Effect of continuous aerobic exercise on the heart of Atlantic salmon

Marianne Eidskrem Gullaksen

This thesis is in partial fulfilment of the requirements for the degree in Master of Science – Aquaculture

Department of Biological Sciences

University of Bergen

June 2023

Supervisors:

Pablo Balseiro Vigo, UIB

Ana Cristina Da Silva Gomes, UIB

Sigurd Olav Handeland, UIB

Ivar Rønnestad, UIB

ACKNOWLEDGEMENTS

First and foremost, I would like to express my gratitude to my main supervisors Pablo Balseiro Vigo and Ana Cristina Da Silva Gomes for excellent guidance, feedback and follow up during this project as well as great guidance throughout the data analyzing process.

I would also like to thank Cindy Pedrosa for teaching me the lab protocols and offering help in the lab when needed. Thank you to Sigurd Olav Handeland for great feedback on the thesis and to Ivar Rønnestad for giving me the opportunity to be part of this project.

A special thanks goes to the Marine Development Biology group at BIO for providing experimental- and research facilities, and to Lerøy Sjøtroll AS, in particularly to Harald Sveier and Julia Fossberg Buhaug, for providing fish material to the experiment as well as experimental facilities.

Finally, my appreciation goes to my family for great support and motivation throughout this study.

This study was part of the project SwimFit “Effekt av svømmetrening hos smolt; En grunnleggende studie” and was financed by the Research Council of Norway (Grant no 309384).

Marianne Eidskrem Gullaksen

Bergen, June 2023

ABSTRACT

The Atlantic salmon farming industry have experienced increasing challenges related to abnormal heart morphology characterized by small, rounded, and fatty hearts. In 2019 and 2020 heart disease was ranged as the most important cause of death during the seawater phase. To reduce the mortality rates, aerobic exercise have been suggested as a method to produce more robust fish and improve the cardiovascular health of the Atlantic salmon (*Salmo salar*). The cardiovascular health is affected by the relative heart size, amount of red blood cells and capillary size to maintain an adequate oxygen supply. This study examined the effect of continuous aerobic exercise on cardiac health as well as growth, through two distinct experimental trials. Experiment 1 (5-month duration) was a constant current experiment that investigated the effect of different exercise regimes (0.5 BL/s, 1.0 BL/s and 1.5 BL/s) during the freshwater phase (11 weeks). The results indicated that fish swimming at moderate and high velocities increased their relative heart size compared to fish swimming at low velocities. Experiment 2 was conducted at a production scale level and compared the performance of fish reared in Preline S-CCS facility to the performance of fish reared in open net pens. After 4 months in different rearing systems, the reference group exhibited a significantly higher transcription level of *gata4*, *mef2c*, *angpt2* and *angpt1* suggesting that seasonal differences including temperature and light, could potentially stimulate the transcription of cardiac genes.

ABBREVIATIONS

AKT - Protein kinase B

angpt1 – Angiopoietin-1

angpt2 – Angiopoietin-2

B-actin – Beta-Actin

BL/s – Body length per second

cDNA – Complementary DNA

Cq – Quantification cycles

CI – Confidence interval

CSI – Cardiosomatic index

CSV – Comma-separated values

CV - Coefficient of variance

DNA – Deoxyribonucleic Acid

DNase – Deoxyribonuclease

eNOS – Endothelial nitric oxide synthase

FOXO1 – Forkhead in rhabdomyosarcoma

gata4 – Transcription factor

GLM – Generalized linear model

GLMM - Generalized linear mixed model

Hct – Hematocrit

HE – Haematoxylin-eosin

HSD – Honest Significant Difference

K – Condition factor

LD – Light, Darkness

mef2c – Myocyte-specific enhancer factor 2C

mRNA – Messenger RNA

NTC – No template control

PCR – Polymerase chain reaction

pedf – Pigment epithelial-derived factor

PIT-tag – Passive Integrated Transponder Tag

qPCR – Quantitative PCR

RAS – Recirculatory Aquaculture System

RG – Relative growth

RNA – Ribonucleic acid

RNase – Ribonucleases

rps20 – Ribosomal Protein S20

S-CCS – Semi-closed raceway system

SD – Standard deviation

SGR – Specific growth rate

Tie-1 – Tyrosine-protein kinase receptor 1

Tie-2 – Tyrosine-protein kinase receptor 2

U_{opt} – Optimal swimming speed

vegf – Vascular endothelial growth factor

TABLE OF CONTENT

1.INTRODUCTION

1.1 BACKGROUND

Aquaculture account for approximately 70% of the global salmonids production (Shahbandeh, 2022) with Norway as the top producer. In 2021 the Norwegian production of farmed Atlantic salmon (*Salmo salar*) reached a total of 1.45 million tons which equals a contribution above 50% on a global scale (FAO, 2022). Norwegian aquaculture began as a small industry in 1970 with the introduction of the sea pen, and became a commercial industry in 1980 (Afewerki et al., 2023). Atlantic salmon has emerged as the domain species among salmonids due to its high demand on the global marked (SSB, 2017), and ease production management.

Atlantic salmon is an anadromous fish, meaning it hatch in freshwater, and migrate to the sea to grow before returning to freshwater to spawn (Hansen and Quinn, 1998). Compared to wild salmon, the farmed salmon display an elevated growth rate. This is a result of selective breeding programs that prioritize growth through several environmental parameters like high fat diets, light manipulations and temperature, which in turn mimic natural conditions (Harvey et al., 2016) In aquaculture facilities, the lifecycle of the Atlantic salmon begins in the hatcheries. The salmon remains in the hatcheries for about 6-12 months where they are reared in freshwater until they attain the smolt stage. Smolts are then transferred to open net pens located at sea, where they undergo a period of accelerated growth for another 12-18 months before being harvested (Persson et al., 2022).

1.2 AQUACULTURE TOWARDS SUSTAINABILITY

Atlantic salmon farming is important for the national economy in Norway as it is the second largest export industry after oil and gas (Fiskeridepartementet, 2021). However, there is still a great potential for growth. For this reason, the Norwegian government has set a goal of a five-fold increase in production towards 2050 and established that the development must be sustainable. According to the Brundtland commission report (chapter 2, point 1), sustainable development can be defined as a "development which meets the needs for current generations without compromising the ability of future generations to meet their needs" (World Commission on Environment and Development, 1987).

In salmon farming one of the most important factors for a sustainable development is fish welfare. An important indicator to determine fish welfare is the mortality rate. In Atlantic salmon farming, a higher mortality rate during the sea water phase compared to the rest of the production process have been reported (Sommerset et al., 2021; Bang Jensen et al., 2020) . This represents a problem towards a sustainable production. According to the 'fish health report' 2021, the median mortality of fish transferred to sea at marine salmon farms in Norway was 17.4% in 2021 (Sommerset et al., 2021). A study by Bang Jensen et al. (2020) found that there is a higher mortality during the three first months after sea-transfer. This transfer happens when the fish have gone through smoltification and become post-smolt. Smoltification is the phase where salmonids undergo both physiological and morphological changes that prepare them for a life in high-salinity water (Aas et al, 2011). This is an energy demanding transformation with a high impact on the immune system causing the fish to become more vulnerable to stress and disease (Johansson et al., 2016). Considering that this is also the phase where the fish are transferred to the sea, it includes several stressful events related to transportation as well as the introduction to marine environments and new pathogens. In addition to the risk of diseases caused by pathogens, there have also been reported on cases of lifestyle diseases such as cardiac failures due to atherosclerosis (narrowing of arteries), hypoplasia (under developing of an organ or tissue) and malformations (Brocklebank and Raverty, 2002; Poppe and Taksdal, 2000), and in 2019 and 2020, the heart disease, cardiomyopathy syndrome (CMS), was ranged as the most important cause of death for Atlantic salmon during the seawater phase (Sommerset, 2021).

Apart from being a welfare issue, high mortality also have economic consequences due to the inability to harvest fish that do not survive till slaughter (Oliveira et al., 2021). To ensure better fish health in addition to reduce economic losses, it has been suggested that farmers should produce more robust fish (Øvrebø et al., 2022). Fish robustness can be defined as the ability to combine fast growth with normal organ development and improved resistance to both diseases and physiological challenges (Castro et al., 2013). Thus, improving the robustness will increase the overall survival rate and welfare of the fish. This can in turn increase profitability by improving the industry´s reputation and meet the consumers demand for sustainable and ethical food. One method which have shown promising results at producing more robust fish is aerobic swimming training (Castro et al., 2011).

1.3 SWIMMING-TRAINING

Swimming is the principal form of locomotion in fish. Migratory species like Atlantic salmon swim almost continuously and are active species. In the fish farms, Atlantic salmon are not allowed to fully display their normal swimming behavior. As a result, farmed fish does not experience the same physiological benefits that swimming provide for the wild salmon. However, these benefits can be achieved by exercising the fish. This can be done by increasing the water velocity and thereby making the fish swim against controlled currents. Such currents can be applied, for e.g., in raceways or in circular and elliptical tanks.

Different fish species and fish sizes swim at different speeds (Moore, 2008). Experiments on active swimmers such as Atlantic salmon (Castro et al., 2011; Timmerhaus et al., 2021), yellowtail (*Seriola quinqueradiata*) (Yogata and Oku, 2000) and Arctic charr (*Salvelinus alpinus*) (Adams et al., 1995) have shown a positive correlation between swimming exercise and improved growth (Castro et al., 2011; Timmerhaus et al., 2021). In addition swimming induced exercise have also been reported to increased disease resistance (Castro et al., 2011) and reduced aggressive behavior (Balseiro et al., 2018) in Atlantic salmon. However, such benefits have not been reported for all species. Species that are typically perceived to be inactive swimmers, such as the Japanese flounder (*Paralichthys olivaceus*) (Ogata and Oku, 2000) and rock carp (*Procypris rabaudi*) (Hou et al., 2022) have not experienced the same benefits. Swimming induced exercise in these species, either had negative- or no effects on growth performance. Similar results have been documented for exercising of Atlantic cod (*Gadus morhua L.*) (Bjørnevik et al., 2003) Although this species is not considered to be as inactive as the previously mentioned, but it is less active than salmonid species.

In addition to being species dependent, exercise induced benefits are dependent on the exercise regimen (Davison, 1997; Davison and Herbert, 2013). The type of swimming can either be aerobic or anaerobic based on the oxygen demand (Pearson et al., 1990). Anaerobic training involves rapid swimming in the form of a sprint training regime where the fish swim to exhaustion (Davison, 1997). In nature this can be represented by the conditions where the fish is chased by a predator or when it is chasing a prey. In a study by Gamperl et al, 1988, the effect of anaerobic exercise was investigated by chasing rainbow trout (*Salmo gairdneri*) to a speed where it became exhausted for 30 s every second day. Compared to the control group, the trained fish experienced less growth. Similar results were presented by Hernandez et al.

(2002) indicating that high speed training is a stressor rather than a growth promoter. A more natural and sustainable way to exercise fish is aerobic exercise. This regime includes continuous training which create a cardiac load that increase the need for internal oxygen transport (Castro et al., 2013). As salmonids are migratory fish, they have a high capacity for aerobic swimming (Timmerhaus et al., 2021). This type of swimming activate fatty acid and glucose oxidation in the red muscle to maintain a stable aerobic metabolism of energy supply, which in turn enable the fish to grow more and improves its survival (Pearson et al., 1990).

1.3.1 Optimal swimming speed for Atlantic salmon

In Atlantic salmon, increased growth, increased disease resistance and reduced aggressive behavior are some of the benefits related to swimming exercise. Increased growth have been linked to an improved feed efficiency, higher feed intake or both (Bjørnevik et al., 2003; Davison, 1997). There are several factors that affect the growth rate including species, swimming speed, temperature, water quality, stress and density of fish (Davison and Herbert, 2013; Fivelstad et al., 2003; Handeland et al., 2008; McCormick et al., 1998; Soderberg et al., 1993). The most influential factors linking growth and exercise are species and swimming speed (Davison and Herbert, 2013). For this reason, growth by exercise can be optimized by utilizing the optimal swimming speed (U_{opt}). The optimal speed is where the fish obtain the benefits of exercise without expending excessive energy and where the growth rate increase without compromising welfare (Timmerhaus et al., 2021). The swimming speed is measured using body length per second (BL/s). This enables a comparison to be made between fish of different lengths (Davison and Herbert, 2013).

For salmonids it has been shown that the U_{opt} is achieved using velocities between 1.0 BL/s and 1.5 BL/s (Castro et al., 2011; Davison, 1997; Timmerhaus et al., 2021). Speeds below the U_{opt} have been reported to have a higher level of spontaneous behavior, which in turn can lead to dominant hierarchies and aggression. In an experiment by Balseiro et al. (2018), it was observed that fish that swim against the current in the semi-closed facility, Preline, had fewer interactions and less aggressive behavior compared to those in the open sea cage. The heterogenous regime of currents in open sea cages, can promote dominant hierarchies in areas of low currents. This can result in an increased aggressive behavior. An environment characterized by aggressive behavior may cause wounds that could potentially

lead to infections, making the fish more vulnerable to diseases. On the opposite, it has been shown that speeds higher than U_{opt} results in reduced growth and can compromise the fish welfare (Solstorm et al., 2015). A higher swimming speed increases the resistance from the water causing an increase in the oxygen demand. As a result, swimming will be more challenging. If the speed remains above optimal over time, the fish will not have enough energy to withstand the high oxygen demand as well as maintaining important life processes such as growth and immune responses. In comparison, moderate exercise at U_{opt} will also increase oxygen needs, but these needs can be fulfilled without the fish becoming exhausted. By transporting oxygen-rich blood throughout the body with every contraction, the heart is an important organ for sufficient oxygen supply (Moore, 2008). To meet the increased oxygen demand during exercise, the heart must respond with an increase in cardiovascular activity. There have been reported several cardiovascular adjustments induced by exercise including elevations in heart rate and stroke volume (Wang et al., 2019), induced hematocrit (Gallaugher et al., 2001) and relative ventricle size (Davison, 1997). These adaptations allow the heart to operate with greater efficiency, to meet the increased oxygen demand during exercise.

1.4 FISH HEART

1.4.1 Heart structure and morphology

As swimming exercise is a form of aerobic exercise, the heart is one of the main target organs for exercise induced benefits (Castro et al., 2011). Atlantic salmon have a teleost heart which is responsible for pumping blood around the body and delivering oxygen to the tissues and organs. Deoxygenated blood is pumped from the heart via the ventral aorta into four gill arch arteries (Kryvi & Poppe, 2016). The blood is oxygenated as it passes through the gills before being pumped into the dorsal aorta, which distributes the oxygenated blood to the rest of the body´s organs and tissues via smaller arteries. Deoxygenated blood is then collected by the venous system and returned to the heart (*Fig. 1.1*).

The teleost heart has an S shape and is located ventrally towards the head and separated from the abdominal cavity by *septum transversum* (Kryvi & Poppe, 2016). It consists of four chambers: *sinus venosus*, atrium, ventricle and *bulbus arteriosus* (*Fig.1.1*)

leading to the ventral aorta (Moore, 2008). Venous blood comes in through the first chamber, *sinus venosus*. *Sinus venosus* functions as a blood-storage and is responsible for initiating and controlling the heartbeat. After coming through sinus venosus, the blood is pumped to the second chamber, the atrium. To prevent blood from returning to *sinus venous*, there are sinoatrial valves consisting of elastic connective tissue between the atrium and the sinus venous. The atrium wall consists of inner walls called trabeculae. These are made up of heart muscle cells that extend into the cavity (lumen). The contractions of the muscles help to move the roof and sides of the atrium towards the opening between the atrium and the ventricle. From the atrium, the blood is further pumped to the ventricle, which is the main pump. The ventricle develops the pressure needed to propel the blood into circulation and is enriched with fibers from the *vagus* nerve that have an inhibitory effect on the heart rate and stroke volume. From the ventricle, the blood is pumped to the final chamber, *bulbus arteriosus*, which is a thickened part of the ventral aorta. *Bulbus arteriosus* functions as a pressure equalizer that is made up of smooth muscle cells and it is equipped with valves that prevent backflow of blood (Kryvi & Poppe, 2016). Muscle contractions further allow blood to be pumped from *bulbus arteriosus* to the gills for oxygen supply.

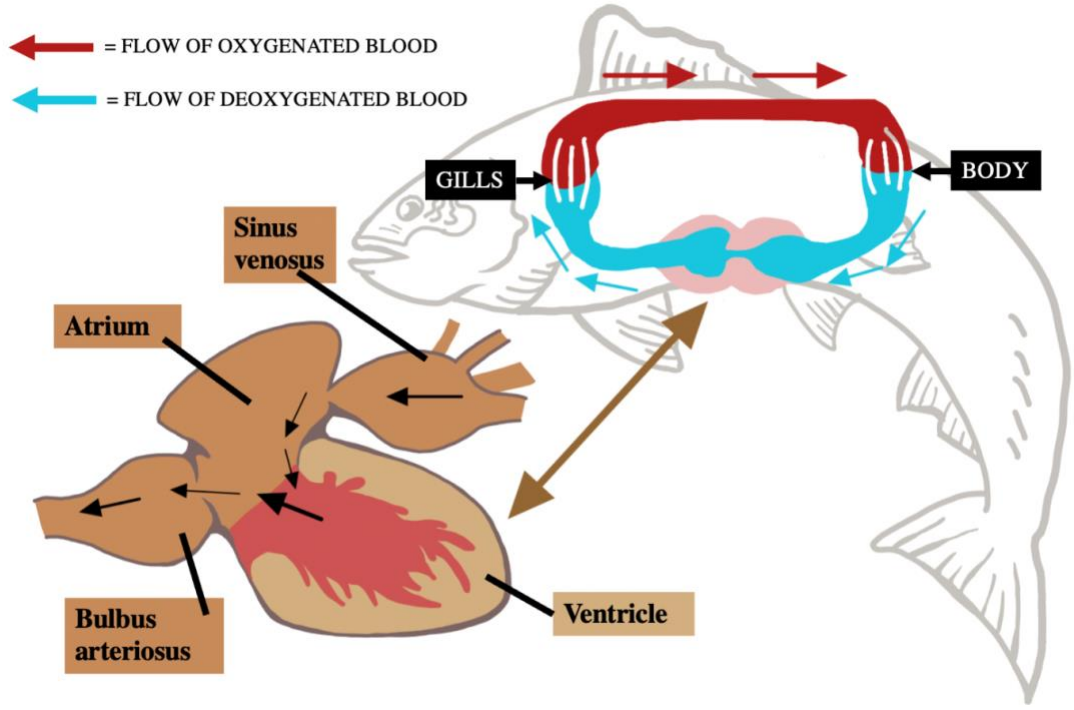

Figure 1.1. Drawing of the structure of the fish circulation system and highlights the four chambers of the heart. Deoxygenated blood from the body comes into the heart via sinus venosus and is pumped through the atrium to the ventricle. The bulbus arteriosus is the final destination before the blood is pumped through the gills for oxygen supply. Oxygenated blood is transported throughout the body before coming back to the heart, and the process is repeated.

Different fish species show great variation in heart morphology, particularly the ventricle (Farrell & Jones 1992; Kryvi & Poppe 2016). The ventricles can be sacular, tubular or pyramidal (*Fig. 1.2A*). The sacular ventricle (*Fig. 1.2B*) is the most common form. This shape is typical in elasmobranchs and many marine teleost. The least common is the tubular ventricle which is only found in fish species with elongated body shape, like eels. However, this does not mean that all species with this body shape have tubular hearts (Farrell & Jones 1992, pp.6). Sacular and tubular ventricles are typical in sedate fish species and often have a ventricle that only consist of spongiosa myocardium (Farrell & Jones 1992, pp. 7). Active species like Atlantic salmon have a pyramid-shaped ventricle (Farrell & Jones 1992; Kryvi & Poppe 2016).

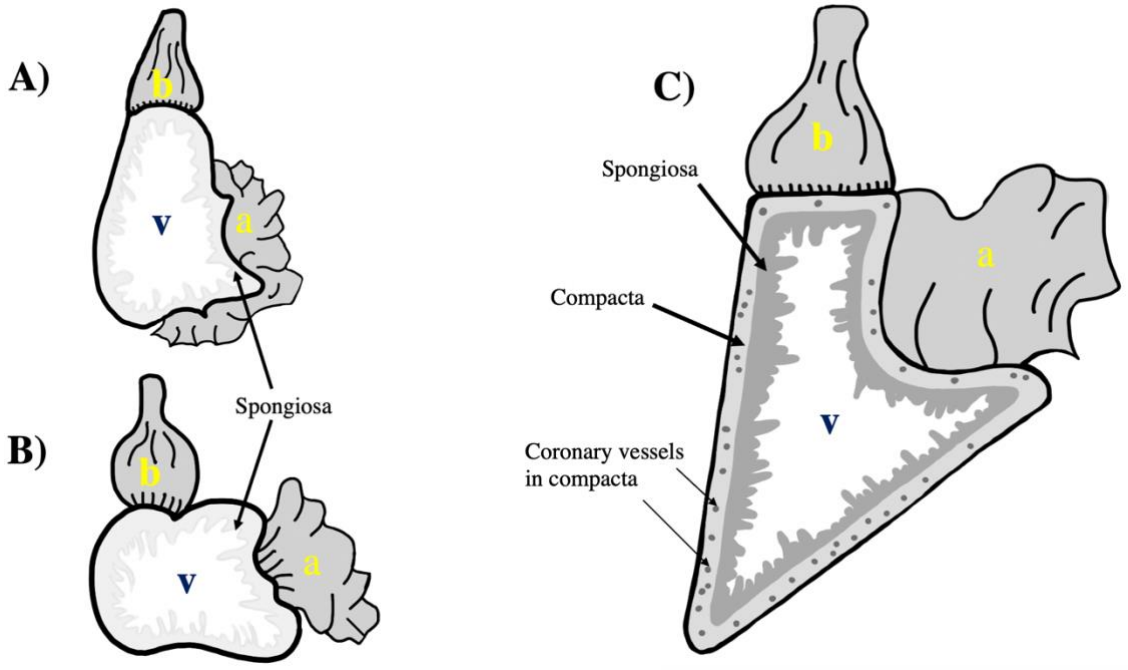

Figure. 1.2: The figure demonstrates the ventricular shapes: A) tubular B) sacular and C) pyramidal. v=ventricle, a=atrium and b=bulbus arteriosus. The figure also demonstrates the position of the typical features of the given ventricle shape including spongiosa, compacta and coronary vessels in compacta. The figure is reproduced and slightly based on the figures from Farrell (2011).

Pyramidal ventricles (*Fig. 1.2C*) consist of a muscular wall of variable thickness and a varying degree of vascularization through coronary circulation. This allows the heart to receive oxygenated blood from the gills in addition to the deoxygenated venous blood from the systemic circulation. Pyramidal ventricles also have an outer compact layer, called compactum. Compactum is arranged with numerous trabeculae enclosing the spongiosa (Kryvi & Poppe, 2016), which gives the heart the potential to act as a pressure pump

(Agnisola and Tota, 1994). Among the teleost that have a compactum, the most active species generally have a higher proportion of compactum (Farrell & Jones 1992, pp. 8). For active swimming species, a pyramidal ventricle is advantageous in terms of this great pumping capacity as the amount of blood entering the heart is determined by the volume ejected from the ventricle at each stroke, and by the heart rate (Moore, 2008).

Optimum cardiac output is generated through a relatively high heart rate, high pressure, and a small stroke volume (Frisk et al., 2020; Poppe et al., 2003). During long sustained aerobic swimming, cardiac output naturally rises to meet increased tissue oxygen demand. To determine the bloods capacity to carry oxygen, hematocrit (Hct) levels can be measured. This is the volume of red blood cells compared to the total blood volume (red blood cells and plasma). Hematocrit is a good health indicator as a low value might indicate blood disorders or an inadequate oxygen carrying capacity. Another indicator used to determine cardiovascular health is the cardiosomatic index (CSI) which is the weight of the heart relative to the body weight. A low CSI may be associated with a reduced cardiac capacity and reduced robustness, as a small heart will have reduced cardiac capacity (Poppe et al., 2003).

1.4.2 Abnormal hearts in salmon farming

A healthy and functional heart is important to maintain good welfare. There have been established a positive correlation between heart morphology and functioning (Poppe et al., 2003). The salmonids heart normally has a pyramidal shape, allowing the heart to effectively move small volumes of blood at a relatively high rate under high pressure. However, the heart of farmed salmon has been reported to have some abnormalities including small and rounded shapes (*Fig.1.3*) (Frisk et al., 2020; Poppe et al., 2003). A small heart reduces the cardiovascular capacity, thereby restricted the cardiac function (Poppe et al., 2003). As a result, fish are less tolerant to stress, which results in increased mortality and reduced growth rate. This is most problematic in the fall when growth is rapid, oxygen demand is high, and levels of dissolved oxygen in water are low. As a result, the cardiac output required to fulfil the oxygen demands will not be met due to abnormal shapes and reduced function.

The study by Frisk et al. 2020 suggested that cardiac deformities could be a result of the accelerated growth rate experienced in farming facilities in early rearing stages. In order to

accelerate the embryonic development, some farmers increase the temperature resulting in earlier first feeding and larger smolts (Poppe and Taksdal, 2000). As a result, the heart might grow slower than the rest of the body making it too small to meet the body´s demands. Another study by Foddai et al. 2022 tested the effect of temperature and dietary energy on the heart of Atlantic salmon in Tasmania and found that there was no correlation between deformities and temperature during the grow-out phase. Based on these findings, rounded and small heart deformities might be related to other causes like breeding program, rearing strategies, over-feeding, and reduced swimming activity, but more research is needed in this area (Foddai et al., 2022).

In addition to a small and rounded heart, there have been reported on high levels of fat deposition in the ventricle (*Fig.1.3*) (Kristensen et al., 2012; Poppe and Taksdal, 2000). This is likely related to the high-energy diet coupled with the nonactive lifestyle found in the fish farms. A high fat deposition might be a reason behind an increasing observation in heart failures in farmed salmon (Robinson et al., 2017). To reduce the problems related to a high fat deposition and a small, rounded shape, exercise has been suggested as a solution. Swimming induced exercise have shown to improve the overall cardiac health by improving the cardiovascular capacity and the overall heart size (Castro et al., 2013; Solstorm et al., 2015) due to factors like increased cardiac output and active metabolic rate, and increased number of capillaries (Solstorm et al., 2015).

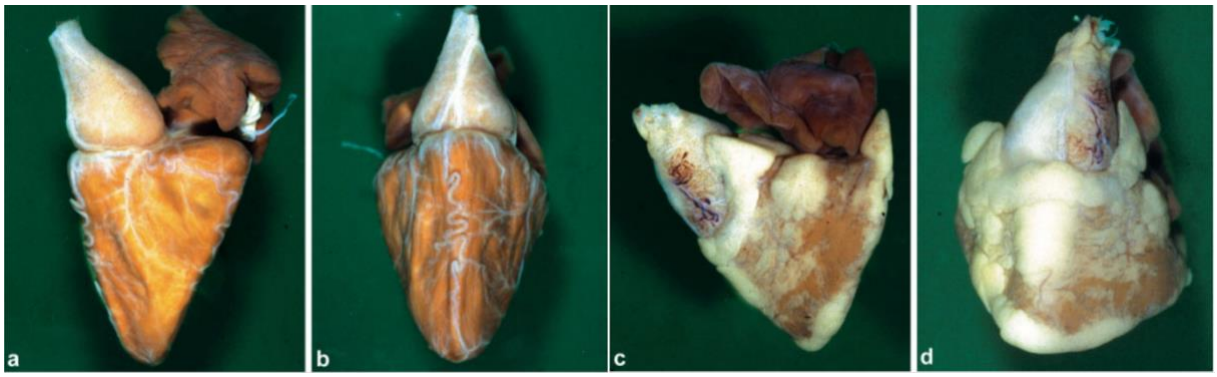

Figure 1.3. Atlantic salmon heart. (a) lateral and (b) cranio-ventral view of heart from wild fish. (c) Lateral and (d) cranio-ventral view of heart from farmed fish. The shape of the ventricle of the wild salmon is pyramidical while the farmed fish have a rounder ventricle. In addition the farmed fish (c,d) have a higher fat deposition than the wild (a,b) as seen by the white layers on the heart. Pictures from Poppe et al. 2003.

1.4.3 Effect of exercise on heart size

Atlantic salmon exposed to high velocities of 1.5 BL/s had a greater increase in heart size compared to those exposed to low (0.2 BL/s) and moderate (0.8BL/s) velocities (Solstorm et al., 2015) . As a response to an increased cardiac workload the cardiac muscle size increase to satisfy the higher energy demand by the swimming muscles (Takle and Castro, 2013). An increase in heart size is thought to be related to fish robustness (Castro et al., 2013). For humans, exercise induced cardiac growth is typically associated with improved physical capacity (Castro et al., 2013). Physical activity causes the heart to undergo cardiac remodeling to improve performance (Fathi et al., 2020). This seems to be similar for Atlantic salmon as a study by Castro et al, 2013 found that genes known as key components for mammalian cardiac growth, were also present in fish and responsive to exercise in Atlantic salmon hearts.

Cardiac growth can result from hypertrophy or hyperplasia or a mix of both (Robinson et al., 2017). Cardiac hypertrophy is an increase of muscle mass by increasing cardiac myocyte size (Fathi et al., 2020), while hyperplasia is an increase in the number of myocytes (Komi, 2008). In contrast to mammals who experience muscle growth by hypertrophy, teleost fish experience muscle growth by both hypertrophy and hyperplasia (Castro et al., 2013; Higgins and Thorpe, 1990). Exercise induced cardiac hypertrophy is referred to as physiological cardiac hypertrophy. In contrast to pathological cardiac hypertrophy, which is associated with heart failure, physiological cardiac hypertrophy improves the cardiovascular function (Fernandes et al., 2015). Exercise increase the workload on cardiac myocytes which stimulate cardiac hypertrophy in order to increase cell size to maintain a normal cardiac output (Ellmers et al., 2007). In response to hypertrophic stimuli, the transcription factor (*gata4*) and the myocyte-specific enhancer factor 2C (*mef2c*) are upregulated (Akazawa and Komuro, 2003; Castro et al., 2013). *Gata4* is part of the GATA family of zinc finger transcription factors that regulate gene transcription by binding to GATA elements (Suzuki, 2011). It has an important role in cardiomyocyte regeneration by promoting transcription of regulatory, structural- and signaling proteins essential in cardiac function and blood pressure regulation (Juhanna et al., 2020). *Mef2c* is part of the MADS domain that have binding sites associated with muscle-specific genes (Wang et al., 2001) and is an essential regulator of muscle development and maintenance of muscle cells (Fathi et al., 2020; Wang et al., 2001).

1.4.4 Capillary growth

In addition to muscle growth by hypertrophy, increased muscle capillarity from aerobic swimming has been found in several fish species (Davie et al., 1986; Ibarz et al., 2011; Pelster et al., 2003). After exercising new capillary growth occurs by sprouting as a response to muscle repair and to ensure greater oxygen extraction capacity and muscle performance. The process in which new blood vessels are developed from pre-existing ones is called angiogenesis (Gustafsson & Kraus, 2001). This is a natural process that occurs in response to inflammation or low oxygen supply, referred to as hypoxia. Two important glycoproteins involved in angiogenesis are angiopoietin-2 (*angpt2*) and angiopoietin-1 (*angpt1*). *Angpt2* is involved in the initiation of angiogenesis while *angpt1* facilitate stabilization of capillaries prior to- and after angiogenesis (Nader et al., 2014). Angpt1 and Angpt2 bind to the same endothelial receptor, Tyrosine-protein kinase receptor 2 (Tie-2). However, Angpt2 possesses antagonist properties, which means they contain opposite physiological properties of Angpt1 *(Fig.1.4)*. In the human- and rat skeletal muscle, exercise have been proven to increase the *angpt2*:*angpt1* ratio (Gustafsson et al., 2007; Lloyd et al., 2003) due to an increase in the transcription of *angpt2* and only a small decrease in the transcription of *angpt1*. This is because higher concentrations of *angpt2* leads to destabilization of the Angpt1/Tie-2 bond which in turn promotes angiogenesis (Gavin, 2009). However, tumor studies suggest that Vascular endothelial growth factor (Vegf) must be present for *angpt2* to induce vessel growth. If it is expressed in the absence of Vegf, destabilization causes vessel regression (Holash et al., 1999; Lloyd et al., 2003).

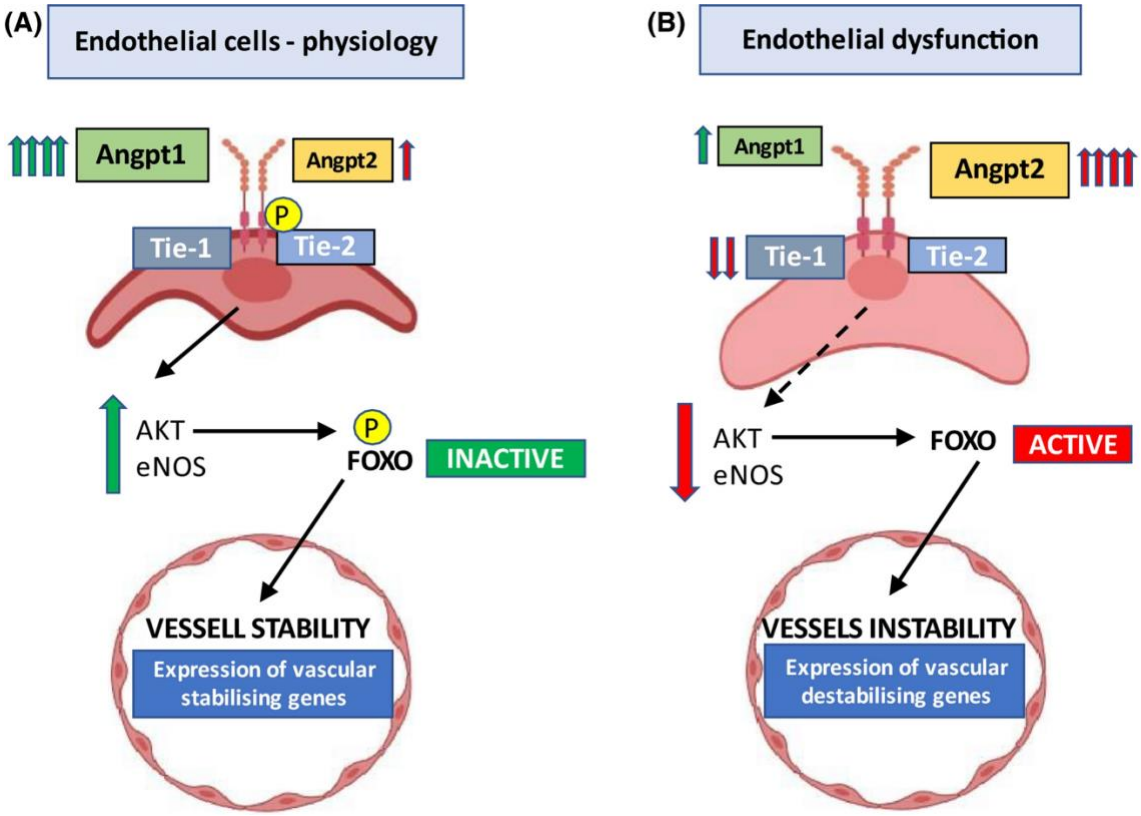

Figure 1.4. The figure demonstrates the angiopoietin signaling pathway in vessel stabilization and destabilization. (A) Activation of angpt1/Tie-2 pathway favors vessel stability in the presence of Tyrosine-protein kinase receptor 1 (Tie-1). Protein kinase B(AKT) and Endothelial nitric oxide synthase (eNOS) are then activated, and AKT promotes the phosphorylation of Forkhead in rhabdomyosarcoma (FOXO1) resulting in its inhibition and thereby stabilizing the vessels. (B) Upregulation of angpt2 and parallel Tie-1 downregulation, results in Tie-2 phosphorylation. AKT is then reduced and FOXO1 is activated (dephosphorylated form), which stimulates vascular destabilization leading to vascular instability. Figure from: Ricciardi & Gnudi, 2021.

Even though angiogenesis plays a vital role in biological systems, excessive or insufficient angiogenesis is associated with several disorders. A medical study on human patients showed that high levels of *angpt2* and low levels of *angpt1* are related to abnormal cardiac structure and heart failure (Tsai et al., 2016). Excessive angiogenesis can lead to the development of atherosclerosis (Rychli et al., 2009) which is a state where the arteries become narrow, making it difficult for blood to flow through. To protect against atherosclerosis and avoid the formation of abnormal vessels, pigment epithelial-derived factor (Pedf) works as an inhibitor of angiogenesis (Tink, 2005). It has been shown that Pedf protect against inflammatory activation and injury on the endothelial cells in humans, which is

thought to be a crucial early event in the development of atherosclerosis (Rychli et al., 2009). Pedf belongs to the non-inhibitory serpin family and is expressed in most tissues of the body, including the heart. In addition to inhibit angiogenesis, Pedf is also involved in neuroprotection and cell differentiation (Tink, 2005). In human hearts it has been shown that the expression of Pedf is higher in healthy hearts than ischemic hearts (Rychli et al., 2010). Cardiac ischemia is a condition of inadequate blood supply and oxygen to the heart muscle due to narrow arteries or blood clot which in turn can lead to a heart attack (Nader et al., 2014). The *pedf* gene is located on chromosome 13 in Atlantic salmon, but there is little knowledge on the effect of the *pedf* gene in the fish heart. However, it is likely that it could have a similar role as it has in the human heart.

1.5 AIM OF THIS THESIS

The aim of this thesis was to test if aerobic exercise increases growth and improves the cardiovascular capacities of Atlantic salmon, by increasing the heart size, capillarization, hematocrit and cardiosomatic index. To test this, two experiments were conducted: one constant current experiment and one production group study. For the constant current experiment, Atlantic salmon were trained in freshwater under three different velocities: 0.5 BL/s, 1.0 BL/s and 1.5 BL/s. For the production group study, a commercial group was divided into two subgroups at the transfer to seawater. One subgroup was transferred to Preline where the fish was exposed to continuous water currents, and the second group was placed in ordinary pens and used as a reference. Furthermore, quantitative polymerase chain reaction (qPCR) was conducted to analyze biomarkers that have been correlated to hypertrophy growth like *gata4* and *mef2c,* as well as angiogenetic- and anti-angiogenetic biomarkers previously described in mammals, *angpt1* and *angpt2* and *pedf.*

For the constant current experiment, the following hypothesis are being tested:

- **H01.1:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the growth rate.
- **Ha1.1:** Rearing Atlantic salmon at different velocities during the freshwater phase has significant effect on the growth rate.

- **H01.2:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the condition factor (K).
- **Ha1.2:** Rearing Atlantic salmon at different velocities during the freshwater phase has significant effect on the condition factor (K).

