Tolerance limits to fluctuating water currents in Atlantic salmon (*Salmo salar*): A novel method to simulate the impact of ocean waves on salmon welfare in the laboratory

"Wave after Wave"

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Thanks for everything, Fiskefolke

Abstract

The Norwegian aquaculture industry aims to expand the production of Atlantic salmon (*Salmo salar)* to offshore locations where the fish will be experiencing more extreme weather conditions compared to traditional sheltered farms. Consequently, acceptable welfare guidelines are needed to ensure that the salmon will thrive in these wavy environments. However, studies have yet to test how the fish will perform in a fluctuating water current compared to a steady current. Therefore, with an updated swim tunnel system, the water flow was programmed to alternate between high and low current speeds, simulating big oceanic waves where groups of six Atlantic post-smolt were tested at 9°C. First, the fish group's critical swimming speed (U_{crit}) was established (94.5 cm s^{-1}). Then, trials with peak current speeds of 80, 100, 120, and 140% of the group mean U_{crit} were conducted for up to four hours or until the fish fatigued. The low current speed interval was set to 20% U_{crit}, and wave periods differed between 30, 60, and 120 s. In peak speeds of 80% and 100% U_{crit}, all fish endured for four hours, while only an average of three fish completed the tests at 120% U_{crit}. At 140% U_{crit}, all fish reached fatigue within 1.5 hours (51 ± 19 minutes). The results indicate that salmon can endure a ≈ 20% higher speed in fluctuating water currents compared to established swimming limits from earlier studies with a constant current. Furthermore, fatigue time did not differ significantly between wave periods, suggesting that the current peak speed is more decisive than the wave period. However, the results of the present study primarily represent an upper threshold value for brief periods of extreme conditions conducted in laboratory settings, and further studies should investigate long-term exposure and more complex wave dynamics under natural conditions.

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Abbreviations

ANOVA	One-way analysis of variance
BL s^{-1}	Body lengths per second
cm s^{-1}	Centimeter per second
CoT	Cost of transport
TukeyHSD	Tukey's Honest Significant Difference test
K	Condition factor
L_f	Fork length
MO_2	Oxygen consumption rate
PLC	Programmable Logic Controller
RPM	Rounds per minute
U_{crit}	Critical swimming speed
W	Weight

1. Introduction

1.1 Introduction to aquaculture production

1.1.1 The global aquaculture production

The world's population has quadrupled during the past century, and the increase is expected to continue. As the population rise, so does the demand for food (Elferink & Schierhorn, 2016). To be able to meet the demand, both The Food and Agriculture Organization of the United Nations (FAO) and The Organization for Economic Co-operation and Development (OECD) has cited aquaculture as a crucial part of the worldwide effort to increase the production of healthy, sustainable food (Regjeringen, 2021a). Due to the high development, the industry has become one of the world's fastest-growing food-producing sectors (FAO, 2018). In 2020, the world's total aquaculture production of aquatic animals reached 87.5 million tons, and the production is forecasted to increase by 14% in 2023 to meet humans' need for nutritious food. This increase will mainly be because of the expansion and intensification of sustainable aquaculture production (FAO, 2022).

1.1.2 The Norwegian salmon industry

The Norwegian aquaculture industry has been continuously developed since the first commercial fish cage was built in 1970 by two brothers on the Island of Hitra (Norsk Industri, 2017). Since then, the production has increased from 200 000 tons in 1994 to almost 1.5 million tons in 2020, resulting in a yearly growth of approximately 8% (Regjeringen, 2021b)**.** Despite the increasing presence of new farmed species, the industry has been and still is dominated by the Atlantic salmon (*Salmo salar*). This species emerged to prominence in aquaculture since it is relatively easy to breed, has favorable nutritional composition, is popular among consumers, and thereby conferring significant commercial value (Sprague et al., 2016). Because of the optimal farming locations and high investment in resources and development, Norway has become the world-leading nation in salmon production (Sjømat Norge, 2021). In 2021, the total market value was 75.78 billion Norwegian kroner, making it the second-largest export industry in Norway (Fiskeridirektoratet, n.d.a; Olaussen, 2018). However, the industry faces several environmental challenges, most notably the spread of sea lice, the risk of escapees interbreeding with the wild population, and eutrophication (Olaussen, 2018), which have led to enforced restrictions on further expanding production since 2012 (Regjeringen, 2021b).

1.1.3 The need for new locations - New technology and production methods

To reach the aquaculture industry's future expansion goal, FAO has pointed at technical developments as a significant contributor (Subasinghe, 2003). Traditionally, the production of Atlantic salmon has been carried out in sheltered fjord environments along the Norwegian coastline. However, due to the massive increase in the number of farms over the past decades, combined with other activities along the coast, such as commercial fisheries and tourism, fewer areas are now available for further expansion (Bjelland et al., 2015). Studies suggest that Norway has the potential to threefold the production by 2030 and fivefold by 2050 (Hersoug et al., 2014), but increased production will depend on several caveats, where two central limitations will be access to novel land or sea areas and the governmental approval to farm there (Bjelland et al., 2015; Nøstbakken & Selle, 2019). In 2015, the Norwegian Government introduced "Developing licenses," a temporary scheme that promoted incentives for technological innovation, allowing the farmers to develop new and more sustainable production methods at lower risk (Regjeringen, 2018; Fiskeridirektoratet, n.d.b). The high investment capacity combined with these licenses has taken the development of the industry in different directions, including exploring offshore aquaculture as a novel way of farming Atlantic salmon that can facilitate further growth (Fiskeridirektoratet, n.d.c).

1.2 Offshore farming

1.2.1 Defining offshore farming

Because of the crowded fjords, moving farms to more exposed locations, or even offshore, has become a considered option in searching for new production sites (Holmer, 2010), and because of the Developing licenses, several aquaculture companies have applied for introducing novel concepts of offshore farming constructions (Fiskeridirektoratet, n.d.c). Consequently, in 2022, the Norwegian Directory of Fisheries published a report recommending three areas for public overall impact assessment to facilitate the new production method, “offshore aquaculture” (Appendix A) (Fiskeridirektoratet, 2022). However, there is an inconsistency between disciplines in how the term offshore is understood, and no agreement is yet reached. FAO defines it as when farming occurs more than two kilometers from the coastline, at a greater depth than fifty meters, and entirely or partially exposed to waves with height above five meters (or more), stronger ocean currents, and winds (Lovatelli et al., 2013). Others define offshore more defuse as an operation located a distance from or remotely off the coast or in the open waters and occasionally exposed to rougher weather conditions, such as bigger oceanic waves and stronger currents (Watson et al., 2022; Hvas et al., 2020; Morro et al., 2022).

When discussing the relatively new phenomenon of establishing new farms away from more protected areas, it is important to acknowledge that terms such as “exposed” and “offshore” can be used interchangeably while other times describe very different locations. Generally, exposed locations may also include more shallow water closer to shore or coastal waters, with occasionally more extreme environmental challenges. Offshore sites, more specifically, refer to deeper open waters away from the coast (Hvas et al., 2020; Morro et al., 2022). Regardless of terminology, the most critical factors will be the environmental conditions that involve stronger currents and bigger waves at the new potential farm sites over a more extended period (Morro et al., 2022).

1.2.2 Potential advantages and challenges of offshore farming

Looking at the environmental factors, moving the production of Atlantic salmon to offshore locations provides several potential advantages. The nutrient and organic enrichment of nearby sediments is one of fish aquaculture's environmental problems. With stronger water currents resulting in rapid water exchange, such waste products are unlikely to accumulate and substantially influence the benthic environment below an offshore farm, and increased dispersal will increase the site's carrying capacity (Soto & Wurmann, 2019). Rapid water exchange should also improve water quality and cause more stable vertical gradients in temperature and oxygen (Hvas et al., 2019a). Furthermore, with an expected greater distance between offshore sites, the risk of transmitting pathogens and parasites between sites will likely be lower than the infection pressure between smaller farms closer together in the fjords (Salama & Murray, 2011).

