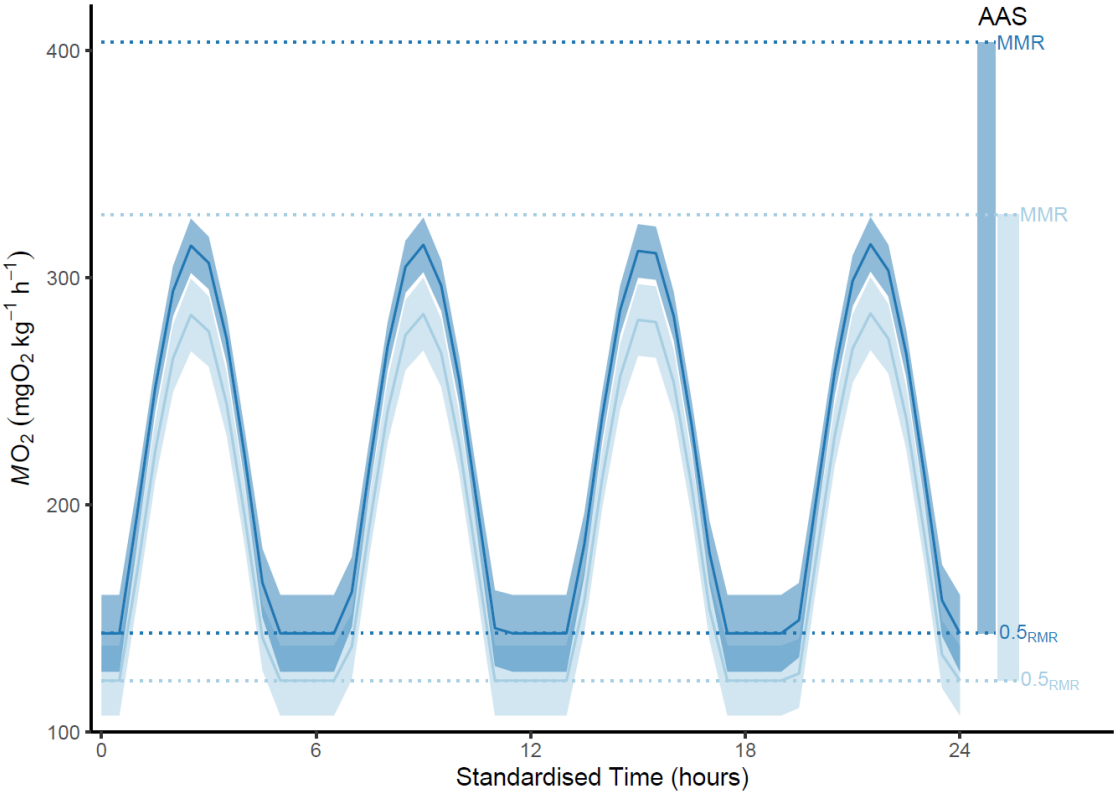


Figure 7.5 Oxygen consumption rates for Chinook salmon farmed in the Blue Endeavour site at 11 – 20 m depth during a full moon phase with low (reared under 0.3 body lengths per second; bl s^{-1}; light blue) or moderate (reared under 0.8 bl s^{-1}; dark blue) exercise training. Lines represent estimated values and shading represents 99% confidence intervals. Vertical bars represent the active aerobic scope (AAS) for Chinook salmon with low (light blue) and moderate (dark blue) exercise training. MMR, maximum metabolic rate; 0.5_{RMR}, routine metabolic rate at 0.5 bl s^{-1}; AAS, active aerobic scope.

Expanding on this, the production performance and chemical composition data collected throughout this thesis (**Chapter 5, 6** and general monitoring) were used to create bioenergetic models estimating the required energy consumption for trained and untrained salmon in the Blue Endeavour site. The models predict the energy demands of Chinook salmon farmed offshore and can be used to assess the viability of prior exercise training to advise the industry. Using an energy consumption model modified from (Lawson et al., 2018; Lawson et al., 2021):

$$C = \frac{ACT+G}{(1-A)}, \quad (1)$$

where C is energy consumption in (KJ day^{-1}), ACT is activity (KJ day^{-1}), G is growth (KJ day^{-1}), and A is assimilation costs, energy demands were estimated. Assimilation is a proportion based on estimates for egestion (0.104), excretion (0.068), and specific dynamic action (0.172; SDA) (Brodie et al., 2016; Lawson et al., 2021). Excess-post oxygen consumption was included in the model for Chinook salmon reared under LFR, as current speeds were predicted to exceed their U_{crit} and, therefore incur an oxygen debt, as measured in **Chapter 6**. To validate this approach, energy demands were modelled for the Chinook salmon reared in tanks under LFR and MFR at ten months under treatment in **Chapter 6** and the observed energy consumption values were included as the daily feed intake measured throughout this study with respect to size and the rearing environment.