- **H01.3:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the cardiosomatic index (CSI).
- **Ha1.3:** Rearing Atlantic salmon at different velocities during the freshwater phase has significant effect on the cardiosomatic index (CSI).

- **H01.4:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on hematocrit levels.
- **Ha1.4:** Rearing Atlantic salmon at different velocities during the freshwater phase has significant effect on hematocrit levels.

- **H01.5:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the relative transcription of *gata4.*
- **Ha1.5:** Rearing Atlantic salmon at different velocities during the freshwater phase has significant effect on the relative transcription of *gata4.*

- **H01.6:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the relative transcription of *mef2c.*
- **Ha1.6:** Rearing Atlantic salmon at different velocities during the freshwater phase has significant effect on the relative transcription of *mef2c.*

- **H01.7:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the relative transcription of *angpt2.*
- **Ha1.7:** Rearing Atlantic salmon at different velocities during the freshwater phase has significant effect on the relative transcription of *angpt2.*

- **H01.8:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the relative transcription of *angpt1*.

- **Ha1.8:** Rearing Atlantic salmon at different velocities during the freshwater phase has significant effect on the relative transcription of *angpt1*.

- **H01.9:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the relative transcription of *pedf.*
- **Ha1.9:** Rearing Atlantic salmon at different velocities during the freshwater phase has significant effect on the relative transcription of *pedf.*

For the production group study, the following hypothesis are being tested:

- **H02.1:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has no significant effect on the mean body weight.
- **Ha2.1:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has significant effect on the mean body weight.

- **H02.2:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has no significant effect on the mean body length.
- **Ha2.2:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has significant effect on the mean body length.

- **H02.3:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has no significant effect on the condition factor (K).
- **Ha2.3:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has significant effect on the condition factor (K).

- **H02.4:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has no significant effect on the cardiosomatic index (CSI).
- **Ha2.4:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has significant effect on the cardiosomatic index (CSI).

- **H02.5:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has no significant effect on the relative transcription of *gata4.*
- **Ha2.5:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has significant effect on the relative transcription of *gata4.*

- **H02.6:** Exposing Atlantic salmon to different rearing conditions (PrelineS-CCS and open net pens) has no significant effect on the relative transcription of *mef2c.*
- **Ha2.6:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has significant effect on the relative transcription of *mef2c.*¨

- **H02.7:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has no significant effect on the relative transcription of *angpt2.*
- **Ha2.7:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has significant effect on the relative transcription of *angpt2.*

- **H02.8:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has no significant effect on the relative transcription of *angpt1*.
- **Ha2.8:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has significant effect on the relative transcription of *angpt1*.

- **H02.9:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has no significant effect on the relative transcription of *pedf.*
- **Ha2.9:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has significant effect on the relative transcription of *pedf.*

2. MATERIAL AND METHODS

2.1 ETHICAL STATEMENT

The experiment complied with the guidelines of the Norwegian Regulation on Animal Experimentation and have been approved by the National Animal Research Authority in Norway (FOTS ID 27401) before start-up.

2.2 THE EXPERIMENTS

Two separate experiments were conducted for this thesis: 1. Constant current experiment; 2. Production group study. The constant current experiment was located at Høyteknologisenteret at the Department of Biological Sciences, University of Bergen (60.38103° N, 5.33314° E). The production group study utilized experimental fish from two different locations. The reference group reared in open net pens was located at 26595 Djupvika, Vestland (60.035817° N, 5.342217° E). The Preline group reared in Preline S-CCS was located at 32137 Sagen 2 in Samnanger commune at Trengereid fjord, Vestland (60,348383° N, 5,644033° E). In addition, the production group study sampled fish from a baseline group, which was reared in a Recirculatory Aquaculture System (RAS) facility, located at 11493 Lerøy Sjøtroll Kjærelva AS (59,899483° N, 5,293883° E). These fish were collected ahead of the production group study to examine the current condition of the fish before the trial. The figure below (*Fig.2.1*) shows the locations of all the experimental facilities in addition to the land-based RAS facility at Kjærelva.

Figure 2.1. The different experimental locations and their respective facilities. ▼ = *Høyteknologisenteret;* ▼ = *Preline S-CCS in Sagen;* ▼ = *Lerøy Sjøtroll Kjærelva, Fitjar;* ▼ = *Djupevika.*

2.2.1 FISH BREED

All the Atlantic salmon used in the two experiments had the same genetic and biological background from Salmobreed strain 0+ and were provided from Lerøy Sjøtroll Kjærelva AS, which produce fish from hatching to smoltification.

Hatching followed standard procedure. The eggs were incubated at 6-7 ºC and hatched after 450-degree days. First feeding began after 250-degree days post hatching. From first feeding and until the fish were collected, the Atlantic salmon from experiment 1; the constant current experiment, were kept in constant day light (LD24:0) with a water temperature of 13.5 ºC (± 0.4 ºC) and oxygen levels above 95 %. The fish from experiment 2; the production group study, were kept in constant day light (LD24:0) until the 10th of August 2021 when the light regime was changed to LD12:12. On the 29th of September 2021 the light regime was

changed back to constant day light (LD24:0). The water temperature remained at 13.7 ºC (± 0.6 ºC) and oxygen was kept above 95% from first feeding.

2.2.2 CONSTANT CURRENT EXPERIMENT

2.2.2.1 EXPERIMENTAL DESIGN

The Constant current experiment conducted at the Department of Biological Sciences lasted for approximately 5 months from 28th September 2021 to 23rd of February 2022. A total of 480 pre-smolt were transferred from Lerøy Kjærelva, Fitjar to the University of Bergen (UiB) on the 1st of September 2023. At arrival, the fish were anesthetized with 80 mg/L of NaCO3-buffered tricaine methane sulfonate (MS-222TM; MSD Animal Health, Netherlands) and individually PIT-tagged (Glass tag 2.12x12 mm 134.2 kHz ISO FDX B, REID solutions) to identify the fish. The weight (g) and length (cm) of the fish were also measured (78.9 ± 18.1 g; 18.6 ± 1.5 cm). On the 25th of October 2023, the fish (98.1 ± 17.6 g; 20.7 ± 1.3 cm) were randomly assigned into six circular experimental tanks with the capacity of 460 L water. Each tank had a height of 57 cm and a diameter of 107 cm. Additionally, a cylinder-shaped separator, with a diameter of 20 cm, was positioned in the centre of the tank to gain better control over the swimming position of the fish. Flow Watch FW450 (General, USA) was used to measure the velocity within the tanks. The velocity was based on an average, which was calculated from both the horizontal positions (1, 2 and 3) and the vertical positions (a, b and c) (*Fig.2.2*). However, point c was not as frequently measured as point a and b.

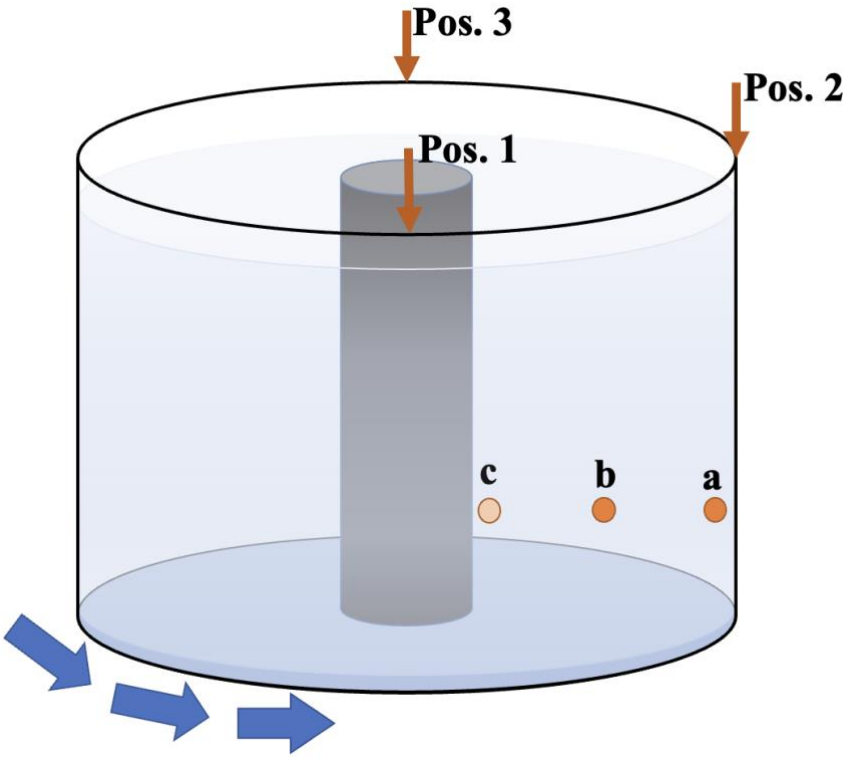

Figure 2.2. The design of the experimental tank. The circles (a, b, c) represent the vertical positions and the numbers (1, 2, 3) represent the horizontal positions. The water flow is represented by the blue pointer (⇒), while the dark circles (•) represent the points usually used for measures of the velocity (point a and b) and the light circle (◦) represent point c which was rarely used for measurements of the velocity. In addition, the dark orange pointers represent the positions where velocities were measured (⇒).

The fish were acclimated to the experimental conditions for 27 days at continuous light (LD24:0) in freshwater with a counterclockwise velocity of 0.5 BL/s. After acclimation, the light regime was changed to 12 h light:12 h dark (LD12:12) to stimulate winter signals. This light regime was maintained for 5 weeks before returning to 24 h light (*Fig. 2.3*). During the freshwater phase the water temperature was 12.5 ± 0.2 °C and oxygen levels were maintained above 80%.

During the trial all conditions remained constant among tanks except for the flow rates. After acclimation, the velocities were increased in four tanks while the remaining two (tank 6 and tank 1) continued at 0.5 BL/s. The velocity in tank 2 and tank 4 were increased to 1.0 BL/s to simulate moderate swimming conditions. Tank 3 and tank 5 had the highest speeds with a velocity of 1.5 BL/s (*Fig. 2.3*). The duration of the swimming exercise lasted for 11 weeks (25th of October 2021 to the 13th of January 2022). On the 17th of January, the

salinity of the water was increased to 26‰, implying a shift from freshwater to brackish water, which in this thesis, it will be referred to as seawater. The water current was also altered to 0.5 BL/s. During the seawater phase temperature was kept at 9.9 ± 0.2°C and oxygen above 90%.

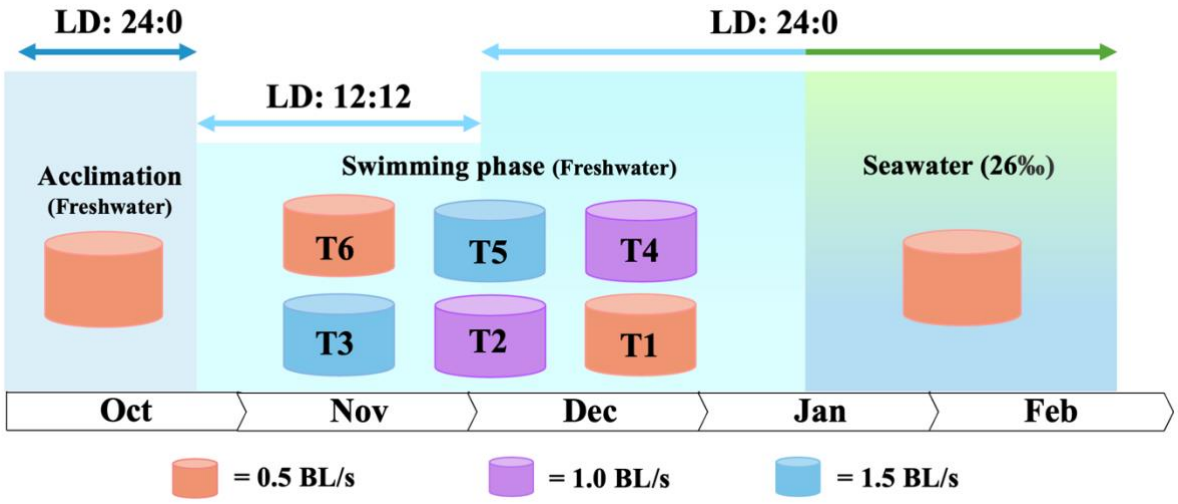

Figure 2.3. Overview of the Constant current experiment design conducted at the University of Bergen. The figure shows the different phases from the acclimation period to the swimming phase before the last phase in seawater. The tanks are color-coded in relation to the induced water currents; ■= 0.5 BL/s, ■ = 1.0 BL/s, ■= 1.5 BL/s. In addition, the light regimes throughout the experiment are represented at the top, with LD12:12 representing winter signals.

2.2.2.2 ENVIRONMENTAL CONDITIONS

Environmental conditions, including oxygen, temperature, and salinity were monitored daily using an OxyGuard standard probe and a salinity probe respectively. Light was monitored by the computer program, OxyGuard Commander (OxyGuard, Denmark) and water velocity was maintained by pumps (Grundfos, Norway) controlled by a Siemens frequency converter which was monitored by the computer program, OxyGuard Commander (OxyGuard, Denmark). These conditions were registered daily through the data program, Visual VIGO (VIGO 5.9). The oxygen levels were kept above 80% throughout the sampling. Oxygen supply was added after the second sampling, specifically on the 10.12.2021, and levels were maintained using an OxyGuard Pacific automated oxygen control system (OxyGuard, Denmark).

All fish were fed commercial dry feed (3 mm, Ewos Clear Fly 80) by automatic feeders once a day between 09:00-15:00 controlled by OxyGuard Commander (OxyGuard, Denmark). The feeding of the fish was adjusted to an amount of 1.9% body weight/day. As the fish grew, the velocities were adjusted every two weeks based on the specific growth rate of the fish length to keep the velocities constant throughout the experiment.

2.2.2.3 SAMPLING

Over the course of the constant current experiment, a total of 210 out of the 480 fish were sampled through four sampling points (*Fig. 2.4*). The initial sampling (25th of October 2021) occurred after 27 days at the end of the acclimation period, shortly before the swimming exercise started. This sampling lasted for one day and involved the collection of 30 fish, with five fish from each tank. The remaining three samplings lasted for three days each, with a total of 60 fish collected for each sampling, with 10 fish from each tank. Two of the remaining sampling points were conducted in freshwater; the second sampling (30th of November – 2nd of December 2021) was conducted at the end of the winter signal (12:12 LD), while the third sampling (11th – 13th of January 2022) was conducted at the end of the swimming training, just before seawater transfer. The final, and fourth, sampling (21st – 23rd of February 2022) was performed after approximately 5 weeks in seawater.

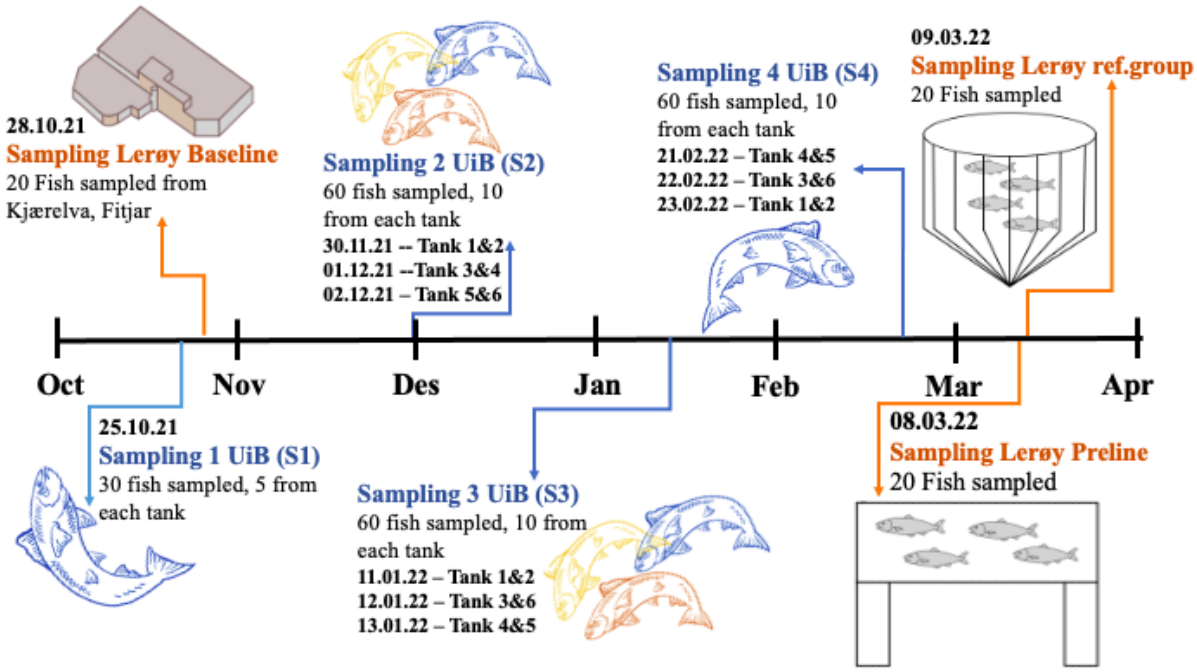

Figure 2.4. Timeline over the two experiments showing all the sampling dates from the constant current experiment (marked in blue) and the production group study (marked orange).

For each sampling, the fish were randomly collected and euthanized using a lethal concentration of 200 mg/L Tricaine methane sulfonate (MS-222). One by one, the fish were numerated, and the PIT-tag was read using a APR600 RFID Handheld Reader (Agrident, USA). To determine the overall growth, measurements of length in cm and weight in grams (OHAUS Valor 2000, USA) were measured for each individual fish. Ahead of opening the fish, 2 mL of blood was collected from the caudal vein using a 23 G needle (BD Microlance™ 3, Spain) with a 2 mL heparinized syringes (BD Emerald Syringe, Spain). Blood was collected into a 75 mm Brand micro hematocrite capillary (Wertheim, Germany) and centrifuged for 5 min at room temperature at max speed of 33869 g using a Sigma 201M centrifuge with microhematocrit rotor nr. 11001 (Osterode am Harz, Germany).

Upon opening the fish, its sex was identified, and the heart was weighed (VWR International LPC-213i, Italy). Tissue samples from the heart was then collected for histological examination and RNA transcription analyses. For RNA, the tissue was collected into RNAlater (Invitrogen, USA), kept at 4°C overnight, and stored at -80°C. For histology the tissue was fixed for 48 h at 4°C in formaldehyde using BiopSafe containers. The tissue was then washed for 30 min using 50% ethanol. The 50% ethanol was removed, and the tissue was stored in 70% ethanol at 4ºC prior to being sent to PHARMAQ Analytiq AS for further processing.

Measurements of weight at the start and the end of each sampling were used to calculate the specific growth rate (SGR) in body weight (g) measured in g-day^{-1} and the relative growth (RG) in body weight (g) measured in percentage as showed in equation 2.1 and equation 2.2.

$$SGR = 100 * \frac{ln(Body\ weight\ (g))_{END} - ln(Body\ weight(g))_{START}}{T_1 - T_0} \quad (2.1)$$

$$RG = 100 * \frac{Body\ weight\ (g)_{END} - Body\ weight\ (g)_{START}}{Body\ weight\ (g)_{START}} \quad (2.2)$$

The relationship between weight and length for each sampling point was used to calculate the condition factor (K) measured in g/cm^3 (Equation 2.3).

$$K = 100 * \frac{Body\ weight\ (g)}{Body\ length\ (cm)^3} \quad (2.3)$$

To find the size of the heart relative to the body, the cardiosomatic index (CSI) was calculated from equation 2.4 and measured in percentage. In addition, the hematocrit (Hct) was calculated through equation 2.5 to find the percentage of red blood cells in the blood.

$$CSI = 100 * \frac{Heart\ weight\ (g)}{Body\ weight\ (g)} \quad (2.4)$$

$$Hct = 100 * \frac{Red\ blood\ cells\ (mm)}{Blood\ (mm)} \quad (2.5)$$

2.2.3 PRODUCTION GROUP STUDY

The production group study consisted of two experimental groups: the Preline group and the reference group. The Preline group was reared in a semi-closed raceway system (S-CCS). This tube shaped (50x12x8 m) facility have a capacity of 780-ton fish and can hold 2000 m^3 of water with a maximum water flow of 400 m^3 min^{-1} that is exchanged every 5–6 min. The water is pumped from a 20-30 m depth and circulate from inlet to outlet creating a constant one-way water current through the system. The water current inside Preline was measured using a Nortek Vector 3D acoustic velocimeter (Nortek AS, Norway) and flow was recorded as 20 cm s^{-1}.

The reference group was reared in open net pens with a total biomass of 5460 tons divided by 12 circular pens. Each pen has a diameter of 160 m with the capacity of 200000 fish. The area itself has a dept that varies from 150 m to 450 m with the pen having an internal dept of 44.5 m.

2.2.3.1 EXPERIMENTAL DESIGN

The fish were followed for approximately 4 months (from 28th October 2021 to 9th of March 2022). All experimental fish were initially based at Lerøy Sjøtroll Kjærelva, Fitjar until smoltification. After smoltification the experimental fish were transferred to two different rearing facilities in seawater on the 31st of October 2021. The Preline group was transferred to the Preline S-CCS facility, while the reference group was transferred to open net pens in Djupevika (*Fig.2.5*). The Preline group swam for 4 months at an initial velocity of approximately 1.1 BL/s and a velocity of approximately 0.5 BL/s by the end of the experiment (constant flow of 20 cm s^{-1}). The reference group, facilitated in open net pens, moved spontaneously.

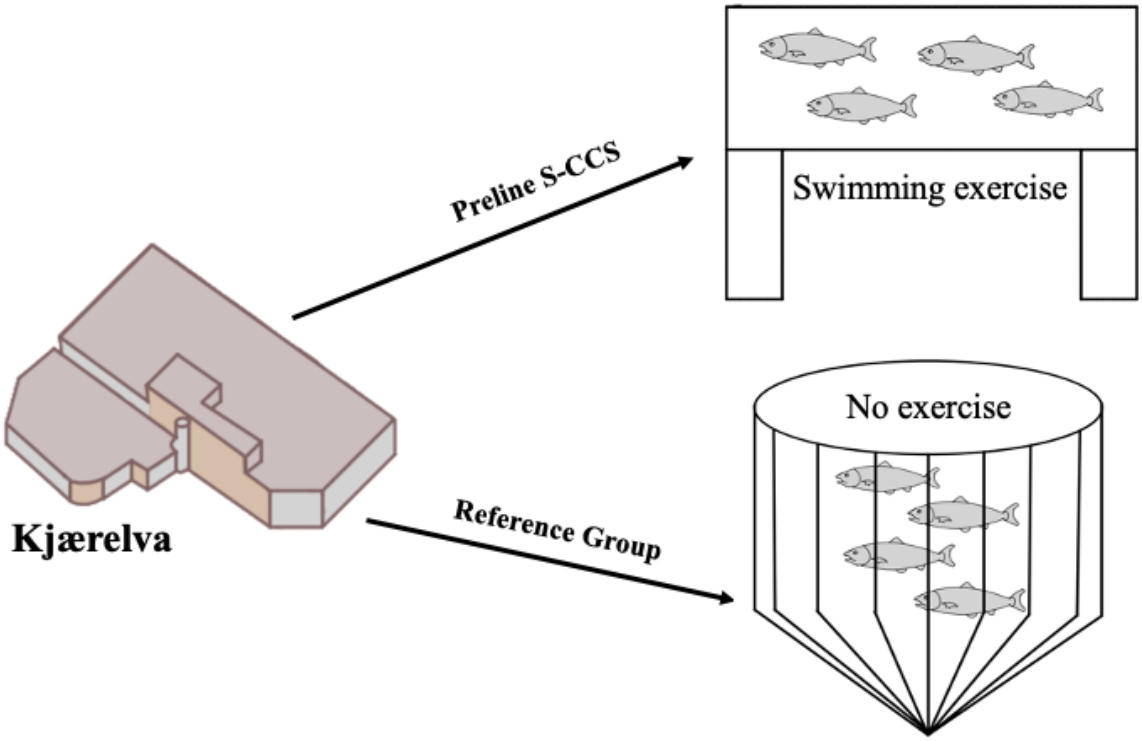

Figure 2.5. Overview of experimental design for the production group study. Fish from Kjærelva were transferred to Preline S-CCS where it swam against a constant current and to open net pens in Djupevika where fish moved spontaneously.

2.2.3.2 ENVIRONMENTAL CONDITIONS

In the Preline facility fish were exposed to 24 h light. The water temperature was 11.0 ± 0.6 ºC, oxygen 82 ± 1 %, and salinity of 34.5 ± 1.1 ‰. At Djupevika, fish were exposed to natural light conditions. The water temperature during the experimental period was 8.1 ± 1.6 ºC (measured at 0-5 m depth), oxygen 95 ± 3.0 %, and salinity of 28.2 ± 2.7 ‰. The environmental conditions were registered daily following standard rearing procedures.

2.2.3.3 SAMPLING

The collection of fish in the production group study, consisted of two sampling points (*Fig. 2.4*). The first sampling (28th of October 2021) was conducted ahead of seawater transfer where 20 fish was collected form Lerøy Sjøtroll Kjærelva represented by the baseline group. The next sampling included the collection of fish from both the Preline (8th of March 2022) and reference group (9th of March 2022) and was conducted approximately 4 months after the baseline sampling.

The sampling took place at the correspondent locations; Sagen and Djupevika. For each sampling, manual feeding was employed to attract fish to the water surface before being netted. Sampled fish were euthanized using a lethal concentration of benzocaine. To determine the overall growth, measurements of length (cm) and weight (g) were measured for each individual fish. Upon opening the fish, its sex was identified, and the heart was kept in dry ice before it was weighed (VWR International LPC-213i, Italy). Tissue samples from the heart was then collected into RNAlater (Invitrogen, USA), kept at 4°C overnight, and stored at -80°C for RNA transcription.

2.3 TRANSCRIPTION OF RNA

2.3.1 RNA EXTRACTION

Step 1

Isolation of ribonucleic acid (RNA) from heart tissue was preformed manually using the TRI reagent (Sigma-Aldrich, USA) method. The standard protocol was followed with a few adjustments to break down the heart tissue. In general, 50-60 mg of heart tissue were used for RNA extraction, except for the samples where the total tissue weight was below 50 mg. For these samples, all the tissue was used. Heart tissue were employed in 1 mL TRI-reagent and homogenized using 5 mm stainless-steel beads (Qiagen, Germany) in Precellys 24 homogenizer (Bertin Instruments, France). To break down the tissue, the samples were run twice in Precellys 24 homogenizer at a speed of 5000 g for 15 s each run. The samples were incubated for 5 min at room temperature, after which 200 µl of chloroform (Sigma-Aldrich, USA) was added and vortex for 30 s. After 15 min centrifugation (Eppendorf 5415R Centrifuge, Germany) at 4°C and 16100 g, the aqueous phase (~ 450 µl) was transferred to a new tube. 500 µl of 2-Propanol (Sigma-Aldrich, France) were added to each tube and the liquids were mixed by inverting the tubes 5 times. This was followed by an incubated period of 10 min at room temperature before being centrifuged for an additional 10 min at 4°C and 16100 g.

Step 2

Supernatant was decanted from tubes, leaving a visible pellet. The pellet was washed with 500 µl 80% cold Ethanol and centrifuged for 5 min at 4°C, 7500 g. Ethanol was removed, and the samples were left to dry at room temperature for 15 min. After drying the samples, nuclease-free water (QIAGEN, Germany) was added, with the amount depending on the initial heart tissue volume used. For the tissue that were less than 30 mg, the sample were reconstituted in 50 µl and for the tissue between 50-60 µl, the sample were reconstituted in 100 µl nuclease-free water. Finally, samples were heated for 10 min at 56°C in an Eppendorf ThermoMixer F1.5. Furthermore, the total RNA purity was examined by Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) with an input of 1.2 µl total RNA. RNA Purity was validated at 260:280 and 260:230 ratios above 1.7. Purified RNA was stored in a -80°C freezer.

2.3.2 RNA PRECIPITATION

Samples with an A260/A230 ratio less than 1.7 were precipitated by adding 1/10 of 3 M Sodium Acetate buffered to pH 5.2, and 2.5 volumes of cold anhydrous Ethanol. The mixture was gently mixed and stored at -80°C for at least one hour. Following this, the samples were centrifuged for 30 minutes and «step 2» of the RNA extraction was repeated.

2.3.3 DNase-TREATMENT

The Ambion TURBO DNA-free kit (Thermo Fisher Scientific, Lithuania) was employed to treat the samples. A total solution of 26 µl mixed with 10 µg RNA and nuclease-free water was added to new tubes. After adding the respective amounts of RNA and nuclease-free water, the dilution was mixed well. A mastermix comprised of 3 µl 10X TURBO DNase Buffer and 1 µl TURBO DNase was compounded for each sample and mixed by vortexing. 4 µl of mastermix were added to each of the diluted RNA tubes and mixed before being incubated for 30 min at 37°C. Thereafter, 3 µl of resuspended DNase Inactivation Reagent were added to each sample, following an incubation of 5 min at room temperature with occasional mixing before being centrifuged for 1.5 min at room temperature and 10 000 g. The resultant liquid was divided into two phases with a supernatant on the top and a precipitate on the bottom. 25 µl of DNase treated total RNA (supernatant) was collected and transferred to new tubes before measuring the concentration.

2.3.4 RNA QUANTIFICATION

Total RNA concentration (ng/µl) was measured using the Qubit 3.0 Fluorometer (Thermo Fisher Scientific, USA) with the Qubit RNA BR assay kit (Thermo Fisher Scientific, USA) using 2 µl total RNA as input. Measurements were conducted following manufacturer instructions (Thermo Fisher Scientific, USA).

2.3.5 RNA INTEGRITY ASSESSMENT

About 25% of the total samples (66 out of 270 samples) were randomly chosen to confirm RNA integrity. To assess the RNA integrity, RNA 6000 Nano LabChip kit was used following the Agilent RNA 6000 Nano Assay Protocol. RNA dye- and marker were equilibrated to room temperature, and the Gel-Dye Mix was prepared by vortexing for 10 s followed by 10 min centrifuging at 13 000 g. 2 µl of DNase-treated RNA was transferred to new tubes. DNase-treated RNA and the ladder were denatured for 2 min at 70°C. 9 µl Gel-Dye Mix were added to the wells marked with a black "G" and 5 µl RNA marker (green) were added to all 12 sample wells in addition to the ladder-well. 1µl of the denatured ladder and 1µl of denatured DNase-treated RNA were added to their respective wells. The chip was then vortexed for 1 min prior to running the Agilent 2100 Bioanalyzer (Agilent Technologies, Germany) using program "2100 expert_Eukaryote Total RNA Nano_DE72901192". Integrity was confirmed with RIN values higher than 7.0.

2.3.6 cDNA SYNTHESIS

Complementary DNA (cDNA) was reversely transcribed using oligo dT primer and the Superscript III kit (Thermo Fisher Scientific, USA). A total of 10 µl DNase treated RNA was added to new tubes and diluted to a 150 ng/µl dilution with RNase free water. 10 µl of the dilution mix was transferred to new tubes, to which 3 µl of mastermix (1 µl RNase free water, 1 µl Oligo dT and 1 µl 10mM dNTP mix) were added. The samples were heated at 65°C for 5 min and placed on ice after. Following this, 7 µl of the second mastermix (4 µl 5X FS buffer, 1 µl DTT, 1 µl RNaseOUT and 1 µl Superscript III RT) was added. The liquid was mixed by pipetting up and down before being incubated for 60 min at 50°C and then heated for 15 min at 70°C. The cDNA samples were stored at -20°C.

2.3.7 QUANTITATIVE PCR (qPCR)

Quantitative PCR (q-PCR) was performed to quantify the messenger RNA (mRNA) expression of selected genes. Prior to performing q-PCR on the samples, various reference genes were evaluated to determine which were the most optimal for heart samples. To check the reference genes, 30 cDNA samples from the production group study (10 from each group) were diluted with nuclease-free water to a 1:50 dilution of 5 ng/µl. The analysis was performed in triplicates and Ribosomal Protein S20 (rps*20)* and Beta-Actin *(b-actin)* were selected as reference genes as they were the most stable across the samples.

After the reference genes were chosen, a series of dilutions was performed for all genes to determine which dilution would provide the best values for quantification cycles as well as testing primer efficiency. A pool of cDNA was made from a total of 42 samples (6 cDNA samples from each group) by pipetting 2.5µl from each cDNA sample into an empty 1.5mL microtube. To ensure proper mixing, the mixture was vortexed. Six two-fold dilution samples were made (1:5, 1:10, 1:20, 1:40, 1:80 and 1:160) in a range of 50.0 ng/µl to 1.5625 ng/µl. Triplicate-two-folded cDNA dilution series were prepared to generate standard curves, which were used to calculate the primer and reference gene efficiency. The 1:20 dilution had the most favourable Cq-values, leading to a 1:20 dilution of 12.5 ng/µl for all cDNA samples by mixing 3.0 µl of cDNA with 57 µl of RNase-free water in empty 0.2 mL PCR tubes.

Ahead of preparing qPCR plates, the forward and reverse primers had to be diluted with RNase free H2O to a 1/10 dilution giving a primer solution of 10 µM. The qPCR was performed in 96-well plates (Bio-Rad, USA) with a total volume of 12.5 µl in each well containing; 6.5 µl iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, USA), 3.0 µl H2O, 0.25 µl of forward primer, 0.25 µl of reverse primer and 2.5 µl of 1/20 diluted cDNA. Duplicates were made for each plate, at which non-template control NTC, consisting of RNase free H_2O, were added to the two initial wells. Diluted samples were added to the following wells, while the two final wells contained cDNA-pool. All plates were sealed with Microseal `B´ seal (Bio-Rad, UK) and centrifuged (Eppendorf 5415 R Centrifuge, Germany) at room temperature for 1 min at 500 g before being ran in Thermocycler (Bio-Rad Laboratories AB, Norway). The thermal protocol consisted of the following steps: an initial denaturation at 95°C for 3 min, followed by a 1-min incubation at 60°C. This was followed by 34 cycles of 15 s at 95°C for denaturation and 1 min at 60°C for annealing and extension.

The final step involved 10 s at 95°C, 5 s at 65°C and 5 s at 95°C. A threshold between duplicates with a coefficient of variance (CV) > 1.5% was set, thus, when duplicates exceeded this threshold, the affected sample, or the whole plate, in the case of the cDNA-pool exceeding the threshold, had to be repeated.

Table 2.1. Primer sequences used for qPCR in the present study of Atlantic salmon (*Salmo salar)*. The accession number for the genes is from the Ensembl database (v.69; EMBL-EBI, 2023).

GENE	*SEQUENCE (5´>3´)*	*ACCESSION NO.*	*REFERENCE*
rps20	F- GCAGACCTTATCCGTGGAGCTA R- TGGTGATGCGCAGAGTCTTG-	BG936672	Olsvik et al 2005
b-actin	F- CCAAAGCCAACAGGGAGAAG R- AGGGACAACACTGCCTGGAT	BG933897	Olsvik et al 2005
gata4	F- AGCCTCGCCAGATAAAG R- GAAACCTGTGTGTGTGTG	ENSSSAT00000017313.1	Present work
mef2c	F- ATGAACGCAACAGACAGG R- AGATGATGAGGGCGATCT	ENSSSAT00000087718.1	Present work
pedf	F- CTTCAAAGTGAAGTGGATGAC R- CTGCTGCATCATGGAAATG	ENSSSAT00000054769.1	Present work
angpt2_ch6	F- GACGCTTATTCAGGGAACTG R- ATACCACACGCCGTTTAAG	ENSSSAT00000024877.1	Present work
angpt2_ch20	F- CCAACAGACTGATGCAGGT R- GTTTCCAAAGCCTTGCTTGT	ENSSSAT00000086001.1	Present work
angpt1	F- TACAAGACGGGCTTTGG R- ACACGAAGGGCATACTG	ENSSSAT00000111499.1	Present work

The quantification cycle (Cq) values from the Thermocycler were imported into a Microsoft excel document. To create the standard curves, the mean value of the triplicates was calculated along with the logarithmic (log) value of the cDNA quantity for each dilution. These values were used to generate scatterplots for each gene by plotting the log value of the cDNA concentration on the x-axis and the mean quantification cycle (Cq) value on the y-axis. The slope of the curve (represented by the equation: $y = mx + b$) from the scatterplot, was used to determine the efficiency (Equation 2.6) of the genes.

$$PCR\ Efficiency = 10^{\frac{-1}{m}} \quad (2.6)$$

To convert the PCR efficiency into a percentage, the following equation was used:

$$\%\ PCR\ Efficiency\ =\ (\text{PCR Efficiency} - 1)\ *\ 100 \quad (2.7)$$

Table 2.1: Overview of the standard curve values and the efficiency (%) for all genes.

GENE	SLOPE (M)	INTERCEPT (B)	R^2	% EFFICIENCY
rps20	-3.5548	21.752	0.999	91.1%
b-actin	-3.3514	21.275	0.9978	98.7%
gata4	-3.4662	25.312	0.9865	94.0%
mef2c	-3.3423	26.079	0.9985	99.0%
pedf	-3.5853	27.926	0.9983	90.0%
angpt2_ch6	-3.3868	31.183	0.9809	94.0%
angpt2_ch20	-3.4967	27.306	0.9993	93.2%
angpt1	-3.6272	30.813	0.9983	88.6%

Prior to calculating the relative quantification of any primer set, a series of calculations needed to be performed ahead. The initial step was to determine the intercalibration factor for each plate. The intercalibration factor was found by dividing the average rounded value of the Cq for the duplicated cDNA pool, by the true mean of the Cq. The following step was to calculate the intercalibrated Cq, which was determined by multiplying the Cq for each sample with the intercalibration factor. Next, the efficiency^{-Cq} was calculated by exponentiating the mean intercalibrated Cq to the power of the efficiency value calculated for each primer set from table 2.1, for each sample. In the final step, the relative quantification (Equation 2.8) was determined by dividing the efficiency^{-Cq} of each sample by the geometric mean of both reference genes to normalize the concentration.

$$Relative\ quantification = \frac{\text{Efficiency}^{-Cq_{Target\ Sample}}}{\sqrt[2]{\text{Efficiency}^{-Cq_{Ref1\ Sample}} * \text{Efficiency}^{-Cq_{Ref2\ Sample}}}} \quad (2.8)$$

2.4 HISTOLOGY

The heart samples used for histology analysis were collected from the S4 sampling group with a total of n= 14 samples. Among these, n= 6 samples were gathered from each group exposed to an exercise regime of 0.5 BL/s and 1.5 BL/s, while n= 5 samples were obtained from the group exercised at 1.0 BL/s. The samples were shipped to PHARMAQ Analytiq AS for histological analysis.