There is still a need for more data on the actual outcome of full-scale production of Atlantic salmon at offshore locations. A traditional open-net cage can have a 30% reduction in cage volume when exposed to steady currents above 60 cm s^{-1}, impacting the fish's behavior and distribution inside the cage (Klebert et al., 2015). Cage deformation has also been observed when exposed to waves (Kristiansen et al., 2015). The farming constructions will therefore likely be larger rearing units and more rigid to counteract the influence of stronger currents and wave forces to avoid cage deformation and attrition (Hvas et al., 2020). However, as the size of the constructions increases, the surface area to volume ratio decreases, resulting in a reduced relative water exchange compared to smaller net cages (Klebert et al., 2013). Consequently, if a location experience extended periods of low current speeds, low dissolved oxygen levels can be a potential challenge in larger cage constructions due to limited water exchange, which contradicts the initial statement of better oxygen levels at offshore locations. Such potential risks of environmental hypoxia can result in reduced feed intake and growth (Oldham et al., 2018; Alver et al., 2023). Additionally, rougher weather conditions at offshore sites are expected to increase the probability of escape events, a key concern in salmon aquaculture. If an escape event were to happen, larger cages would increase the possibility of more salmon escaping as they hold more fish than a traditional cage (Morro et al., 2022).

In addition to the requirement of technological development and new constructions that can withstand harsher conditions, fish welfare is a central factor that needs to be considered at offshore sites (Hvas et al., 2020). It must be confirmed that the fish can thrive in more extreme conditions, as the biological needs of the salmon must come first. Considering the potential impact of waves on fish welfare will therefore be essential.

1.3 General waves

Various water waves exist, including surface waves (wind-, and ship-generated waves), ocean swells, tidal waves, deep-water waves, and tsunamis (UiO, 2019). The most common are surface waves, and these are often caused by winds and will be affected by the winds' strength and stability regarding speed, direction, and the size of the area the wind acts. After a wave is created, it can travel a great distance before it hits the shore (NauticalCampus, 2019). The wave does not cause much net transport of water particles forward, but its shape propagates across the water's surface. Between the crest (peak of the wave) and the through (wave's bottom point), the water particles in the upper part of the water column will move up and down, forward and back, creating circular movements (Fig. 1A) (Vistnes, 2009; NauticalCampus, 2019), and the time it takes for two successive wave crests to pass a fixed point is defined as the wave period (UiO, 2019). The circular pattern will propagate vertically through the water column, and perceptibility diminishes with increasing depth in deeper waters (Fig. 1B) (Vistnes, 2009, Det Norske Veritas, 2007). A wave will range in size from small ripples to several meters in height, and the wave period will typically vary between 1 and 30 seconds (Wright et al., 1999). The characteristics of a wave are often irregular, including variations in shape, period, height, length, and speed of propagation (Det Norske Veritas, 2007). Distance to the bottom will impact the wave's size, whereas deeper water, with less influence from the seabed, generally results in larger waves (Albretsen et al., 2019).

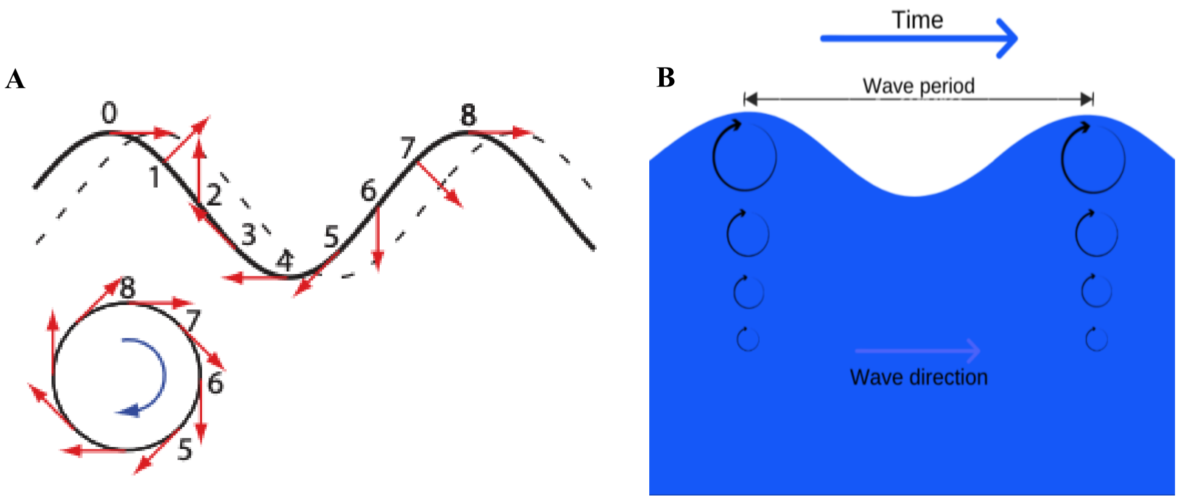


Figure 1*. As a wave passes towards the right, the water particles on the surface will create circular movements and cause (almost) no net transport of particles forward (A). The circular movements will gradually diminish with increasing depth until it becomes negligible (B). Illustration A obtained from Vistnes (2009).*

1.4 Welfare at offshore locations

1.4.1 Welfare indicators

Fish welfare is a central key issue in commercial aquaculture and plays a crucial role in many important decisions made by farmers in their daily animal husbandry practices and the planning of future production strategies (Noble et al., 2018). Improving fish welfare is anticipated to result in better production performance through more efficient feed conversion, less susceptibility to diseases and parasites, and reduced mortality rates. These outcomes will contribute to optimized and ethical production practices that meet consumers' expectations, as good fish welfare has become a prominent topic in recent years (Noble et al., 2018). Several feasible indicators have been suggested to ensure that all welfare-related aspects are considered within offshore aquaculture, including fish size, swimming behaviors, and swimming capacity (Stien et al., 2013; Hvas et al., 2020).

1.4.2 Swimming activity and swimming behavior in sea cages

A typical sea cage in Norwegian production today contains up to 200,000 individual salmon (Akvakulturdriftsforskriften, 2008 §47a), and the swimming behavior is often observed as a group structure, where individual fish usually swim in the same direction forming a circular school pattern at their preferred swimming speed (Juell, 1995). Various environmental and biological factors such as ambient oxygen, temperature, and health status can affect swimming behavior, even though the values are well within the tolerance limits (Johansson et al., 2006; Johansson et al., 2007; Føre et al., 2013). Strong currents and powerful waves will also heavily affect swimming behavior (Hvas et al., 2020), requiring fish to adapt their swimming activity to the varying experienced speeds.

The swimming activity of fish can be classified into three major categories; sustained swimming speed, prolonged swimming speed, and burst swimming speed, where swimming speeds often are expressed on a relative scale as body lengths per second (BL s^{-1}) or on absolute scales such as cm s^{-1}. The relative scale is usually more sensitive to size differences, while the absolute scale is less sensitive and appears approximately constant within limited size ranges. Moreover, attainable swimming speeds are higher in smaller fish when expressed in relative scales and higher in larger fish when expressed in absolute scales, and consequently, a static relative speed (i.e., 2 BL s^{-1}) will signify a drastically different work intensity across life stages (Brett, 1964; Remen et al., 2016; Hvas et al., 2018a). The swimming categories are based on how long a given swimming speed can be maintained before the stage of fatigue is reached, where propulsion is gained by contractions of either slow red muscle fibers, fast white muscle fibers, or a combination (Wilson & Egginton, 1994). By definition, sustained swimming is derived entirely from aerobic processes with no lactate accumulation and, in theory, can be maintained indefinitely. This type of swimming is powered by slow red muscle fibers, which are present as a thin layer along the lateral line of the salmon (Beamish, 1978; Kiessling et al., 2006). The red muscle fibers have a high mitochondrial volume density and a dense network of capillaries. This is associated with a high aerobic capacity necessary for longer periods of sustained swimming activity (Beddow & McKinley, 1999). Burst or sprint swimming represents the highest level of speed performance, often associated with predator–prey interactions (Jones, 1982), and the movements are mainly fueled by the white muscle fibers. The white fast muscle fibers depend on anaerobic energy sources and cannot be sustained for long (Beddow & McKinley, 1999), leading to fatigue within a short timeframe. The prolonged swimming speed falls between sustained and burst swimming and has been defined as swimming that can be continued between 2 and 200 minutes involving both red and white muscle fibers (Jones, 1982; Hammer, 1995; Kiessling et al., 2006).