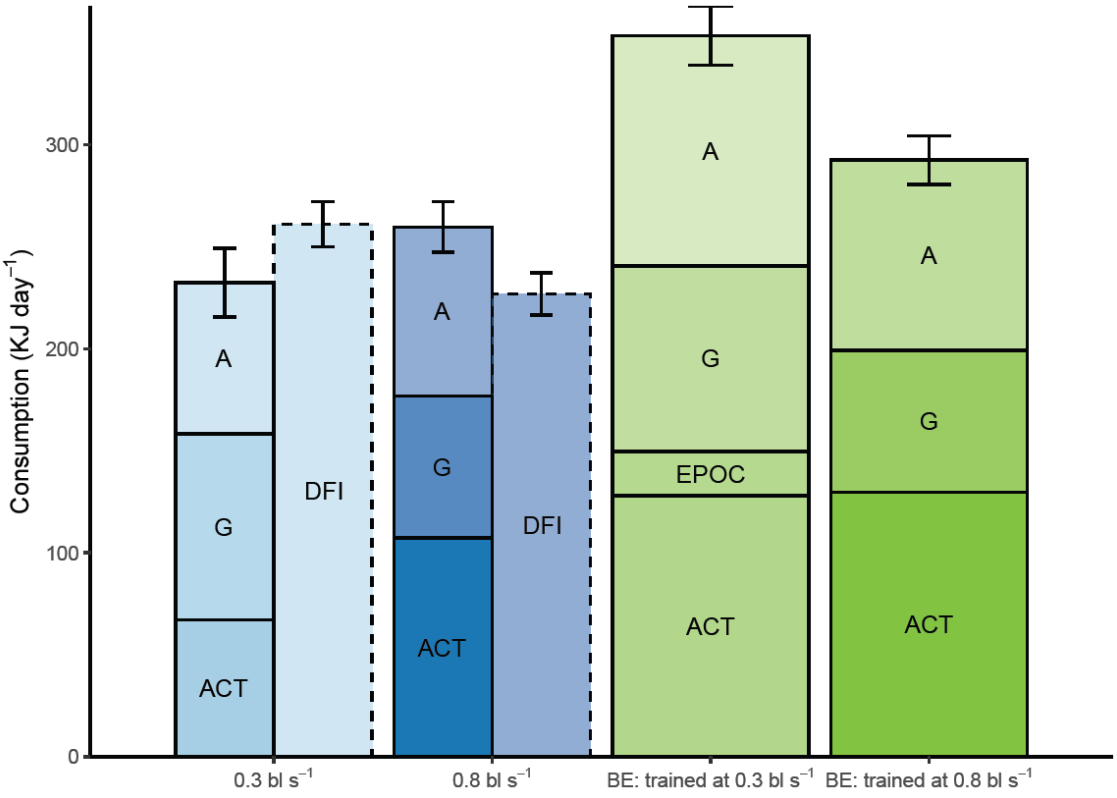


Figure 7.6 Daily energy consumption rate of New Zealand farmed Chinook salmon reared under low (0.3 body lengths per second; bl s^{-1}; light blue bars), moderate (0.8 bl s^{-1}; dark blue bars) flow regimes in a recirculating aquaculture system, and farmed in the Blue Endeavour (BE) offshore farm site with no (reared under 0.3 bl s^{-1}; light green bars) or prior (reared under 0.8 bl s^{-1}; dark green bars) exercise training. For Chinook salmon reared under low and moderate flow regimes, observed daily feed intake (DFI) rates are presented by the adjacent bar. Error bars represent 99% confidence intervals derived from the models used. A, assimilation; G, growth; ACT, activity; DFI, daily feed intake; EPOC, excess post-exercise oxygen consumption.

The energy demands estimated for Chinook salmon reared in tanks with LFR and MFR present similar values to the observed feed intake values, providing some confidence in the model approach. Some error in the model could be associated with using a constant derived from the literature to estimate assimilation (A) and that A is not based on Chinook salmon or aquaculture fish. The model also considers SDA as a constant embedded in A; however, SDA is complex and protein metabolism changes in exercising fish where muscle growth becomes more efficient (Carter and Houlihan, 2001; Huang et al., 2021; Timmerhaus et al., 2021). Error could also occur in the growth estimate as growth was derived from an assessment that occurred up to one month prior to swimming respirometry experiments taking place, thus the growth rate may have reduced over this short time and could explain why the observed energy intake for Chinook salmon reared under MFR was lower than the estimated energy demand. However, this difference could be linked to potential trade-offs between feeding and maintaining position in the current, as discussed in section 7.2. Based on this, the industry may consider matching feeding times to low periods of current speeds, and to feed below the surface to avoid the strongest currents.

Furthermore, the bioenergetic models quantify the potential for exercise trained Chinook salmon to have better performance in the Blue Endeavour site. Specifically, the analysis shows that the required energy consumption is less in exercise-trained salmon, and that additional energy is not required to support recovery as exhaustion is not reached (Figure 7.6). This information provides insights into the energy demands for Chinook salmon in the Blue Endeavour site and how prior exercise training could greatly improve their performance and the success of farming offshore. By improving aerobic efficiency through exercise training, less energy is needed for swimming and recovery, allowing more energy to be allocated to growth.

7.4 Beyond currents: other high energy challenges of offshore farming

In the preceding chapters, particularly in **Chapter 2**, there is information outlining the influence of sustained swimming on many aspects of salmon physiology. This information has allowed predictions of how salmon may respond when farmed offshore and under higher sustained swimming pressures. A missing area to best inform these predictions is understanding how salmon will respond to oscillating current speeds and wave action, both of which will influence swimming behaviour and initiate periods of unsteady swimming. This area of research demands attention as it can ensure high welfare standards are met.

Recently, insights into energetic performance of fish in unsteady or turbulent conditions have been assessed (Agbeti et al., 2024; Athammer et al., 2024; Barbier et al., 2024; Zhang et al., 2024; Zhang and Lauder, 2024). These studies show promising results for performance in more variable high

energy environments, where fish in group settings and individual assessments of salmon showed energy savings under varying conditions (Agbeti et al., 2024; Zhang et al., 2024; Zhang and Lauder, 2024). Using a swim tunnel set up, Atlantic salmon (837 g, 411 mm) were exposed to different peak current speeds that oscillated over a 1 min period. Atlantic salmon that experienced peak currents speeds equivalent to 140% of U_{crit} (133 cm s^{-1}) resulted in 100% fatigue within 1.5 h and the majority (14 out of 18) that experienced peak currents speeds equivalent to 120% of U_{crit} (114 cm s^{-1}) fatigued within 3.5 h (Athammer et al., 2024). Atlantic salmon were also tested at 120% of U_{crit} (114 cm s^{-1}) but across different periods (i.e., 0.5, 1, and 2 mins). Fatigue time was not different between the different periods, where fatigue started to occur after 1 h and for the majority within 4 h (Athammer et al., 2024). In a separate study, Atlantic salmon were raised in tank environments with wave-generating equipment to simulate turbulent conditions for eight weeks. There were no observed differences in feed intake, growth, swimming behaviour (post-acclimation), swimming performance, or welfare (Barbier et al., 2024). Together, these studies provide early insights into how salmon may respond when farmed in high-energy environments, and show that salmonids are robust against challenging conditions, suggesting they are ideal candidates for offshore farming.