At PHARMAQ Analytiq the samples were processed in a Thermo Scientific Excelsior tissue processor (Thermo Fisher Scientific, UK) and embedded in paraffin (Histowax (56-58 °C)) using a Tissue – Tek, TEC 5 (Sakura Finetek Europe B.V., The Netherlands) embedding centre. Embedded tissue was sectioned at 1.5-2 µm using a Leica RM 2255 Microtome, sections were mounted on glass slides and stained with haematoxylin-eosin (HE). Stained slides were scanned in an Aperio ScanScope AT Turbo slide scanner and read using Aperio ImageScope (Leica Biosystems, USA).

Sections were observed using QuPath (v.0.4.3; Bankhead et al., 2017) to find the size of the whole heart (*Fig.2.6A*), spongy myocardium (*Fig.2.6A*), and blood vessel lumen (*Fig.2.6B-D*). After creating a project, all histology images were imported into QuPath (v.0.4.3; Bankhead et al., 2017). Size of the whole heart, spongy myocardium, and blood vessel lumen were measured and categorized into different classes to organize annotations. The data was then transferred to a comma-separated value (csv) file to import the data into R studio for analysis.

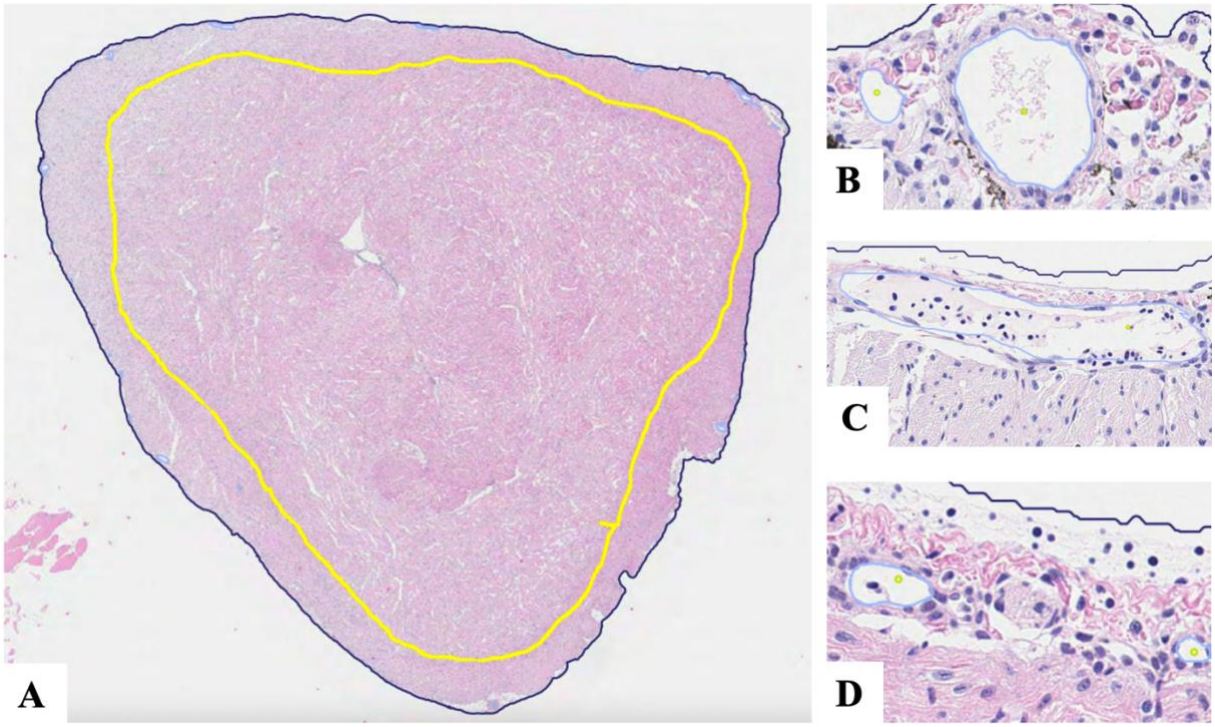

Figure. 2.6. Examples of histology annotations from QuPath. (A) Whole heart and spongy myocardium (from yellow line to the outer line), (B-D) vessels of different sizes are marked out with light blue line.

Furthermore, the compact myocardium area (%), the vessel total area and the vessel mean area, were calculated from the following equations:

$$Vessel\ total\ area\ (\%) = \frac{\sum Vessel\ area\ (\mu m^2)}{Whole\ heart\ area\ (\mu m^2)} * 100 \quad (2.9)$$

$$Vessel\ mean\ area\ (\%) = \frac{mean(Vessel\ area\ (\mu m^2))}{Whole\ heart\ area\ (\mu m^2)} * 100 \quad (2.10)$$

$$Compact\ myocardium\ (\%) = \frac{Spongy\ miocardium\ area\ (\mu m^2)}{Whole\ heart\ area\ (\mu m^2)} * 100 \quad (2.11)$$

2.5 STATISTICAL ANALYSIS

Statistical analysis and data visualization of the histology-, morphometric- and gene transcription data were performed using RStudio (v4.2.3; Posit team 2023, Boston) and R (v4.2.3; R Core Team 2023, Austria). The readxl (v.1.4.2; Wickham H & Bryan J, 2023) and readr (v.2.1.4; Wickham et al., 2023) packages were used to import excel- and csv files and tidyverse (Wickham et al., 2019) was used to manipulate and tidy the data. Furthermore, the packages glmmTMB (Brooks et al., 2017) and DHARMa (v.0.4.6; Hartig, 2022) which was used for model fit and evaluation of residual distribution, and emmeans (v.1.8.6; Lenth, 2023) for post-hoc testing, were also used. To plot and save the data, ggpubr (v.0.6.0; Kassambara, 2023) and ggplot2 (Wickham, 2016) were used.

Histology

The csv file generated from QuPath (v.0.4.3; Bankhead et al., 2017) was imported to Rstudio (v4.2.3; Posit team 2023, Boston) and merged with an excel file that contained information regarding the histology images and the assigned samples and treatments. Following that, boxplots were created to visualize the results from each treatment within the S4 sampling group with treatment (water velocity: 0.5 BL/s, 1.0 BL/s & 1.5 BL/s) as an explanatory variable.

Morphometric- and gene analysis

Density plots were generated to check if the data was normally distributed and if it contained any zero values. Afterward, boxplots were created to visualize the distribution of the data across groups and to detect outliers. All clear outliers were removed. For the constant current experiment, generalized linear mixed models (GLMM) were used to analyze the data in R. The GLMM was applied to both the gene transcription data and the morphometrics data and utilized a Gamma distribution (log-link function) with treatment (water velocity: 0.5 BL/s, 1.0 BL/s & 1.5 BL/s) and sampling time (S1, S2, S3 & S4) as categorical explanatory variables. Additionally, an interaction between these two fixed variables was included in the model. To account for potential within-cluster correlation, tanks were added as random intercepts. As for the production group study, the gene transcription and morphometric data were modelled using a generalized linear model (GLM). The GLM was applied to both the gene transcription data and the morphometrics data and utilized a Gamma distribution (log-

link function) with rearing facility groups (Baseline, Preline and reference) as categorical explanatory variables. To determine significant differences ($p < 0.05$) across treatments and sampling points in the constant current experiment as well as significant differences ($p < 0.05$) between rearing facility groups in the production group study, Tukey's Honest Significant Difference (HSD) post-hoc test was applied. Following that, the fitted model was used to make predictions by generating 95% confidence intervals (CI) which was used to visualize the results in plots.

For the gene analysis, the relative quantification values (Equation 2.8) from each sample were used to show the differences in gene transcription between sampling points and treatments for each gene. For the morphometric analysis several calculations were made ahead of the analysis. Both the constant current experiment and production group study analyzed the condition factor (K) and the cardiosomatic index (CSI). In addition, the constant current experiment analyzed the hematocrit (Hct), specific growth rate (SGR) and relative growth (RG).

3. RESULTS

3.1 CONSTANT CURRENT EXPERIMENT

3.1.1 SOMATIC PARAMETERS

Somatic growth parameters

Specific growth rate (SGR) was used to quantify the increase in individual body weight over a specific time. From the GLMM analyses, a weak tank effect was observed for SGR (SD = 0.0173, Appendix I Figure IA), and all fixed variables, including treatment, sampling and interaction between both, are important to explain the SGR (Appendix I Table I*)*.

Additionally, Tukey's post hoc analyses (Appendix I Table VII) revealed that at the final sampling (S4), after 6 weeks in seawater, a significantly higher SGR were observed in the groups that exercised at moderate- and high velocities of 1.0 BL/s ($p < 0.01$) and 1.5 BL/s ($p < 0.05$) compared to than the group exercised at the low velocity of 0.5 BL/s *(Fig.3.1).* Significant differences were also observed between sampling points for SGR *(*Appendix I Table VII). There was a significant increase in SGR from the first sampling to the second sampling for all velocities of 0.5 BL/s ($p < 0.01$), 1.0 BL/s ($p < .0001$) and 1.5 BL/s ($p < 0.01$) before a small decline in SGR from the second to the third sampling. From the third to the fourth sampling there was a significant decrease in SGR ($p < .0001$) at all exercise regimes.

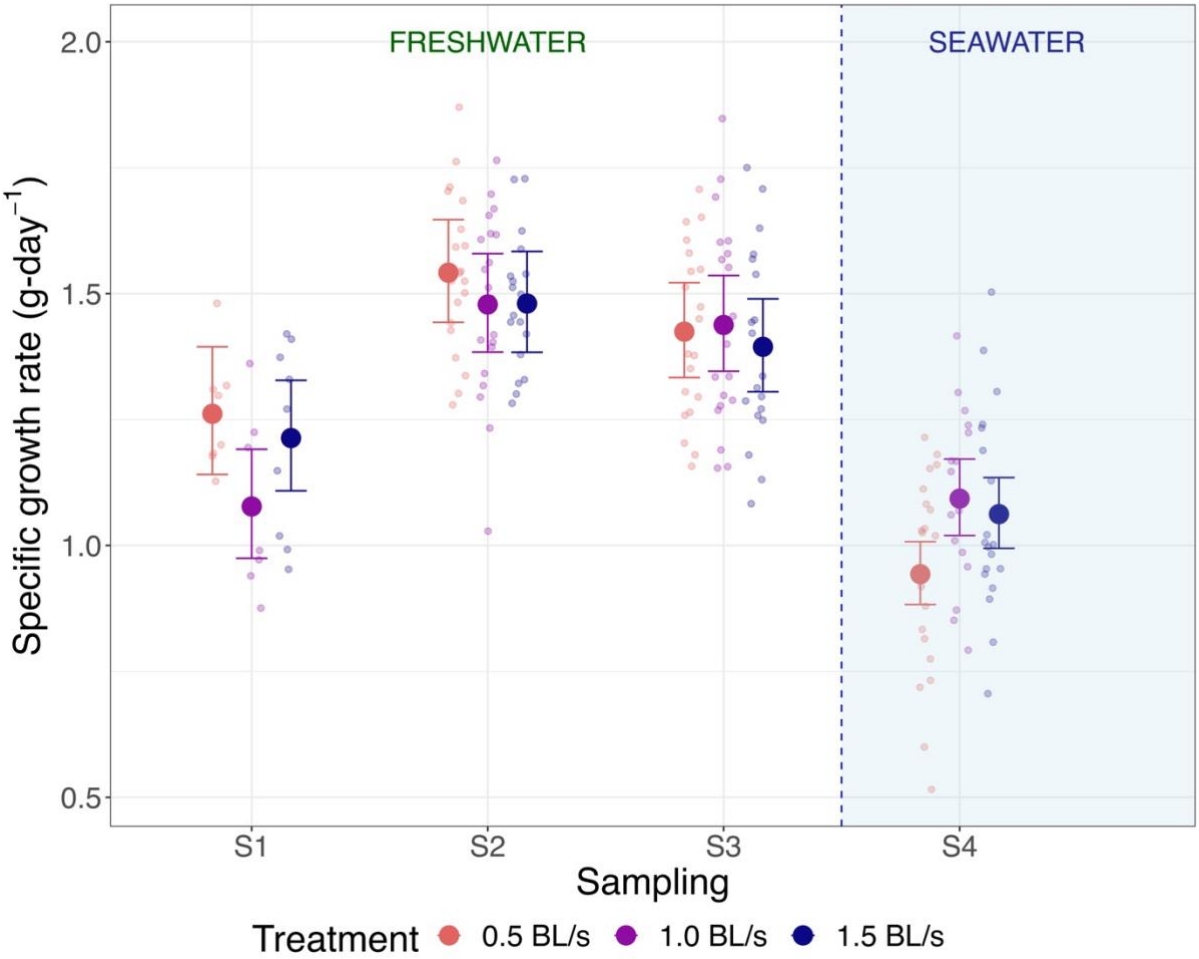

Figure 3.1. Specific growth rate (SGR) of Atlantic salmon weight measured in g-day^{-1}. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The freshwater phase lasted for 15 weeks (28th of September to 13th of January) and consisted of 3 sampling points (S1, S2 and S3). The last sampling (S4) was conducted after 5 weeks in seawater (23rd of February) where the initial weight for SGR was based on the weight at the end of the freshwater phase (S3). The different treatments are also visualized (● = 0.5 BL/s, ● = 1.0 BL/s, ● = 1.5 BL/s).

The relative growth (RG) in this experiment refers to a change in weight over a specific time relative to the initial weight. From GLMM analysis a weak tank effect (SD = 0.03, Appendix I Figure IB) for RG was observed. Sampling was the main variable explaining the RG differences, however, treatment and interaction between the fixed variables, also contributed to explain the data (Appendix I Table II). Between sampling points in freshwater, an upward trend was observed *(Fig. 3.2*) as well as post hoc analysis (Appendix I Table X) revealing significant differences between sampling points. A significant increase was

observed for all velocities from the initial sampling to the second sampling (p <.0001) and from the second to the third sampling (p <.0001). When the fish was transferred to seawater, there was a significant decrease (p <.0001) in RG.

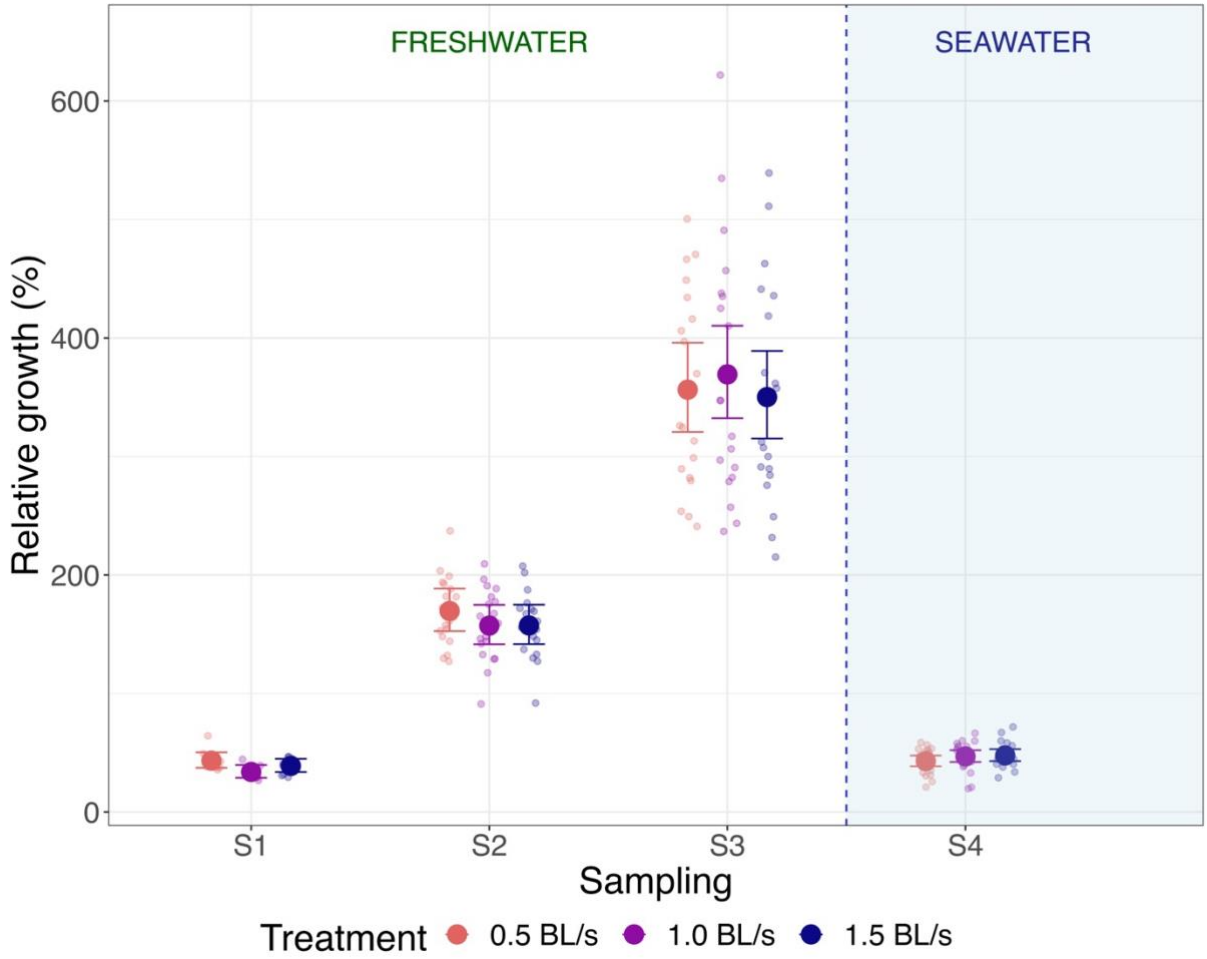

Figure 3.2. Relative growth (RG) of Atlantic salmon weight measured in %. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The freshwater phase lasted for 15 weeks (28th of September to 13th of January) and consisted of 3 sampling points (S1, S2 and S3). The last sampling (S4) was conducted after 5 weeks in seawater (23rd of February) where the initial weight for RG was based on the weight at the end of the freshwater phase (S3). The different treatments are also visualized (● = 0.5 BL/s, ● = 1.0 BL/s, ● = 1.5 BL/s).

The condition factor (K) represents the relationship between length and weight. GLMM analyses revealed that the tank random intercepts were negligible (SD = 1.1e-6, Appendix I Figure IC). In terms of fixed variables, sampling is important to explain K (Appendix I Table III). Within each sampling point, post hoc analysis revealed (Appendix I

Table XI) no significant difference was observed between treatments. Throughout the experiment, there was a consistent upward trend in K, with the overall highest values observed in the last sampling (S4) in seawater (*Fig 3.3*), but only a few significant differences were observed through post hoc analysis between sampling points (Appendix I Table XII). A significant increase in K were observed between the initial sampling and the second sampling in freshwater for the group exercising at a moderate velocity ($p < 0.01$) and at the high velocity ($p < 0.05$). In addition, a significant increase in K was observed in the group exercising at a high velocity from the last sampling in freshwater to the seawater sampling ($p < 0.05$).

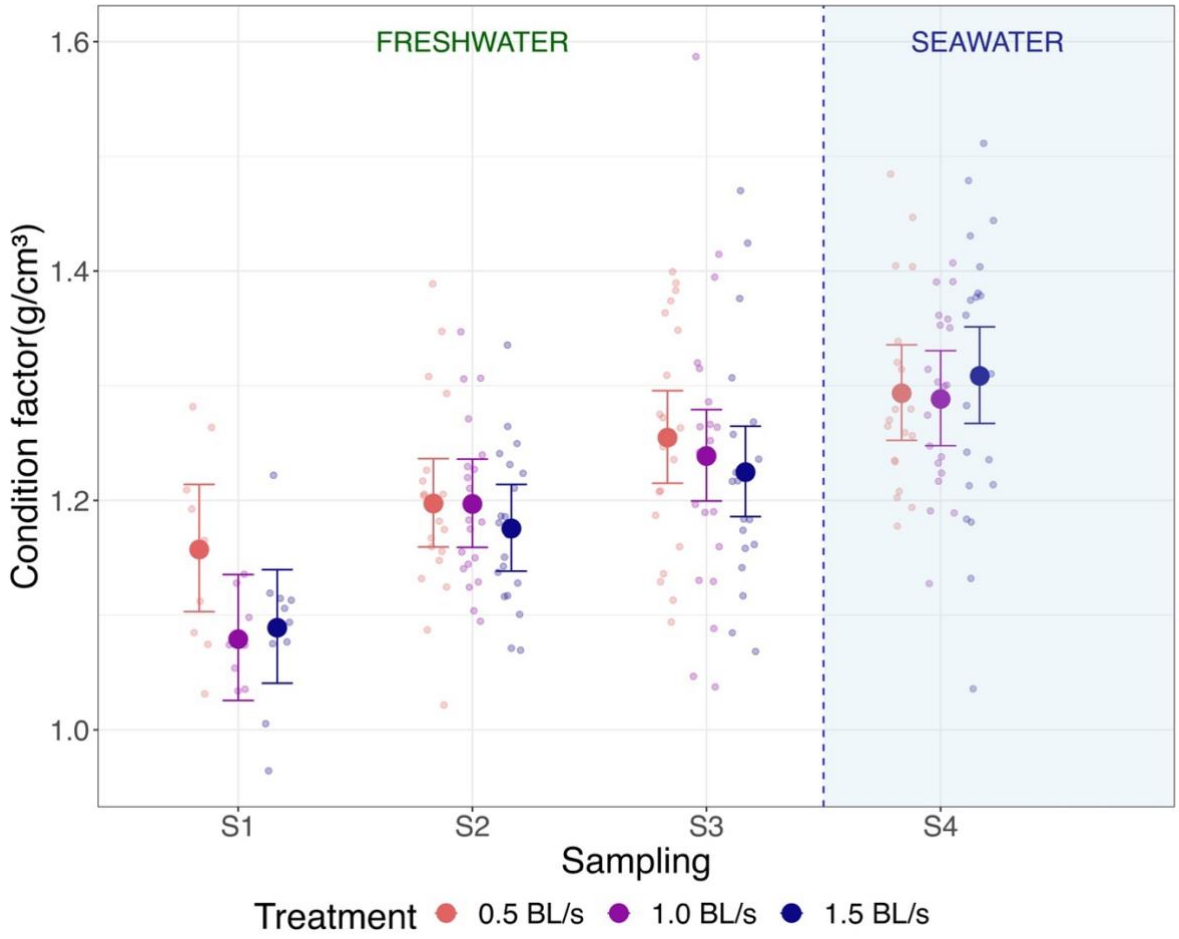

Figure 3.3. Condition factor (K) of Atlantic salmon measured in g/cm^3. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The freshwater phase lasted for 15 weeks (28th of September to 13th of January) and consisted of 3 sampling points (S1, S2 and S3). The last sampling (S4) was conducted after 5 weeks in seawater (23rd of February). The different treatments are also visualized (● = 0.5 BL/s, ● = 1.0 BL/s, ● = 1.5 BL/s).

Cardiovascular parameters

The cardiosomatic index (CSI) measures the weight of the heart relative to the body weight. GLMM statistical analysis displayed a negligible effect of the random parameter tank for CSI (SD = 2.5e-6, Appendix I Figure ID). As for the fixed variables, sampling was important to explain the CSI, while treatment was only marginally significant (Appendix I, Table IV). Furthermore, post hoc analyses revealed a significantly lower CSI for the group exercised at a low velocity of 0.5 BL/s compared to the groups exercised at the moderate- and high velocities of 1.0 BL/s ($p < 0.05$) and 1.5 BL/s ($p < 0.001$) (*Fig.3.4*). Through the experiment, there was a significant decline from the second sampling to the third sampling at all exercise regimes of 0.05 BL/s ($p < 0.01$), 1.0 BL/s ($p < .0001$) and 1.5 BL/s ($p < .0001$).

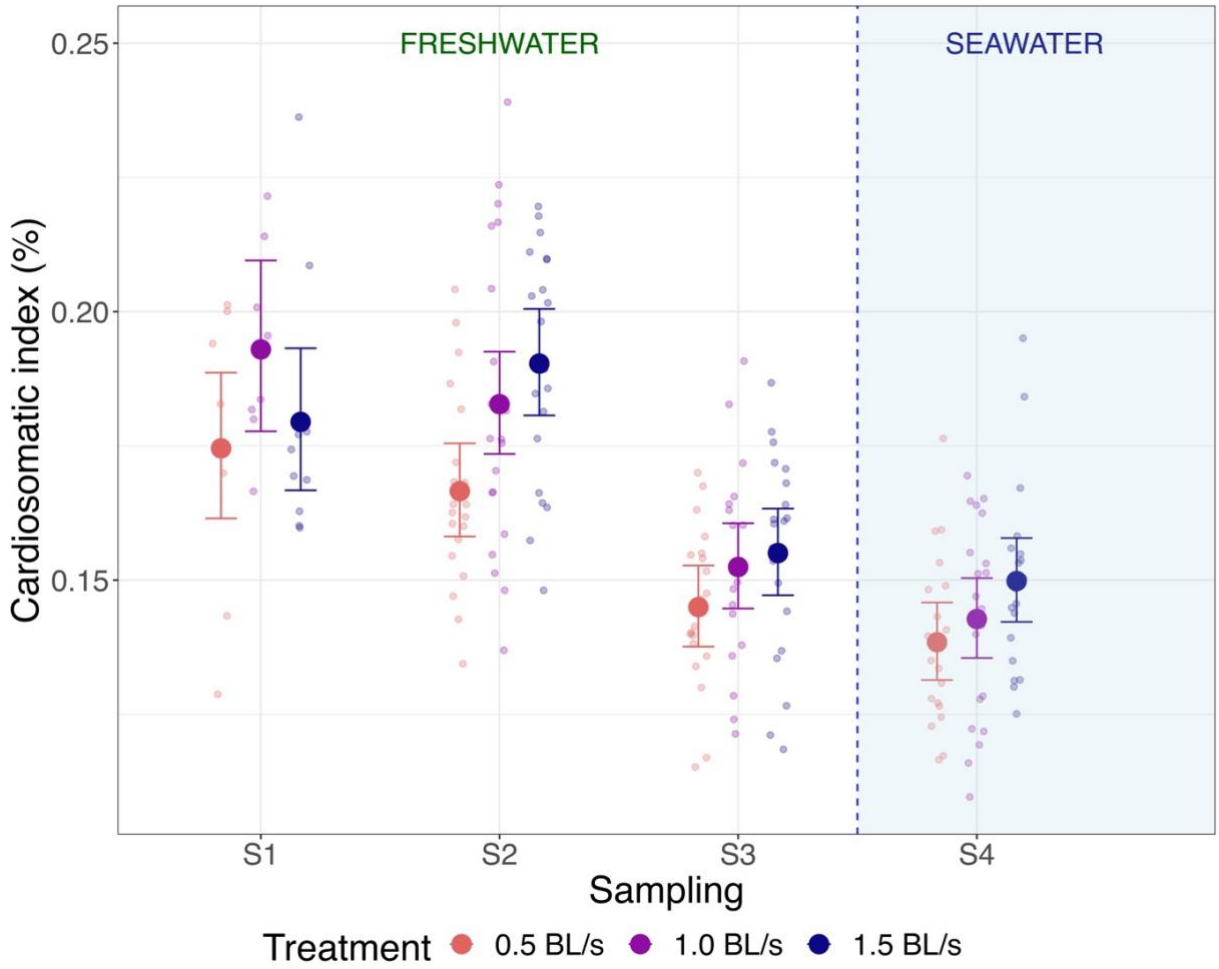

Figure 3.4. Cardiosomatic index (CSI) of Atlantic salmon measured in %. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The freshwater phase lasted for 15 weeks (28th of September to 13th of January) and consisted of 3 sampling points (S1, S2 and S3). The last sampling (S4) was conducted after 5 weeks in seawater (23rd of February). The different treatments are also visualized (● = 0.5 BL/s, ● = 1.0 BL/s, ● = 1.5 BL/s).

Hematocrit (Hct) measures the percentage of red blood cells in the blood. Statistical analyses revealed a weak tank effect (SD = 0.0212, Appendix I Figure IE) for the Hct and, as for the fixed variables, the interaction between sampling and treatment is important to explain the Hct data (Appendix I Table V). Indeed, a significant decline in Hct was observed through post hoc analysis (Appendix I Table XVI) from the initial- to the second sampling for the group exercised at the highest velocity of 1.5 BL/s ($p < 0.05$), while the following sampling points showed no significant differences in Hct levels *(Fig.3.5*).

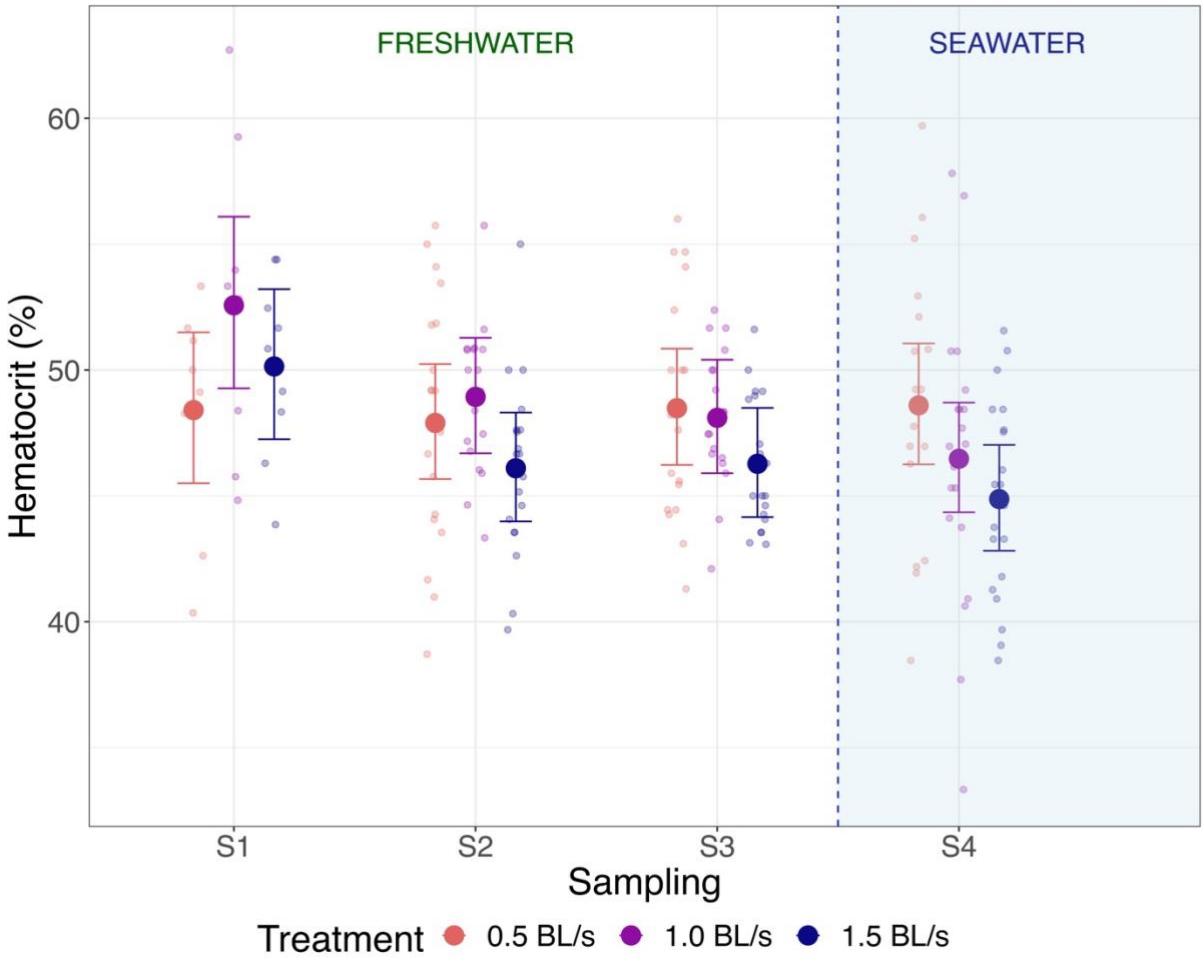

Figure 3.5. Hematocrit (Hct) of Atlantic salmon measured in %. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The freshwater phase lasted for 15 weeks (28th of September to 13th of January) and consisted of 3 sampling points (S1, S2 and S3). The last sampling (S4) was conducted after 5 weeks in seawater (23rd of February). The different treatments are also visualized (● = 0.5 BL/s, ● = 1.0 BL/s, ● = 1.5 BL/s).

3.1.2 GENE TRANSCRIPTION

Hypertrophic growth

Statistical analysis using GLMM showed an inexistent tank effect in *gata4* mRNA expression (SD = 9.6e-6, Appendix I Figure IIA). In addition, only the variable sampling did significantly affect the data (Appendix I Table XVII). Over the course of the experiment, a weak non-significant upward trend was observed for the transcription of *gata4* at the low velocity of 0.5 BL/s, while stable values were observed for the moderate- and high velocity of 1.0 BL/s and 1.5 BL/s *(Fig.3.6)*.

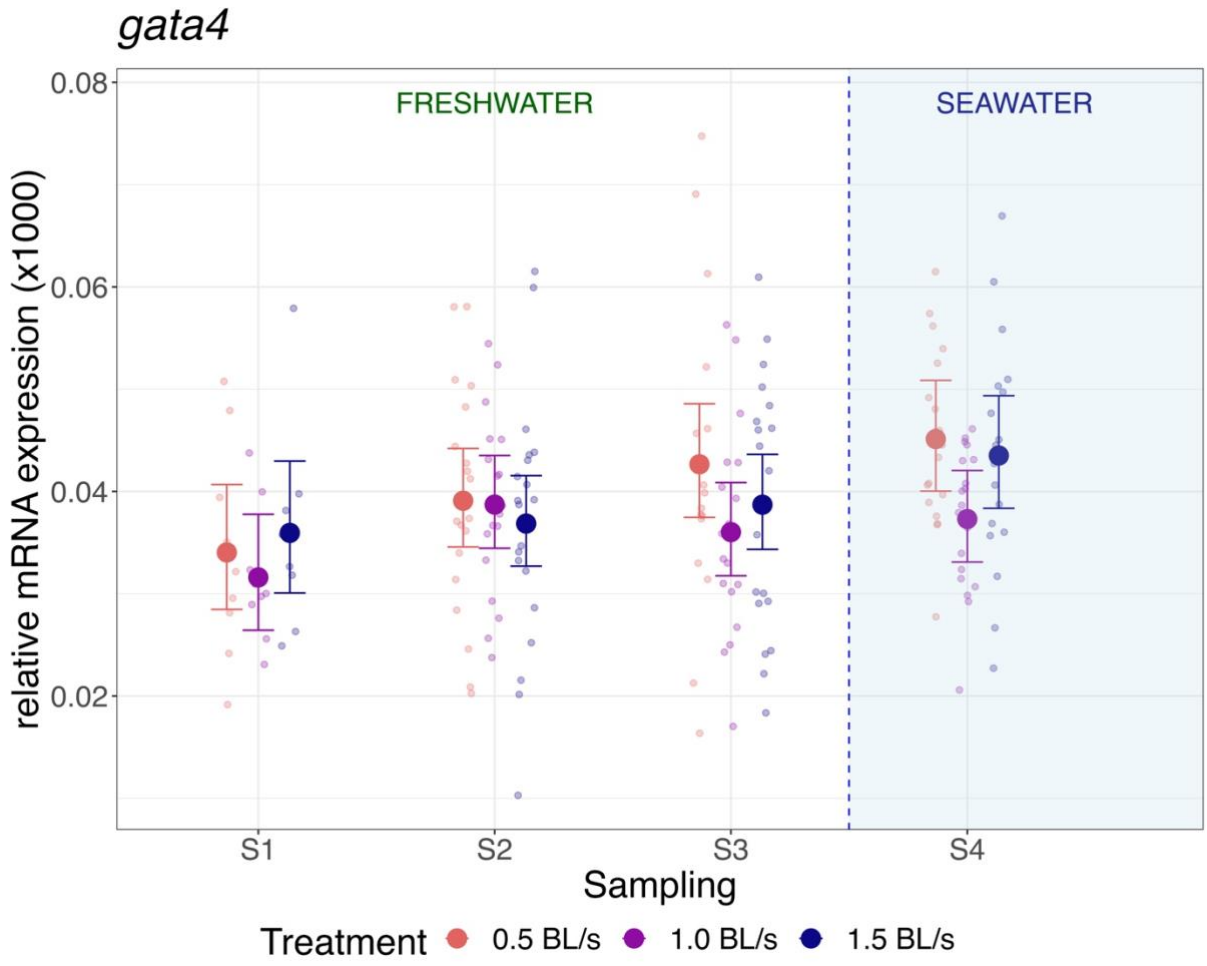

Figure 3.6. Relative gene transcription of gata4 in Atlantic salmon. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The freshwater phase lasted for 15 week (28th of September to 13th of January) and consisted of 3 sampling points (S1, S2 and S3). The last sampling (S4) was conducted after 5 weeks in seawater (23rd of February). The different treatments are also visualized (● = 0.5 BL/s, ● = 1.0 BL/s, ● = 1.5 BL/s).

Similar, to *gata4*, no tank random effect was observed for the transcription of *mef2c* (SD = 1.63e-5, Appendix I Figure IIB). The fixed variables treatment and interaction between treatment and sampling only marginally significantly affected the *mef2c* mRNA expression (Appendix I Table XVII). The post hoc analysis showed no significant differences between treatments (Appendix I Table XXVI) or sampling points (Appendix I Table XXVII) (*Fig. 3.7*).