In most remote farm sites, the average current speed outside the cage is generally below 20 cm s^{-1}, where the fish chooses its swimming speed independent of the environment (Johansson, 2007). Surprisingly, this is close to the average median current speeds measured at the three recommended areas for offshore aquaculture in Norway, where the highest average median was measured at 0.26 cm s^{-1} (Appendix A, Table 6). Under this circumstance at sheltered farms, the average swimming speed of salmon is observed to range from 0.3 - 1.1 BL s^{-1} (Sutterlin et al., 1979; Juell, 1995). This preferred swimming speed has been associated with the wild salmons' migratory nature and linked to the optimal speed for minimum cost of transport (CoT), where CoT is defined as the cost required to move a unit mass (of the fish) over a certain distance (Tudorache et al., 2011; Hvas et al., 2021a). As the current speed increases, the schooling structure will gradually get disrupted, and more fish will begin to stand against the current, where the fish will maintain its position in the cage with little to no forward movement (Johansson et al., 2014). Finally, the school is wholly dispersed at even stronger currents where all fish will stand against the current, as illustrated in Fig. 2 (Hvas et al., 2019a).

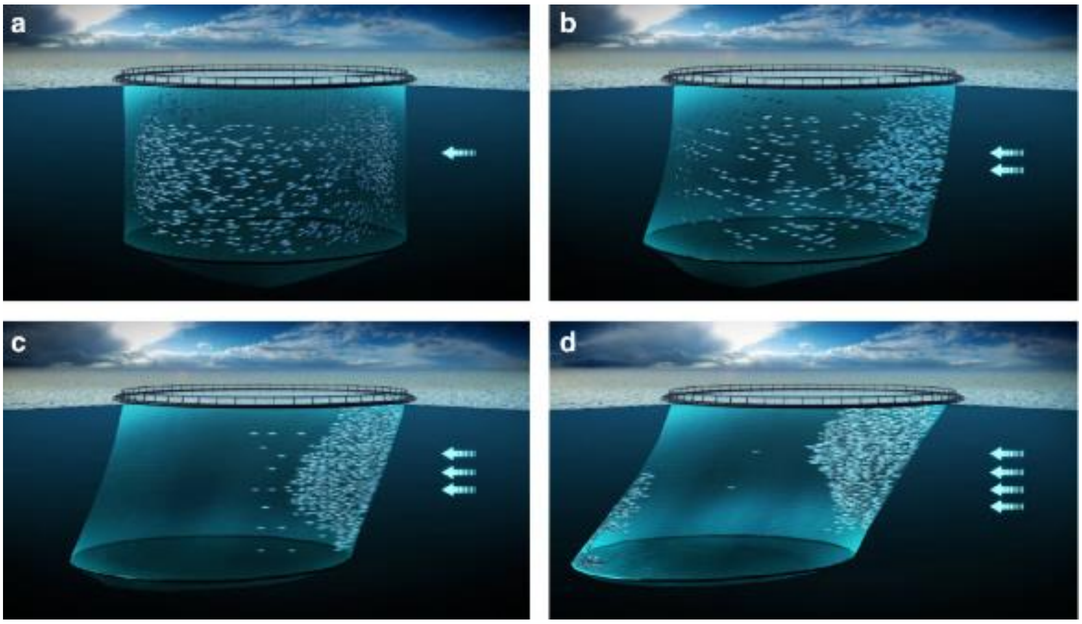


Figure 2*. Atlantic salmon exhibit different swimming behaviors in an open net cage depending on the experienced current speed. The fish determines its swimming speed at relatively modest currents and forms a circular schooling pattern (a). As the speed increases, the schooling structure will begin to disperse, and more fish will stand against the current (b) until the current is so strong that standing against it is the fish's only option (c). If the current increases even further and exceed the fish's swimming capacity, the fish will become fatigued and fall back on the posterior net wall (d). The illustration is obtained from Hvas et al. (2020).*

1.4.3 Biological consequences of higher current

When the salmon can no longer stand against the current, it will eventually become physiologically fatigued. Consequently, it will get trapped on the rear cage wall and likely be inflicted injuries by collision with the surroundings and with other fish (Hvas et al., 2020). Even more important, the state also causes the fish to undergo significant acid-base, metabolic, and ionic disturbances (Davison, 1997; Kieffer, 2000). When white muscle fibers power the swimming, there will be a depletion of glycogen stores in the muscles and an associated buildup of the byproduct lactate as the process is anaerobic. This lactate will leak into the bloodstream, causing metabolic and raspatory acidosis, subsequently causing a decrease in blood pH (Wood, 1991; Kieffer, 2000). A decrease in blood pH has been observed to diminish the oxygen-carrying capacity of hemoglobin, affect metabolic processes, and disrupt ion homeostasis in fish (Fromm, 1980). Studies have shown that the lactate concentration in the blood usually reaches a peak two to four hours after an exhaustive physical activity. Consequently, fish may experience a delayed response resulting in death several hours after the exercise (Fromm, 1980; Wood et al., 1983:).

Because of exhaustive exercise stress, forcing a fish to swim over its swimming capacity will also lead to high production of stress hormones such as catecholamines and cortisol (Kieffer, 2000; Davison, 1997). In a particularly strong stressor, such as fatigue, the fish will struggle to maintain its vital equilibria, with death as the worst-case scenario (Norges Forskningsråd, 2009). The effect of elevated levels of acid-base disturbances and stress hormones can last for several hours, and the recovery depends on calmer conditions so that the fish have time to reestablish their baseline levels (Kieffer, 2000; Davison, 1997). Moreover, osmotic and ionic disturbances can take even longer to recover from, where research has demonstrated that fish exposed to strenuous swim challenges had not fully recovered their plasma osmolality after 24 hours in full-strength seawater (Hvas et al., 2021b). In offshore farms characterized by harsher weather conditions where fish are required to swim at speeds above their sustained swimming speed repeatedly, an increased osmoregulatory burden could therefore be a potential limiting factor.

1.4.4 Swimming capacity of Atlantic salmon

Wild Atlantic salmon naturally migrate from the river, where they hatch, to the open ocean for feeding and growth. Then, they migrate back to the same river when they are ready to spawn. Because of their anadromous life cycle, salmonids are generally seen as athletic species with high sustained swimming capacities (Aas et al., 2010).

To be able to describe swimming capacity in fish, different methods have been established. The most common concept in the literature is the work of Brett (1964), which was the first to develop the method of critical swimming speed (U_{crit}). This method is performed in a swim tunnel system and involves an incremental increase in speed until the fish reaches fatigue, where the final speed indicates the highest swimming speed a fish can sustain for a certain amount of time (often in minutes) (Brett, 1964; Plaut, 2001). The U_{crit} can therefore provide a rough estimate of Atlantic salmon's maximum capacity and is often used to measure the prolonged swimming capacity since it eventually involves both types of muscle fibers and is performed over a moderate duration (Farrell, 2007). This method has subsequently been used as a starting point to formulate welfare guidelines related to acceptable current velocity in offshore aquaculture farming (Remen et al., 2016, Hvas et al., 2017a). However, U_{crit} is a tolerance limit of the speed fish can endure for a brief period and offers little insight into whether the fish can withstand or get exhausted in an intermediate current velocity for a longer duration (Hvas et al., 2020). Sustained swimming is therefore an alternative measure to consider. Previous studies have shown that aerobically fueled swimming is predominating at 70-80% of the U_{crit} in salmonid species (Webb, 1971; Jones, 1982). More recent studies by Hvas et al. (2017c, 2021a) conducted sustained swimming trials on farmed Atlantic salmon where fish were forced to swim for 4 and 72 hours (or until they fatigued) with a current speed based on the knowledge of aerobic swimming. Most of the salmon successfully completed the trials at 80% and 85 % U_{crit}, indicating that the transition between fully aerobic to a combination of aerobic and anaerobic metabolism occurs at swimming speeds exceeding ≈ 80% of the U_{crit} (Hvas et al., 2017c, 2021a).