The combination of tides (changing flow direction) with current speeds and wave action need addressing simultaneously to improve understanding around how salmon will cope offshore and to ensure salmon will thrive when transitioned into these high-energy locations. Although developing research environments with multiple factors may be very technically difficult to do in unison, exploring these factors individually in laboratory settings and comparing to responses in existing farm sites would be possible. The interaction between currents and waves presents interesting insights, as strong waves and currents have been shown to influence Atlantic salmon differently when assessed in nearshore sea pens. Fish tend to avoid the surface and pen edge when large waves are apparent and during daylight (Johansson et al., 2014; Hvas et al., 2020; Johannesen et al., 2022). Conversely, when current speeds change the swimming behaviour of farmed salmon to holding position into the current, fish tend to favour the pen edge, positioning themselves directly into the incoming current (Johansson et al., 2014; Hvas et al., 2020; Johannesen et al., 2022). It is also important to mention that when currents are strong, the sea pen can change shape, where strong currents can push the bottom of the pen upwards and force fish closer to the surface. The impact of these variables and their interactions needs to be addressed to provide detailed guidelines of cage structure, design, and size for when waves and currents are at extremes.

7.5 Next research questions for farming robust and resilient salmon from nursery to harvest

Translational science is defined as a process of transferring empirical findings into commercial outcomes to improve practices (Austin, 2021; Zohar, 2021). Several research programs in AUS and NZ (outlined in **Chapter 1**) including the experiments performed in this thesis were and are set out to achieve translational science by working closely with industry and developing relevant R&D projects. Realising that the link between experimental and commercial setups is not always easily achieved, future research can be designed to strengthen this link and identify how valuable experimental outcomes can be translated into industry.

The role of exercise in farming fish has been identified as a valuable experimental outcome, requiring attention for translation into industry (Davison and Herbert, 2013; Palstra et al., 2015a; McKenzie et al., 2020; Rodgers and Gomez Isaza, 2023). This is because studies involving exercise training fish, in particular salmon, present improvements in production performance and resilience, a potential tool to enhance finfish farming offshore and an immediate solution to farming fish in the Anthropocene. Research is now needed to optimise training regimes for industry settings and to better understand the role of pre-conditioning (early production shaping) to prepare for offshore farming conditions, including understanding how challenging hydrodynamic conditions impact salmon physiology. This research will contribute to some of the challenges needed in developing a sustainable offshore blue economy (Novaglio et al., 2021). Some specific points of interest for future research are illustrated in Figure 7.7 and discussed below.

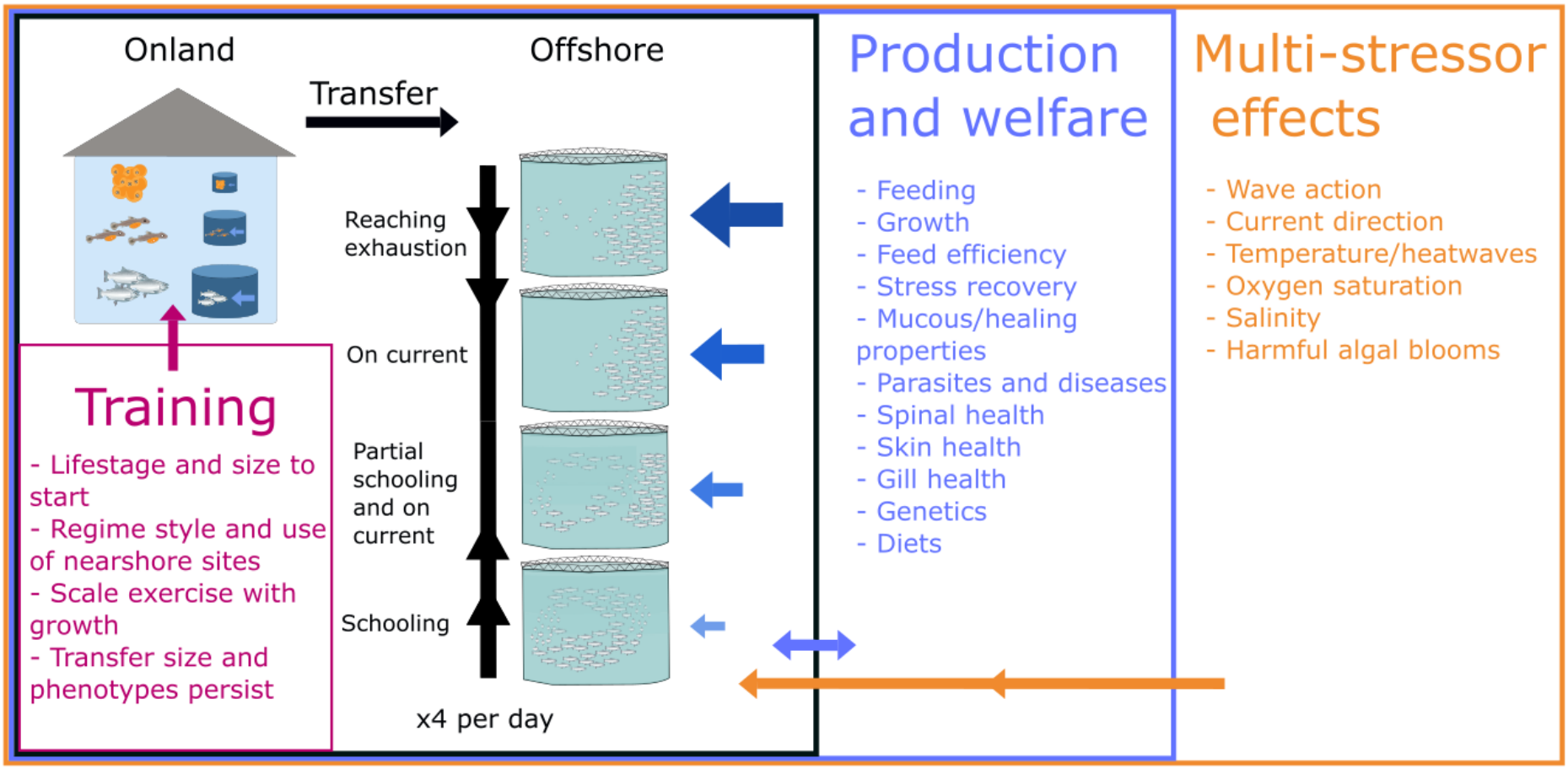


Figure 7.7 Schematic outlining next research questions for farming salmon offshore. Schematic highlights the how oscillating current speeds (blue arrows) will impact swimming behaviour (depicted by fish changing from schooling to maintaining position into the current).