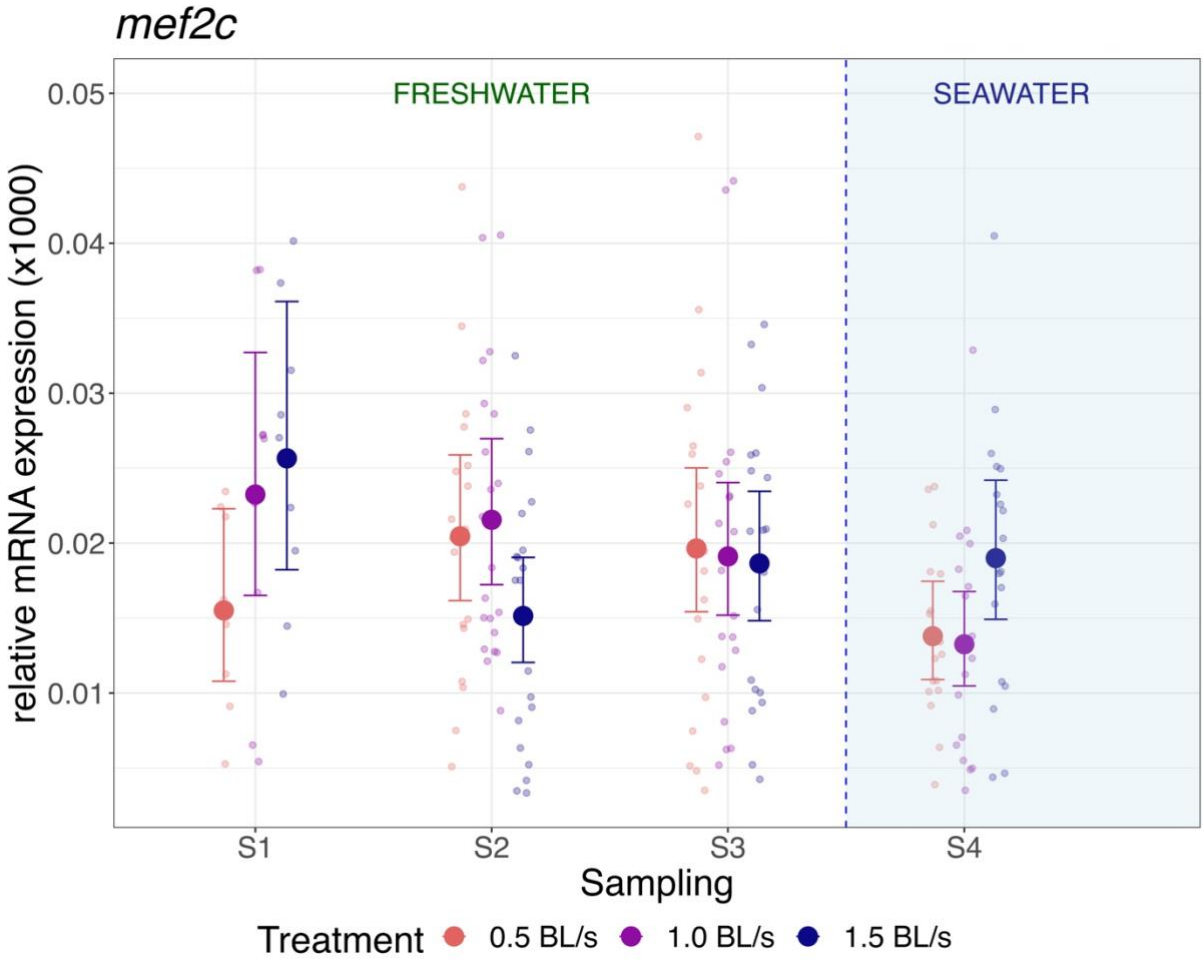

Figure 3.7. Relative gene transcription of mef2c in Atlantic salmon. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The freshwater phase lasted for15 weeks (28th of September to 13th of January) and consisted of 3 sampling points (S1, S2 and S3). The last sampling (S4) was conducted after 5 weeks in seawater (23rd of February). The different treatments are also visualized (● = 0.5 BL/s, ● = 1.0 BL/s, ● = 1.5 BL/s).

Pro-Angiogenic genes

For *angpt2_ch6* GLMM analysis showed a minor tank effect (standard deviation = 0.0624, Appendix I Figure IIC), and only the fixed variable sampling explains the *angpt2_ch6* data (Appendix I Table XIX). In fact, post hoc analysis showed that there was a significant increase in the transcription of *angpt2_ch6* between the last sampling in freshwater and the sampling in seawater (Appendix I Table XXIX) for all velocities: 0.5 BL/s ($p<0.001$), 1.0 BL/s ($p<0.01$) and 1.5 BL/s ($p<0.05$). Overall, the transcription of *angpt2_ch6* was higher in seawater than freshwater (*Fig. 3.8*).

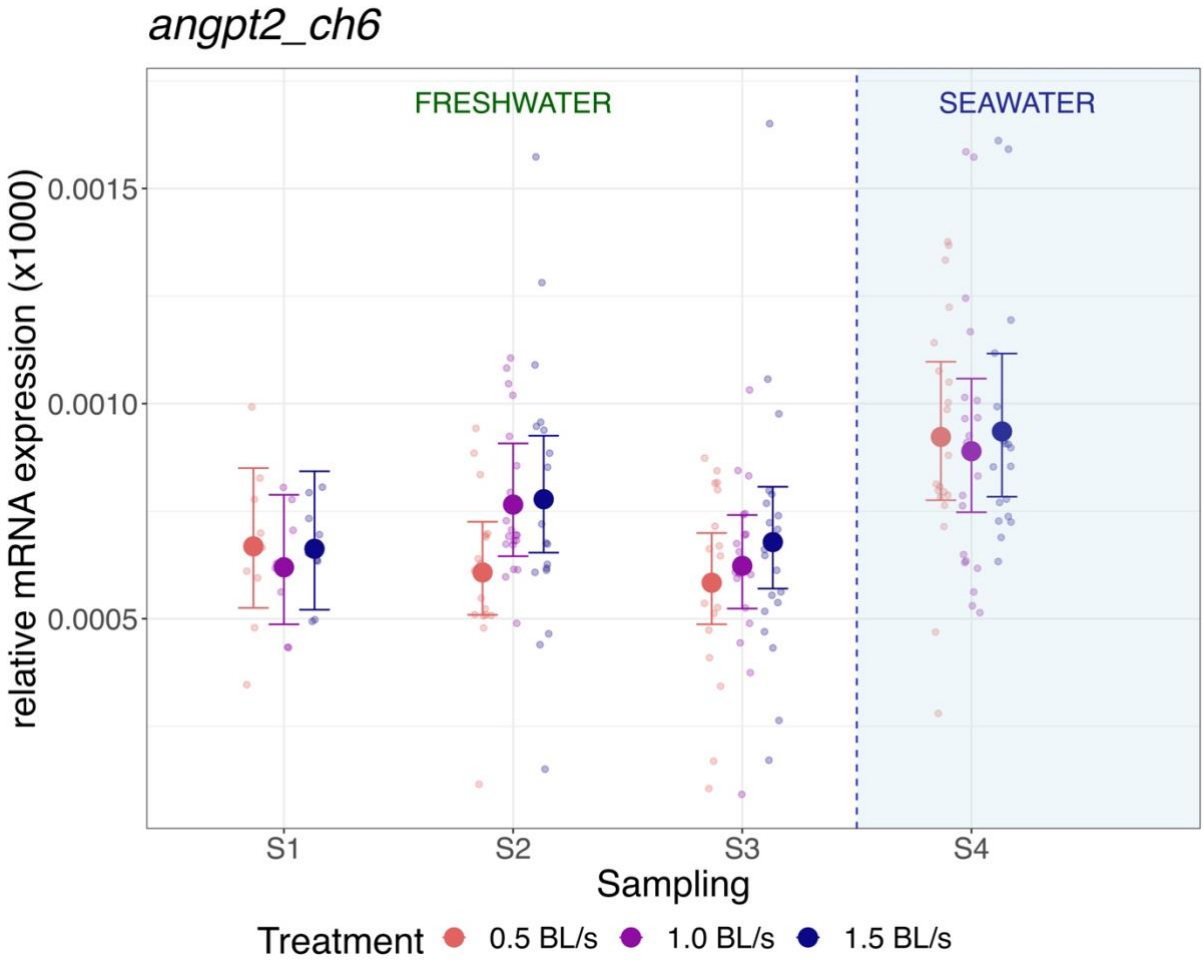

Figure 3.8. Relative gene transcription of angpt2_ch6 in Atlantic salmon. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The freshwater phase lasted for 15 weeks (28th of September to 13th of January) and consisted of 3 sampling points (S1, S2 and S3). The last sampling (S4) was conducted after 5 weeks in seawater (23rd of February). The different treatments are also visualized (● = 0.5 BL/s, ● = 1.0 BL/s, ● = 1.5 BL/s).

For *angpt2_ch20* GLMM analysis showed no tank random effect (SD = 1.9e-5, Appendix I Figure IID), and none of the fixed variables had a significant effect on the *angpt2_ch20* expression (Appendix I Table XXX, Appendix I Table XXXI *Fig. 3.9*).

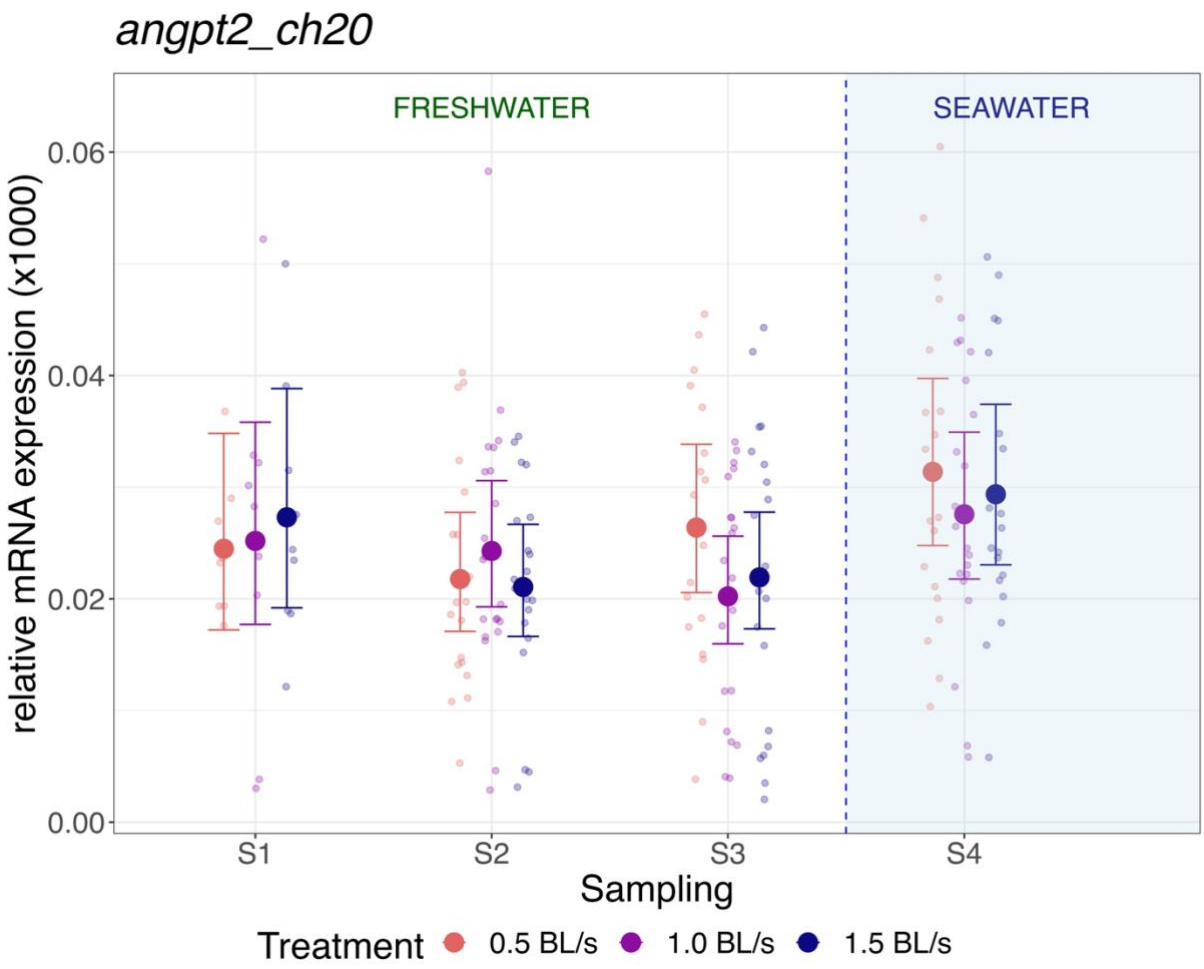

Figure 3.9. Relative gene transcription of angpt2_ch20 in Atlantic salmon. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The freshwater phase lasted for 15 weeks (28th of September to 13th of January) and consisted of 3 sampling points (S1, S2 and S3). The last sampling (S4) was conducted after 5 weeks in seawater (23rd of February). The different treatments are also visualized (● = 0.5 BL/s, ● = 1.0 BL/s, ● = 1.5 BL/s).

Anti-Angiogenic genes

Statistical analysis using GLMM showed no tank effect (SD = 7.5e-5, Appendix I Figure IIE), and only sampling had a marginally significant effect on *angpt1* (Appendix I Table XXI). A downward trend in the transcription of *angpt1*was observed across the sampling points with the lowest observed transcription in seawater (*Fig.3.10*). Post hoc analysis for the fixed variable for sampling (Appendix I Table XXXIII), showed no significant decline in the transcription of *angpt1* between sampling points, but there was a significant decline from the two groups exercising at the moderate- and high velocity of 1.0 BL/s ($p < 0.05$) and 1.5 BL/s ($p < 0.01$) from the initial sampling in freshwater to the sampling in seawater.

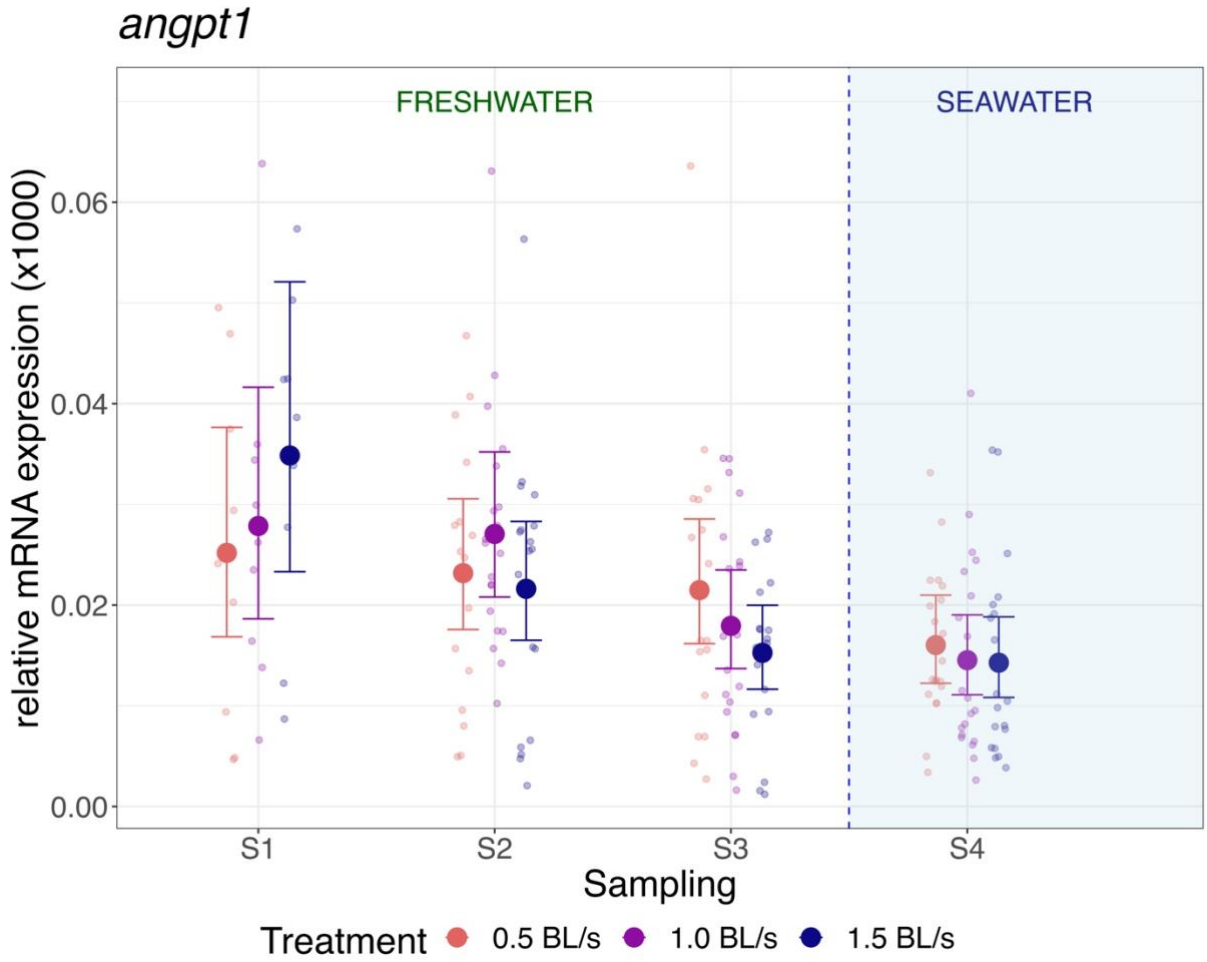

Figure 3.10. Relative gene transcription of angpt1 in Atlantic salmon. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The freshwater phase lasted for 15 weeks (28th of September to 13th of January) and consisted of 3 sampling points (S1, S2 and S3). The last sampling (S4) was conducted after 5 weeks in seawater (23rd of February). The different treatments are also visualized (● = 0.5 BL/s, ● = 1.0 BL/s, ● = 1.5 BL/s).

There was a weak tank effect (SD = 0.0778, Appendix I Figure IIF) and only the fixed variable sampling had a significant effect on *pedf* (Appendix I Table XXII). For the transcription of *pedf*, post hoc analysis for the fixed variable of sampling (Appendix I Table XXXV), revealed that there was a significant decrease between the third sampling in freshwater and the sampling in seawater for all treatments of 0.5 BL/s ($p < .0001$), 1.0 BL/s ($p < 0.01$) and 1.5 BL/s ($p < 0.01$). Overall, the transcription of *pedf* was lower in the seawater phase compared to the freshwater phase (*Fig. 3.11*).

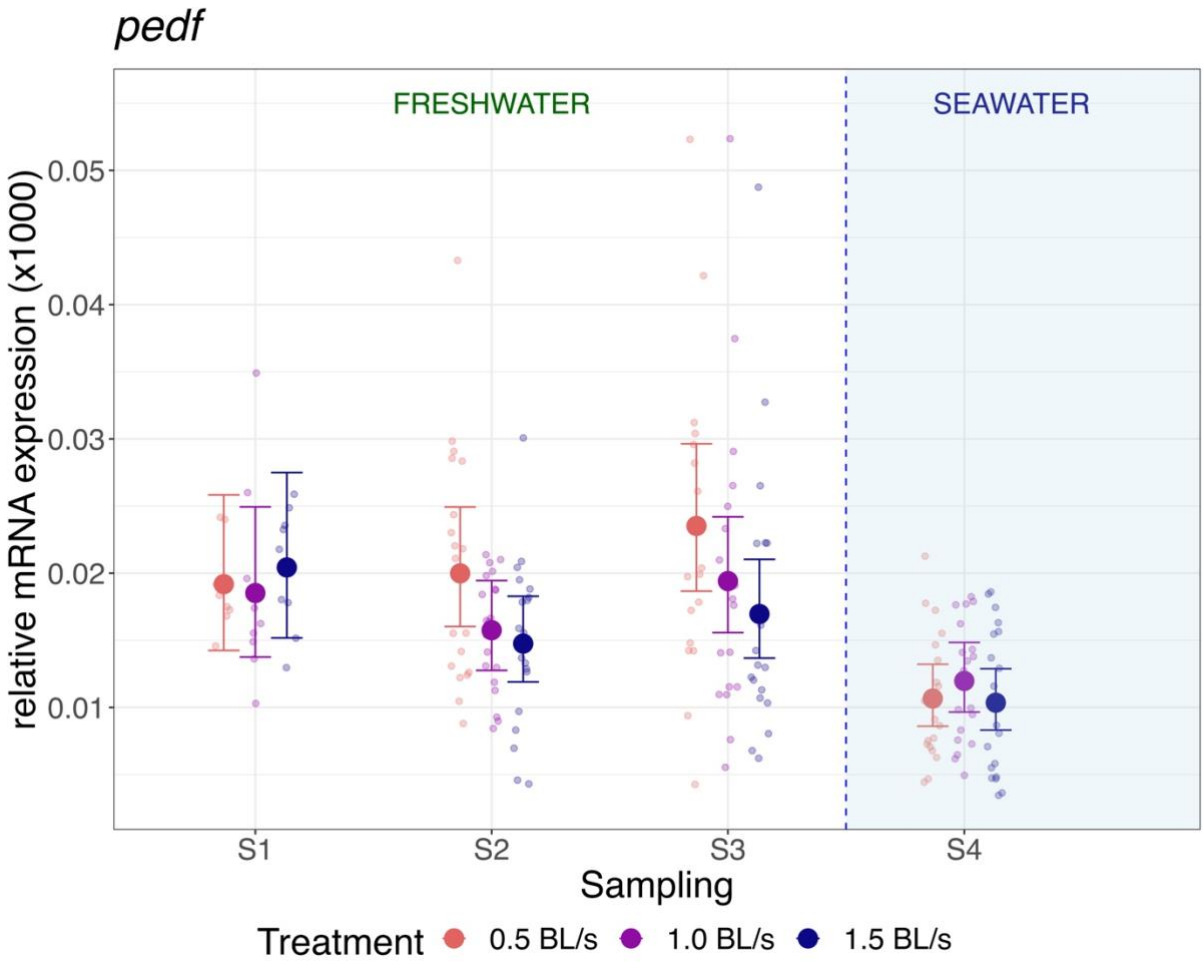

Figure 3.11. Relative gene transcription of pedf in Atlantic salmon. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The freshwater phase lasted for 15 weeks (28th of September to 13th of January) and consisted of 3 sampling points (S1, S2 and S3). The last sampling (S4) was conducted after 5 weeks in seawater (23rd of February). The different treatments are also visualized (● = 0.5 BL/s, ● = 1.0 BL/s, ● = 1.5 BL/s).

3.1.3 HISTOLOGY

Histology analysis was only done for the last sampled group, S4. The fourth sampling took place after 6 weeks in seawater where all fish swam at the same velocity of 0.5 BL/s to see the aftereffects of exercise.

The coronary network was estimated comparing the relative coronaries total area to the whole heart (*Fig. 3.12A*). The group that exercised at the low velocity of 0.5 BL/s had the highest mean of the coronary total area compared to whole heart, with a median percentage of 0.201 ± 0.0389 %. This was closely followed by the group exercised at the highest velocity of 1.5 BL/s with a median of 0.170 ± 0.0567 %. The lowest median of 0.157 ± 0.0468% was observed for the group exercised at the moderate velocity of 1.0 BL/s.

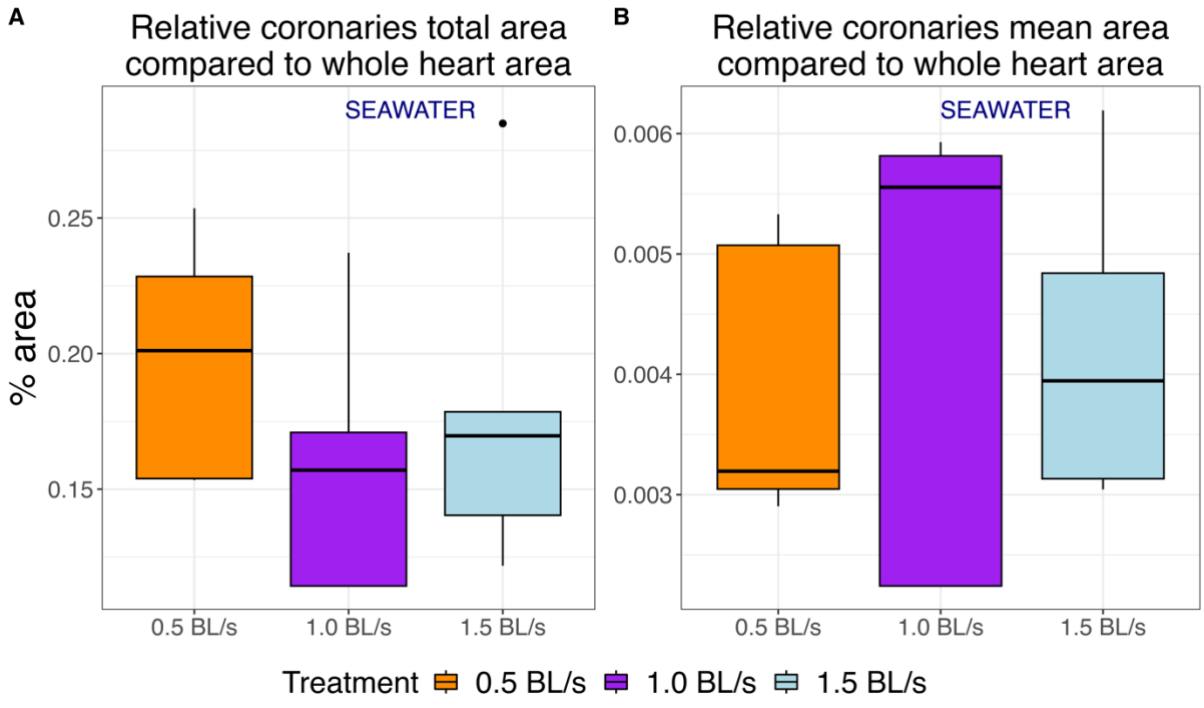

Figure 3.12. Histology results for the last sampling (S4) conducted in seawater (17th of January to 23rd of February). (A) Relative coronaries total area compared to whole heart area, (B) Relative coronaries mean area compared to whole heart area. The y-axis shows the % area while the x-axis shows the different treatments (■= 0.5 BL/s, ■ = 1.0 BL/s, ■= 1.5 BL/s). The box represents the interquartile range with whiskers extending from the upper and lower quartiles and the black horizontal line representing the median. Outliers that differ significantly from the rest of the dataset are plotted as individual points as seen in Fig. 3.12A.

The relative coronary mean area was also measured and compared to the whole heart area. The group exercised at moderate velocity of 1.0BL/s had the highest median value for the relative coronary mean of 0.00555 ± 0.00155% as well as the largest variability compared to the two other groups (*Fig.3.12B*). The lowest median values were found in the group exercised at the low velocity of 0.5BL/s with 0.00319 ± 0.00089%, while the group exercised at the high velocity had a slightly higher value of 0.00395 ± 0.00121%.

The compact myocardium was also measured from the last sampling (*Fig.3.13*). The median percentage of the compact myocardium was found to be highest for the least exercised group, measuring 28.1 ± 1.34 %. This was closely followed by the group exercised at the highest velocity of 1.5 BL/s with a median percentage of 27.3 ± 4.51 %. The moderate exercised group displayed the lowest median of the compact myocardium with 25.9 ± 4.46 %.

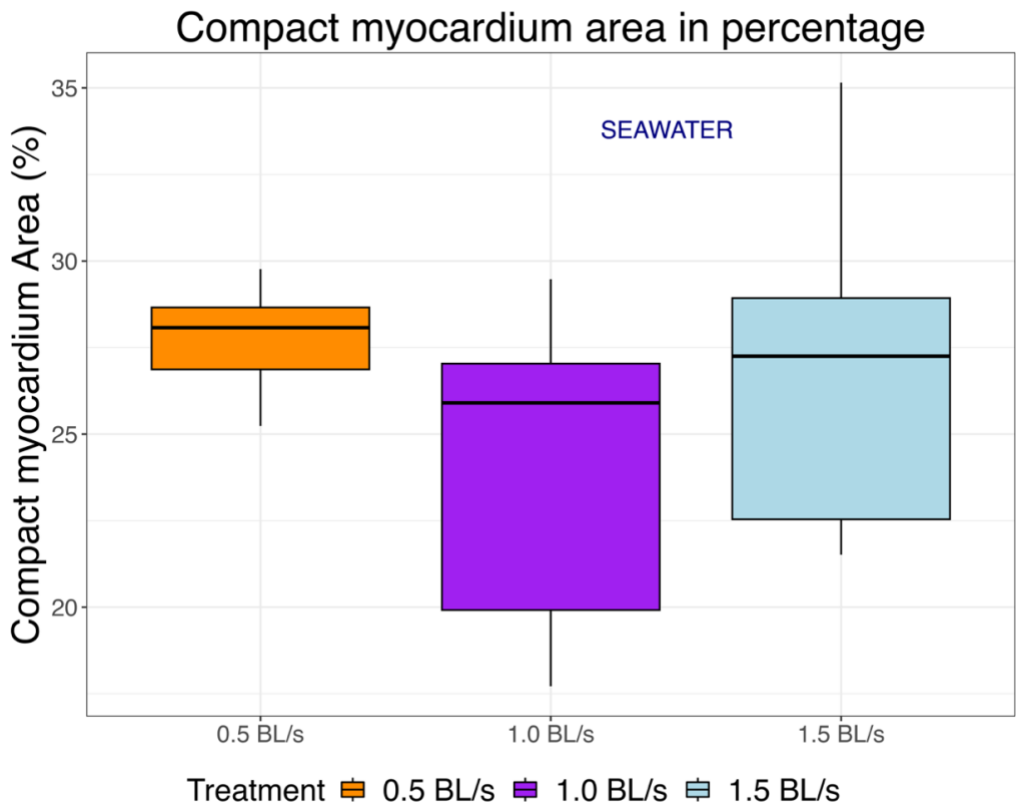

Figure 3.13. Histology results for the last sampling (S4) conducted in seawater (17th of January to 23rd of February) for the compact myocardium area in percentage. The y-axis of A & B shows the % area while the y-axis of C shows the compact myocardium area in percentage. The x-axis shows the different treatments (■= 0.5 BL/s, ■ = 1.0 BL/s, ■= 1.5 BL/s). The box represents the interquartile range with whiskers extending from the upper and lower quartiles and the black horizontal line representing the median.

3.2 PRODUCTION GROUP STUDY

3.2.1. SOMATIC PARAMETERS

Average weight and length were used to measure the growth for the production group study. For the baseline group, the mean weight (*Fig 3.13A*) was 124.9 ± 26.0 g, while the mean length (*Fig 3.13B*) was 22.1 ± 1.3 cm. After 4 months in seawater, measurements of mean weight and mean length were taken for both experimental groups, Preline and reference. The mean weight for the reference group was 615.4 ± 135.0 g while the mean length was 36.9 ± 2.3 cm. For Preline, the mean weight was 658.5 ± 142.9 g while the mean length was 37.5 ± 2.3 cm. GLM analysis showed significant differences for the fixed variables of rearing facilities groups (Appendix II Table I) and length (Appendix II Table II). Post hoc analysis revealed a significant increase in mean weight (Appendix II Table IV) from the baseline group to the Preline group ($p < .0001$) and to the reference group ($p < .0001$). In addition, post hoc analysis showed a significant increase in length (Appendix II Table VI) from the baseline- to the Preline group ($p < .0001$) and from the baseline- to the reference group ($p < .0001$) . There were no significant differences in neither the weight nor the length between the two experimental groups, Preline and Reference group.

GLM analysis showed significant differences in condition factor (K) between the fixed variable of rearing facility groups (Appendix II Table III). Post hoc analysis (Appendix I Table VII) revealed a significant increase from the freshwater phase to seawater phase, where K increased significantly from a mean of 1.14 ± 0.059 in freshwater to a mean of 1.22 ± 0.058 in the Preline group ($p < 0.01$) and to a mean of 1.20 ± 0.116 in the reference group ($p < 0.05$). After 4 months in seawater, the K was not significantly different between the two experimental groups (*Fig. 3.13C).*

The cardiosomatic index (CSI) was highest for the baseline group, i.e., before the group was split *(Fig. 3.13D)*. Statistical analysis through GLM and post hoc analysis (Appendix II Table IV, Table IX) showed a significant decrease from the baseline group with a mean CSI of 0.177 ± 0.017 to a mean of 0.134 ± 0.014 in Preline ($p < .0001$) and to a mean of 0.144 ± 0.018 in the reference group ($p < .0001$). Between the experimental groups there was no significant difference (*Fig. 3.8D*).

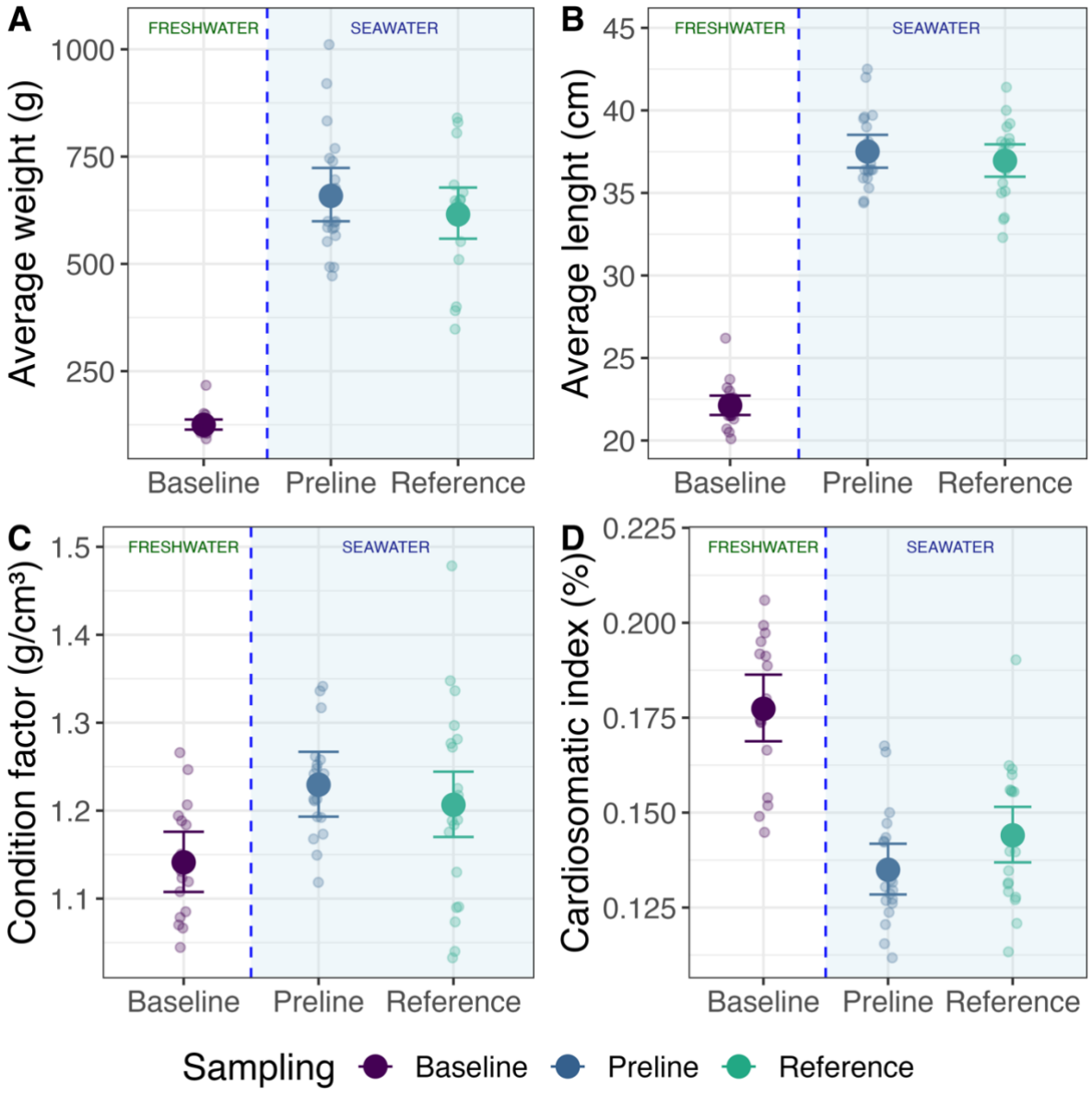

Figure 3.13. (A) Mean weight (g), (B) Mean length (cm) (C) condition factor (g/cm³), (D) cardiosomatic index (%). The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The first sampling (28th of October) was conducted in freshwater right before the experimental fish was transferred to seawater. The seawater phase was conducted during wintertime and lasted for 4 months (28th of October – 9th of March). The different groups are represented by colors (● = Baseline, ● = Preline, ● = Reference).

3.2.2 GENE TRANSCRIPTION

Hypertrophic growth

Statistical analysis using GLM revealed significant differences in the fixed variable of rearing facilities for the relative transcription of *gata4* (Appendix I Table IX). Post hoc analysis (Appendix II Table XV) revealed that the transcription of *gata4* increased significantly from the freshwater phase, represented by the baseline group, to the seawater phase for the Preline group ($p < 0.05$) and the reference group ($p < .0001$). There was also a significant difference between the two experimental groups where the reference group had a significantly higher transcription of *gata4* compared to the Preline group ($p < .0001$).

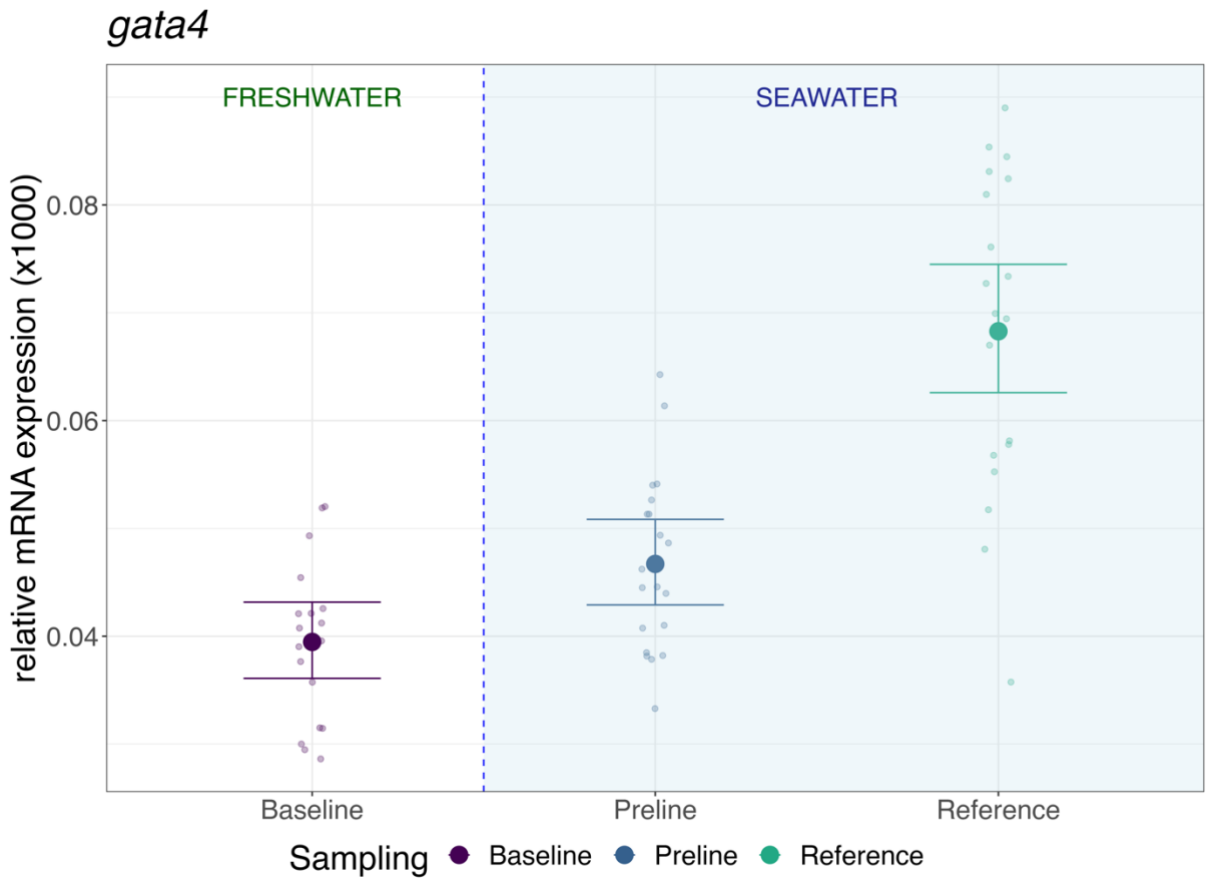

Figure 3.14. Relative gene transcription of gata4. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The first sampling (28th of October) was conducted in freshwater right before the experimental fish was transferred to seawater. The seawater phase was conducted during wintertime and lasted for 4 months (28th of October – 9th of March). The different groups are represented by colors (● = Baseline, ● = Preline, ● = Reference).