1.4.5 Wave impacts on swimming behavior

Fluctuating water currents (i.e., waves) present other challenges for the fish compared to constant current speeds. As shown in Fig. 1, waves follow an orbit pattern with two distinct phases: an active phase (moving against the current) and a passive phase (moving with the current) (Vistnes, 2009). In a wave-exposed environment, fish are required to adjust their swimming speeds to counteract the water movements. During the active phase of the wave, fish must accelerate, while in the passive phase, they need to decelerate (Marcoux & Korsmeyer, 2019). The frequency of accelerations will depend on wave periods, with shorter periods resulting in a higher number of accelerations. However, waves exhibit irregular and unpredictable characteristics (DNV, 2007), necessitating the fish to continually adapt their swimming to maintain their position in the water. This alternating swimming pattern deviates from the more constant swimming required in a steady current. However, previous studies examining the swimming capacity of Atlantic salmon have been conducted with a constant current speed. Until now, only a limited number of published studies have investigated the effect of waves on Atlantic salmon at exposed locations in the Faro Islands (Dam, 2015; Johannesen et al., 2020). In the study by Dam (2015), it was observed that exposure to higher waves temporarily disrupted the group behavior of fish, transitioning from schooling to a more chaotic state. However, the impact of wave periods, wave height, or vertical net displacement, how it affected the time spent in the chaotic state, and the consecutive number of incidents, could not be definitively determined. Johannesen et al. (2020) found that the impact of waves on salmon behavior depended on the time of day, where hydrodynamic conditions had a greater influence during the day. The salmon were observed to adjust their swimming to align with the direction of a wave and responded differently to various wave periods, where the salmon tended to disperse widely in the water column and move away from the net cages in larger waves. However, the impacts of waves on salmon behavior were influenced by various factors, including the current and cage deformations, making it difficult to establish a clear consensus on the exact outcomes (Johannesen et al., 2020).

Even though there is substantial knowledge regarding threshold values of swimming capacity in Atlantic salmon at constant current speeds, the impacts of waves on their behavior and welfare remain a significant knowledge gap. Understanding how ocean waves impact fish welfare is crucial to ensure that the fish will thrive in novel offshore farms. It will be essential to determine whether salmon can maintain a controlled position in the water or if it will collide with other fish and the cage wall, resulting in unacceptable welfare and potentially high mortality rates (Hvas et al., 2020).

1.5 Aim of the study

The study's aims were first to develop a robust lab-based method for testing Atlantic salmon swimming capacity in a large swim tunnel with the possibility of varying the water flow to simulate waves. Then, by conducting trials in the swim tunnel based on U_{crit} and sub-U_{crit} values, the fish’s behavior and swimming capacity were observed and measured to produce relevant threshold values that can be used in the work of establishing welfare guidelines concerning acceptable conditions at offshore locations. A third objective was to investigate the effect of different wave periods, as waves with a shorter wave period result in more frequent accelerations for the fish than waves with longer wave periods. It was, therefore, of interest to gain knowledge of whether the wave period would affect fatigue time as the wave period naturally will vary in an offshore farm.

In the present study, two main research questions were examined:

Question 1*: Will fluctuating water currents affect the tolerance limits of Atlantic salmon?*

H_{01}: Tolerance limits to fluctuating water currents will be the same as constant current exposure of similar peaks.

H_{A1}: Tolerance limits to fluctuating water currents will differ from constant current exposure of similar peaks.

Question 2*: Will different wave periods significantly affect fatigue time?*

H_{02}: There will be a significant difference in fatigue time regarding wave periods.

H_{A2}: There will not be a significant difference in fatigue time regarding wave periods.

2. Material and Method

2.1 Animal husbandry

Reared Atlantic salmon post-smolts *Salmo salar* (Aquagen strain) were held in three indoor circular tanks in the Tank Environmental Laboratory at the Institute of Marine Research Matre, Norway. The holding tanks had a total volume of 5.3 m^3 (3m in diameter) with a continuous inflow of 130 liters min^{-1} of 9°C full-strength (34ppt) seawater. The seawater was filtered, aerated, and treated with UV-C light before being pumped into the holding tanks. Dissolved oxygen was monitored, and the oxygen levels were consistently above 80% saturation owing to the high inflow rate, which also prevented the accumulation of unwanted waste products such as ammonia and CO_2.

450 fish, 150 in each tank, with an average weight of 434 grams, were moved to the described tank environment approximately one month before the experimental trials began so that the fish had time to acclimatize. This resulted in a stocking density of ≈12.3 kg m^{-3}. The fish were held under a natural light regime and continuously fed standard commercial feed (Skretting, 4.5mm pellet size) from an automated feeder in excess daily.

The experimental trials were performed between September and November 2022. The Norwegian Food Safety Authorities approved all use of animals in this study in accordance with the Norwegian laws and regulations for procedures and experiments on live animals (permit number 29323).

2.2 Swim tunnel setup

The present study used an extensive Brett-type swim tunnel system to assess swimming performance. The setup and technical specification of the swim tunnel are provided in detail in previously published articles such as Remen et al. (2016), but the main specification will briefly be summarized. The swim tunnel setup is illustrated in Fig. 3.

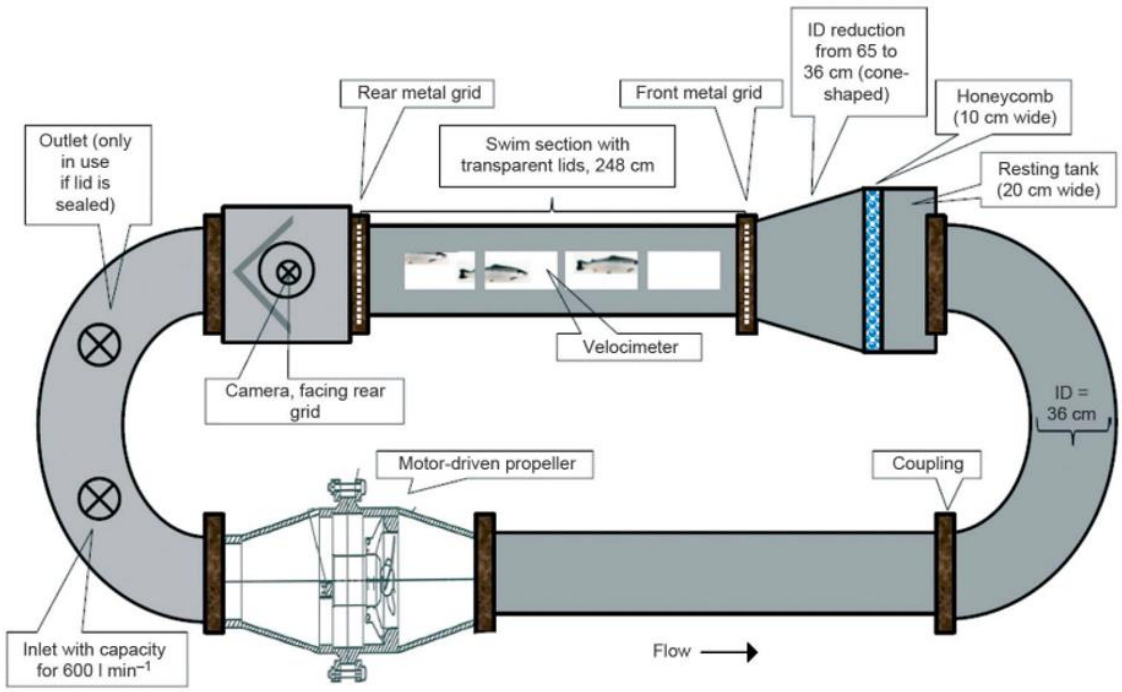


Figure 3. *Schematic drawing of essential components of the large swimming tunnel used in the study. ID: inner diameter. The illustration is obtained from Remen et al. (2016).*

This custom-made tunnel was built of polypropylene pipes with an internal diameter of 36 cm and two longer sides, resulting in a stadium shape. The total volume of the entire swim tunnel set up was 1905 liters. A motor-driven propeller (Flygt 4630, 11° propeller blade, Xylem Water Solutions Norge AS) was mounted inside the tunnel to generate the current flow. Using a frequency converter (ITT Monitoring and Control), the current could be controlled and set to a preferable velocity.