Preparing salmon before sea transfer. Determining when exercise training should begin (e.g., parr, pre- or post-smolt), how to scale exercise regimes with larger fish and in commercial setups, and what type of training and duration is needed, all demands attention to better condition salmon before sea transfer. Most studies investigating exercise-enhanced traits used continuous swimming regimes at low sustainable swimming speeds, i.e., targeting endurance. For offshore farming, training regimes may benefit from targeting training at higher speeds with subsequent recovery periods at slower swimming speeds to improve both swimming endurance and intensity. Measuring additional markers of production performance and health alongside swimming performance is needed to ensure animal welfare can be met (Macaulay et al., 2022).

Do exercise-enhanced phenotypes persist when transferred offshore? Laboratory-based experiments are required to better understand how salmon will perform when farmed under exposed offshore conditions. Future experiments may involve implementing training regimes during early production stages and rearing salmon under these conditions until they reach a larger post-smolt size (e.g., < 500 g). Defining the most appropriate size to transfer fish offshore is needed, and therefore how long training regimes need to last for and whether existing nearshore sites are needed as an intermediate phase before moving offshore. To determine the effectiveness of prior training for offshore farming, assessing trained fish under simulated offshore conditions, such as strong current and wave action, is recommended. Pairing these assessments of challenging hydrodynamic conditions with other stressors (e.g., increased temperature, reduced oxygen saturation, salinity, parasites and disease, and harmful algal blooms; HABs) would be more realistic, informative, and could identify the most threatening stressors.

Production performance and welfare in offshore farming. Similar to testing the effectiveness of prior training, rearing salmon under these challenging hydrodynamic conditions is needed in the lab to better predict production performance, resilience, and welfare. Farming offshore is suggested to lessen the threats from anthropogenic stressors, but exposure to warming waters, heatwaves, and other biotic stressors are still real and in combination with high energy expenditure could have greater effects. Simulating exposed offshore farm conditions in laboratory settings and assessing fish production and health performance is needed, but also with other factors (e.g., increased temperature, reduced oxygen saturation, salinity, parasites and disease, and HABs) to understand multi-stressor interactions. Developing a framework of stressors and applying a criterion to assess fish performance could help determine if higher swimming speeds places salmon under greater threat to anthropogenic factors, can identify which stressors are most concerning, and how to avoid these scenarios when farming offshore. Thus, this information can improve decisions in finding suitable offshore locations, designing offshore infrastructure, as well as implementing best farming practices.

Selective breeding and offshore farming. Selective breeding for genetic gains is a huge aspect in farming of any species. Minimal attention has been paid to how existing selective breeding programs may have to change as offshore farming becomes more common. It is unknown if swimming performance will need to be a priority trait in the selection criteria, or if swimming performance is heritable and if there are any unfavourable genetic and phenotypic correlations with other important traits. To appropriately measure the role of selective breeding in offshore farming, an evaluation of whether swimming performance is better improved through genetic gains or training regimes, or whether these two approaches can work synergistically is needed. Knowing the importance of broodstock conditioning in terms of training for improved cardiovascular and locomotory performance and if any inter-, transgenerational, or epigenetic effects exist would also highly benefit how selective breeding will aid salmon farming offshore.

Diet and animal nutrition. Offshore environments can present drastically different conditions from existing farm sites, where the energetic requirements offshore will be considerably greater. Increased energy demands associated with offshore performance need to be considered in terms of diet formulation and animal nutrition. Presently, diets are energy dense and have been designed to maximise weight gain, which, in combination with low exercise and selective breeding, is linked to poor body shape and health (i.e., spinal curvature, fatty livers) (Araújo et al., 2022a; Esmaeili et al., 2022; Prescott et al., 2023; Young et al., 2023; Prescott et al., 2024). Diet formulations need to be reconsidered to improve fish health, but to also enable the potential of achieving robust fish with exercise regimes and support evolving farming practices.

To aid the success of transitioning salmon farming offshore, research is needed to ensure farmed fish are equipped with the physiological needs to thrive in exposed offshore conditions and to avoid negative perceptions. Gaining and sharing this knowledge openly would underpin the advancements of the global salmonid aquaculture industry and finfish farming in general, as offshore farming becomes reality.

7.6 Conclusion

Anthropogenic climate change is shaping environments at unprecedented rates, and these changes are significantly impacting existing aquaculture productions, particularly those in open environments. Strategies for commercial aquaculture need to evolve and include implementation of adaptive planning measures to improve industry sustainability. For salmonid farming and other aquaculture industries, offshore farming presents a viable opportunity to avoid many of these constraints (anthropogenic impacts, restricted farming space, resource expensive) and enable significant production expansion. Offshore environments are drastically different from most

existing farm conditions, and strong currents and waves present significant challenges to farming infrastructure, operations, and animal welfare.

This thesis investigated the influence of sustained swimming on Chinook salmon form and function to determine if exercise can enhance production performance and improve respiratory and locomotory processes needed for exposed offshore farm environments, while gaining insight into the physiological changes Chinook salmon may experience when farmed offshore in high energy environments. The key finding conveyed from the results of this thesis is that moderate exercise directly benefits farmed fish by equipping them with the physiological abilities to thrive in an exposed offshore farm site. The data presented in this thesis contributes directly to the NZ salmon aquaculture industry and the global salmonid aquaculture industry by sharing essential knowledge for developing offshore farming practices. Furthermore, the research presented in this thesis has provided new insights into the fundamental physiology and bioenergetics of Chinook salmon, contributing valuable knowledge to the broader fields of salmonid and teleost biology.