The transcription of *mef2c* had a similar trend to *gata4* (*Fig.3.15*). GLM analysis showed significant differences in the transcription of *mef2c* of the fixed variable of rearing facilities (Appendix I Table X). After 4 months in seawater, post hoc analysis (Appendix II Table XVI) showed that the relative transcription of *mef2c* had increased significantly from the freshwater phase to the seawater phase for both the Preline group ($p < 0.05$) and the reference group ($p < .0001$). Between the Preline group and reference group, the reference group exhibited a significantly higher transcription of *mef2c* ($p < 0.001$).

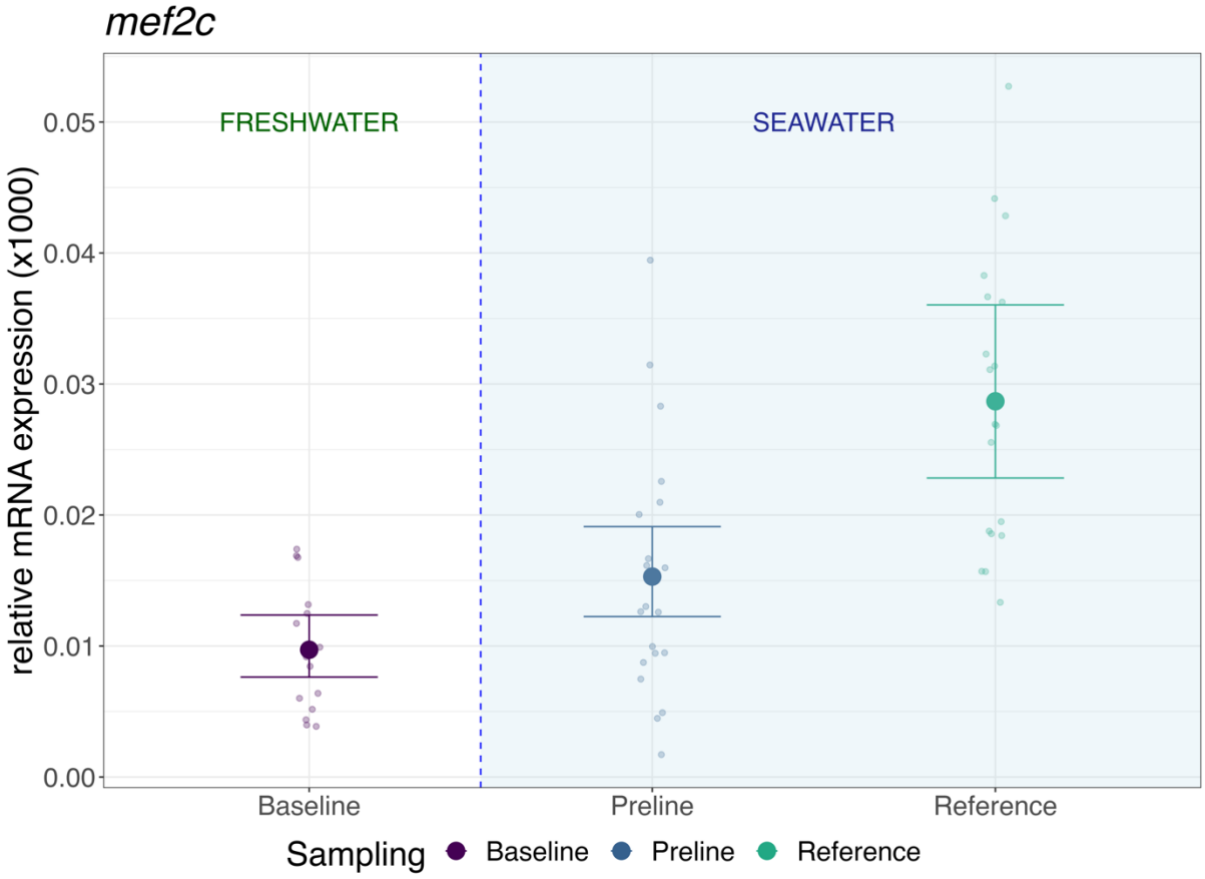

Figure 3.15. Relative gene transcription of mef2c. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The first sampling (28th of October) was conducted in freshwater right before the experimental fish was transferred to seawater. The seawater phase was conducted during wintertime and lasted for 4 months (28th of October – 9th of March). The different groups are represented by colors (● = Baseline, ● = Preline, ● = Reference).

Pro-Angiogenic genes

For *angpt2_ch6* (*Fig. 3.16*) statistical analysis through GLM showed some significant differences between the fixed variable of rearing facilities (Appendix II Table XI). Post hoc tests (Appendix II Table XVII) revealed that the relative transcription, after 4 months in seawater, had a weak, non-significant increase from the baseline group to the Preline group and a significant increase from the baseline group to the reference group ($p < .0001$). Between the Preline group and the reference group, there was a significantly higher transcription of *angipt2_ch6* in the reference group ($p < .0001$).

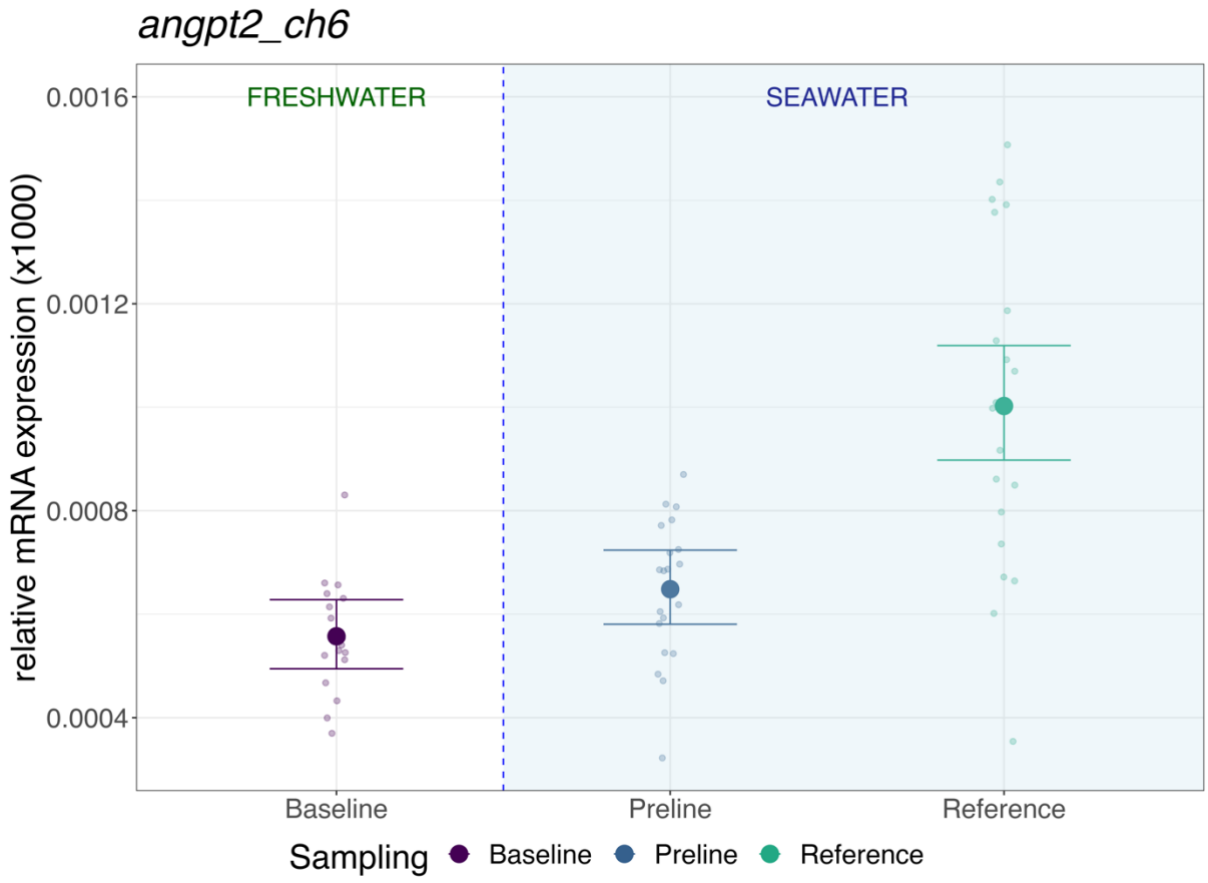

Figure 3.16. Relative gene transcription of angpt2_ch6. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The first sampling (28th of October) was conducted in freshwater right before the experimental fish was transferred to seawater. The seawater phase was conducted during wintertime and lasted for 4 months (28th of October – 9th of March). The different groups are represented by colors (● = Baseline, ● = Preline, ● = Reference).

For *angipt2_ch20 (Fig.3.17)* GLM analysis showed some significant differences between the fixed variable of rearing facilities (Appendix II Table XII) with the lowest transcription was found in the Preline group. Post hoc analysis (Appendix II Table XVII) revealed that the Preline group had a significantly lower relative transcription compared to the baseline group ($p < 0.05$) and the reference group ($p < 0.01$). No significant difference in relative transcription was observed between the baseline group to the reference group.

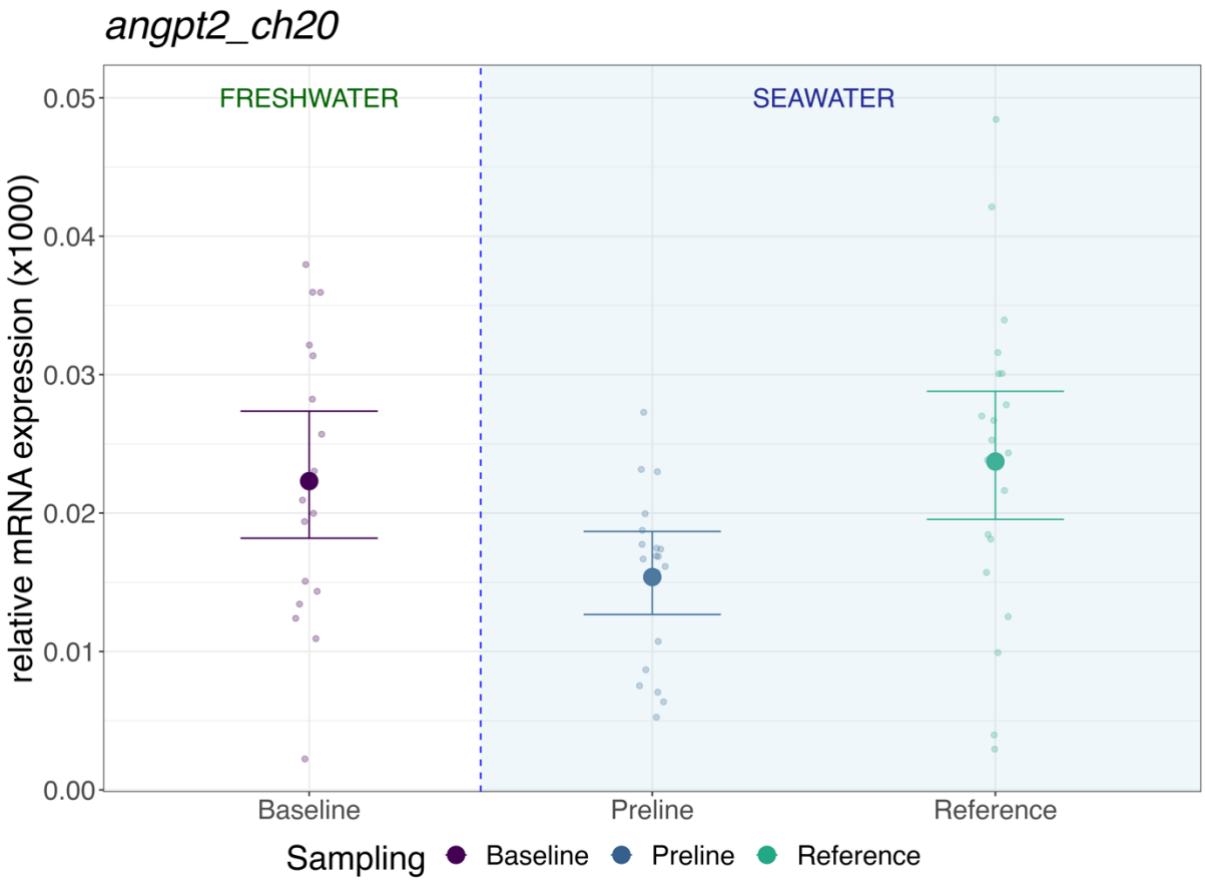

Figure 3.17. Relative gene transcription of angpt2_ch20. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The first sampling (28th of October) was conducted in freshwater right before the experimental fish was transferred to seawater. The seawater phase was conducted during wintertime and lasted for 4 months (28th of October – 9th of March). The different groups are represented by colors (● = Baseline, ● = Preline, ● = Reference).

Anti-Angiogenic genes

There were some significant differences between the fixed variable of rearing facilities from GLM analysis (Appendix II Table XIII). Post hoc analysis (Appendix II Table XIX) showed no difference in the relative transcription of *angpt1* between the baseline- and reference group, but there was a significantly lower relative transcription of *angipt1* in the Preline group compared to the reference group ($p < 0.05$) as well as a non-significantly lower relative transcription compared to the baseline group (*Fig. 318*).

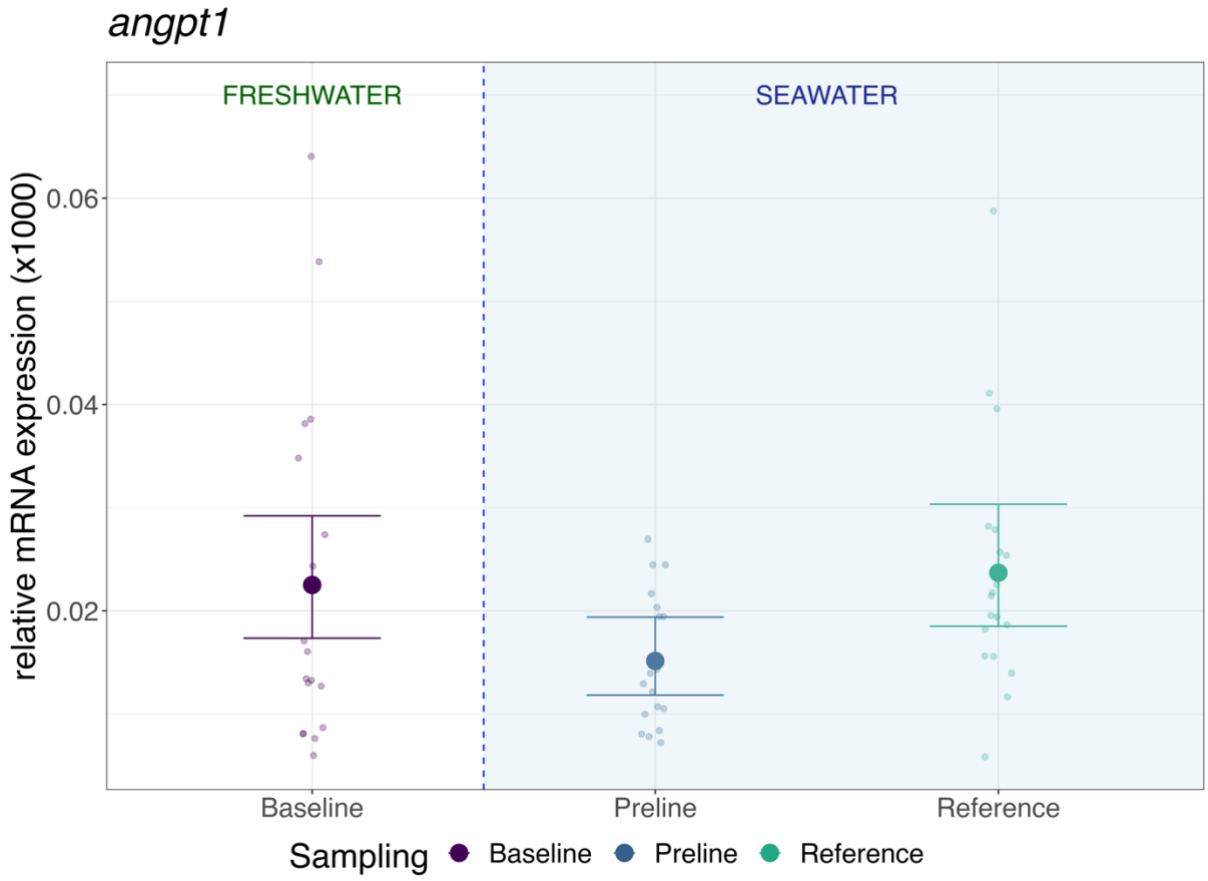

Figure 3.18. Relative gene transcription of angpt1. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The first sampling (28th of October) was conducted in freshwater right before the experimental fish was transferred to seawater. The seawater phase was conducted during wintertime and lasted for 4 months (28th of October – 9th of March). The different groups are represented by colors (● = Baseline, ● = Preline, ● = Reference).

GLM analysis (Appendix II Table XIV) and post hoc tests (Appendix I Table XX) reveal that there was no significant difference between any rearing facilities for the relative transcription of *pedf*. However, there was a higher, non-significant, relative transcription of *pedf* in the Preline group compared to the baseline group and reference group.

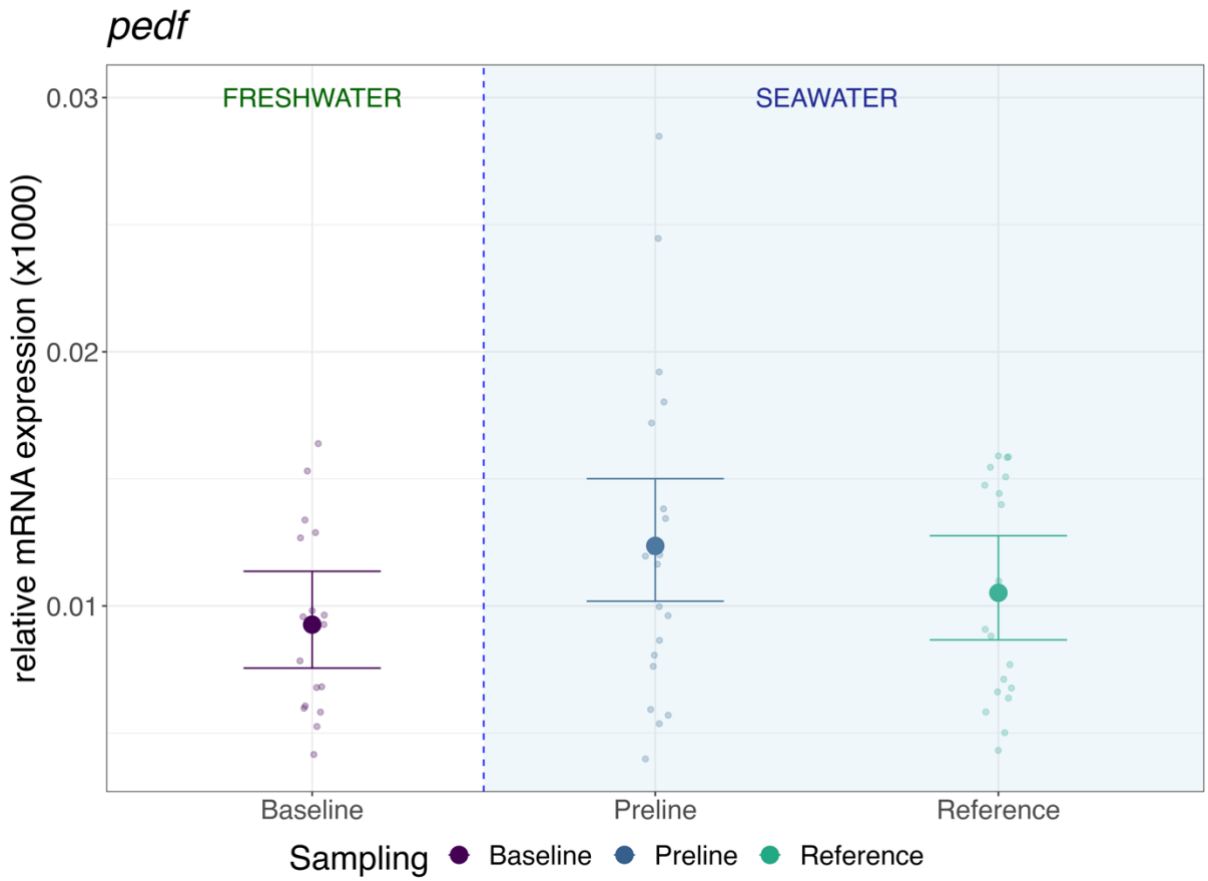

Figure 3.19. Relative gene transcription of pedf. The plot displays the mean of the model predicted values (large dot) and the 95% CI with the raw values represented as smaller dots. The first sampling (28th of October) was conducted in freshwater right before the experimental fish was transferred to seawater. The seawater phase was conducted during wintertime and lasted for 4 months (28th of October – 9th of March). The different groups are represented by colors (● = Baseline, ● = Preline, ● = Reference).

4. DISCUSSION

4.1 DISCUSSION OF METHODS

4.1.1 EXPERIMENTAL FISH

All Atlantic salmon used in both experiments originated from the same genetic background. This was important to ensure that all fish had the same foundation before the experimental start. During the baseline sampling in the production group study, fish numbered 1 to 5 were highly stressed due to oxygen deficiency from an extended time spent out of water. The stress itself should not affect the relative gene transcription of the fish, but RNA could start degrading if the fish were dead for too long before it was sampled. However, as clear outliers were removed from the data, the stressed fish should not cause significant changes in the results.

4.1.2 EXPERIMENTAL DESIGN

Constant current experiment

The constant current trial employed a factorial design. This allowed for complete control over all environmental conditions, thereby making it possible to observe the effect of exercise at different velocities. However, it was not possible to keep a uniform velocity within circular tanks as the current near the outer wall exceeded the current near the inner region of the tank. This represents a limitation of the experimental design, as any change in the fish position expose them to varying velocities depending on their position relative to the outer wall. For this reason, it is difficult to maintain a homogenous current, which, in turn, has the potential to influence the results. It is possible that certain fish might exercise at lower velocities than intended, if they move close to the inner circular wall as the velocity averaged based on mainly the velocity close to the outer wall and the middle of the tank. In addition, there was no real control as all fish were exposed to velocities of different strengths. Even though a small velocity would be needed to keep a sufficient water quality, a velocity of 0.5 BL/s is considered a modest training regime.

Another limitation is related to the sampling approach. While the results from freshwater was based on three sampling points, the results from seawater were only based on one. This represents a challenge, as the absence of multiple samplings hinders the ability to observe true patterns and random variations in the data from the seawater phase, thereby reducing the reliability of the findings. However, it would be difficult to prolong the seawater phase as the fish would continue to grow making it difficult for a growing biomass to thrive in such small tanks. A large biomass within a small tank would cause the water quality to drop, thereby reducing the fish welfare and probably affect the results in a negative way. If more sampling points were to be executed, a bigger tank would be needed. However, this demands more space as well as being costly.

It is also important to note that the initial sampling conducted in freshwater followed the acclimation period, during which all fish swam at 0.5 BL/s. Consequently, no significant differences were expected across treatments for this group. To examine the after-effect of exercise, the velocity was set to 0.5 BL/s in the final sampling which was conducted in seawater. Additionally, it is important to highlight that the temperature was changed from 12 ºC in freshwater to 10 ºC in seawater which likely had an impact on the results as temperature is among the most influential environmental parameters.

Production group study

In contrast to the constant current study, it was not possible to have complete control over the environmental parameters, in the production group study. The Preline group were reared in a semi closed facility where fish are exposed to a constant current to maintain water quality. The reference group, which was not a control group, represented the standard way of producing salmon in the industry. These rearing methods represent the true conditions found in fish farming and were compared to explore the outcomes of swimming induced exercise in a production scenario.

In addition to the inability to control environmental conditions, the production group study only consisted of one replication and two samplings: one in freshwater and one in seawater. The sampling in seawater involved the collection of fish from both experimental groups; Preline and reference. However, the lack of replicates is regarded as a weakness as

replicates would have strengthened the ability to validate of the results. The lack of replicates was a consequence of financial limitations associated with additional replicates. Even though the absence of replicates represents a limitation, previous studies have examined the effects of Preline compared to open net pens, which makes it possible to compare results.

Another limitation is temperature. The Preline facility extracted water from a dept between 20-30 m, providing a more consistent temperature throughout the seasons. In contrast, the reference group, reared in open net pens, experienced temperature fluctuations influenced by seasonal changes. In addition, while the Preline group remained in the same position as they swam against a constant current, the fish in the reference group had the freedom to distribute themselves throughout the open net pens within a depth of 44.5 m. This can pose a challenge in determining the specific parameters individual fish was exposed to, especially in terms of activity level, temperature, and light. The reference group was also located in an area exposed to tidal water currents. This can influence the experiment as the fish experience periods of higher velocities. For this reason, the reference fish might have experienced periods of exercise where it had to swim against currents.

4.1.3 TRANSCRIPTION OF RNA

Compared to measurements like weight and length which do not fluctuate much with time, genes are vulnerable to instant stimulators.This makes the gene transcription vulnerable to fluctuating environmental conditions (Pascual-Ahuir et al., 2020). Gene transcription provides information about specific RNA molecules produced in a gene (Guo, 2014), in contrast to gene expression which refers to the overall activity of the gene. For this reason, examining gene transcriptions can contribute to improve our understanding of gene operations and the factors affecting them. However, as there are many steps involved in the transcription process, errors can occur.

One important matter when extracting RNA is to validate if the RNA is pure. To accomplish this, we utilized the 260:230 ratio and the 280:260 ratio from the Nanodrop spectrophotometer (Thermo Fisher Scientific, USA). These ratios can determine contaminants absorbed at 230 nm and 260 nm respectively. The generally accepted limit as “pure” for RNA

is ~2.0 with a lower ratio indicating a presence of contaminants (Thermo Scientific, 2012). However, our limit was set to 1.7, but most samples did have values around 2.0 or above. Following DNase treatment, the 260:230 and the 280:260 ratio decreased. This was expected as Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) is prone to DNA contaminants as both RNA and DNA absorb at 260 nm. As the ratio decreased this indicates that DNA contamination was present before the DNase treatment further cleaned the samples. Furthermore, RNA quantification was measured utilizing Qubit 3.0 Fluorometer (Thermo Fisher Scientific, USA). Qubit 3.0 Fluorometer was chosen over the Nanodrop spectrophotometer due to the fact that Qubit 3.0 Fluorometer uses different kits with specific probes (in this case against RNA), making it less affected by the presence of contaminants compared to the Nanodrop spectrophotometer.

Furthermore, the transcription of specific genes from cDNA treated samples were detected by conducting qPCR. However, as variations in RNA quantity and quality can cause differences in qPCR results, due to differences in sampling (Olsvik et al., 2005), it was necessary to normalize the values against a reference gene. The optimal reference gene is expressed at constant levels among different tissues of in an organism at all stages of development and should not be affected by experimental treatment (Olsvik et al., 2005; Radonić et al., 2004). However, the concept of an unregulated gene is not supported as all genes exhibit some level of regulation (Radonić et al., 2004). Thus, it is optimal to select multiple reference genes (Olsvik et al., 2005). We decided on two different reference genes based on the results from the dilution series. For every gene, including the reference genes, the CV limit for variation between samples from the same individual, was set to 1.5, to ensure that the random fluctuations were small.

During the transcription process of RNA, a total of seven samples were dismissed, most of which were due to errors in the RNA extraction process. Errors during the RNA extraction resulted in RNA concentrations that were too low for DNase treatment. Consequently, six samples were dismissed as they did not have any more heart tissue to repeat RNA isolation. Furthermore, there were some errors during the cDNA synthesis resulting in one additional dismissal.

4.1.4 HISTOLOGY

The histology was performed by PHARMAQ Analytiq AS. One limitation in the histology results was that the only adequate images we received was from the seawater sampling. For this reason, histological analysis has only been done for this one sampling, making it difficult to compare histological results across samplings, and observe potential trends. The reason for inadequate images was that multiple hearts were divided during the sampling which limited the possibility of doing standard cutting of the tissue by PHARMAQ Analytiq. This resulted in some of the images we did use to exhibiting large white areas due to the loss of tissue in the compact myocardium (*Fig. 4.1A & 4.1B*) which might have affected the results as it was not possible to spot vessels in these areas. Another important limitation is based on the fact that capillary size is easily affected by environmental stimuli. When fish are stressed, stress hormones as catecholamines are released which in turn can stimulate a narrowing of the blood vessels called vasoconstrictions (Paravati et al., 2023). As sampling represents a stressful event for the fish, potential occurring of vasoconstrictions could have affected the results. In addition, only a few individual fish were used for histological examination, including six fish from the low exercise regime at 0.5 BL/s and five fish for each of the groups exercising at 1.0 BL/s and 1.5 BL/s. This consequently prevented the use of statistics in the results and only allowed for the use of relative measures.

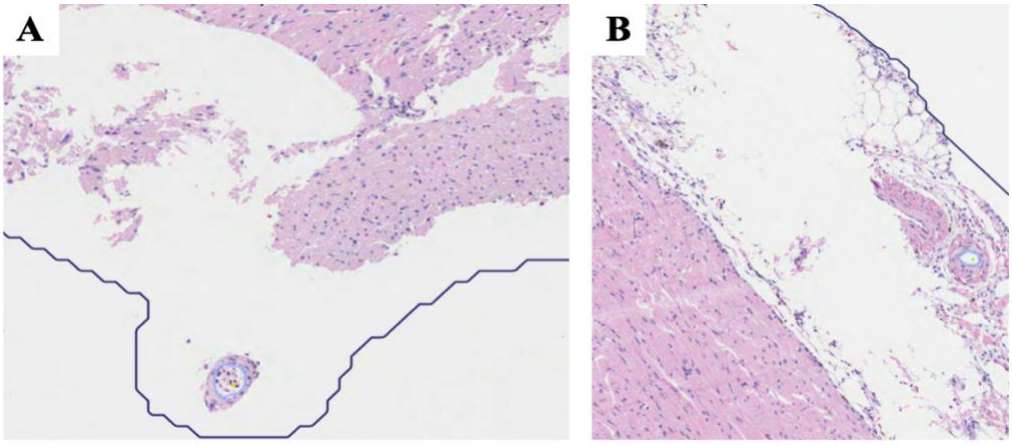

Figure 4.1. Example pictures of (A & B) white areas in the compact myocardium.

4.1.5 TANK EFFECT

Random tank effect was measured for all morphometric parameters and for all genes in the constant current experiment. Potential random tank effects were accounted for in the statistical model but were found to be very minor to not relevant (Appendix Figure, and table).

4.2 DISCUSSION OF RESULTS

4.2.1 EFFECT OF EXERCISE AND TIME ON SOMATIC GROWTH

Growth rate in body weight

Rapid growth is an important factor in salmon farming as it increases production efficiency and reduces production costs. Exercise at higher velocities have been reported to increase the growth rate in Atlantic salmon (Jørgensen and Jobling, 1993, 1994). Through exercise, the fish swim against currents and the oxygen supply increase through ram ventilation (Solstorm et al., 2015). For this reason, the fish can use less energy on pumping oxygen through the gills, which grantes more energy to growth. In our study, no significant increase in growth rate in body weight were observed among exercise regimens in freshwater. These findings contradict previous studies (Jørgensen and Jobling, 1993, 1994; Waldrop et al., 2018). However, the fish from these experiments had a start weight of 14 g and 20 g (Jørgensen and Jobling, 1994, 1993) and 10 g (Waldrop et al., 2018), while the fish in our experiment had a start weight of 78 g. In addition, there was a larger difference in swimming speed in the study by Waldrop et al. (2018), with the velocities of 0.5 BL/s and 2.0 BL/s that lasted till 440-days post-hatching, while Jørgensen and Jobling, (1994;1993) compared a control group, which did not swim, to a minimum velocity of 1.0 BL/s for 9 weeks in the experiment from 1993 and 122 days for the experiment from 1994. This could indicate that the velocities used in our experiment were too similar. The lack of significance in growth was also presented by Castro et al. (2013), who had a start weight of 40.7±0.2 g and a minimum velocity of 0.32 BL/s used as a control and a maximum velocity of 1.31 BL/s. The difficulties related to the maintenance of homogeneous current velocities within the tanks could also affect the effect of different velocities as the velocity will be higher at the outer wall of the tank (Duarte et al., 2011; Solstorm et al., 2015). However, this is a larger issue when the tanks are completely open without any closed area in the middle of the tank, which was not the case in our study.

In contradiction to the results from freshwater, exercise did have a significant effect on the specific growth rate (SGR) after seawater transfer. The groups exercised at a moderate- and high velocity exhibited a higher SGR compared to the group exercising at a low velocity. There was a similar trend in the relative growth (RG), but it was not significantly different. Increasing growth in seawater for exercised groups at higher velocities, have also been reported in other studies (Jørgensen and Jobling, 1994; Timmerhaus et al., 2021; Totland et

al., 1987; Ytrestøyl et al., 2020). A potential reason for the faster growth in seawater, could be due to improved stress tolerance (Woodward and Smith, 1985). The transfer to seawater can represent a stressor as it involves handling as well as physical adaptions to increasing salinity. Esbaugh et al. (2014) observed that exercise upregulated the expression of seawater relevant genes in the gills. This suggests that aerobic exercise allows Atlantic salmon to adapt to seawater faster which provides more energy for growth. Another reason can be caused by larger fiber sizes of red- and white muscle in relation to exercise. Previous studies have found that the overall body weight in exercised individuals can be correlated to an increasing size in muscle fibers (Timmerhaus et al., 2021; Totland et al., 1987). However, it does take time before these differences appear, which would explain why the differences among treatments first become apparent at the final sampling in our study.

In the production group study, growth rate was not measured as several sampling points would be necessary. PIT-tagging was not possible as the number of fish were huge in addition to most being attended for human consumption. As the experimental period for the production group study was conducted during the winter seasons (31st of October – 9th of March), the temperature was averaged to 8.1 ± 1.6 ºC for the reference group, while it remained stable at 11.0 ± 0.6 ºC for the Preline group. However, there was only a slightly, and not significantly higher weight and length in the Preline group compared to the reference group. Similar results were presented in the study by Øvrebø et al, (2022) where the Preline group exhibited an overall higher growth performance during the fall season. This study also observed a higher growth in the control group during the spring season, which strengthen the claim that differences in temperature may be the main cause of the differences in growth between Preline and reference. However, as the differences in our study was not significant it could indicate that the temperature difference was not large throughout the experimental period.

Through time, in the present study, the growth rate in SGR increased significantly from the initial to the second sampling, while RG had a significant increase all though the freshwater phase. Following the transition to seawater, there was a significant decline in both SGR and RG. Despite the velocity being reduced to 0.5 BL/s for all experimental groups in seawater, this reduction in velocity cannot account for the observed decrease in SGR and RG. If growth rate were to diminish in correlation with decreasing velocity, one would expect the group, initially exercising at 0.5 BL/s to maintain a consistent growth rate, while only the

groups exercising at 1.0 BL/s and 1.5 BL/s would experience a reduction in growth rate. However, as the growth for the higher exercise regimes were significantly larger than the less exercised group, it is evident that the decline in growth rate after seawater transfer, was a cause of other factors. One such factor could include the decrease in temperature from 12 ºC in freshwater to 10 ºC in seawater as temperature is among the most influential factors related to growth (Balseiro et al., 2018; Fry, 1971). However, as the decline in growth rate was quite significant, this might not be the only responsible factor. As a declining growth rate after seawater transfer have been observed in previous studies (Jørgensen and Jobling, 1994; Ytrestøyl et al., 2020), this suggests that growth decreases with increasing salinity. Handeland et al. (2003) presented that the decreasing growth rate after seawater transfer is likely related to appetite as the daily feed intake in their study went from 2% before transfer to a range between 0.27% and 0.85% in early seawater phase. A decrease in appetite can be a result of the physiological challenges associated with the adaption to seawater (Handeland et al., 2003; Jørgensen and Jobling, 1994).

Condition factor

The condition factor (K) assesses the general health of the fish by measuring the nutritional status. For this reason, the fish can exhibit a good nutritional status even though it has a low condition factor. In Atlantic salmon farming, a high condition factor is desirable as it indicates that the fish have a more rounded shape, while a low condition factor could suggests that the fish are thin and not feeding properly (Noble et al., 2018). However, it could also suggest that the fish have grown more in length compared to weight as the condition factor measures the weight relative to the length.

Some previous studies have found associations between a higher K and exercise at higher velocities in Atlantic salmon (Castro et al., 2011; Nilsen et al., 2019). Our study, did however, not observe any significant difference in K across treatments, neither in the constant current experiment nor the production group study. Similar results have been found in other studies investigating the effect of exercise on Atlantic salmon (Kiessling et al., 1994; Solstorm et al., 2015; Waldrop et al., 2018). This indicates that the weight: length ratio was similar across treatments and was not affected by the exercise regimes.

Through time, there was a consequent and significant increase in K from the freshwater phase to the seawater phase in both the constant current experiment and the production group study. The observed results from both experiments resemblance the results presented by Solstorm et al. (2015), who observed a small, non-significant increase in K after 6 weeks in seawater. The consistent increase in condition factor through time could suggest inadequate smoltification of the fish, as smoltification is associated with a decline in the condition factor coupled with a rapid escalation in growth rate (Jørgensen and Jobling, 1994). However, as the transition to seawater was conducted by changing the inlet water, the fish did not experience stress caused by transportation, pumping, or moving as it does in commercial salmon farming. In addition, the level of salinity was quite low at 26‰ while the normally range of salinity in the ocean is between 33‰ – 37‰ (NOAA, 2023). The low salinity used in our experiment, which can be considered brackish water, contributes to a less challenging transition to seawater. As there were no dead fish and the condition factor continued to increase after seawater transfer this suggests that the transitioning to seawater did not limit the welfare of the fish even if smoltification was not adequate. Furthermore, a higher condition factor is linked to a larger increase in weight compared to length, which might have been the case in our study, given the observed growth rate in weight as previously discussed.