To minimize turbulence, the water was sent through a honeycomb with a cell diameter of 5 mm before entering the swim section of the tunnel. The swim section was a 248 cm long part of the system with a volume of 252 liters, where the fish swam during a trial. At the rear of the swim chamber, the top opening was partially removable so that fish could be transferred into the tunnel and taken out after a trial. Behind the rare grid in the same section, a camera was placed to observe the fish's activity during each trial without disturbing them. The same water source supplying the holding tanks was provided into the swim tunnel from an adjustable intake between the swim chamber and the motor section.

2.3 Update of the swim tunnel system

2.3.1 Testing of the system with a constant water current

For the purpose of this study, the swim tunnel system was updated with a Programmable Logic Controller (PLC) programmed and delivered by Xylem Water Solutions Norge AS. With the PLC, the current speed could be adjusted to a fluctuating rather than a constant flow, alternating between high and low current speeds of various durations.

Initially, to determine which pump speed in rounds per minute (RPM) corresponded to the speed experienced in the tunnel, several tests with a handheld flow meter (Höntzsch Flow Measuring Technology) were conducted. The flow sensor was attached to the inside of the swim tunnel, with the flow meter taking measurements every other second for five minutes at one specific pump speed. Thirteen tests with different RPM values were conducted. After each test, the data were uploaded from the flow meter to the associated computer program (Software UCOM for Configuring Höntzsch Transducers) to determine which current speed it corresponded to. These tests were done without any fish in the swim tunnel system. The obtained data were calibrated to calculate the specific RPM value that gave the wanted speed for the different current speed protocols and plotted with a linear regression line (Fig. 4).

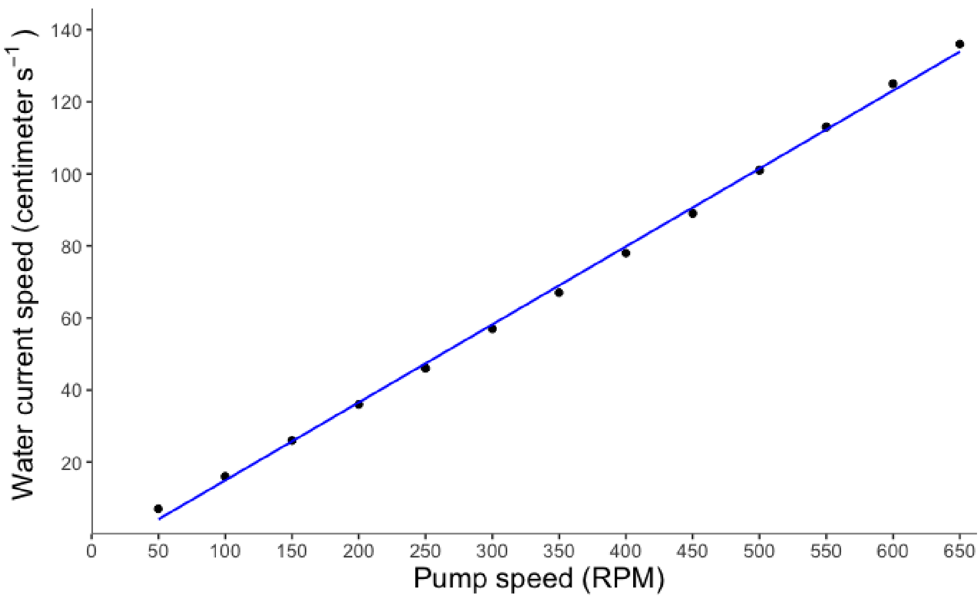


Figure 4. *The linear regression line from the calibration of obtained data from the flow meter when testing the updated swim tunnel system. The measurements were taken without any fish in the swim tunnel.*

2.3.2 The frequency converter's acceleration time

Since the water flow now would be fluctuating between high and low current speed, the frequency converter's acceleration time needed to be calculated to ensure that the decided rotation frequency, equaling the wanted current speed, was reached after a certain number of seconds. The acceleration time was therefore calculated so that the peak speed was reached just before the "high-speed interval" ended, and the current speed decreased (See 2.5 Calculations). Likewise, the lowest speed was reached just before the "low-speed interval" ended. This enabled the ability to create automatic fluctuating current speeds of a desired magnitude and periodicity to simulate oceanic waves.

2.4 Swim trials

The day before each trial, six randomly selected fish were gently netted and transferred from the holding tanks into the swim tunnel to acclimatize overnight. Since the swim tunnel was situated in the same room as the holding tanks, the fish could be moved efficiently to minimize handling stress. The current speed overnight was set to 15 cm s^{-1}, which was too slow to initiate constant swimming efforts in the size class of Atlantic salmon tested. A constant moderate inflow of water was supplied into the tunnel to ensure normoxic conditions and a constant temperature of 9ºC during the swim trials.

2.4.1 Critical swim speed protocol

To obtain a baseline of general swimming capacities for defining the protocols in the subsequent fluctuating water current trials, a representative average critical swimming speed (U_{crit}) of the fish group needed to be established. The method of Brett (1964) was used: a stepwise increase in prescribed intervals of 15 cm s^{-1} every 30 min until the fish became fatigued. Fish were defined as fatigued when they, despite tactile stimulation applied by the experimenter's hand, could no longer swim against the current and ended up trapped on the rear grid. At this point, the salmon were taken out of the tunnel and quickly euthanized with a blow to the head, whereafter, the elapsed time was noted. The swim trial continued until all six fish reached fatigue. Then fork length (L_f) and weight (W) were measured for every individual.

The procedure was repeated for all three holding tanks, providing 18 individual U_{crit} measurements. As a supplementary observation, the tail beat frequency at each velocity interval up to 90 cm s^{-1} was determined by counting the time required to complete 30-50 tail beats of the six fish.

2.4.2 Pilot testing with fluctuating water current

The updated swim tunnel had not been used previously. The method and protocol therefore needed to be developed and tested before the fluctuating water current trials with fish in the tunnel could begin to ensure the swim tunnel system could provide the desired regimes. The high- and low-intensity interval speed for the central part of the study was chosen based on a defined percentage of the mean U_{crit} value obtained from the U_{crit} trials, where the data from the calibration of RPM values were used as guidelines. Since the water current would fluctuate between high and low current speeds, new tests had to be conducted to ensure that the required speed was reached within the interval (especially for the low-intensity interval). The same method as the first speed calibration with the constant speed was used. However, the test was expanded to eigth minutes and conducted with different RPM values on both the high- and low-intensity interval to determine the RPM required for the wanted speed for all treatment groups.

In addition to the RPM value and corresponding speed, the duration of an interval (equivalent to the wave period) had to be considered. Of particular interest was how short an interval could be obtained when attempting to mimic relevant wave action. This would depend on how fast the motor was able to accelerate the water current within a given timeframe. Further tests were therefore conducted, varying both the speed and the duration of the high and the low intervals to establish the physical limitations of the swim tunnel setup with regards to simulating wave periods. These pilot tests concluded that the shortest wave period that could be consistently achieved with the desired peak current was 30 s (15 s at high and 15 s at low intensity). See Appendix B for an elaboration of the method development.

2.4.3 Fluctuating water currents protocols

The main experiment consisted of two parts, A and B, both with fluctuating water currents. The two parts consisted of six different treatment groups, where three trials were conducted per treatment group to account for statistical validity. All fluctuating water current trials continued for four hours or until the fish became fatigued, where fatigued was defined similarly to the U_{crit} trials.

Like the U_{crit} test, six random fish were netted and transferred to the swim tunnel in each trial, resulting in eighteen individual fish per treatment group. The fish were transferred to the swim tunnel the day before so they had time to acclimatize overnight at a modest speed of 15 cm s^{-1}. Each trial began with a warm-up before the fluctuating water current tests started. The warm-up was a 15 cm s^{-1} increase every five minutes from the overnight velocity to a speed slightly below the peak speed in each treatment group or up to 90 cm s^{-1} (Fig. 5). 90 cm s^{-1} was the highest speed used in the warm-up because any further rise of 15 cm s^{-1} would have caused the critical swimming speed to be surpassed.