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Appendix

Table A.1 Swimming Speeds of Chinook salmon under low and moderate flow regimes.

Flow Regime	0-4 weeks			4-8 weeks			8-12 weeks		
	7am	**3pm**	**11pm**	**7am**	**3pm**	**11pm**	**7am**	**3pm**	**11pm**
Low	2.71 ± 0.61	2.39 ± 0.48	2.57 ± 0.64	2.68 ± 0.68	2.32 ± 0.55	2.27 ± 0.50	2.60 ± 0.61	2.47 ± 0.54	2.57 ± 0.58
Moderate	2.83 ± 0.11	2.30 ± 0.49	2.30 ± 0.58	2.59 ± 0.75	2.24 ± 0.59	1.87 ± 0.59	2.53 ± 0.69	2.36 ± 0.56	1.51 ± 0.74

Values are means ± S.D.

Table A.2 Linear and linear mixed effects model statistical output for short-term effects of low and moderate flow regimes on growth and feed performance in Chinook salmon.

Parameters	**Model**	**Factors**	**D.F.**	***F*-value**	***P*-value**
Mass (g)	LMER	Sampling timepoints	2, 6171	57994.45	**<0.0001**
		Flow regime	1, 10	0.53	0.49
Fork length (mm)	LMER	Sampling timepoints	2, 6145	234789.4	**<0.0001**
		Flow regime	1, 10	0.35	0.57
Girth (mm)	LMER	Sampling timepoints	1, 2986	48264.07	**<0.0001**
		Flow regime	1, 10	0.73	0.41
Condition factor	LMER	Sampling timepoints	2, 6154	24842.44	**<0.0001**
		Flow regime	1, 10	1.98	0.19
Specific growth rate (%)	LMER	Sampling timepoints	2, 6139	29927.23	**<0.0001**
		Flow regime	1, 10	1.00	0.34
Specific feed rate (%)	LMER	Sampling timepoints	1, 3092.2	1813.28	**<0.0001**
		Flow regime	1, 10	0.01	0.95
Feed conversion ratio	LM	Flow regime	1, 2948	4.10	**<0.05**

Bold text indicates significant *P*-values ($\alpha = 0.05$).

Table A.3 Linear mixed effects model statistical output for short-term effects of low and moderate flow regimes on tissue metrics in Chinook salmon.

Parameters	**Factors**	**D.F.**	***F*-value**	***P*-value**
Viscerosomatic Index	Sampling timepoints	1, 455	94.70	**<0.0001**
	Flow regime	1, 10	0.06	0.81
Heart Index	Sampling timepoints	1,743	99.50	**<0.0001**
	Flow regime	1, 10	03.28	0.10
Hepatosomatic Index	Sampling timepoints* Flow regime	1, 446	21.08	**<0.0001**
Gonadosomatic Index	Sampling timepoints	1, 451	19.38	**<0.0001**
	Flow regime	1, 10	0.82	0.39
Belly-flap thickness:				
Pectoral (mm)	Flow regime	1, 10	0.13	0.73
Pelvic (mm)	Flow regime	1, 10	1.37	0.27
Anal (mm)	Flow regime	1, 10	0.01	0.93
Hct (%)	Flow regime	1, 2.39	1.04	0.40
Hb (g L^{-1})	Flow regime	1, 10	0.17	0.69
White Blood Cell Count (10^9 L^{-1})	Flow regime	1, 10	0.87	0.37
Neutrophils (%)	Flow regime	1, 10	3.82	0.08
Lymphocytes (%)	Flow regime	1, 10	2.67	0.13
Monocytes (%)	Flow regime	1, 10	1.24	0.29

Bold text indicates significant *P*-values ($\alpha = 0.05$).

Table A.4 Linear and linear mixed effects model statistical output for short-term effects of low and moderate flow regimes on FT-NIR proximate composition in Chinook salmon.

Parameters		**Model**	**Factors**	**D.F.**	***F*-value**	***P*-value**
Lipid (g $100g^{-1}$)	Liver	LMER	Sampling timepoints	1, 457	18.91	**<0.0001**
			Flow regime	1, 9	5.87	**0.02**
	Viscera	LMER	Sampling timepoints	1, 459	11.70	**<0.001**
			Flow regime	1, 10	0.85	0.40
	Fillet	LMER	Sampling timepoints	1, 461	155.38	**<0.0001**
			Flow regime	1, 10	0.05	0.83
	Whole-body	LMER	Sampling timepoints	1, 457	141.42	**<0.0001**
			Flow regime	1, 10	0.08	0.78
Protein (g $100g^{-1}$)	Fillet	LMER	Sampling timepoints	1, 455	19.89	**<0.0001**
			Flow regime	1, 10	0.49	0.5
	Whole-body	LMER	Sampling timepoints	1, 460	17.80	**<0.0001**
			Flow regime	1, 10	0.0002	0.99
Ash (g $100g^{-1}$)	Fillet	LM	Sampling timepoints	1, 464	5.26	**0.02**
			Flow regime		0.18	0.67
	Whole-body	LM	Sampling timepoints	1,464	10.59	**<0.0001**
			Flow regime		4.43	**0.04**
Moisture (g $100g^{-1}$)	Fillet	LMER	Sampling timepoints	1, 455	78.45	**<0.0001**
			Flow regime	1, 10	0.02	0.90
	Whole-body	LMER	Sampling timepoints	1, 455	154.23	**<0.0001**
			Flow regime	1, 10	0.15	0.71

Bold text indicates significant *P*-values ($\alpha = 0.05$).