4.2.2 EFFECT OF EXERCISE AND TIME ON CARDIOVASCULAR HEALTH

Relative heart size

A larger heart is desirable as it pumps oxygen more efficiently through the body, which potentially improves stress tolerance and health. Overall heart size is also related to a thicker compact myocardium (Incardona et al., 2015). Compact myocardium only exist in active fish species (Takle and Castro, 2013) and its thickness is related to higher swimming speed (Incardona et al., 2015). However, our histological analysis did not show much difference in the compact myocardium across treatments. The exception was for the moderate group, which did have a noticeably lower mean. However, this group also had a large spread of the data, which indicates a high individual variability. This can, however, be highly impacted by the fact that only a few individuals were used for the histological analysis: six

fish from the group exercising at a low velocity and five from the two others, making it difficult to draw a conclusion.

Another parameter used to measure the relative heart size is the cardiosomatic index (CSI). In the constant current experiment, a weak trend was found where the groups exercising at a moderate- and high velocities had a consistently higher CSI compared to the group exercising at a low velocity, with the only significantly higher CSI observed in the second sampling in freshwater. This corresponds with the study by Good et al, (2016), who observed a significantly higher CSI for the exercised group in freshwater compared to the unexercised group. Other studies (Timmerhaus et al. 2021; Ytrestøyl et al 2020) have reported on similar trends, although not significant. Overall, this indicates that higher velocities result in a higher relative heart size.

In contrast to the commonly observed increase in heart size related to higher exercise regimes, no significant differences in CSI were observed for the experimental groups in the production group study. This could be a consequence of the swimming speed being too low in the Preline group as some significant differences was observed in the constant current experiment. However, it can also be a cause of other factors as including the temperature differences between the Preline and reference, the duration of the experiment or potentially the occurrence of tidal currents in the open net pens.

Over time there was little change in CSI between the two initial samplings in freshwater in the constant current experiment. This was followed by a significant decline in CSI with the lowest value observed in the seawater phase. Similar results were observed in the production group study, as the CSI had the highest value in the freshwater phase before decreasing significantly after 4 months in seawater. These results correspond with Ytrestøyl et al, (2020) who observed a lower CSI at higher salinity. However, the significant decline in CSI during the constant current experiment, occurred before seawater transfer, indicating that the decline in CSI was likely affected by another parameter than salinity. It is more likely that the decreasing CSI was a result of a higher growth rate in body size compared to the growth rate of the heart considering that CSI is affected by the overall size of the animal itself.

Oxygen delivery capacity

The ability to provide adequate oxygen around the body is affected by the amount of red blood cells as these cells carry oxygen. The amount of red blood cells is measured by hematocrit (Hct). Increasing hematocrit levels implies that there is a higher amount of red blood cells in the bloodstream, resulting in reduced requirement for blood to support aerobic metabolism during swimming. This allows for unhindered digestion (Davison, 1997; Thorarensen et al., 1993). Elevated Hct, consequently allow the fish to swim continuously at higher speeds without compromising growth and maintenance functions (Thorarensen et al., 1993).

In our study the Hct was only measured in the constant current experiment and no significant difference in Hct was found across treatments in neither the freshwater nor the seawater phase. Other studies on Atlantic salmon (Solstorm et al., 2015) and Chinook salmon *(Oncorhynchus tshawytscha)* (Hoffnagle et al., 2006) have also reported on a lack of significant differences in Hct across treatments. As the mean range remains stable within a range of apporximately 44-53% throughout our study, it suggests that the absence of differences across treatments could be a result of reaching a plateau in the Hct value. This can be an effect of the automatic oxygen control with an oxygen setpoint of setpoint of 90%. When the fish exercise, the oxygen demand will increase which decrease the oxygen saturation. This stimulates erythrocyte production in the fish to compensate for reduced oxygen levels.

Another reason as presented by Solstorm et al. (2015) drawed on the findings of Djordjevic et al. (2012), suggests that hematocrit values exceeding 30% might have been affected by sampling stress, consequently leading to the absence of variations among treatments. Studies reporting on Hct levels around- and below 30% (Gallaugher et al., 2001; Thorarensen et al., 1993) have found a significantly higher Hct in exercised chinook salmon. One of the authors presented that increasing Hct was related to the blood flow in the intestinal artery, but not related to the rate of oxygen consumtion as this remained the same (Thorarensen et al., 1993). Due to reduced blood requirement at higher Hct levels, exercised fish are able to maintain optimal oxygen transportation while preserving regular digestion during high-velocity swimming. In contrary to the expectations, improved aerobic swimming performance was not correlation to higher Hct levels according to the study by Thorarensen et

al. (1993). This suggests that swimming performance is not limited by the oxygen-carrying capacity as long as Hct remains within a normal range.

Hypertrophic growth

Exercise increases the workload on cardiac myocytes which in turn stimulate cardiac hypertrophy by upregulating *gata4* and *mef2c*. These genes, will in turn increase the cell size to maintain normal cardiac output (Akazawa and Komuro, 2003; Castro et al., 2013). Our study found no significant differences in the transcription of *gata4* and *mef2c* across treatments for the constant current experiment. However, there was a weak trend in the transcription of *gata4* where the group exercised at a low velocity exhibited the highest transcription through the experiment. These results contradict the study by Castro et al. (2013) who found a significantly higher transcriptions of *gata4* and *mef2c* observed in groups exercised at higher velocities. This was suggested to be related to the training induced cardiac enlargement. Based on the histology results from our study, which did not observe a clear difference between treatments, this could be one reason for why our study did not have significantly higher transcription levels of *gata4* and *mef2c*. However, as the groups exercising at the higher velocities exhibited the highest CSI, the absence of differences in relative gene transcription of *gata4* and *mef2c*, could potentially be due to a downregulation of the genes at the time of sampling. Compared to weight measurement which represent a stable integrated value that changes through time, gene transcription only provides a snapshot of the moment and fluctuate based on environmental stimuli.

For the production group study, it was found that the transcription of *gata4* and *mef2c* was significantly higher in the reference group compared to the Preline group. This suggests that other factors than exercise had a higher impact on the transcription of *gata4* and *mef2c* or maybe that the swimming velocity in the Preline facility was too low to stimulate hypertrophic growth. Based on the fact that opposite results was found in Balseiro et al. (2018), which conducted their experiment in spring- and summer time, it could suggest that seasons have an impact on the transcription levels of these genes. In this case this would suggests that higher transcription levels of *gata4* and *mef2c* could be affected by lower temperatures and less light as our experiment conduced in wintertime found significantly higher transcription levels of *gata4* and *mef2c* in the reference group. However, the study by Castro et al. (2013) found a higher relative transcription of *gata4* and *mef2c* at 10 ºC, which is

more similar to the temperature found in Preline at 11.0 ºC than the mean temperature found in the reference group at 8.1 ºC. To gain a better understanding of which parameters affect the relative transcription of *gata4* and *mef2c*, further research is needed.

Capillary growth

Enlarged capillaries serve an important role in facilitating adequate oxygen to meet the growing demand as fish grow. In the human- and rat skeletal muscle, exercise have been proven to increase the amount of coronary capillaries through angiogenesis which can be induced by a higher relative transcription of *angpt2* and a lower relative transcription of *angpt1* (Gustafsson et al., 2007; Lloyd et al., 2003). However, when angiogenesis is initiated antiangiogenic genes like *pedf* might be upregulated to inhibit excessive angiogenesis as this can lead to the development of abnormal vessels (Rychli et al., 2009). In this study we investigated four genes associated with angiogenesis, aiming to determine whether exercise influences the transcription of these genes, as demonstrated in previous studies on humans (Walton et al., 2015). The two paralogues of *angpt2* were compared to *angpt1* to investigate if angiogenesis might have occurred. In addition, the transcription of *pedf* was observed to see if angiogenesis was inhibited.

In the constant current experiment, there was no significant differences across treatments for *angpt2*, *angpt1* nor *pedf* in neither the freshwater nor the seawater phase suggesting that there was no difference in capillary growth in any exercise regime. There were also little differences between treatments represented by the histology result with the largest total area in the group exercised at a low velocity, while the mean area was highest in the moderate group. However as previously mentioned these results are only based on a few individual fish and the vessel area can be modulator by vasoconstrictor. Potential reasons for the absence of variation in the relative gene transcription among treatments, could be due to the velocities of the exercise regimes being too similar or the duration of exercise being too short.

For the production group study there was little change in the relative transcription of *angpt2*, *angp1* and *pedf* between the baseline group and the reference group. The exception was observed for *angpt2_ch6*, where the reference group had a significantly higher relative transcription of *angpt2_ch6* compared to the two other groups. However, this gene exhibited

relatively low transcription values measured up against the other genes. Hence, it is possible that this low transcription level could have a notable effect on angiogenesis, but this will depend on the sensitivity of angiogenesis. The Preline group exhibited a lower transcription level of both *angpt1* and *angpt2* compared to the reference group and the baseline group. However, considering the coincident decrease in both genes, combined with a negligible upregulation of *pedf*, it is likely that no angiogenesis occurred. These results could suggest that capillary growth through angiogenesis was not favored in neither of the rearing systems.

To our knowledge, no prior studies have examined the relative transcription of *angpt2*, *angpt1* and *pedf* in relation to exercise in salmonids. However, there have been studies investigating *vegf* in relation to exercise, which is another gene promoting angiogenesis. Exercise at higher velocities have been found to upregulate mRNA levels of *vegf* in the ventricle in Atlantic salmon (Castro et al., 2013a) and in liver and white muscle in largemouth bass (*Micropterus salmoides*) (Zhao et al., 2023). These studies suggests that higher exercised individuals did experience increased capillarization with exercise. A large-scale study by Balseiro et al. (2018) did, however, not find any difference in the expression of *vegf* in Atlantic salmon between fish reared in S-CCS and fish reared in open net pens. It was suggested that this might be an effect of the currents were too low to induce any differences.

Through time in the constant current experiment, the group exercised at the highest velocity had a significant decline in the relative transcription of the antiangiogenic gene, *angpt1*, between the initial sampling and the third sampling. This suggests that exercise could have had a small effect on angiogenesis. However, mammalian studies have found that induced capillary growth through angiogenesis have resulted from an increasing relative transcription of *angpt2* coupled with a small decrease in *angpt1* (Gustafsson et al., 2007; Lloyd et al., 2003). As there was no significant increase in *angpt2* in the third sampling, it is not clear if angiogenesis was induced. Nevertheless, given the significant change in the relative transcription of *angpt1* and limiting literature on the impact of these genes in salmonids and other fish species, it is not possible to exclude the possibility that angiogenesis might have occurred. The sensibility of angiogenesis to these genes, could possibly be determine in future studies with the access of adequate histology analysis.

When the fish from the constant current experiment went from freshwater to seawater, an increase in *angpt2* and a reduction in *angpt1* was observed in all exercised groups. This was coupled with a decline in *pedf* suggesting that angiogenesis was induced. The stimulation

of angiogenesis in the seawater phase, provides further support to the previous suggestion that detectable differences in capillary growth through angiogenesis required a significant amount of time to become apparent. However, it could also be induced by other factors including temperature, muscle growth and salinity, but more research is needed in this area to reach a better understanding.

5. CONCLUSION

To conclude, exercise at higher velocities in the constant current experiment demonstrated improved growth in body weight and enlargement of the relative heart size. For the production group study fish reared in open net pens, exhibited a higher relative gene transcription of *gata4*, *mef2c*, the two paralogues of *angpt2* and *angpt1*, compared to the fish reared in semi-closed containment systems.

- **H01.1:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the growth rate, is **rejected.** At the final sampling in seawater, the groups exercising at a moderate velocity of 1.0 BL/s and a high velocity group of 1.5 BL/s exhibited a higher specific growth rate compared to the group exercised at the low velocity of 0.5 BL/s. Rearing Atlantic salmon at different velocities during the freshwater phase has significant effect on the growth rate, **Ha1.1** is **accepted.**

- **H01.2:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the condition factor (K), is **accepted.**

- **H01.3:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the cardiosomatic index (CSI), **is rejected.** In the second sampling in freshwater, the groups exercising at the moderate- and high velocities of 1.0 BL/s and 1.5 BL/s had a higher CSI than the group exercising at the low velocity of 0.5 BL/s. Rearing Atlantic salmon at different velocities during the freshwater phase has significant effect on the cardiosomatic index (CSI), **Ha1.3** is **accepted.**

- **H01.4:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on hematocrit levels, is **accepted.**

- **H01.5:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the relative transcription of *gata4*, is **accepted.**

- **H01.6:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the relative transcription of *mef2c*, is **accepted.**

- **H01.7:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the relative transcription of *angpt2*, is **accepted.**

- **H01.8:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the relative transcription of *angpt1*, is **accepted.**

- **H01.9:** Rearing Atlantic salmon at different velocities during the freshwater phase has no significant effect on the relative transcription of *pedf,* is **accepted.**

- **H02.1:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has no significant effect on the mean body weight, is **accepted**.

- **H02.2:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens) has no significant effect on the mean body length, is **accepted**.

- **H02.3:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens), has no significant effect on the condition factor (K), is **accepted**.

- **H02.3:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens), has no significant effect on the cardiosomatic index (CSI), is **accepted**.

- **H02.4:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens), has no significant effect on the relative transcription of *gata4*, is **rejected**. The reference group reared in open net pens had a significantly higher transcription of *gata4* compared to the Preline group reared in S-CCS. Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens), has significant effect on the relative transcription of *gata4*, **Ha2.4** is **accepted**.

- **H02.5:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens), has no significant effect on the relative transcription of *mef2c,* is **rejected**. The reference group reared in open net pens had a significantly higher

transcription of *mef2c* compared to the Preline group reared in S-CCS. Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens), has significant effect on the relative transcription of *mef2c*, **Ha2.5** is **accepted**.

- **H02.6:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens), has no significant effect on the relative transcription of *angpt2*, is **rejected**. The reference group reared in open net pens had a significantly higher relative transcription of both paralogues of *angpt2* compared to the Preline group reared in S-CCS. Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens), has significant effect on the relative transcription of *angpt2*, **Ha2.6** is **accepted**.

- **H02.7:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens), has no significant effect on the relative transcription of *angpt1*, is **rejected**. The reference group reared in open net pens had a significantly higher transcription of *angpt1* compared to the Preline group reared in S-CCS. Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens), has significant effect on the relative transcription of *angpt1*, **Ha2.7** is **accepted**.

- **H02.8:** Exposing Atlantic salmon to different rearing conditions (Preline S-CCS and open net pens), has no significant effect on the relative transcription of *pedf*, is **accepted**.

FUTURE PERSPECTIVES

Future studies should consider several key aspects to further our understanding of the effect of exercise at different velocities on Atlantic salmon. It is recommended to conduct multiple samplings over a longer time period during the seawater phase after the fish have exercised in freshwater. This approach enables the examination of beneficial impacts over a prolonged timeframe, as well as detecting if these benefits will fade or sustain after a longer time period without exercise.

In terms of understanding the differences between rearing systems, more studies are needed to spot a trend due to a lack of control over the environmental parameters. It would be interesting to further investigate whether seasonal differences have a significant effect on the relative transcription of *gata4* and *mef2c* and if this could be a consequence of different temperatures or light or any other parameter. In addition, there is an overall need for more studies on the pro-angiogenic genes, *angpt2* and the anti-angiogenic genes *angpt1* and *pedf* as these are mostly studied in mammals and not in fish. To gain such knowledge it would be beneficial to conduct multiple small-scale studies to test the effects of different environmental parameters, one by one, on the relative gene transcription. This could extend our knowledge in terms of what influences the genes.

REFERENCES

ADAMS, C.E., HUNTINGFORD, F.A., KRPAL, J., JOBLING, M., BURNETT, S.J., 1995. Exercise, agonistic behaviour and food acquisition in Arctic charr,Salvelinus alpinus. Environ Biol Fish 43, 213–218.

AFEWERKI, S., ASCHE, F., MISUND, B., THORVALDSEN, T., TVETERAS, R., 2023. Innovation in the Norwegian aquaculture industry. Reviews in Aquaculture 15, 759–771.

AGNISOLA, C., TOTA, B., 1994. Structure and function of the fish cardiac ventricle: flexibility and limitations. Cardioscience 5, 145–153.

AKAZAWA, H., KOMURO, I., 2003. Roles of Cardiac Transcription Factors in Cardiac Hypertrophy. Circulation Research 92, 1079–1088.

BALSEIRO, P., MOE, Ø., GAMLEM, I., SHIMIZU, M., SVEIER, H., NILSEN, T.O., KANEKO, N., EBBESSON, L., PEDROSA, C., TRONCI, V., NYLUND, A., HANDELAND, S.O., 2018. Comparison between Atlantic salmon Salmo salar post-smolts reared in open sea cages and in the Preline raceway semi-closed containment aquaculture system. Journal of Fish Biology 93, 567–579.

BANG JENSEN, B., QVILLER, L., TOFT, N., 2020. Spatio-temporal variations in mortality during the seawater production phase of Atlantic salmon (Salmo salar) in Norway. Journal of Fish Diseases 43, 445–457.

BANKHEAD, P., LOUGHREY, M.B., FERNÁNDEZ, J.A., DOMBROWSKI, Y., MCART, D.G., DUNNE, P.D., MCQUAID, S., GRAY, R.T., MURRAY, L.J., COLEMAN, H.G., JAMES, J.A., SALTO-TELLEZ, M., HAMILTON, P.W., 2017. QuPath: Open source software for digital pathology image analysis. Sci Rep 7, 16878.

BJØRNEVIK, M., KARLSEN, Ø., JOHNSTON, I.A., KIESSLING, A., 2003. Effect of sustained exercise on white muscle structure and flesh quality in farmed cod (Gadus morhua L.). Aquaculture Research 34, 55–64.

BONE, Q. & MOORE, R. 2008. *Biology of fishes*, Taylor & Francis.

BROCKLEBANK, J., RAVERTY, S., 2002. Sudden mortality caused by cardiac deformities following seining of preharvest farmed Atlantic salmon (Salmo salar) and by cardiomyopathy of postintraperitoneally vaccinated Atlantic salmon parr in British Columbia. Can Vet J 43, 129–130.

CASTRO, V., GRISDALE-HELLAND, B., HELLAND, S.J., KRISTENSEN, T., JØRGENSEN, S.M., HELGERUD, J., CLAIREAUX, G., FARRELL, A.P., KRASNOV, A., TAKLE, H., 2011. Aerobic training stimulates growth and promotes disease resistance in Atlantic salmon (Salmo salar). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 160, 278–290.

CASTRO, V., GRISDALE-HELLAND, B., HELLAND, S.J., TORGERSEN, J., KRISTENSEN, T., CLAIREAUX, G., FARRELL, A.P., TAKLE, H., 2013. Cardiac Molecular-Acclimation Mechanisms in Response to Swimming-Induced Exercise in Atlantic Salmon. PLOS ONE 8, e55056.

CRITERIA, I. OF M. (US) C. ON S.S.C.D., 2010. Ischemic Heart Disease, Cardiovascular Disability: Updating the Social Security Listings. National Academies Press (US).

DAVIE, P.S., WELLS, R.M.G., TETENS, V., 1986. Effects of sustained swimming on rainbow trout muscle structure, blood oxygen transport, and lactate dehydrogenase isozymes: Evidence for increased aerobic capacity of white muscle. Journal of Experimental Zoology 237, 159–171.

DAVISON, W., 1997. The Effects of Exercise Training on Teleost Fish, a Review of Recent Literature. Comparative Biochemistry and Physiology Part A: Physiology 117, 67–75.

DAVISON, W., HERBERT, N.A., 2013. Swimming-Enhanced Growth, in: Palstra, A.P., Planas, J.V. (Eds.), Swimming Physiology of Fish: Towards Using Exercise to Farm a

Fit Fish in Sustainable Aquaculture. Springer, Berlin, Heidelberg, pp. 177–202.
DUARTE, S., REIG, L., MASALÓ, I., BLANCO, M., OCA, J., 2011. Influence of tank geometry and flow pattern in fish distribution. Aquacultural Engineering 44, 48–54.
ELLMERS, L., SCOTT, N., PIUHOLA, J., MAEDA, N., SMITHIES, O., FRAMPTON, C., RICHARDS, M., CAMERON, V., 2007. Npr1-regulated gene pathways contributing to cardiac hypertrophy and fibrosis. Journal of molecular endocrinology 38, 245–57.
ESBAUGH, A.J., KRISTENSEN, T., TAKLE, H., GROSELL, M., 2014. The effects of sustained aerobic swimming on osmoregulatory pathways in Atlantic salmon Salmo salar smolts. Journal of Fish Biology 85, 1355–1368.
FAO (2022). Globefish highlights. International markets for fisheries and aquaculture products. First issue 2022.
FARRELL AP, JONES DR (1992) The heart. In: Hoar WS, Randall DJ, Farrell AP (eds) Fish physiology, Vol. XIIA. Academic Press, San Diego, CA, p 1–21.
FATHI, M., GHARAKHANLOU, R., REZAEI, R., 2020. The Changes of Heart miR-1 and miR-133 Expressions following Physiological Hypertrophy Due to Endurance Training. Cell J 22, 133–140.
FERNANDES, T., BARAÚNA, V.G., NEGRÃO, C.E., PHILLIPS, M.I., OLIVEIRA, E.M., 2015. Aerobic exercise training promotes physiological cardiac remodeling involving a set of microRNAs. American Journal of Physiology-Heart and Circulatory Physiology 309, H543–H552.
FISKERIDEPARTEMENTET. 2021. Norsk havbruksnæring. Regjeringen.no. 3.20.23.
FIVELSTAD, S., WAAGBØ, R., ZEITZ, S.F., HOSFELD, A.C.D., OLSEN, A.B., STEFANSSON, S., 2003. A major water quality problem in smolt farms: combined effects of carbon dioxide, reduced pH and aluminium on Atlantic salmon (Salmo salar L.) smolts: physiology and growth. Aquaculture 215, 339–357.
FODDAI, M., CARTER, C.G., HILDER, P.E., GURR, H., RUFF, N., 2022. Combined effects of elevated rearing temperature and dietary energy level on heart morphology and growth performance of Tasmanian Atlantic salmon (Salmo salar L.). Journal of Fish Diseases 45, 301–313.
FRISK, M., HØYLAND, M., ZHANG, L., VINDAS, M.A., ØVERLI, Ø., JOHANSEN, I.B., 2020. Intensive smolt production is associated with deviating cardiac morphology in Atlantic salmon (Salmo salar L.). Aquaculture 529, 735615.
FRY, F.E.J., 1971. 1 - The Effect of Environmental Factors on the Physiology of Fish, in: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology, Environmental Relations and Behavior. Academic Press, pp. 1–98.
GALLAUGHER, P.E., THORARENSEN, H., KIESSLING, A., FARRELL, A.P., 2001. Effects of high intensity exercise training on cardiovascular function,oxygen uptake, internal oxygen transport and osmotic balance in chinook salmon(oncorhynchus tshawytscha) during critical speed swimming. Journal of experimental biology 204, 2861–2872.
GAVIN, T.P., 2009. Basal and exercise-induced regulation of skeletal muscle capillarization. Exerc Sport Sci Rev 37.
GUO, J., 2014. Transcription: the epicenter of gene expression. J Zhejiang Univ Sci B 15, 409–411.
GUSTAFSSON, T., RUNDQVIST, H., NORRBOM, J., RULLMAN, E., JANSSON, E., SUNDBERG, C.J., 2007. The influence of physical training on the angiopoietin and VEGF-A systems in human skeletal muscle. Journal of Applied Physiology 103, 1012–1020.
HANDELAND, S.O., BJÖRNSSON, ARNESEN, A.M., STEFANSSON, S.O., 2003. Seawater adaptation and growth of post-smolt Atlantic salmon (Salmo salar) of wild

and farmed strains. Aquaculture 220, 367–384.
HANDELAND, S.O., IMSLAND, A.K., STEFANSSON, S.O., 2008. The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. Aquaculture 283, 36–42.
HANSEN, L.P., & QUINN, T.P. 1998. The marine phase of the Atlantic salmon (Salmo salar) life cycle, with comparisons to Pacific salmon. *Canadan Journal of Fisheries and Aquantic Sciences*, Vol. 55.
HARVEY, A.C., SOLBERG, M.F., TROIANOU, E., CARVALHO, G.R., TAYLOR, M.I., CREER, S., DYRHOVDEN, L., MATRE, I.H., GLOVER, K.A., 2016. Plasticity in growth of farmed and wild Atlantic salmon: is the increased growth rate of farmed salmon caused by evolutionary adaptations to the commercial diet? BMC Evolutionary Biology 16, 264.
HIGGINS, P.J., THORPE, J.E., 1990. Hyperplasia and hypertrophy in the growth of skeletal muscle in juvenile Atlantic salmon, Salmo salar L. Journal of Fish Biology 37, 505–519.
HOFFNAGLE, T.L., CARMICHAEL, R.W., KENIRY, P.J., 2006. The Effect of Moderately Increased and Variable Raceway Flow Rates on Juvenile Physiology, Survival, and Adult Return of Hatchery-Reared Chinook Salmon. Transactions of the American Fisheries Society 135, 1567–1577.
HOLASH, J., MAISONPIERRE, P.C., COMPTON, D., BOLAND, P., ALEXANDER, C.R., ZAGZAG, D., YANCOPOULOS, G.D., WIEGAND, S.J., 1999. Vessel Cooption, Regression, and Growth in Tumors Mediated by Angiopoietins and VEGF. Science 284, 1994–1998.
HOU, Q., FU, S., HUANG, T., LI, X., SHI, X., 2022. Effects of Aerobic Exercise Training on the Growth, Swimming Performance, Antipredation Ability and Immune Parameters of Juvenile Rock Carp (Procypris rabaudi). Animals 12, 257.
IBARZ, A., FELIP, O., FERNÁNDEZ-BORRÀS, J., MARTÍN-PÉREZ, M., BLASCO, J., TORRELLA, J.R., 2011. Sustained swimming improves muscle growth and cellularity in gilthead sea bream. J Comp Physiol B 181, 209–217.
INCARDONA, J.P., CARLS, M.G., HOLLAND, L., LINBO, T.L., BALDWIN, D.H., MYERS, M.S., PECK, K.A., TAGAL, M., RICE, S.D., SCHOLZ, N.L., 2015. Very low embryonic crude oil exposures cause lasting cardiac defects in salmon and herring. Sci Rep 5, 13499.
JOHANSSON, L.-H., TIMMERHAUS, G., AFANASYEV, S., JØRGENSEN, S.M., KRASNOV, A., 2016. Smoltification and seawater transfer of Atlantic salmon (Salmo salar L.) is associated with systemic repression of the immune transcriptome. Fish & Shellfish Immunology 58, 33–41.
JØRGENSEN, E.H., JOBLING, M., 1994. Feeding and growth of exercised and unexercised juvenile Atlantic salmon in freshwater, and performance after transfer to seawater. Aquacult Int 2, 154–164.
JØRGENSEN, E.H., JOBLING, M., 1993. The effects of exercise on growth, food utilisation and osmoregulatory capacity of juvenile Atlantic salmon, Salmo salar. Aquaculture 116, 233–246.
JUHANNA, I., ADIPUTRA, I., ADIATMIKA, I.P., MULIARTA, I.M., LINAWATI, M., GRIADHI, A., 2020. Exercise and cardiomyocyte regeneration. Bali Medical Journal 9, 947.
KIESSLING, A., HIGGS, D.A., DOSANJH, B.S., EALES, J.G., 1994. Influence of Sustained Exercise at Two Ration Levels on Growth and Thyroid Function of All-Female Chinook Salmon (*Oncorhynchus tshawytscha*) in Seawater. Can. J. Fish. Aquat. Sci. 51, 1975–1984.

KOMI, P., 2008. Strength and Power in Sport. John Wiley & Sons.
KRISTENSEN, T., URKE, H.A., POPPE, T.T., H.TAKLE, 2012. Atrial natriuretic peptide levels and heart morphology in migrating Atlantic salmon (Salmo salar) smolts from 4 rivers with different environmental conditions. Aquaculture, Smolt 2009: Proceedings of the 8th International Workshop on Smoltification. 362–363, 172–176.
KRYVI, H. P., TRYGVE 2016. *Fiskeanatomi*, Bergen, Fagbokforlaget.
LLOYD, P.G., PRIOR, B.M., YANG, H.T., TERJUNG, R.L., 2003. Angiogenic growth factor expression in rat skeletal muscle in response to exercise training. American Journal of Physiology-Heart and Circulatory Physiology 284, H1668–H1678.
MCCORMICK, S.D., SHRIMPTON, J.M., CAREY, J.B., O'DEA, M.F., SLOAN, K.E., MORIYAMA, S., BJÖRNSSON, B.T. 1998. Repeated acute stress reduces growth rate of Atlantic salmon parr and alters plasma levels of growth hormone, insulin-like growth factor I and cortisol. Aquaculture 168, 221–235.
NADER, M., YAQINUDDIN, A., KVIETYS, P. 2014. Cardiac Angiogenesis: Role of Cardiomyocytes and Macrophages and Possible Therapeutic Approaches. Current Angiogenesis 3, 11–18.
NILSEN, A., HAGEN, Ø., JOHNSEN, C.A., PRYTZ, H., ZHOU, B., NIELSEN, K.V. BJØRNEVIK, M., 2019. The importance of exercise: Increased water velocity improves growth of Atlantic salmon in closed cages. Aquaculture 501, 537–546.
NOAA. 2023. Sea Water. [Online]. Available: https://www.noaa.gov/jetstream/ocean/sea-water. [5.29.23].
NOBLE, E.C., GISMERVIK, K., IVERSEN, M.H., KOLAREVIC, J., NILSSON, J., STIEN, L.H., TURNBULL, J.F., n.d. Welfare Indicators for farmed Atlantic salmon - tools for assessing fish welfare.
OGATA, H.Y., OKU, H., 2000. Effects of Water Velocity on Growth Performance of Juvenile Japanese Flounder Paralichthys olivaceus. Journal of the World Aquaculture Society 31, 225–231.
OLIVEIRA, V.H.S., DEAN, K.R., QVILLER, L., KIRKEBY, C., BANG JENSEN, B., 2021. Factors associated with baseline mortality in Norwegian Atlantic salmon farming. Sci Rep 11, 14702.
OLSVIK, P.A., LIE, K.K., JORDAL, A.-E.O., NILSEN, T.O., HORDVIK, I., 2005. Evaluation of potential reference genes in real-time RT-PCR studies of Atlantic salmon. BMC Molecular Biology 6, 21.
PARAVATI, S., ROSANI, A., WARRINGTON, S.J., 2023. Physiology, Catecholamines, in: StatPearls. StatPearls Publishing, Treasure Island (FL).
PASCUAL-AHUIR, A., FITA-TORRÓ, J., PROFT, M., 2020. Capturing and Understanding the Dynamics and Heterogeneity of Gene Expression in the Living Cell. Int J Mol Sci 21, 8278.
PEARSON, M.P., SPRIET, L.L., STEVENS, E.D., 1990. Effect of sprint training on swim performance and white muscle metabolism during exercise and recovery in rainbow trout (Salmo Gairdneri). Journal of Experimental Biology 149, 45–60. https://doi.org/10.1242/jeb.149.1.45
PELSTER, B., SÄNGER, A.M., SIEGELE, M., SCHWERTE, T., 2003. Influence of swim training on cardiac activity, tissue capillarization, and mitochondrial density in muscle tissue of zebrafish larvae. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 285, R339–R347.
PERSSON, D., NØDTVEDT, A., AUNSMO, A. & STORMOEN, M. 2022. Analysing mortality patterns in salmon farming using daily cage registrations. *Journal of Fish Diseases*, 45: 335-347.
POPPE, T., JOHANSEN, R., GUNNES, G., TØRUD, B., 2003. Heart morphology in wild

and farmed Atlantic salmon Salmo salar and rainbow trout Oncorhynchus mykiss. Dis. Aquat. Org. 57, 103–108.
POPPE, T.T., TAKSDAL, T., 2000. Ventricular hypoplasia in farmed Atlantic salmon Salmo salar. Diseases of Aquatic Organisms 42, 35–40.
RADONIĆ, A., THULKE, S., MACKAY, I.M., LANDT, O., SIEGERT, W., NITSCHE, A., 2004. Guideline to reference gene selection for quantitative real-time PCR. Biochemical and Biophysical Research Communications 313, 856–862.
ROBINSON, N.A., TIMMERHAUS, G., BARANSKI, M., ANDERSEN, Ø., TAKLE, H., KRASNOV, A., 2017. Training the salmon's genes: influence of aerobic exercise, swimming performance and selection on gene expression in Atlantic salmon. BMC Genomics 18, 971.
RYCHLI, K., HUBER, K., WOJTA, J., 2009. Pigment epithelium-derived factor (PEDF) as a therapeutic target in cardiovascular disease. Expert Opinion on Therapeutic Targets 13, 1295–1302.
RYCHLI, K., KAUN, C., HOHENSINNER, P.J., DORFNER, A.J., PFAFFENBERGER, S., NIESSNER, A., BAUER, M., DIETL, W., PODESSER, B.K., MAURER, G., HUBER, K., WOJTA, J., 2010. The anti-angiogenic factor PEDF is present in the human heart and is regulated by anoxia in cardiac myocytes and fibroblasts. Journal of Cellular and Molecular Medicine 14, 198–205.
SHAHBANDEH, M. (2023) Salmon Industry - statistics & facts. [Online]. Available: https://www.statista.com/topics/7411/salmon-industry/#topicOverview [4.18.23].
SODERBERG, R.W., MEADE, J.W., REDELL, L.A., 1993. Growth, Survival, and Food Conversion of Atlantic Salmon Reared at Four Different Densities with Common Water Quality. The Progressive Fish-Culturist 55, 29–31.
SOLSTORM, F., SOLSTORM, D., OPPEDAL, F., FERNÖ, A., FRASER, T., OLSEN, R., 2015. Fast water currents reduce production performance of post-smolt Atlantic salmon Salmo salar. Aquacult. Environ. Interact. 7, 125–134.
SOMMERSET I, WALDE C S, BANG JENSEN B, WIIK-NIELSEN J, BORNØ G, OLIVEIRA VHS, HAUKAAS A OG BRUN E. 2022. Fiskehelserapporten 2021, Veterinærinstituttet, 2a/2022, 1890-3290.
STATISTICS NORWAY. 2017. Frå attåtnæring til milliardindustri. [Online]. Available: https://www.ssb.no/jord-skog-jakt-og-fiskeri/artikler-og-publikasjoner/fra-attatnaering-til-milliardindustri [4.18.23].
SUZUKI, Y.J., 2011. Cell signaling pathways for the regulation of GATA4 transcription factor: Implications for cell growth and apoptosis. Cellular Signalling 23, 1094–1099.
TAKLE, H., CASTRO, V., 2013. Molecular Adaptive Mechanisms in the Cardiac Muscle of Exercised Fish, in: Palstra, A.P., Planas, J.V. (Eds.), Swimming Physiology of Fish: Towards Using Exercise to Farm a Fit Fish in Sustainable Aquaculture. Springer, Berlin, Heidelberg, pp. 257–274.
THERMO SCIENTIFIC. 2012. Interpretation of Nucleic Acid 260/280 Ratios. 302-479.
THORARENSEN, H., GALLAUGHER, P.E., KIESSLING, A.K., FARRELL, A.P., 1993. Intestinal Blood Flow in Swimming Chinook Salmon Oncorhynchus Tshawytscha and the Effects of Haematocrit On Blood Flow Distribution. Journal of Experimental Biology 179, 115–129.
TIMMERHAUS, G., LAZADO, C.C., CABILLON, N.A.R., REITEN, B.K.M., JOHANSEN, L.-H., 2021. The optimum velocity for Atlantic salmon post-smolts in RAS is a compromise between muscle growth and fish welfare. Aquaculture 532, 736076.
TOTA, B., GATTUSO, A., 1996. Heart ventricle pumps in teleosts and elasmobranchs: A morphodynamic approach. Journal of Experimental Zoology 275, 162–171.
TOTLAND, G.K., KRYVI, H., JØDESTØL, K.A., CHRISTIANSEN, E.N., TANGERÅS,

A., SLINDE, E., 1987. Growth and composition of the swimming muscle of adult Atlantic salmon (Salmo salar L.) during long-term sustained swimming. Aquaculture 66, 299–313.
TSAI, Y.-C., LEE, C.-S., CHIU, Y.-W., KUO, H.-T., LEE, S.-C., HWANG, S.-J., KUO, M.-C., CHEN, H.-C., 2016. Angiopoietin-2, Angiopoietin-1 and subclinical cardiovascular disease in Chronic Kidney Disease. Sci Rep 6, 39400.
WALDROP, T., SUMMERFELT, S., MAZIK, P., GOOD, C., 2018. The effects of swimming exercise and dissolved oxygen on growth performance, fin condition and precocious maturation of early-rearing Atlantic salmon Salmo salar. Aquaculture Research 49, 801–808.
WALTON, R.G., FINLIN, B.S., MULA, J., LONG, D.E., ZHU, B., FRY, C.S., WESTGATE, P.M., LEE, J.D., BENNETT, T., KERN, P.A., PETERSON, C.A., 2015. Insulin-resistant subjects have normal angiogenic response to aerobic exercise training in skeletal muscle, but not in adipose tissue. Physiological Reports 3, e12415.
WANG, D.-Z., VALDEZ, M.R., MCANALLY, J., RICHARDSON, J., OLSON, E.N., 2001. The Mef2c gene is a direct transcriptional target of myogenic bHLH and MEF2 proteins during skeletal muscle development. Development 128, 4623–4633.
WANG, T., JOYCE, W., HICKS, J.W., 2019. Similitude in the cardiorespiratory responses to exercise across vertebrates. Current Opinion in Physiology, Exercise Physiology 10, 137–145.
WOODWARD, J.J., SMITH, L.S., 1985. Exercise training and the stress response in rainbow trout, Salmo gairdneri Richardson. Journal of Fish Biology 26, 435–447.
SPECIAL WORKING SESSION. *World Commission on Environment and Development*; Oxford University Press: London, UK, 1987
YOGATA, H., OKU, H., 2000. The effects of swimming exercise on growth and wholebody protein and fat contents of fed and unfed fingerling yellowtail. Fisheries science 66, 1100–1105.
YTRESTØYL, T., TAKLE, H., KOLAREVIC, J., CALABRESE, S., TIMMERHAUS, G., ROSSELAND, B.O., TEIEN, H.C., NILSEN, T.O., HANDELAND, S.O., STEFANSSON, S.O., EBBESSON, L.O.E., TERJESEN, B.F., 2020. Performance and welfare of Atlantic salmon, Salmo salar L. post-smolts in recirculating aquaculture systems: Importance of salinity and water velocity. Journal of the World Aquaculture Society 51, 373–392.
ZHAO, L., XU, L., YANG, Y., HE, Q., LIU, Q., LUO, J., LUO, W., ZHANG, X., YAN, T., YANG, S., 2023. Exercise training promotes growth through hypertrophy and enhances capillarization and antioxidant capacity in juvenile largemouth bass (Micropterus salmoides). Aquaculture 562, 738850.
ØVREBØ, T.K., BALSEIRO, P., IMSLAND, A.K.D., STEFANSSON, S.O., TVETERÅS, R., SVEIER, H., HANDELAND, S., 2022. Investigation of growth performance of post-smolt Atlantic salmon (Salmo salar L.) in semi closed containment system: A big-scale benchmark study. Aquaculture Research 53, 4178–4189.