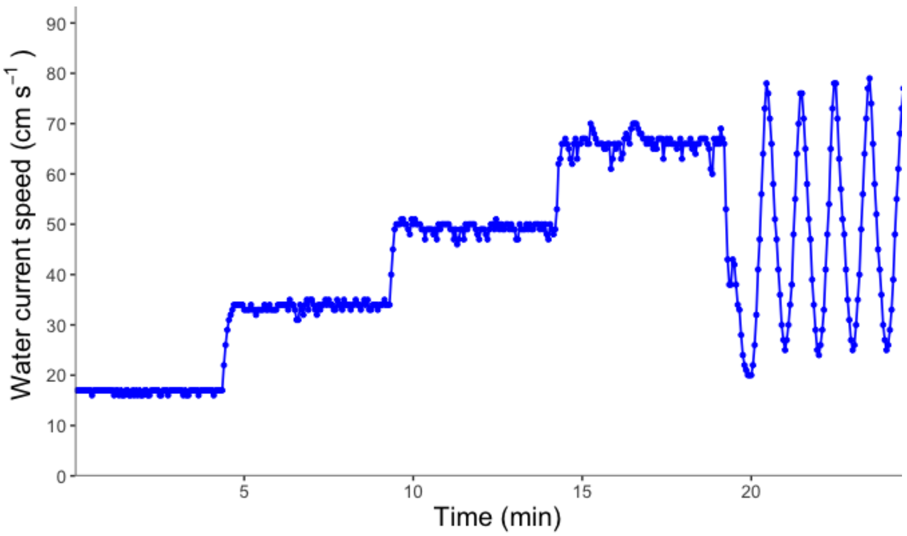


Figure 5. *The warm-up with a stepwise increase of 15 cm s^{-1} in current speed every five minutes from the overnight speed at 15 cm s^{-1} until a speed just below the peak current speed in the trial (or until 90 cm s^{-1}) was reached. These measurements were taken from a trial with a peak current speed of 80% of the mean U_{crit} and a wave trough at 20% of the mean U_{crit} with a wave period of 60 s. The measurements were taken with six fish in the swim tunnel.*

Part A consisted of four treatment groups with current peak speeds of 80, 100, 120, and 140% of the determined mean U_{crit}. All four groups' high-intensity interval was 30 s, followed by a 30 s low-intensity interval at 20% of the mean U_{crit}. Therefore, the wave period of the simulated waves in part A was 60 s. An illustration of the different treatment groups in A is shown in Fig. 6.

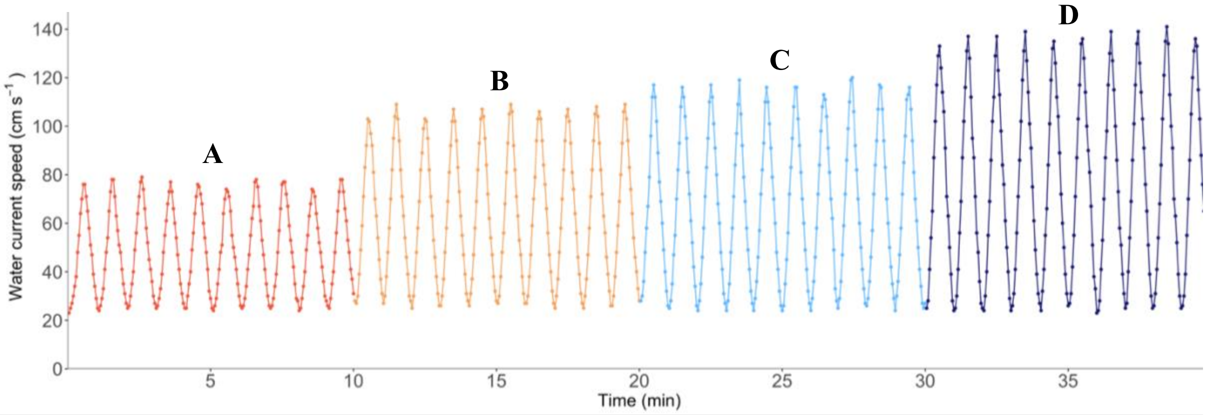


Figure 6. *The four treatment groups with a high-intensity interval with peak speeds of 80 (A), 100 (B), 120 (C), and 140% (D) of mean U_{crit}, followed by the same low-intensity interval with a current speed of 20% of mean U_{crit} for all treatment groups. The trials continued for four hours or until all fish were fatigued. The measurements of the different peak speeds were taken with six fish in the tunnel.*

In part B, with the two last treatment groups in addition to the one group from A (120% U_{crit} with a wave period of 60 s), the high- and low-intensity interval durations were set to 15 s and 60 s, resulting in wave periods of 30 s and 120 s, respectively. The wave peak was set to 120% U_{crit}, and the low-intensity interval was set to 20% U_{crit}. An illustration of the different treatment groups in B is shown in Fig. 7.

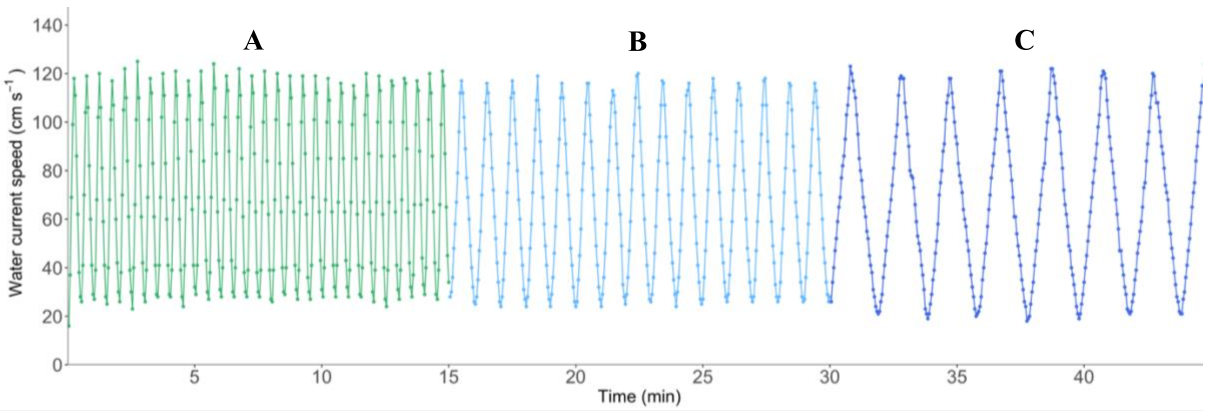


Figure 7. *The three treatment groups with wave periods of 30 (A), 60 (B), and 120 s (C) with a peak speed of 120% of mean U_{crit}, followed by a lower current speed at 20% of mean U_{crit}. Plot B is the same treatment group (120% U_{crit}, 60s wave period) described in part A. The trials continued for four hours or until all fish were fatigued. The measurements of the different wave periods were taken with six fish in the tunnel.*

An overview of the experimental setup is given in Table 1.

Table 1. *An overview of the experimental setup of the fluctuation water current trials. The trials were divided into parts A and B, where different peak speeds were examined in A, and different wave periods with the same peak speed were examined in B. The 120% U_{crit} group with a wave period of 60 s is considered in both parts.*

A

Part	**Max speed warm-up (cm s^{-1})**	**High-intensity interval (% of U_{crit})**	**Low-intensity interval (% of U_{crit})**	**Time on each interval (s)**
A	60	80	20	30
A	75	100	20	30
A/B	90	120	20	30
A	90	140	20	30

B

Part	**Max speed warm-up (cm s^{-1})**	**High-intensity interval (% of U_{crit})**	**Low-intensity interval (% of U_{crit})**	**Time on each interval (s)**
B	90	120	20	15
A/B	90	120	20	30
B	90	120	20	60

At the point where the Atlantic salmon approached exhaustion, it was observed that some of the fish tried to rest against the rear grid. To determine whether the Atlantic salmon was truly fatigued, a brief touch applied by the experimenter's hand was necessary, and often the fish would resume the swimming efforts. Fish that endured for all four hours were noted to have completed the swim test and were quickly removed from the setup and euthanized once the trial had concluded. After removal from the tunnel, the L_f and W were measured on all fish.