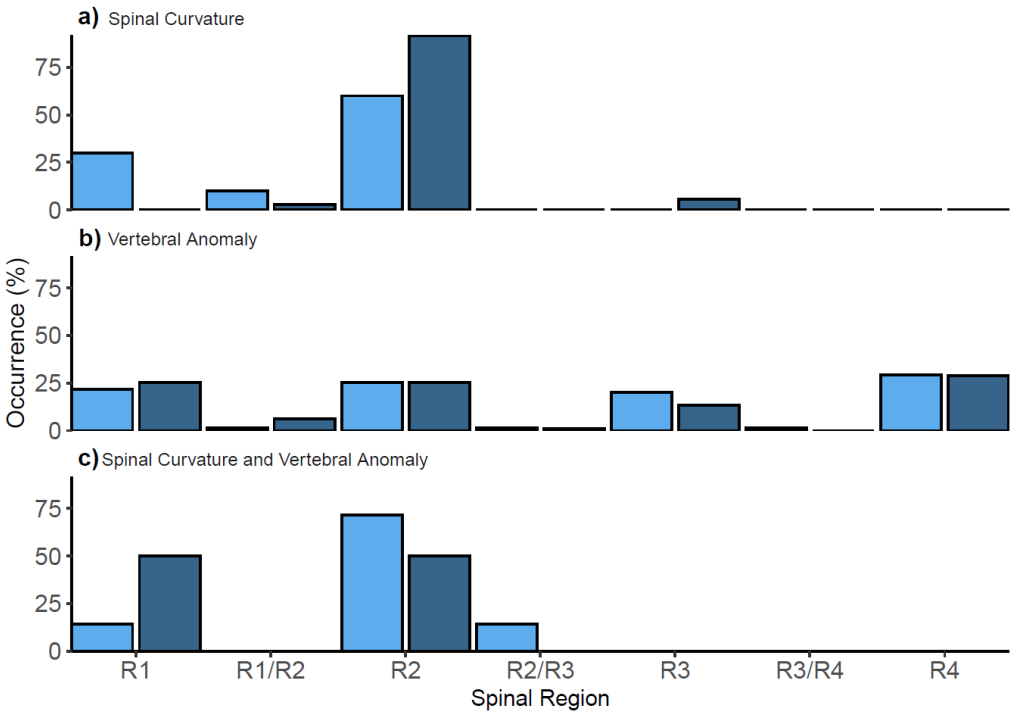

Figure A.1 Occurrence of spinal curvature a), vertebral anomaly b), and spinal curvature and vertebral anomaly c), across spinal regions in Chinook salmon raised under low (light blue) and moderate (dark blue) flow regimes.

Table A.5 Estimated marginal means output for short-term effects of spinal curvature and vertebral anomalies on feed and growth performance in Chinook salmon.

Parameters		**Estimate ± S.E.**	**D.F.**	**t-ratio**	***P*-value**
Specific feed rate (%)	NS - SC	-0.19 ± 0.08	2973	-2. 48	0.06
	NS – VA	-0.001 ± 0.03	2971	1.01	0.74
	NS – SC & VA	0.13 ± 0.10	2972	1.70	0.32
Mass (g)	NS - SC	-107.86 ± 13.81	2972	-7.81	**<0.0001**
	NS – VA	37.47 ± 8.95	2971	4.19	**<0.001**
	NS – SC & VA	-9.69 ± 33.47	2971	-0.29	1.00
Fork length (mm)	NS - SC	-5.97 ± 1.98	2969	-3.01	**0.01**
	NS – VA	7.41 ± 1.28	2968	5.79	**<0.0001**
	NS – SC & VA	5.28 ± 4.98	2968	1.06	0.71
Condition factor	NS - SC	-0.26 ± 0.02	2975	-13.61	**<0.0001**
	NS – VA	-0.01 ± 0.1	2972	-0.43	0.97
	NS – SC & VA	-0.07 ± 0.05	2973	-1.56	0.40
Specific growth rate (%)	NS - SC	-0.04 ± 0.02	2962	-1.54	0.42
	NS – VA	0.11 ± 0.02	2961	7.13	**<0.0001**
	NS – SC & VA	0.13 ± 0.06	2962	2.33	0.09
Feed conversion ratio	NS - SC	-0.07 ± 0.04	2943	-1.87	0.24
	NS – VA	-0.09 ± 0.03	2936	-3.33	**<0.01**
	NS – SC & VA	0.01 ± 0.10	2942	0.12	1.0

NS = normal spinal health; SC = spinal curvature; VA = vertebral anomaly
Bold text indicates significant *P*-values ($\alpha = 0.05$).

Table A.6 Tissue weights and hematocrit from Chinook salmon reared under low and moderate flow regimes for 167 days.

Parameters	Low flow regime	Moderate flow regime	*P*-value
Belly-flap thickness:			
Pectoral (mm)	9.22 ± 0.35	9.14 ± 0.33	> 0.05
Pelvic (mm)	9.99 ± 0.53	8.74 ± 0.38	> 0.05
Anal (mm)	3.36 ± 0.16	3.14 ± 0.12	> 0.05
Viscera (g)	84.52 ± 3.87	78.95 ± 3.53	> 0.05
Heart (g)	1.32 ± 0.05	1.26 ± 0.05	> 0.05
Liver (g)	11.95 ± 0.56	11.23 ± 0.59	> 0.05
Gonad (g)	1.08 ± 0.07	1.11 ± 0.06	> 0.05
Hct (%)	42.4 ± 0.80	42.8 ± 0.70	> 0.05

Values are means ± S.E.M.

Table A.7 FT-NIR chemical composition in Chinook salmon under low and moderate flow regimes for 167 days.