APPENDIX I
THE CONSTANT CURRENT EXPERIMENT

MORPHOMETRIC DATA

MODEL SUMMARY

TABLE I. *Model summary output for the specific growth rate (SGR) from GLMM analysis utilizing gamma distribution (log-link function) with treatment (0.5 BL/s. 1.0 BL/s and 1.5 BL/s) and sampling time (S1. S2. S3. S4) as categorical variables.*

```
     AIC      BIC   logLik deviance df.resid
   -91.1    -44.7     59.5   -119.1      189

Random effects:

Conditional model:
 Groups Name        Variance Std.Dev.
 fTank  (Intercept) 0.000299 0.01729
Number of obs: 203, groups:  fTank, 6

Dispersion estimate for Gamma family (sigma^2): 0.0197

Conditional model:
                              Estimate Std. Error z value Pr(>|z|)
fTreatment0.5 BL/s             0.232209   0.051144   4.540 5.62e-06 ***
fTreatment1.0 BL/s             0.074534   0.051186   1.456 0.145352
fTreatment1.5 BL/s             0.193332   0.046070   4.196 2.71e-05 ***
fSamplingS2                    0.200623   0.058761   3.414 0.000640 ***
fSamplingS3                    0.121611   0.058759   2.070 0.038486 *
fSamplingS4                   -0.290834   0.058760  -4.950 7.44e-07 ***
fTreatment1.0 BL/s:fSamplingS2 0.115833   0.083109   1.394 0.163391
fTreatment1.5 BL/s:fSamplingS2 -0.001686  0.080402  -0.021 0.983273
fTreatment1.0 BL/s:fSamplingS3 0.166979   0.083122   2.009 0.044555 *
fTreatment1.5 BL/s:fSamplingS3 0.017537   0.080075   0.219 0.826649
fTreatment1.0 BL/s:fSamplingS4 0.305282   0.083755   3.645 0.000267 ***
fTreatment1.5 BL/s:fSamplingS4 0.157897   0.080076   1.972 0.048628 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TABLE II. *Model summary output for the relative growth (RG) from GLMM analysis utilizing gamma distribution (log-link function) with treatment (0.5 BL/s. 1.0 BL/s and 1.5 BL/s) and sampling time (S1. S2. S3. S4) as categorical variables.*

```
     AIC      BIC   logLik deviance df.resid
  1958.8   2005.4   -965.4   1930.8      193

Random effects:

Conditional model:
 Groups Name        Variance  Std.Dev.
 fTank  (Intercept) 0.0009024 0.03004
Number of obs: 207, groups:  fTank, 6

Dispersion estimate for Gamma family (sigma^2): 0.0489

Conditional model:
                              Estimate Std. Error z value Pr(>|z|)
fTreatment0.5 BL/s             3.76852    0.07672   49.12  < 2e-16 ***
fTreatment1.0 BL/s             3.52108    0.08106   43.44  < 2e-16 ***
fTreatment1.5 BL/s             3.66114    0.07305   50.12  < 2e-16 ***
fSamplingS2                    1.36522    0.08873   15.39  < 2e-16 ***
fSamplingS3                    2.10742    0.08875   23.74  < 2e-16 ***
fSamplingS4                   -0.01032    0.08880   -0.12  0.90748
fTreatment1.0 BL/s:fSamplingS2 0.17183    0.12818    1.34  0.18007
fTreatment1.5 BL/s:fSamplingS2 0.03260    0.12329    0.26  0.79146
fTreatment1.0 BL/s:fSamplingS3 0.28301    0.12822    2.21  0.02730 *
fTreatment1.5 BL/s:fSamplingS3 0.08983    0.12331    0.73  0.46632
fTreatment1.0 BL/s:fSamplingS4 0.33748    0.12823    2.63  0.00849 **
fTreatment1.5 BL/s:fSamplingS4 0.21474    0.12335    1.74  0.08170 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TABLE III. *Model summary output for the condition factor (K) from GLMM analysis utilizing gamma distribution (log-link function) with treatment (0.5 BL/s. 1.0 BL/s and 1.5 BL/s) and sampling time (S1. S2. S3. S4) as categorical variables.*

```
     AIC      BIC   logLik deviance df.resid
  -383.4   -336.7    205.7   -411.4      193

Random effects:

Conditional model:
 Groups Name        Variance  Std.Dev.
 fTank  (Intercept) 1.662e-12 1.289e-06
Number of obs: 207, groups:  fTank, 6

Dispersion estimate for Gamma family (sigma^2): 0.00538

Conditional model:
                              Estimate Std. Error z value Pr(>|z|)
fTreatment0.5 BL/s             0.14600    0.02446   5.969 2.38e-09 ***
fTreatment1.0 BL/s             0.07612    0.02594   2.934 0.003345 **
fTreatment1.5 BL/s             0.08524    0.02320   3.674 0.000239 ***
fSamplingS2                    0.03405    0.02945   1.156 0.247594
fSamplingS3                    0.08093    0.02945   2.748 0.006000 **
fSamplingS4                    0.11131    0.02945   3.779 0.000157 ***
fTreatment1.0 BL/s:fSamplingS2 0.06957    0.04254   1.635 0.101956
fTreatment1.5 BL/s:fSamplingS2 0.04247    0.04093   1.038 0.299423
fTreatment1.0 BL/s:fSamplingS3 0.05701    0.04254   1.340 0.180198
fTreatment1.5 BL/s:fSamplingS3 0.03652    0.04093   0.892 0.372208
fTreatment1.0 BL/s:fSamplingS4 0.06603    0.04254   1.552 0.120630
fTreatment1.5 BL/s:fSamplingS4 0.07238    0.04093   1.768 0.076995 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TABLE IV. *Model summary output for the cardiosomatic index (CSI) from GLMM analysis utilizing gamma distribution (log-link function) with treatment (0.5 BL/s. 1.0 BL/s and 1.5 BL/s) and sampling time (S1. S2. S3. S4) as categorical variables.*

```
     AIC      BIC   logLik deviance df.resid
 -1026.4   -979.7    527.2  -1054.4      193

Random effects:

Conditional model:
 Groups Name        Variance  Std.Dev.
 fTank  (Intercept) 8.584e-12 2.93e-06
Number of obs: 207, groups:  fTank, 6

Dispersion estimate for Gamma family (sigma^2): 0.0141

Conditional model:
                                Estimate Std. Error z value Pr(>|z|)
fTreatment0.5 BL/s             -1.745595   0.039615  -44.06  < 2e-16 ***
fTreatment1.0 BL/s             -1.645210   0.042018  -39.16  < 2e-16 ***
fTreatment1.5 BL/s             -1.717760   0.037582  -45.71  < 2e-16 ***
fSamplingS2                    -0.046739   0.047702   -0.98    0.327
fSamplingS3                    -0.185595   0.047702   -3.89 1.00e-04 ***
fSamplingS4                    -0.231762   0.047702   -4.86 1.18e-06 ***
fTreatment1.0 BL/s:fSamplingS2 -0.007545   0.068900   -0.11    0.913
fTreatment1.5 BL/s:fSamplingS2  0.105533   0.066288    1.59    0.111
fTreatment1.0 BL/s:fSamplingS3 -0.050118   0.068900   -0.73    0.467
fTreatment1.5 BL/s:fSamplingS3  0.039294   0.066288    0.59    0.553
fTreatment1.0 BL/s:fSamplingS4 -0.069694   0.068900   -1.01    0.312
fTreatment1.5 BL/s:fSamplingS4  0.051268   0.066288    0.77    0.439
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TABLE V. *Model summary output for the hematocrit (Hct) from GLMM analysis utilizing gamma distribution (log-link function) with treatment (0.5 BL/s. 1.0 BL/s and 1.5 BL/s) and sampling time (S1. S2. S3. S4) as categorical variables.*

```
     AIC      BIC   logLik deviance df.resid
  1164.4   1210.7   -568.2   1136.4      188

Random effects:

Conditional model:
 Groups Name        Variance  Std.Dev.
 fTank  (Intercept) 0.0004482 0.02117
Number of obs: 202, groups:  fTank, 6

Dispersion estimate for Gamma family (sigma^2): 0.00694

Conditional model:
                                Estimate Std. Error z value Pr(>|z|)
fTreatment0.5 BL/s              3.879652   0.031571  122.89  < 2e-16 ***
fTreatment1.0 BL/s              3.962157   0.033054  119.87  < 2e-16 ***
fTreatment1.5 BL/s              3.914860   0.030309  129.17  < 2e-16 ***
fSamplingS2                    -0.010571   0.033791   -0.31  0.75440
fSamplingS3                     0.001584   0.033726    0.05  0.96255
fSamplingS4                     0.003889   0.034440    0.11  0.91010
fTreatment1.0 BL/s:fSamplingS2 -0.061148   0.048564   -1.26  0.20798
fTreatment1.5 BL/s:fSamplingS2 -0.073504   0.046728   -1.57  0.11571
fTreatment1.0 BL/s:fSamplingS3 -0.090415   0.048512   -1.86  0.06235 .
fTreatment1.5 BL/s:fSamplingS3 -0.081836   0.046682   -1.75  0.07959 .
fTreatment1.0 BL/s:fSamplingS4 -0.127110   0.049017   -2.59  0.00951 **
fTreatment1.5 BL/s:fSamplingS4 -0.114890   0.047201   -2.43  0.01493 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TANK EFFECT

TABLE VI. *Tank effect for each morphometric parameter from the constant current experiment measured in SD.*

MORPHOMETRIC	*SD*
Condition factor (K)	1.29e-06
Specific growth rate (SGR)	0.02
Relative growth (RG)	0.03
Cardiosomatic index (CSI)	2.93e-06
Hematocrit (Hct)	0.02

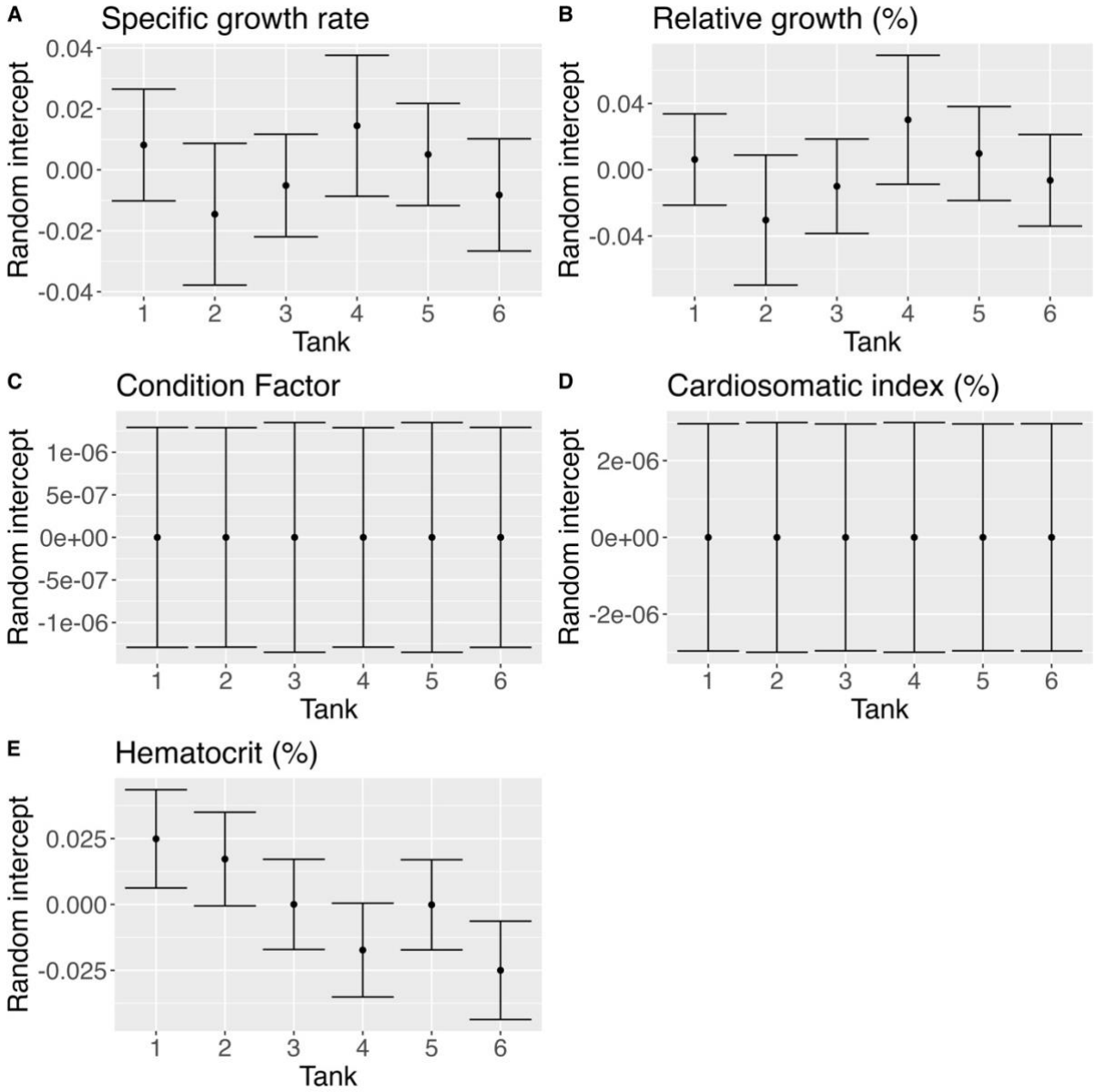

FIGURE I*. Turkey´s post-hoc test for the Tank effect for morphologic parameters. (A) Specific growth rate, (B) Relative growth, (C) Condition factor, (D) Cardiosomatic index, (E) Hematocrit. Tank 1 and 6 have a velocity of 0.5 BL/s, tank 2 and 4 have a velocity of 1.0 BL/s, tank 3 and 5 have a velocity of 1.5 BL/s. The plot displays the mean ± SD.*

TABLE VII. *Turkey´s post-hoc test for specific growth rate (SGR) across treatments for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Sampling	estimate	SE	z.ratio	p.value
(0.5 BL/s) - (1.0 BL/s)	S1	0.1577	0.0724	2.1792	0.0748
(0.5 BL/s) - (1.5 BL/s)	S1	0.0389	0.0688	0.5648	0.8388
(1.0 BL/s) - (1.5 BL/s)	S1	-0.1188	0.0689	-1.7251	0.1958
(0.5 BL/s) - (1.0 BL/s)	S2	0.0418	0.0477	0.8778	0.6544
(0.5 BL/s) - (1.5 BL/s)	S2	0.0406	0.0482	0.8413	0.6773
(1.0 BL/s) - (1.5 BL/s)	S2	-0.0013	0.0482	-0.0265	0.9996
(0.5 BL/s) - (1.0 BL/s)	S3	-0.0093	0.0477	-0.1952	0.9792
(0.5 BL/s) - (1.5 BL/s)	S3	0.0213	0.0477	0.4477	0.8954
(1.0 BL/s) - (1.5 BL/s)	S3	0.0306	0.0477	0.6429	0.7964
(0.5 BL/s) - (1.0 BL/s)	S4	-0.1476	0.0489	-3.0211	**0.0071****
(0.5 BL/s) - (1.5 BL/s)	S4	-0.1190	0.0477	-2.4967	**0.0335**
(1.0 BL/s) - (1.5 BL/s)	S4	0.0286	0.0489	0.5849	0.8282

TABLE VIII. *Turkey´s post-hoc test for specific growth rate across sampling points for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Treatment	estimate	SE	z.ratio	p.value
S1 - S2	0.5 BL/s	-0.2006	0.0588	-3.4142	**0.0036***
S1 - S3	0.5 BL/s	-0.1216	0.0588	-2.0696	0.1631
S1 - S4	0.5 BL/s	0.2908	0.0588	4.9495	**4.44e-06****
S2 - S3	0.5 BL/s	0.0790	0.0444	1.7788	0.2835
S2 - S4	0.5 BL/s	0.4915	0.0444	11.0621	**2.04e-14****
S3 - S4	0.5 BL/s	0.4124	0.0444	9.2846	**4.24e-14****
S1 - S2	1.0 BL/s	-0.3165	0.0588	-5.3836	**4.37e-07****
S1 - S3	1.0 BL/s	-0.2886	0.0588	-4.9080	**5.48e-06****
S1 - S4	1.0 BL/s	-0.0144	0.0597	-0.2421	0.9950
S2 - S3	1.0 BL/s	0.0279	0.0444	0.6273	0.9233
S2 - S4	1.0 BL/s	0.3020	0.0457	6.6102	**2.30e-10****
S3 - S4	1.0 BL/s	0.2741	0.0457	5.9963	**1.21e-08****
S1 - S2	1.5 BL/s	-0.1989	0.0549	-3.6251	**0.0016***
S1 - S3	1.5 BL/s	-0.1391	0.0544	-2.5578	0.0515
S1 - S4	1.5 BL/s	0.1329	0.0544	2.4437	0.0691
S2 - S3	1.5 BL/s	0.0598	0.0450	1.3286	0.5446
S2 - S4	1.5 BL/s	0.3319	0.0450	7.3744	**1.02e-12****
S3 - S4	1.5 BL/s	0.2721	0.0444	6.1256	**5.42e-09****

TABLE IX. *Turkey´s post-hoc test for relative growth across treatments for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Sampling	estimate	SE	z.ratio	p.value
(0.5 BL/s) - (1.0 BL/s)	S1	0.2474	0.1116	2.2165	0.0684
(0.5 BL/s) - (1.5 BL/s)	S1	0.1074	0.1059	1.0137	0.5682
(1.0 BL/s) - (1.5 BL/s)	S1	-0.1401	0.1091	-1.2835	0.4045
(0.5 BL/s) - (1.0 BL/s)	S2	0.0756	0.0761	0.9936	0.5809
(0.5 BL/s) - (1.5 BL/s)	S2	0.0748	0.0761	0.9828	0.5877
(1.0 BL/s) - (1.5 BL/s)	S2	-0.0008	0.0761	-0.0109	0.9999
(0.5 BL/s) - (1.0 BL/s)	S3	-0.0356	0.0761	-0.4675	0.8865
(0.5 BL/s) - (1.5 BL/s)	S3	0.0176	0.0761	0.2307	0.9711
(1.0 BL/s) - (1.5 BL/s)	S3	0.0531	0.0761	0.6982	0.7645
(0.5 BL/s) - (1.0 BL/s)	S4	-0.0900	0.0761	-1.1832	0.4632
(0.5 BL/s) - (1.5 BL/s)	S4	-0.1074	0.0761	-1.4109	0.3352
(1.0 BL/s) - (1.5 BL/s)	S4	-0.0173	0.0761	-0.2275	0.9719

TABLE X. *Turkey´s post-hoc test for relative growth across sampling points for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Treatment	estimate	SE	z.ratio	p.value
S1 - S2	0.5 BL/s	-1.3652	0.0887	-15.3859	**0*** **
S1 - S3	0.5 BL/s	-2.1074	0.0888	-23.7449	**0*** **
S1 - S4	0.5 BL/s	0.0103	0.0888	0.1162	0.9994
S2 - S3	0.5 BL/s	-0.7422	0.0699	-10.6180	**3.71E-14*** **
S2 - S4	0.5 BL/s	1.3755	0.0699	19.6708	**0*** **
S3 - S4	0.5 BL/s	2.1177	0.0699	30.2937	**0*** **
S1 - S2	1.0 BL/s	-1.5371	0.0925	-16.6186	**0*** **
S1 - S3	1.0 BL/s	-2.3904	0.0925	-25.8390	**0*** **
S1 - S4	1.0 BL/s	-0.3272	0.0925	-3.5375	**0.0023***
S2 - S3	1.0 BL/s	-0.8534	0.0699	-12.2087	**0*** **
S2 - S4	1.0 BL/s	1.2099	0.0699	17.3102	**0*** **
S3 - S4	1.0 BL/s	2.0633	0.0699	29.5166	**0*** **
S1 - S2	1.5 BL/s	-1.3978	0.0856	-16.3290	**0*** **
S1 - S3	1.5 BL/s	-2.1972	0.0856	-25.6677	**0*** **
S1 - S4	1.5 BL/s	-0.2044	0.0856	-2.3879	0.0794
S2 - S3	1.5 BL/s	-0.7994	0.0699	-11.4368	**0*** **
S2 - S4	1.5 BL/s	1.1934	0.0699	17.0721	**0*** **
S3 - S4	1.5 BL/s	1.9928	0.0699	28.5117	**0*** **

TABLE XI. *Turkey´s post-hoc test for the condition factor across treatments for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Sampling	estimate	SE	z.ratio	p.value
(0.5 BL/s) - (1.0 BL/s)	S1	0.0699	0.0357	1.9601	0.1222
(0.5 BL/s) - (1.5 BL/s)	S1	0.0608	0.0337	1.8024	0.1688
(1.0 BL/s) - (1.5 BL/s)	S1	-0.0091	0.0348	-0.2620	0.9629
(0.5 BL/s) - (1.0 BL/s)	S2	0.0003	0.0232	0.0135	0.9999
(0.5 BL/s) - (1.5 BL/s)	S2	0.0183	0.0232	0.7885	0.7101
(1.0 BL/s) - (1.5 BL/s)	S2	0.0180	0.0232	0.7750	0.7184
(0.5 BL/s) - (1.0 BL/s)	S3	0.0129	0.0232	0.5549	0.8440
(0.5 BL/s) - (1.5 BL/s)	S3	0.0242	0.0232	1.0448	0.5485
(1.0 BL/s) - (1.5 BL/s)	S3	0.0114	0.0232	0.4899	0.8761
(0.5 BL/s) - (1.0 BL/s)	S4	0.0039	0.0232	0.1663	0.9849
(0.5 BL/s) - (1.5 BL/s)	S4	-0.0116	0.0232	-0.5004	0.8711
(1.0 BL/s) - (1.5 BL/s)	S4	-0.0155	0.0232	-0.6666	0.7829

TABLE XII. *Turkey´s post-hoc test for the condition factor across sampling points for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Treatment	estimate	SE	z.ratio	p.value
S1 - S2	0.5 BL/s	-0.0341	0.0295	-1.1562	0.6545
S1 - S3	0.5 BL/s	-0.0809	0.0295	-2.7478	**0.0306**
S1 - S4	0.5 BL/s	-0.1113	0.0295	-3.7792	**0.0009****
S2 - S3	0.5 BL/s	-0.0469	0.0232	-2.0202	0.1805
S2 - S4	0.5 BL/s	-0.0773	0.0232	-3.3293	**0.0048***
S3 - S4	0.5 BL/s	-0.0304	0.0232	-1.3092	0.5570
S1 - S2	1.0 BL/s	-0.1036	0.0307	-3.3759	**0.0041***
S1 - S3	1.0 BL/s	-0.1379	0.0307	-4.4937	**4.14E-05*****
S1 - S4	1.0 BL/s	-0.1773	0.0307	-5.7772	**4.55E-08*****
S2 - S3	1.0 BL/s	-0.0343	0.0232	-1.4788	0.4504
S2 - S4	1.0 BL/s	-0.0737	0.0232	-3.1766	**0.0081***
S3 - S4	1.0 BL/s	-0.0394	0.0232	-1.6978	0.3248
S1 - S2	1.5 BL/s	-0.0765	0.0284	-2.6927	**0.0357**
S1 - S3	1.5 BL/s	-0.1174	0.0284	-4.1329	**0.0002****
S1 - S4	1.5 BL/s	-0.1837	0.0284	-6.4634	**6.14E-10*****
S2 - S3	1.5 BL/s	-0.0409	0.0232	-1.7638	0.2909
S2 - S4	1.5 BL/s	-0.1072	0.0232	-4.6182	**2.30E-05*****
S3 - S4	1.5 BL/s	-0.0662	0.0232	-2.8544	**0.0224**

TABLE XIII. *Turkey´s post-hoc test for the cardiosomatic index across treatments for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Sampling	estimate	SE	z.ratio	p.value
(0.5 BL/s) - (1.0 BL/s)	S1	-0.1004	0.0577	-1.7383	0.1909
(0.5 BL/s) - (1.5 BL/s)	S1	-0.0278	0.0546	-0.5098	0.8666
(1.0 BL/s) - (1.5 BL/s)	S1	0.0726	0.0564	1.2870	0.4025
(0.5 BL/s) - (1.0 BL/s)	S2	-0.0928	0.0376	-2.4703	**0.0360**
(0.5 BL/s) - (1.5 BL/s)	S2	-0.1334	0.0376	-3.5487	**0.0011***
(1.0 BL/s) - (1.5 BL/s)	S2	-0.0405	0.0376	-1.0784	0.5275
(0.5 BL/s) - (1.0 BL/s)	S3	-0.0503	0.0376	-1.3375	0.3743
(0.5 BL/s) - (1.5 BL/s)	S3	-0.0671	0.0376	-1.7862	0.1742
(1.0 BL/s) - (1.5 BL/s)	S3	-0.0169	0.0376	-0.4487	0.8950
(0.5 BL/s) - (1.0 BL/s)	S4	-0.0307	0.0376	-0.8166	0.6927
(0.5 BL/s) - (1.5 BL/s)	S4	-0.0791	0.0376	-2.1048	0.0888
(1.0 BL/s) - (1.5 BL/s)	S4	-0.0484	0.0376	-1.2882	0.4018

TABLE XIV. *Turkey´s post-hoc test for the cardiosomatic index across sampling points for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Treatment	estimate	SE	z.ratio	p.value
S1 - S2	0.5 BL/s	0.0467	0.0477	0.9798	0.7610
S1 - S3	0.5 BL/s	0.1856	0.0477	3.8907	**0.0006****
S1 - S4	0.5 BL/s	0.2318	0.0477	4.8585	**7.04e-06*****
S2 - S3	0.5 BL/s	0.1389	0.0376	3.6948	**0.0013***
S2 - S4	0.5 BL/s	0.1850	0.0376	4.9232	**5.07e-06*****
S3 - S4	0.5 BL/s	0.0462	0.0376	1.2284	0.6087
S1 - S2	1.0 BL/s	0.0543	0.0497	1.0919	0.6945
S1 - S3	1.0 BL/s	0.2357	0.0497	4.7412	**1.26e-05*****
S1 - S4	1.0 BL/s	0.3015	0.0497	6.0636	**7.98e-09*****
S2 - S3	1.0 BL/s	0.1814	0.0376	4.8276	**8.23e-06*****
S2 - S4	1.0 BL/s	0.2472	0.0376	6.5769	**2.88e-10*****
S3 - S4	1.0 BL/s	0.0657	0.0376	1.7493	0.2982
S1 - S2	1.5 BL/s	-0.0588	0.0460	-1.2774	0.5774
S1 - S3	1.5 BL/s	0.1463	0.0460	3.1785	**0.0081***
S1 - S4	1.5 BL/s	0.1805	0.0460	3.9214	**0.0005****
S2 - S3	1.5 BL/s	0.2051	0.0376	5.4573	**2.89e-07*****
S2 - S4	1.5 BL/s	0.2393	0.0376	6.3671	**1.16e-09*****
S3 - S4	1.5 BL/s	0.0342	0.0376	0.9098	0.7996

TABLE XV. *Turkey´s post-hoc test for hematocrit across treatments for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Sampling	estimate	SE	z.ratio	p.value
(0.5 BL/s) - (1.0 BL/s)	S1	-0.0825	0.0457	-1.8048	0.1680
(0.5 BL/s) - (1.5 BL/s)	S1	-0.0352	0.0438	-0.8045	0.7002
(1.0 BL/s) - (1.5 BL/s)	S1	0.0473	0.0448	1.0546	0.5424
(0.5 BL/s) - (1.0 BL/s)	S2	-0.0214	0.0341	-0.6264	0.8056
(0.5 BL/s) - (1.5 BL/s)	S2	0.0383	0.0341	1.1233	0.4997
(1.0 BL/s) - (1.5 BL/s)	S2	0.0597	0.0338	1.7647	0.1816
(0.5 BL/s) - (1.0 BL/s)	S3	0.0079	0.0341	0.2321	0.9707
(0.5 BL/s) - (1.5 BL/s)	S3	0.0466	0.0341	1.3684	0.3576
(1.0 BL/s) - (1.5 BL/s)	S3	0.0387	0.0338	1.1454	0.4861
(0.5 BL/s) - (1.0 BL/s)	S4	0.0446	0.0347	1.2844	0.4040
(0.5 BL/s) - (1.5 BL/s)	S4	0.0797	0.0347	2.2944	0.0566
(1.0 BL/s) - (1.5 BL/s)	S4	0.0351	0.0338	1.0376	0.5531

TABLE XVI. *Turkey´s post-hoc test for hematocrit across sampling points for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Treatment	estimate	SE	z.ratio	p.value
S1 - S2	0.5 BL/s	0.0106	0.0338	0.3128	0.9894
S1 - S3	0.5 BL/s	-0.0016	0.0337	-0.0470	1.0000
S1 - S4	0.5 BL/s	-0.0039	0.0344	-0.1129	0.9995
S2 - S3	0.5 BL/s	-0.0122	0.0271	-0.4488	0.9699
S2 - S4	0.5 BL/s	-0.0145	0.0278	-0.5197	0.9544
S3 - S4	0.5 BL/s	-0.0023	0.0279	-0.0827	0.9998
S1 - S2	1.0 BL/s	0.0717	0.0349	2.0569	0.1674
S1 - S3	1.0 BL/s	0.0888	0.0349	2.5477	0.0529
S1 - S4	1.0 BL/s	0.1232	0.0349	3.5342	**0.0023***
S2 - S3	1.0 BL/s	0.0171	0.0264	0.6493	0.9158
S2 - S4	1.0 BL/s	0.0515	0.0264	1.9543	0.2056
S3 - S4	1.0 BL/s	0.0344	0.0264	1.3050	0.5597
S1 - S2	1.5 BL/s	0.0841	0.0323	2.6049	**0.0454**
S1 - S3	1.5 BL/s	0.0803	0.0323	2.4864	0.0620
S1 - S4	1.5 BL/s	0.1110	0.0323	3.4390	**0.0033***
S2 - S3	1.5 BL/s	-0.0038	0.0264	-0.1451	0.9989
S2 - S4	1.5 BL/s	0.0269	0.0264	1.0217	0.7367
S3 - S4	1.5 BL/s	0.0307	0.0264	1.1668	0.6478

GENE DATA

MODEL SUMMARY

TABLE XVII. *Model summary output for the relative transcription of gata4 from GLMM analysis utilizing gamma distribution (log-link function) with treatment (0.5 BL/s. 1.0 BL/s and 1.5 BL/s) and sampling time (S1. S2. S3. S4) as categorical variables.*

```
     AIC      BIC   logLik deviance df.resid
 -1233.7  -1187.5    630.8  -1261.7      186

Random effects:

Conditional model:
 Groups Name        Variance Std.Dev.
 fTank  (Intercept) 9.3e-11  9.644e-06
Number of obs: 200, groups:  fTank, 6

Dispersion estimate for Gamma family (sigma^2): 0.0746

Conditional model:
                                Estimate Std. Error z value Pr(>|z|)
fSamplingS1                     -3.38052    0.09104  -37.13   <2e-16 ***
fSamplingS2                     -3.24187    0.06266  -51.74   <2e-16 ***
fSamplingS3                     -3.15459    0.06624  -47.62   <2e-16 ***
fSamplingS4                     -3.09839    0.06107  -50.73   <2e-16 ***
fTreatment1.0 BL/s              -0.07429    0.12875   -0.58    0.564
fTreatment1.5 BL/s               0.05475    0.12875    0.43    0.671
fSamplingS2:fTreatment1.0 BL/s   0.06459    0.15510    0.42    0.677
fSamplingS3:fTreatment1.0 BL/s  -0.09475    0.15846   -0.60    0.550
fSamplingS4:fTreatment1.0 BL/s  -0.11599    0.15504   -0.75    0.454
fSamplingS2:fTreatment1.5 BL/s  -0.11360    0.15567   -0.73    0.466
fSamplingS3:fTreatment1.5 BL/s  -0.15204    0.15714   -0.97    0.333
fSamplingS4:fTreatment1.5 BL/s  -0.09113    0.15637   -0.58    0.560
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TABLE XVIII. *Model summary output for the relative transcription of mef2c from GLMM analysis utilizing gamma distribution (log-link function) with treatment (0.5 BL/s. 1.0 BL/s and 1.5 BL/s) and sampling time (S1. S2. S3. S4) as categorical variables.*

```
     AIC      BIC   logLik deviance df.resid
 -1308.2  -1262.0    668.1  -1336.2      186

Random effects:

Conditional model:
 Groups Name        Variance  Std.Dev.
 fTank  (Intercept) 2.644e-10 1.626e-05
Number of obs: 200, groups:  fTank, 6

Dispersion estimate for Gamma family (sigma^2): 0.274

Conditional model:
                              Estimate Std. Error z value Pr(>|z|)
fSamplingS1                    -4.1663     0.1851  -22.51  < 2e-16 ***
fSamplingS2                    -3.8896     0.1201  -32.39  < 2e-16 ***
fSamplingS3                    -3.9300     0.1234  -31.85  < 2e-16 ***
fSamplingS4                    -4.2835     0.1201  -35.67  < 2e-16 ***
fTreatment1.0 BL/s              0.4044     0.2543    1.59  0.11185
fTreatment1.5 BL/s              0.5032     0.2543    1.98  0.04788 *
fSamplingS2:fTreatment1.0 BL/s -0.3520     0.3036   -1.16  0.24627
fSamplingS3:fTreatment1.0 BL/s -0.4319     0.3060   -1.41  0.15808
fSamplingS4:fTreatment1.0 BL/s -0.4444     0.3058   -1.45  0.14619
fSamplingS2:fTreatment1.5 BL/s -0.8038     0.3046   -2.64  0.00833 **
fSamplingS3:fTreatment1.5 BL/s -0.5553     0.3060   -1.81  0.06955 .
fSamplingS4:fTreatment1.5 BL/s -0.1831     0.3071   -0.60  0.55097
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TABLE XIX. *Model summary output for the relative transcription of angpt2_ch6 from GLMM analysis utilizing gamma distribution (log-link function) with treatment (0.5 BL/s. 1.0 BL/s and 1.5 BL/s) and sampling time (S1. S2. S3. S4) as categorical variables.*

```
     AIC      BIC   logLik deviance df.resid
 -2787.2  -2740.8   1407.6  -2815.2      190

Random effects:

Conditional model:
 Groups Name        Variance Std.Dev.
 fTank  (Intercept) 0.003888 0.06235
Number of obs: 204, groups:  fTank, 6

Dispersion estimate for Gamma family (sigma^2): 0.118

Conditional model:
                               Estimate Std. Error z value Pr(>|z|)
fSamplingS1                    -7.31072    0.12282  -59.52   <2e-16 ***
fSamplingS2                    -7.40584    0.09030  -82.02   <2e-16 ***
fSamplingS3                    -7.44607    0.09220  -80.76   <2e-16 ***
fSamplingS4                    -6.98829    0.08855  -78.92   <2e-16 ***
fTreatment1.0 BL/s             -0.07580    0.17362   -0.44    0.662
fTreatment1.5 BL/s             -0.00849    0.17358   -0.05    0.961
fSamplingS2:fTreatment1.0 BL/s  0.30642    0.19517    1.57    0.116
fSamplingS3:fTreatment1.0 BL/s  0.14096    0.19662    0.72    0.473
fSamplingS4:fTreatment1.0 BL/s  0.03914    0.19506    0.20    0.841
fSamplingS2:fTreatment1.5 BL/s  0.25532    0.19598    1.30    0.193
fSamplingS3:fTreatment1.5 BL/s  0.15850    0.19670    0.81    0.420
fSamplingS4:fTreatment1.5 BL/s  0.02226    0.19588    0.11    0.910
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TABLE XX. *Model summary output for the relative transcription of angpt2_ch20 from GLMM analysis utilizing gamma distribution (log-link function) with treatment (0.5 BL/s. 1.0 BL/s and 1.5 BL/s) and sampling time (S1. S2. S3. S4) as categorical variables.*

```
     AIC      BIC   logLik deviance df.resid
 -1197.4  -1150.9    612.7  -1225.4      190

Random effects:

Conditional model:
 Groups Name        Variance  Std.Dev.
 fTank  (Intercept) 3.567e-10 1.889e-05
Number of obs: 204, groups:  fTank, 6

Dispersion estimate for Gamma family (sigma^2): 0.291

Conditional model:
                               Estimate Std. Error z value Pr(>|z|)
fSamplingS1                    -3.70979    0.17970 -20.644   <2e-16 ***
fSamplingS2                    -3.82726    0.12368 -30.945   <2e-16 ***
fSamplingS3                    -3.63508    0.12707 -28.607   <2e-16 ***
fSamplingS4                    -3.46173    0.12055 -28.717   <2e-16 ***
fTreatment1.0 BL/s              0.02826    0.25414   0.111    0.911
fTreatment1.5 BL/s              0.10898    0.25414   0.429    0.668
fSamplingS2:fTreatment1.0 BL/s  0.08111    0.30614   0.265    0.791
fSamplingS3:fTreatment1.0 BL/s -0.29404    0.30865  -0.953    0.341
fSamplingS4:fTreatment1.0 BL/s -0.15741    0.30602  -0.514    0.607
fSamplingS2:fTreatment1.5 BL/s -0.14212    0.30727  -0.463    0.644
fSamplingS3:fTreatment1.5 BL/s -0.29417    0.30865  -0.953    0.341
fSamplingS4:fTreatment1.5 BL/s -0.17520    0.30727  -0.570    0.569
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TABLE XXI. *Model summary output for the relative transcription of angpt1 from GLMM analysis utilizing gamma distribution (log-link function) with treatment (0.5 BL/s. 1.0 BL/s and 1.5 BL/s) and sampling time (S1. S2. S3. S4) as categorical variables.*

```
     AIC      BIC   logLik deviance df.resid
 -1249.4  -1203.0    638.7  -1277.4      190

Random effects:

Conditional model:
 Groups Name        Variance  Std.Dev.
 fTank  (Intercept) 5.607e-09 7.488e-05
Number of obs: 204, groups:  fTank, 6