2.5 Calculations

Critical swimming speed. Calculated according to Brett (1964):

$$U_{crit} = U_f + \frac{t_f U_i}{t_i},$$

where U_f is the highest completed current speed (cm s^{-1}), t_f is the time endured on the final speed before reaching fatigue (min), U_i is the velocity increment (15 cm s^{-1}), and t_i is the time increment interval (30min).

Acceleration time. The frequency converter's acceleration time was calculated as follows:

$$a_t = \frac{r}{705} * (RPM_{high} - RPM_{low}),$$

where a_t is the amount of time needed to attain the wanted speed (s), r is acceleration time (s), RPM_{high} is the RPM value for the high-speed interval, and RPM_{low} is the RPM value for the low-speed interval.

Condition factor. Calculated according to Fulton's formula:

$$K = 100 * \frac{W}{{L_f}^3},$$

where K is the condition factor, W is weight (g), and L_f is fork length (cm).

2.6 Data analysis

To determine the desired RPM value for the current speed protocols, a regression analysis was performed in Microsoft Excel (version 16.73), and the two variables were plotted with a linear regression line to model the relationship. A one-way ANOVA with a subsequent Tukey's Honest Significant Difference (HSD) post hoc test was conducted to test for differences in size parameters between all treatment groups.

A Pearson correlation test was performed to investigate the strength and direction of the linear relationship between swimming performance (in cm s^{-1} and BL s^{-1}) and L_f in the U_{crit} trials. The two variables were plotted in a scatter plot with a linear regression line to visualize the trend. A linear regression was also conducted for the salmon's tail beat frequency as a function of swimming speed. In part A, a one-way ANOVA with a subsequent TukeyHSD was conducted to test for differences in fatigue time between the four treatment groups. In part A and B, linear regression was used to analyze the relative swimming speed as a function of fatigue time and the difference between fatigue time and the treatment groups. Furthermore, in addition to linear regression, Pearson correlation tests were conducted to explore the relationship between fatigue time and L_f and K to investigate whether the size parameters affected fatigue time in both parts. A Kruskal-Walli's test was performed in part B to examine how wave periods affected fatigue time.

All data analyses (excluding the regression analysis for the RPM calibration) were performed in RStudio (version 2021.09.2, RStudio, PBC, Boston, MA, USA) using R statistical software (version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria), and R coding outputs of the results are presented in Appendix C. P-values below 0.05 were considered significant, and data are reported as mean ± s.e.m unless specified otherwise.

3. Results

3.1 General measurements and observations

L_f, W, and K of all fish tested were 41.1 ± 0.19 cm, 837 ± 13.6 g, and 1.19 ± 0.01, receptively (N = 125), and the average for the U_{crit} trials and the treatment groups are given in Table 2. It was a significant difference between the first group tested, the U_{crit} trials, and the last treatment group, 140% U_{crit}, for all three size parameters (One-way ANOVA with TukeyHSD, p-value < 0.05). The fish in all treatment groups initially exhibited chaotic swimming and collisions as they acclimated to the fluctuating water current. In the 80% and 100% U_{crit} trials, collisions were minimal after the initial period, and the fish distributed themselves well within the tunnel. However, in the 120% and 140% U_{crit} trials, fish had to exert burst swimming to avoid being trapped at the rear grid. This behavior was occasionally observed in the 80% and 100% trials but became more frequent as the current approached or exceeded 100% U_{crit}. When the fish approached fatigue, the swimming became more unsteady, especially as more fish got dragged back to the end of the tunnel simultaneously. When the current decreased, the fish were often observed to rest at the bottom of the tunnel.

Table 2. *The different treatment groups' size parameters in the fluctuating water current trials. The data is divided into two; part A, including the critical swimming speed (U_{crit}) trials and trials with peak speeds based on a percentage of the obtained mean U_{crit}, with a constant wave period (A), and part B, with different wave periods at the same peak speed (B). The 120% treatment group with a wave period of 60 s is considered in both parts.*

A

Treatment group (% of U_{crit})	L_f (cm)	W (g)	K	N	Wave period (s)
U_{crit} trials	39.8 ± 0.52	719 ± 32.5	1.13 ± 0.02	17	-
80	40.5 ± 0.57	767 ± 34.5	1.14 ± 0.02	18	60
100	41.1 ± 0.35	831 ± 24.0	1.19 ± 0.02	18	60
120	41.6 ± 0.52	868 ± 36.7	1.19 ± 0.02	18	60
140	42.3 ± 0.42	951 ± 33.7	1.25 ± 0.01	18	60

B

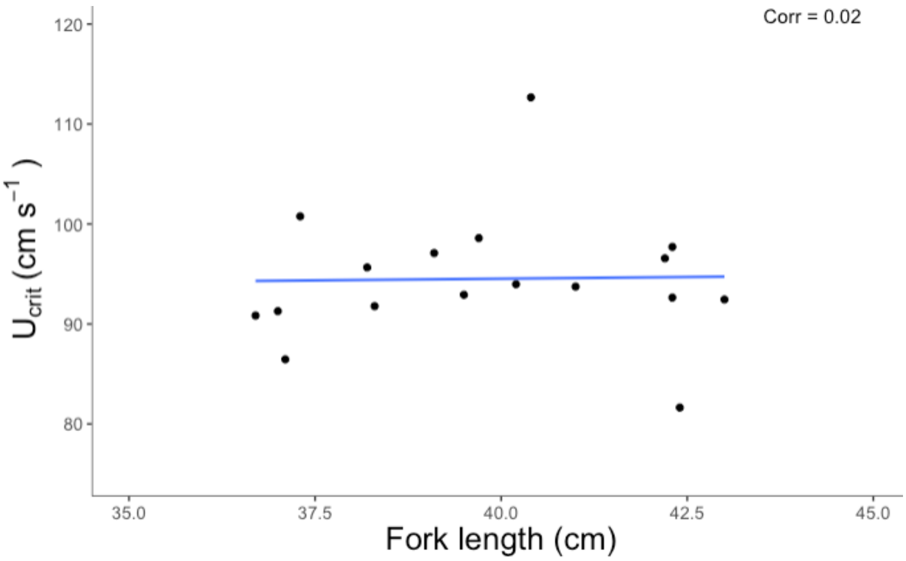
Treatment group (% of U_{crit})	L_f (cm)	W (g)	K	N	Wave period (s)
120	41.4 ± 0.46	864 ± 36.4	1.20 ± 0.02	18	30
120	41.6 ± 0.52	868 ± 36.7	1.19 ± 0.02	18	60
120	41.2 ± 0.47	853 ± 30.3	1.21 ± 0.02	18	120

3.2 Critical swim speed trials

The U_{crit} of the fish group was 94.5 ± 1.6 cm s^{-1}, corresponding to 2.4 ± 0.05 body lengths (BL) per second (s^{-1}) (n = 17). One fish was deemed an outlier and excluded from the data set due to obvious underperformance, presumably caused by abdominal skin wounds.

The absolute U_{crit} in cm s^{-1} and the relative U_{crit} in BL s^{-1} of each individual fish versus their L_f are shown in Fig. 8. When expressing U_{crit} in absolute units, L_f was not correlated with U_{crit} (Pearson, Coeff = 0.022, p-value = 0.932, n = 17). However, expressed in relative units as BL s^{-1} was negatively correlated with L_f (Pearson, Coeff = -0.607, p-value = 0.010, n = 17).