Response variable	Tissue	Low flow regime	Moderate flow regime	*P* - values	
				Side	Flow regime
Lipid (g 100 g $^{-1}$)	Liver	2.72 ± 0.13	3.07 ± 0.20		> 0.05
	Viscera	45.65 ± 2.15	47.22 ± 2.23		> 0.05
	Fillet left	16.96 ± 0.67	17.50 ± 0.79	> 0.05	> 0.05
	Fillet right	18.12 ± 0.92	18.46 ± 0.57		
	Whole-body	19.77 ± 0.43	19.71 ± 0.39		> 0.05
Protein (g 100 g $^{-1}$)	Fillet left	18.48 ± 0.16	18.61 ± 0.18	**< 0.05**	> 0.05
	Fillet right	17.96 ± 0.21	18.34 ± 0.12		
	Whole-body	16.05 ± 0.05	16.06 ± 0.05		> 0.05
Ash (g 100 g $^{-1}$)	Fillet left	1.09 ± 0.01	1.10 ± 0.01	> 0.05	> 0.05
	Fillet right	1.08 ± 0.01	1.09 ± 0.01		
	Whole-body	1.40 ± 0.00	1.39 ± 0.00		> 0.05
Moisture (g 100 g $^{-1}$)	Fillet left	61.79 ± 0.46	61.52 ± 0.49	> 0.05	> 0.05
	Fillet right	61.26 ± 0.63	60.92 ± 0.45		
	Whole-body	60.46 ± 0.32	60.58 ± 0.39		> 0.05

Values are means ± S.E.M.

Letters indicate significant differences between experimental treatments.

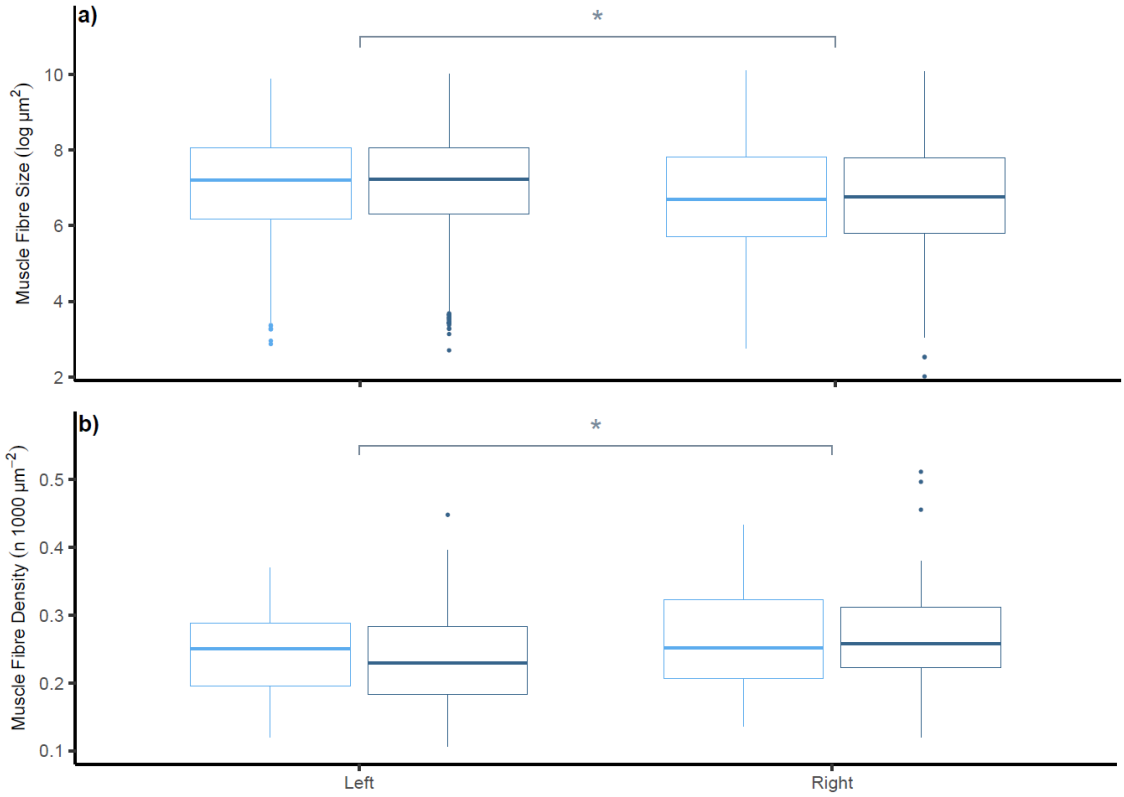

Figure A.2 Muscle fibre size a) and muscle fibre density b) of the left and right white muscle in Chinook salmon reared under low (light blue) and moderate (dark blue) flow regimes for 167 days. Boxplots present the median (middle bar), first and third quartiles (upper and lower bars), the largest and smallest value within 1.5 interquartile range (IQR; vertical bars) and outliers (i.e., > third quartile + 1.5*IQR, < first quartile + 1.5* IQR; points). Brackets and asterisks represent significant differences (P < 0.05) between left and right fillets.*

Table A.8 Fish size and deformity counts from a routine assessment of Chinook salmon raised under low and moderate flow regimes for nine months.

Parameter	Low flow regime	Moderate flow regime
Sample size	74	77
Mass (kg)	1.610 ± 0.052	1.354 ± 0.041
Fork length (mm)	425.20 ± 3.5	408.51 ± 2.7
Girth (mm)	320.85 ± 4.24	301.42 ± 3.89
Condition factor	2.04 ± 002	1.94 ± 0.03
Counts of spinal scoliosis		
Absent	52	39
Mild	7	25
Moderate	9	9
Severe	6	4

Values are means ± S.E.M.