Dispersion estimate for Gamma family (sigma^2): 0.378

Conditional model:
                               Estimate Std. Error z value Pr(>|z|)
fSamplingS1                    -3.68150    0.20507 -17.953   <2e-16 ***
fSamplingS2                    -3.76485    0.14114 -26.675   <2e-16 ***
fSamplingS3                    -3.84032    0.14500 -26.484   <2e-16 ***
fSamplingS4                    -4.13334    0.13756 -30.047   <2e-16 ***
fTreatment1.0 BL/s              0.10071    0.29001   0.347   0.7284
fTreatment1.5 BL/s              0.32493    0.29001   1.120   0.2625
fSamplingS2:fTreatment1.0 BL/s  0.05455    0.34935   0.156   0.8759
fSamplingS3:fTreatment1.0 BL/s -0.28123    0.35221  -0.798   0.4246
fSamplingS4:fTreatment1.0 BL/s -0.19890    0.34922  -0.570   0.5690
fSamplingS2:fTreatment1.5 BL/s -0.39437    0.35064  -1.125   0.2607
fSamplingS3:fTreatment1.5 BL/s -0.66712    0.35221  -1.894   0.0582 .
fSamplingS4:fTreatment1.5 BL/s -0.44059    0.35064  -1.257   0.2089
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TABLE XXII. *Model summary output for the relative transcription of pedf from GLMM analysis utilizing gamma distribution (log-link function) with treatment (0.5 BL/s. 1.0 BL/s and 1.5 BL/s) and sampling time (S1. S2. S3. S4) as categorical variables.*

```
     AIC      BIC   logLik deviance df.resid
 -1444.2  -1397.8    736.1  -1472.2      188

Random effects:

Conditional model:
 Groups Name        Variance Std.Dev.
 fTank  (Intercept) 0.006046 0.07775
Number of obs: 202, groups:  fTank, 6

Dispersion estimate for Gamma family (sigma^2): 0.179

Conditional model:
                                Estimate Std. Error z value Pr(>|z|)
fSamplingS1                     -3.95371    0.15176  -26.05   <2e-16 ***
fSamplingS2                     -3.91262    0.11272  -34.71   <2e-16 ***
fSamplingS3                     -3.75003    0.11797  -31.79   <2e-16 ***
fSamplingS4                     -4.54100    0.10957  -41.44   <2e-16 ***
fTreatment1.0 BL/s              -0.03505    0.21475   -0.16    0.870
fTreatment1.5 BL/s               0.06286    0.21439    0.29    0.769
fSamplingS2:fTreatment1.0 BL/s -0.20292    0.24242   -0.84    0.403
fSamplingS3:fTreatment1.0 BL/s -0.15684    0.24491   -0.64    0.522
fSamplingS4:fTreatment1.0 BL/s  0.15086    0.24141    0.62    0.532
fSamplingS2:fTreatment1.5 BL/s -0.36656    0.24246   -1.51    0.131
fSamplingS3:fTreatment1.5 BL/s -0.38970    0.24428   -1.60    0.111
fSamplingS4:fTreatment1.5 BL/s -0.09235    0.24167   -0.38    0.702
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TANK EFFECT

TABLE XXIII. *Tank random effect standard derivation (SD) for each model in the constant current experiment.*

GENE	***SD***
gata4	9.64e-06
mef2c	1.63e-05
angpt2_ch6	0.062
angpt2_ch20	1.89e-05
angpt1	7.49e-05
pedf	0.08

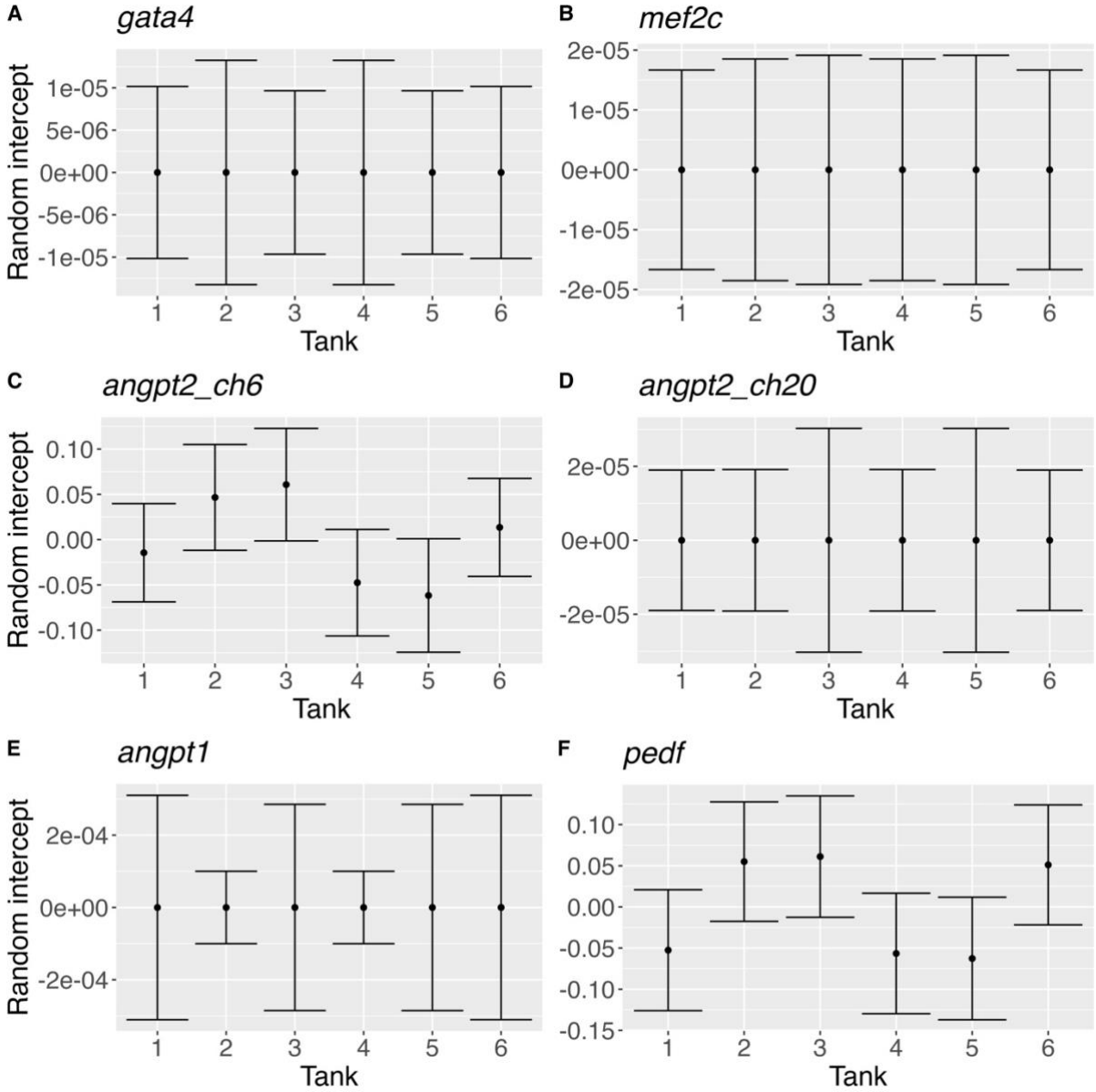

FIGURE II. *Turkey´s post-hoc test for the Tank effect for morphologic parameters. (A) gata4, (B) mef2c, (C) angpt2_ch6, (D) angpt2_ch20, (E) angpt1 (F) pedf. Tank 1 and 6 have a velocity of 0.5 BL/s, tank 2 and 4 have a velocity of 1.0 BL/s, tank 3 and 5 have a velocity of 1.5 BL/s. The plot displays the mean ± SD.*

TABLE XXIV. *Turkey´s post-hoc test for gata4 across treatments for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Sampling	estimate	SE	z.ratio	p.value
(0.5 BL/s) - (1.0 BL/s)	S1	0.0743	0.1287	0.5770	0.8324
(0.5 BL/s) - (1.5 BL/s)	S1	-0.0548	0.1287	-0.4253	0.9051
(1.0 BL/s) - (1.5 BL/s)	S1	-0.1290	0.1287	-1.0023	0.5754
(0.5 BL/s) - (1.0 BL/s)	S2	0.0097	0.0865	0.1122	0.9931
(0.5 BL/s) - (1.5 BL/s)	S2	0.0588	0.0875	0.6725	0.7795
(1.0 BL/s) - (1.5 BL/s)	S2	0.0491	0.0853	0.5759	0.8330
(0.5 BL/s) - (1.0 BL/s)	S3	0.1690	0.0924	1.8301	0.1598
(0.5 BL/s) - (1.5 BL/s)	S3	0.0973	0.0901	1.0798	0.5266
(1.0 BL/s) - (1.5 BL/s)	S3	-0.0718	0.0887	-0.8086	0.6977
(0.5 BL/s) - (1.0 BL/s)	S4	0.1903	0.0864	2.2031	0.0706
(0.5 BL/s) - (1.5 BL/s)	S4	0.0364	0.0887	0.4100	0.9115
(1.0 BL/s) - (1.5 BL/s)	S4	-0.1539	0.0887	-1.7343	0.1924

TABLE XXV. *Turkey´s post-hoc test for gata4 across sampling points for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Treatment	estimate	SE	z.ratio	p.value
S1 - S2	0.5 BL/s	-0.1386	0.1105	-1.2545	0.5920
S1 - S3	0.5 BL/s	-0.2259	0.1126	-2.0066	0.1854
S1 - S4	0.5 BL/s	-0.2821	0.1096	-2.5735	**0.0494**
S2 - S3	0.5 BL/s	-0.0873	0.0912	-0.9572	0.7737
S2 - S4	0.5 BL/s	-0.1435	0.0875	-1.6398	0.3562
S3 - S4	0.5 BL/s	-0.0562	0.0901	-0.6238	0.9245
S1 - S2	1.0 BL/s	-0.2032	0.1088	-1.8677	0.2420
S1 - S3	1.0 BL/s	-0.1312	0.1115	-1.1764	0.6418
S1 - S4	1.0 BL/s	-0.1661	0.1096	-1.5155	0.4281
S2 - S3	1.0 BL/s	0.0721	0.0877	0.8214	0.8444
S2 - S4	1.0 BL/s	0.0371	0.0853	0.4347	0.9725
S3 - S4	1.0 BL/s	-0.0350	0.0887	-0.3940	0.9793
S1 - S2	1.5 BL/s	-0.0250	0.1096	-0.2285	0.9958
S1 - S3	1.5 BL/s	-0.0739	0.1096	-0.6739	0.9070
S1 - S4	1.5 BL/s	-0.1910	0.1115	-1.7129	0.3168
S2 - S3	1.5 BL/s	-0.0488	0.0864	-0.5655	0.9423
S2 - S4	1.5 BL/s	-0.1659	0.0887	-1.8701	0.2410
S3 - S4	1.5 BL/s	-0.1171	0.0887	-1.3198	0.5503

TABLE XXVI. *Turkey´s post-hoc test for mef2c across treatments for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Sampling	estimate	SE	z.ratio	p.value
(0.5 BL/s) - (1.0 BL/s)	S1	-0.4044	0.2543	-1.5899	0.2499
(0.5 BL/s) - (1.5 BL/s)	S1	-0.5032	0.2543	-1.9784	0.1175
(1.0 BL/s) - (1.5 BL/s)	S1	-0.0988	0.2468	-0.4004	0.9154
(0.5 BL/s) - (1.0 BL/s)	S2	-0.0524	0.1657	-0.3162	0.9464
(0.5 BL/s) - (1.5 BL/s)	S2	0.3006	0.1677	1.7923	0.1721
(1.0 BL/s) - (1.5 BL/s)	S2	0.3530	0.1635	2.1583	0.0785
(0.5 BL/s) - (1.0 BL/s)	S3	0.0275	0.1701	0.1616	0.9857
(0.5 BL/s) - (1.5 BL/s)	S3	0.0521	0.1701	0.3062	0.9496
(1.0 BL/s) - (1.5 BL/s)	S3	0.0246	0.1655	0.1485	0.9879
(0.5 BL/s) - (1.0 BL/s)	S4	0.0400	0.1698	0.2356	0.9699
(0.5 BL/s) - (1.5 BL/s)	S4	-0.3201	0.1722	-1.8590	0.1508
(1.0 BL/s) - (1.5 BL/s)	S4	-0.3601	0.1722	-2.0914	0.0916

TABLE XXVII. *Turkey´s post-hoc test for mef2c across sampling points for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Treatment	estimate	SE	z.ratio	p.value
S1 - S2	0.5 BL/s	-0.2767	0.2206	-1.2543	0.5921
S1 - S3	0.5 BL/s	-0.2363	0.2224	-1.0623	0.7125
S1 - S4	0.5 BL/s	0.1172	0.2206	0.5312	0.9515
S2 - S3	0.5 BL/s	0.0405	0.1722	0.2350	0.9954
S2 - S4	0.5 BL/s	0.3939	0.1698	2.3194	0.0936
S3 - S4	0.5 BL/s	0.3534	0.1722	2.0529	0.1688
S1 - S2	1.0 BL/s	0.0753	0.2085	0.3609	0.9839
S1 - S3	1.0 BL/s	0.1956	0.2101	0.9310	0.7882
S1 - S4	1.0 BL/s	0.5616	0.2118	2.6514	**0.0400**
S2 - S3	1.0 BL/s	0.1204	0.1635	0.7359	0.8826
S2 - S4	1.0 BL/s	0.4863	0.1657	2.9344	0.0176
S3 - S4	1.0 BL/s	0.3660	0.1677	2.1825	0.1281
S1 - S2	1.5 BL/s	0.5270	0.2101	2.5085	0.0586
S1 - S3	1.5 BL/s	0.3190	0.2101	1.5184	0.4264
S1 - S4	1.5 BL/s	0.3003	0.2137	1.4054	0.4959
S2 - S3	1.5 BL/s	-0.2080	0.1655	-1.2568	0.5906
S2 - S4	1.5 BL/s	-0.2267	0.1701	-1.3331	0.5417
S3 - S4	1.5 BL/s	-0.0187	0.1701	-0.1098	0.9995

TABLE XXVIII. *Turkey´s post-hoc test for angpt2_ch6 across treatments for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	fSampling	estimate	SE	z.ratio	p.value
(0.5 BL/s) - (1.0 BL/s)	S1	0.0758	0.1736	0.4366	0.9003
(0.5 BL/s) - (1.5 BL/s)	S1	0.0085	0.1736	0.0489	0.9987
(1.0 BL/s) - (1.5 BL/s)	S1	-0.0673	0.1735	-0.3879	0.9204
(0.5 BL/s) - (1.0 BL/s)	S2	-0.2306	0.1254	-1.8397	0.1567
(0.5 BL/s) - (1.5 BL/s)	S2	-0.2468	0.1265	-1.9507	0.1247
(1.0 BL/s) - (1.5 BL/s)	S2	-0.0162	0.1242	-0.1305	0.9907
(0.5 BL/s) - (1.0 BL/s)	S3	-0.0652	0.1278	-0.5097	0.8666
(0.5 BL/s) - (1.5 BL/s)	S3	-0.1500	0.1279	-1.1730	0.4693
(1.0 BL/s) - (1.5 BL/s)	S3	-0.0848	0.1252	-0.6774	0.7766
(0.5 BL/s) - (1.0 BL/s)	S4	0.0367	0.1253	0.2926	0.9539
(0.5 BL/s) - (1.5 BL/s)	S4	-0.0138	0.1265	-0.1089	0.9935
(1.0 BL/s) - (1.5 BL/s)	S4	-0.0504	0.1265	-0.3987	0.9161

TABLE XXIX. *Turkey´s post-hoc test for angpt2_ch6 across sampling points for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Treatment	estimate	SE	z.ratio	p.value
S1 - S2	0.5 BL/s	0.0951	0.1392	0.6834	0.9035
S1 - S3	0.5 BL/s	0.1353	0.1402	0.9652	0.7692
S1 - S4	0.5 BL/s	-0.3224	0.1380	-2.3358	0.0900
S2 - S3	0.5 BL/s	0.0402	0.1130	0.3559	0.9846
S2 - S4	0.5 BL/s	-0.4175	0.1100	-3.7961	**0.0008****
S3 - S4	0.5 BL/s	-0.4578	0.1116	-4.1009	**0.0002****
S1 - S2	1.0 BL/s	-0.2113	0.1368	-1.5447	0.4107
S1 - S3	1.0 BL/s	-0.0056	0.1378	-0.0407	1.0000
S1 - S4	1.0 BL/s	-0.3616	0.1378	-2.6236	**0.0432**
S2 - S3	1.0 BL/s	0.2057	0.1073	1.9174	0.2206
S2 - S4	1.0 BL/s	-0.1503	0.1073	-1.4005	0.4990
S3 - S4	1.0 BL/s	-0.3560	0.1086	-3.2776	**0.0058***
S1 - S2	1.5 BL/s	-0.1602	0.1380	-1.1605	0.6518
S1 - S3	1.5 BL/s	-0.0232	0.1380	-0.1678	0.9983
S1 - S4	1.5 BL/s	-0.3447	0.1390	-2.4793	0.0632
S2 - S3	1.5 BL/s	0.1370	0.1086	1.2621	0.5871
S2 - S4	1.5 BL/s	-0.1845	0.1100	-1.6768	0.3360
S3 - S4	1.5 BL/s	-0.3215	0.1100	-2.9230	**0.0182**

TABLE XXX. *Turkey´s post-hoc test for angpt2_ch20 across treatments for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Sampling	estimate	SE	z.ratio	p.value
(0.5 BL/s) - (1.0 BL/s)	S1	-0.0283	0.2541	-0.1112	0.9932
(0.5 BL/s) - (1.5 BL/s)	S1	-0.1090	0.2541	-0.4288	0.9036
(1.0 BL/s) - (1.5 BL/s)	S1	-0.0807	0.2541	-0.3176	0.9459
(0.5 BL/s) - (1.0 BL/s)	S2	-0.1094	0.1707	-0.6407	0.7976
(0.5 BL/s) - (1.5 BL/s)	S2	0.0331	0.1727	0.1919	0.9799
(1.0 BL/s) - (1.5 BL/s)	S2	0.1425	0.1684	0.8461	0.6743
(0.5 BL/s) - (1.0 BL/s)	S3	0.2658	0.1752	1.5175	0.2826
(0.5 BL/s) - (1.5 BL/s)	S3	0.1852	0.1752	1.0573	0.5407
(1.0 BL/s) - (1.5 BL/s)	S3	-0.0806	0.1705	-0.4728	0.8841
(0.5 BL/s) - (1.0 BL/s)	S4	0.1292	0.1705	0.7576	0.7290
(0.5 BL/s) - (1.5 BL/s)	S4	0.0662	0.1727	0.3834	0.9222
(1.0 BL/s) - (1.5 BL/s)	S4	-0.0629	0.1727	-0.3644	0.9294

TABLE XXXI. *Turkey´s post-hoc test for angpt2_ch20 across sampling points for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Treatment	estimate	SE	z.ratio	p.value
S1 - S2	0.5 BL/s	0.1175	0.2181	0.5384	0.9496
S1 - S3	0.5 BL/s	-0.0747	0.2201	-0.3395	0.9865
S1 - S4	0.5 BL/s	-0.2481	0.2164	-1.1464	0.6607
S2 - S3	0.5 BL/s	-0.1922	0.1773	-1.0838	0.6995
S2 - S4	0.5 BL/s	-0.3655	0.1727	-2.1164	0.1478
S3 - S4	0.5 BL/s	-0.1734	0.1752	-0.9897	0.7553
S1 - S2	1.0 BL/s	0.0364	0.2148	0.1692	0.9983
S1 - S3	1.0 BL/s	0.2193	0.2164	1.0136	0.7415
S1 - S4	1.0 BL/s	-0.0907	0.2164	-0.4190	0.9753
S2 - S3	1.0 BL/s	0.1830	0.1684	1.0863	0.6979
S2 - S4	1.0 BL/s	-0.1270	0.1684	-0.7540	0.8750
S3 - S4	1.0 BL/s	-0.3100	0.1705	-1.8183	0.2646
S1 - S2	1.5 BL/s	0.2596	0.2164	1.1996	0.6271
S1 - S3	1.5 BL/s	0.2195	0.2164	1.0142	0.7411
S1 - S4	1.5 BL/s	-0.0729	0.2181	-0.3340	0.9872
S2 - S3	1.5 BL/s	-0.0401	0.1705	-0.2354	0.9954
S2 - S4	1.5 BL/s	-0.3325	0.1727	-1.9249	0.2175
S3 - S4	1.5 BL/s	-0.2923	0.1727	-1.6926	0.3275

TABLE XXXII. *Turkey´s post-hoc test for angpt1 across treatments for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Sampling	estimate	SE	z.ratio	p.value
(0.5 BL/s) - (1.0 BL/s)	S1	-0.1007	0.2900	-0.3473	0.9357
(0.5 BL/s) - (1.5 BL/s)	S1	-0.3249	0.2900	-1.1204	0.5014
(1.0 BL/s) - (1.5 BL/s)	S1	-0.2242	0.2900	-0.7732	0.7195
(0.5 BL/s) - (1.0 BL/s)	S2	-0.1553	0.1948	-0.7971	0.7048
(0.5 BL/s) - (1.5 BL/s)	S2	0.0694	0.1971	0.3523	0.9339
(1.0 BL/s) - (1.5 BL/s)	S2	0.2247	0.1922	1.1690	0.4718
(0.5 BL/s) - (1.0 BL/s)	S3	0.1805	0.1999	0.9032	0.6383
(0.5 BL/s) - (1.5 BL/s)	S3	0.3422	0.1999	1.7121	0.2006
(1.0 BL/s) - (1.5 BL/s)	S3	0.1617	0.1945	0.8311	0.6837
(0.5 BL/s) - (1.0 BL/s)	S4	0.0982	0.1945	0.5048	0.8690
(0.5 BL/s) - (1.5 BL/s)	S4	0.1157	0.1971	0.5869	0.8272
(1.0 BL/s) - (1.5 BL/s)	S4	0.0175	0.1971	0.0886	0.9957

TABLE XXXIII. *Turkey´s post-hoc test for angpt1 across sampling points for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Treatment	estimate	SE	z.ratio	p.value
S1 - S2	0.5 BL/s	0.0834	0.2489	0.3348	0.9871
S1 - S3	0.5 BL/s	0.1588	0.2512	0.6323	0.9216
S1 - S4	0.5 BL/s	0.4518	0.2469	1.8298	0.2592
S2 - S3	0.5 BL/s	0.0755	0.2024	0.3729	0.9823
S2 - S4	0.5 BL/s	0.3685	0.1971	1.8697	0.2412
S3 - S4	0.5 BL/s	0.2930	0.1999	1.4660	0.4582
S1 - S2	1.0 BL/s	0.0288	0.2451	0.1175	0.9994
S1 - S3	1.0 BL/s	0.4400	0.2469	1.7820	0.2820
S1 - S4	1.0 BL/s	0.6507	0.2469	2.6353	0.0418
S2 - S3	1.0 BL/s	0.4112	0.1922	2.1395	0.1407
S2 - S4	1.0 BL/s	0.6219	0.1922	3.2357	0.0067
S3 - S4	1.0 BL/s	0.2107	0.1945	1.0830	0.6999
S1 - S2	1.5 BL/s	0.4777	0.2469	1.9346	0.2135
S1 - S3	1.5 BL/s	0.8259	0.2469	3.3448	0.0046
S1 - S4	1.5 BL/s	0.8924	0.2489	3.5849	0.0019
S2 - S3	1.5 BL/s	0.3482	0.1945	1.7900	0.2781
S2 - S4	1.5 BL/s	0.4147	0.1971	2.1042	0.1517
S3 - S4	1.5 BL/s	0.0665	0.1971	0.3374	0.9868

TABLE XXXIV. *Turkey´s post-hoc test for pedf across treatments for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Sampling	estimate	SE	z.ratio	p.value
(0.5 BL/s) - (1.0 BL/s)	S1	0.0351	0.2147	0.1632	0.9854
(0.5 BL/s) - (1.5 BL/s)	S1	-0.0629	0.2144	-0.2932	0.9537
(1.0 BL/s) - (1.5 BL/s)	S1	-0.0979	0.2145	-0.4564	0.8915
(0.5 BL/s) - (1.0 BL/s)	S2	0.2380	0.1559	1.5263	0.2785
(0.5 BL/s) - (1.5 BL/s)	S2	0.3037	0.1571	1.9330	0.1294
(1.0 BL/s) - (1.5 BL/s)	S2	0.0657	0.1535	0.4281	0.9039
(0.5 BL/s) - (1.0 BL/s)	S3	0.1919	0.1635	1.1734	0.4691
(0.5 BL/s) - (1.5 BL/s)	S3	0.3268	0.1615	2.0235	0.1066
(1.0 BL/s) - (1.5 BL/s)	S3	0.1350	0.1569	0.8600	0.6656
(0.5 BL/s) - (1.0 BL/s)	S4	-0.1158	0.1550	-0.7469	0.7355
(0.5 BL/s) - (1.5 BL/s)	S4	0.0295	0.1564	0.1885	0.9806
(1.0 BL/s) - (1.5 BL/s)	S4	0.1453	0.1564	0.9288	0.6221

TABLE XXXV. *Turkey´s post-hoc test for pedf across sampling points for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	Treatment	estimate	SE	z.ratio	p.value
S1 - S2	0.5 BL/s	-0.0411	0.1731	-0.2374	0.9953
S1 - S3	0.5 BL/s	-0.2037	0.1748	-1.1651	0.6490
S1 - S4	0.5 BL/s	0.5873	0.1705	3.4454	**0.0032***
S2 - S3	0.5 BL/s	-0.1626	0.1454	-1.1180	0.6784
S2 - S4	0.5 BL/s	0.6284	0.1362	4.6147	**2.34e-05****
S3 - S4	0.5 BL/s	0.7910	0.1415	5.5913	**1.35e-07****
S1 - S2	1.0 BL/s	0.1618	0.1691	0.9571	0.7738
S1 - S3	1.0 BL/s	-0.0468	0.1714	-0.2732	0.9929
S1 - S4	1.0 BL/s	0.4364	0.1706	2.5589	0.0514
S2 - S3	1.0 BL/s	-0.2087	0.1350	-1.5453	0.4103
S2 - S4	1.0 BL/s	0.2746	0.1323	2.0751	0.1613
S3 - S4	1.0 BL/s	0.4833	0.1370	3.5287	**0.0024***
S1 - S2	1.5 BL/s	0.3255	0.1701	1.9139	0.2221
S1 - S3	1.5 BL/s	0.1860	0.1704	1.0919	0.6945
S1 - S4	1.5 BL/s	0.6796	0.1714	3.9660	**0.0004****
S2 - S3	1.5 BL/s	-0.1394	0.1341	-1.0401	0.7258
S2 - S4	1.5 BL/s	0.3542	0.1357	2.6100	**0.0448**
S3 - S4	1.5 BL/s	0.4936	0.1360	3.6298	**0.0016***

APPENDIX II
PRODUCTION GROUP STUDY

MORPHOMETRIC DATA

MODEL SUMMARY

TABLE I. *Model summary output for the mean weight from GLM analysis utilizing gamma distribution (log-link function) with rearing facility groups (Baseline. Preline and Reference) as categorical variables.*

```
Deviance Residuals:
     Min        1Q    Median        3Q       Max
-0.52071  -0.11853  -0.01068   0.06646   0.60799

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
fSamplingBaseline   4.82775    0.04808   100.4   <2e-16 ***
fSamplingPreline    6.48996    0.04808   135.0   <2e-16 ***
fSamplingReference  6.42231    0.04932   130.2   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Gamma family taken to be 0.0462242)

    Null deviance: 53910.9158  on 59  degrees of freedom
Residual deviance:     2.4722  on 56  degrees of freedom
  (1 observation deleted due to missingness)
AIC: 682.96

Number of Fisher Scoring iterations: 4
```

TABLE II. *Model summary output for the mean length from GLM analysis utilizing gamma distribution (log-link function) with rearing facility groups (Baseline. Preline and Reference) as categorical variables.*

```
Deviance Residuals:
     Min        1Q    Median        3Q       Max
-0.13155  -0.03371  -0.00350   0.03213   0.17395

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
fSamplingBaseline   3.09671    0.01354   228.7   <2e-16 ***
fSamplingPreline    3.62461    0.01354   267.7   <2e-16 ***
fSamplingReference  3.60957    0.01354   266.6   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Gamma family taken to be 0.00366663)

    Null deviance: 3330.37027  on 60  degrees of freedom
Residual deviance:    0.20552  on 57  degrees of freedom
AIC: 250.74

Number of Fisher Scoring iterations: 3
```

TABLE III. *Model summary output for the condition factor (K) from GLM analysis utilizing gamma distribution (log-link function) with rearing facility groups (Baseline. Preline and Reference) as categorical variables.*

```
Deviance Residuals:
      Min         1Q     Median         3Q        Max
-0.151743  -0.038526  -0.005067   0.038773   0.210185

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
fSamplingBaseline   0.13215    0.01529   8.643 6.84e-12 ***
fSamplingPreline    0.20662    0.01529  13.514  < 2e-16 ***
fSamplingReference  0.18785    0.01569  11.975  < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Gamma family taken to be 0.004675497)

    Null deviance: 2.25438  on 59  degrees of freedom
Residual deviance: 0.25883  on 56  degrees of freedom
  (1 observation deleted due to missingness)
AIC: -124.41

Number of Fisher Scoring iterations: 3
```

TABLE IV. *Model summary output for the cadiosomatic index (CSI) from GLM analysis utilizing gamma distribution (log-link function) with rearing facility groups (Baseline. Preline and Reference) as categorical variables.*

```
Deviance Residuals:
     Min        1Q    Median        3Q       Max
-0.23036  -0.07621  -0.01627   0.07853   0.29190

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
fSamplingBaseline  -1.72964    0.02524  -68.53   <2e-16 ***
fSamplingPreline   -2.00277    0.02524  -79.35   <2e-16 ***
fSamplingReference -1.93778    0.02589  -74.83   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Gamma family taken to be 0.01273953)

    Null deviance: 123.6008  on 59  degrees of freedom
Residual deviance:   0.7037  on 56  degrees of freedom
  (1 observation deleted due to missingness)
AIC: -309.39

Number of Fisher Scoring iterations: 4
```

SOMATIC GROWTH PARAMETERS

TABLE V. *Turkey´s post-hoc test for average weight across rearing facilities for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	estimate	SE	t.ratio	p.value
Baseline - Preline	-1.6622	0.0680	-24.4485	**7.31e-12*** **
Baseline - Reference	-1.5946	0.0689	-23.1507	**7.31e-12*** **
Preline - Reference	0.0677	0.0689	0.9823	0.5910

TABLE VI. *Turkey´s post-hoc test for average length across rearing facilities for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	estimate	SE	t.ratio	p.value
Baseline - Preline	-0.5279	0.0191	-27.5688	**1.12e-11*** **
Baseline - Reference	-0.5129	0.0191	-26.7832	**1.12e-11*** **
Preline - Reference	0.0150	0.0191	0.7855	0.7134

TABLE VII *Turkey´s post-hoc test for Condition factor (K) across rearing facilities for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	estimate	SE	t.ratio	p.value
Baseline - Preline	-0.0745	0.0216	-3.4440	**0.0031***
Baseline - Reference	-0.0557	0.0219	-2.5426	**0.0362**
Preline - Reference	0.0188	0.0219	0.8570	0.6694

CARDIOVASCULAR PARAMETERS

TABLE VIII *Turkey´s post-hoc test for cardiosomatic index across rearing facilities for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	estimate	SE	t.ratio	p.value
Baseline - Preline	0.2731	0.0357	7.6524	**8.73e-10*** **
Baseline - Reference	0.2081	0.0362	5.7563	**1.12e-06*** **
Preline - Reference	-0.0650	0.0362	-1.7973	0.1798

GENE DATA

MODEL SUMMARY

TABLE IX. *Model summary output for the relative transcription of gata4 from GLM analysis utilizing gamma distribution (log-link function) with rearing facility groups (Baseline. Preline and Reference) as categorical variables.*

```
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.5843  -0.1790   0.0169   0.1220   0.3372

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
fSamplingBaseline  -3.23221    0.04562  -70.86   <2e-16 ***
fSamplingPreline   -3.06392    0.04328  -70.80   <2e-16 ***
fSamplingReference -2.68406    0.04440  -60.45   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Gamma family taken to be 0.03745675)

    Null deviance: 234.9328  on 57  degrees of freedom
Residual deviance:   2.1383  on 54  degrees of freedom
AIC: -360.07

Number of Fisher Scoring iterations: 4
```

TABLE X. *Model summary output for the relative transcription of mef2c from GLM analysis utilizing gamma distribution (log-link function) with rearing facility groups (Baseline. Preline and Reference) as categorical variables.*

```
Deviance Residuals:
     Min        1Q    Median        3Q       Max
-1.61421  -0.44338  -0.05982   0.26723   1.12378

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
fSamplingBaseline   -4.6344     0.1232  -37.61   <2e-16 ***
fSamplingPreline    -4.1799     0.1136  -36.80   <2e-16 ***
fSamplingReference  -3.5515     0.1166  -30.47   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Gamma family taken to be 0.2580999)

    Null deviance: 365.031  on 56  degrees of freedom
Residual deviance:  15.277  on 53  degrees of freedom
AIC: -378.28

Number of Fisher Scoring iterations: 5
```

TABLE XI. *Model summary output for the relative transcription of angpt2_ch6 from GLM analysis utilizing gamma distribution (log-link function) with rearing facility groups (Baseline. Preline and Reference) as categorical variables.*

```
Deviance Residuals:
     Min        1Q    Median        3Q       Max
-0.88737  -0.17099  -0.00435   0.14117   0.43746

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
fSamplingBaseline  -7.49239    0.06096  -122.9   <2e-16 ***
fSamplingPreline   -7.34114    0.05620  -130.6   <2e-16 ***
fSamplingReference -6.90535    0.05620  -122.9   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Gamma family taken to be 0.06317947)

    Null deviance: 714.554  on 57  degrees of freedom
Residual deviance:   3.868  on 54  degrees of freedom
AIC: -811.41

Number of Fisher Scoring iterations: 4
```

TABLE XII. *Model summary output for the relative transcription of angpt2_ch20 from GLM analysis utilizing gamma distribution (log-link function) with rearing facility groups (Baseline. Preline and Reference) as categorical variables.*

```
Deviance Residuals:
     Min        1Q    Median        3Q       Max
-1.67363  -0.38145   0.04041   0.24617   0.80989

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
fSamplingBaseline  -3.80292    0.10427  -36.47   <2e-16 ***
fSamplingPreline   -4.17478    0.09892  -42.20   <2e-16 ***
fSamplingReference -3.74128    0.09892  -37.82   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Gamma family taken to be 0.1957164)

    Null deviance: 355.890  on 58  degrees of freedom
Residual deviance:  15.975  on 55  degrees of freedom
AIC: -369.06

Number of Fisher Scoring iterations: 5
```

TABLE XIII. *Model summary output for the relative transcription of angpt1 from GLM analysis utilizing gamma distribution (log-link function) with rearing facility groups (Baseline. Preline and Reference) as categorical variables.*

```
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.1367  -0.4832  -0.1261   0.2463   1.2645

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
fSamplingBaseline   -3.7937     0.1329  -28.55   <2e-16 ***
fSamplingPreline    -4.1900     0.1261  -33.24   <2e-16 ***
fSamplingReference  -3.7424     0.1261  -29.69   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Gamma family taken to be 0.3178227)

    Null deviance: 356.735  on 58  degrees of freedom
Residual deviance:  16.502  on 55  degrees of freedom
AIC: -367.93

Number of Fisher Scoring iterations: 5
```

TABLE XIV. *Model summary output for the relative transcription of pedf from GLM analysis utilizing gamma distribution (log-link function) with rearing facility groups (Baseline. Preline and Reference) as categorical variables.*

```
Deviance Residuals:
     Min        1Q    Median        3Q       Max
-0.95389  -0.40617  -0.03091   0.34514   0.96838

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
fSamplingBaseline  -4.68143    0.10416  -44.94   <2e-16 ***
fSamplingPreline   -4.39296    0.09882  -44.46   <2e-16 ***
fSamplingReference -4.55472    0.09882  -46.09   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Gamma family taken to be 0.1952906)

    Null deviance: 422.634  on 58  degrees of freedom
Residual deviance:  10.946  on 55  degrees of freedom
AIC: -459.68

Number of Fisher Scoring iterations: 4
```

HYPERTROPHIC GROWTH

TABLE XV *Turkey´s post-hoc test for gata4 across rearing facilities for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	estimate	SE	t.ratio	p.value
Baseline - Preline	-0.1683	0.0629	-2.6764	**0.0262**
Baseline - Reference	-0.5481	0.0637	-8.6108	**3.14e-11*** **
Preline - Reference	-0.3799	0.0620	-6.1265	**3.18e-07*** **

TABLE XVI. *Turkey´s post-hoc test for mef2c across rearing facilities for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	estimate	SE	t.ratio	p.value
Baseline - Preline	-0.4544	0.1676	-2.7115	**0.0241**
Baseline - Reference	-1.0829	0.1696	-6.3845	**1.31e-07*** **
Preline - Reference	-0.6284	0.1628	-3.8612	**0.0009** **

PRO-ANGIOGENIC PARAMETERS

TABLE XVII. *Turkey´s post-hoc test for angpt2_ch6 across rearing facilities for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	estimate	SE	t.ratio	p.value
Baseline - Preline	-0.1512	0.0829	-1.8241	0.1714
Baseline - Reference	-0.5870	0.0829	-7.0798	**9.14e-09*** **
Preline - Reference	-0.4358	0.0795	-5.4827	**3.37e-06*** **

TABLE XVIII. *Turkey´s post-hoc test for angpt2_ch20 across rearing facilities for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	estimate	SE	t.ratio	p.value
Baseline - Preline	0.3719	0.1437	2.5872	**0.0326**
Baseline - Reference	-0.0616	0.1437	-0.4288	0.9038
Preline - Reference	-0.4335	0.1399	-3.0987	**0.0085***

TABLE XIX. *Turkey´s post-hoc test for angpt1 across rearing facilities for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	estimate	SE	t.ratio	p.value
Baseline - Preline	0.3962	0.1832	2.1633	0.0867
Baseline - Reference	-0.0513	0.1832	-0.2801	0.9577
Preline - Reference	-0.4475	0.1783	-2.5103	**0.0393**

TABLE XX. *Turkey´s post-hoc test for pedf across rearing facilities for the constant current experiment. Significant results (p < 0.05) are marked in red with "*", "**" and "***" representing the grade of significance; p<0.01, p<0.001 and p<0.0001.*

contrast	estimate	SE	t.ratio	p.value
Baseline - Preline	-0.2885	0.1436	-2.0092	0.1195
Baseline - Reference	-0.1267	0.1436	-0.8826	0.6535
Preline - Reference	0.1618	0.1397	1.1575	0.4834