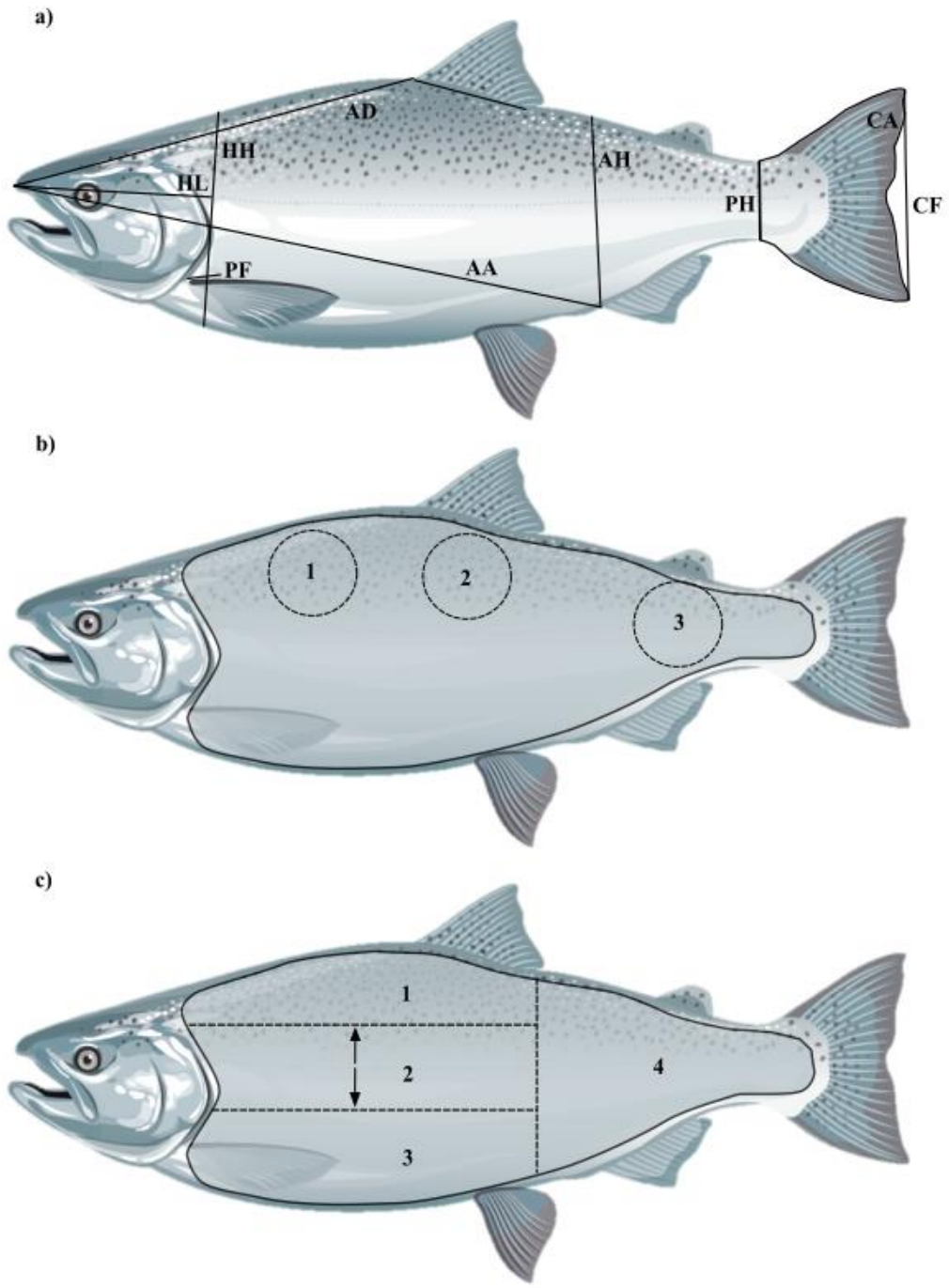

Figure A.3

a) Body shape morphometric measurements following protocols from (Pakkasmaa and Piironen, 2000). HL, head length; HH, head height; AD, antero-dorsal length; AA, antero-anal length; DF, dorsal fin base; PF, pectoral fin base; DH, dorsal body height; AH, anal body height; PH, peduncle body height; CF, caudal fin height; CA, caudal fin area.

b) Fillet colour was measurements across three locations on both left and right fillets. Using a CR-410 chroma meter, fillet colour was measured (1) anterior dorsal, (2) dorsal, and (3) posterior dorsal locations.

c) Fillet regions for FT-NIR composition analysis, regions modified from (Miller et al., 2019). 1, Dorsal region; 2, Lateral line region; 3, Visceral region; 4, Posterior dorsal to caudal region.

Table A.9 Fish size and tissue metrics from Chinook salmon without and with mild scoliosis raised under low and moderate flow regimes.

Response variable	Low flow regime		Moderate flow regime		*P*-values	
	Without scoliosis	With scoliosis	Without scoliosis	With scoliosis	Exercise treatment	Spinal scoliosis
Head length (mm)	82.20 ± 1.96	82.12 ± 3.02	83.12 ± 1.80	86.14 ± 1.56	0.2653	0.4094
Head height (mm)	108.84 ± 2.24	11066 ± 2.90	106.57 ± 2.20	110.02 ± 2.03	0.5852	0.2548
Antero-dorsal length (mm)	209.58 ± 4.31	208.99 ± 5.79	205.26 ± 3.73	210.34 ± 3.68	0.5298	0.8850
Antero-anal length (mm)	316.03 ± 6.38	314.67 ± 7.66	302.20 ± 4.40	308.48 ± 5.84	0.1702	0.8880
Dorsal fin base (mm)	56.95 ± 1.06	58.15 ± 1.60	56.65 ± 1.38	57.01 ± 1.57	0.8064	0.8932
Pectoral fin base (mm)	19.74 ± 0.70	19.84 ± 1.55	20.06 ± 0.61	20.11 ± 0.83	0.0783	0.5092
Dorsal body height (mm)	143.38 ± 4.15	143.23 ± 4.60	139.81 ± 3.02	143.47 ± 3.57	0.1265	0.7548
Anal body height (mm)	94.10 ± 2.04	94.02 ± 2.75	94.04 ± 1.98	95.09 ± 1.76	0.0750	0.6471
Peduncle body height (mm)	37.81 ± 0.80	38.11 ± 0.47	37.73 ± 0.75	38.08 ± 0.71	0.3463	0.9689
Caudal fin height (mm)	80.32 ± 2.88^{a}	82.78 ± 1.35^{a}	77.80 ± 1.28^{b}	74.50 ± 2.56^{b}	**<0.05**	0.7378
Caudal fin area (mm^2)	4263.56 ± 131.96	4124.30 ±79.45	4300.90 ± 193.06	4223.52 ± 129.06	0.2864	0.2607

Values are means ± S.E.M.

Boldness indicates indicate significant *P*-values. Letters indicate significant differences between experimental treatments.