A



B

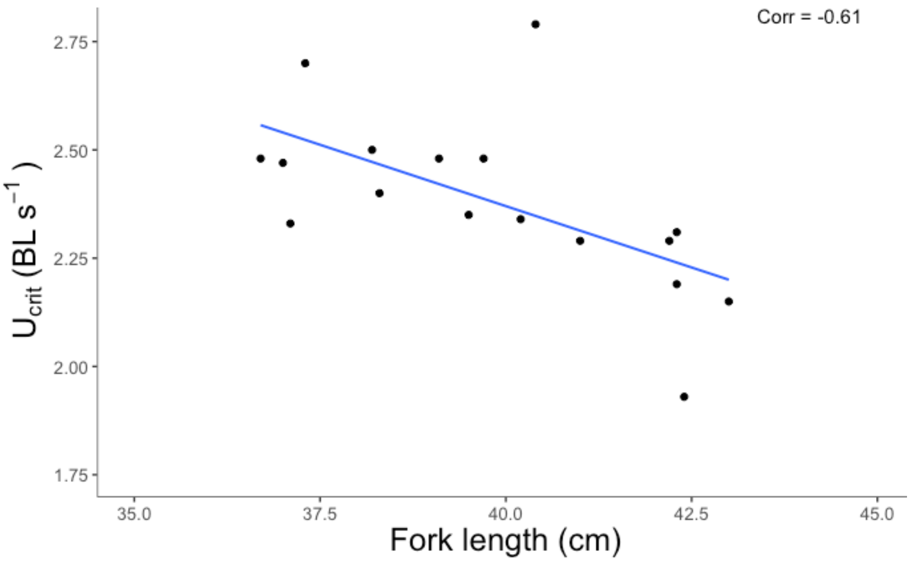


Figure 8. *Scatterplots of the absolute U_{crit} (cm s^{-1}) (A) and relative U_{crit} (BL s^{-1}) (B) as a function of L_f. The blue lines indicate linear regressions, showing an almost horizontal slope in plot A and a negative slope in plot B with n = 17.*

In the U_{crit} trials, the tail beat frequency (TBF) increased linearly with swimming speed from 1.58 ± 0.06 to 4.24 ± 0.04 beats s^{-1} between 30 and 90 cm s^{-1} (Fig. 9).

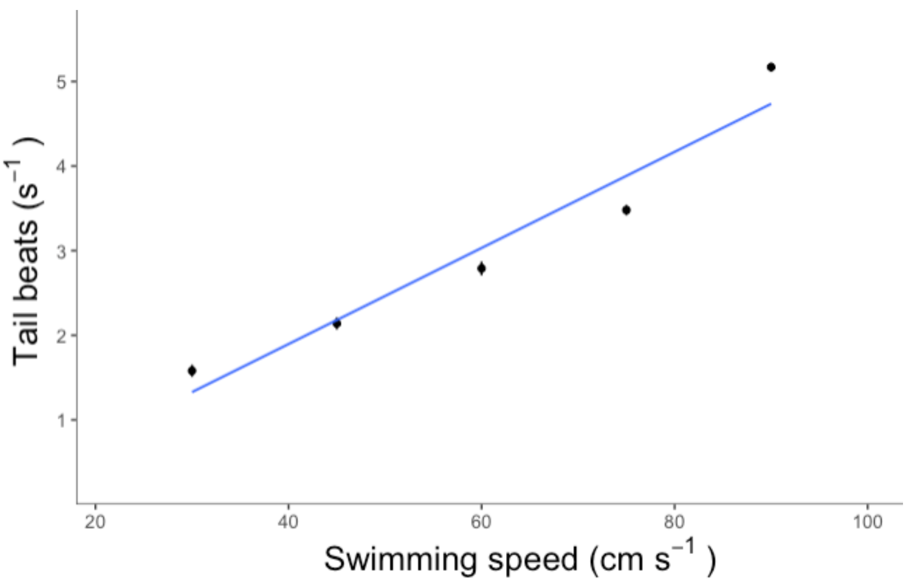


Figure 9. *The tail beat frequency as a function of swimming speed in the critical swimming speed trials with a linear regression line.*

Based on the fish groups' mean U_{crit}, peak current speeds for the fluctuating water current trials at 80, 100, 120, and 140% U_{crit} were set to 76, 95, 114, and 133 cm s^{-1}, respectively.

3.3 Fluctuating water currents part A: Different peak speeds

In the swim trials with peak currents of 80 and 100% U_{crit} and a wave period of 60 s, no fish fatigued during the four hours of testing. However, at 120%, only 22% of the fish (4 out of 18) endured for four hours, while the rest fatigued after 119 ± 10 minutes. At 140% U_{crit}, all the fish reached fatigue within 1.5 hours with a mean fatigue time of 51 ± 4 minutes (Table 3).

Table 3. *The results of part A in the fluctuating water current trials using different peak speeds and a constant periodicity of 60 s. NA = not applicable.*

Treatment group (% of U_{crit})	**Peak current speed (cm s^{-1})**	**Fish completed (%)**	**Fatigue time (of those that fatigues)**	**Wave period (s)**
80	76	100	NA	60
100	95	100	NA	60
120	114	22	119 ± 10 minutes	60
140	133	0	51 ± 4 minutes	60

As the peak current speed increased from 80 and 100% to 120 and 140% U_{crit}, the fatigue time significantly decreased (One-way ANOVA with TukeyHSD, p-value < 0.001) (Fig. 10).

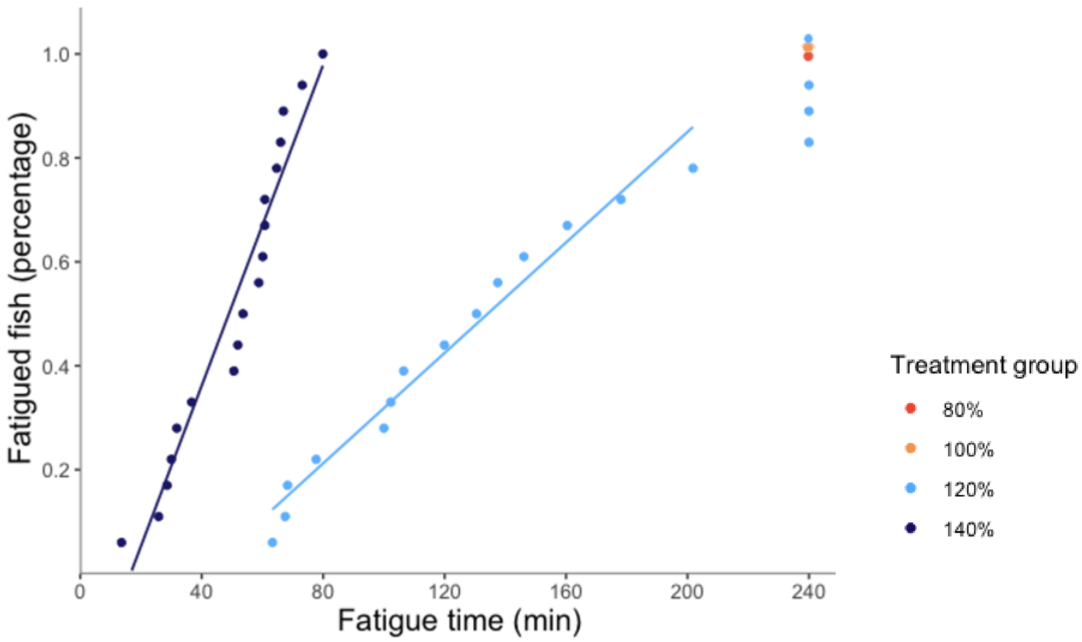


Figure 10. *The percentage of fatigued fish versus fatigue time in part A of the fluctuating water current trials, where each data point represents one individual fish. The lines for treatment groups 120% and 140% U_{crit} indicate linear regressions of the fatigued fish, showing a significantly shorter swimming time at 120 and 140% U_{crit} in contrast to 80% and 100% U_{crit}. The data points at the 240 minutes mark represent fish that completed the four-hour test.*

There was no significant linear relationship between fatigue time and relative swimming speed (in BL s^{-1}) for the fatigued fish in the 120% ($R^2 = 7.31*10^{-5}$, p-value = 0.977) and 140% U_{crit} ($R^2 = 5.0*10^{-2}$, p-value = 0.374) treatment group (Fig. 11).

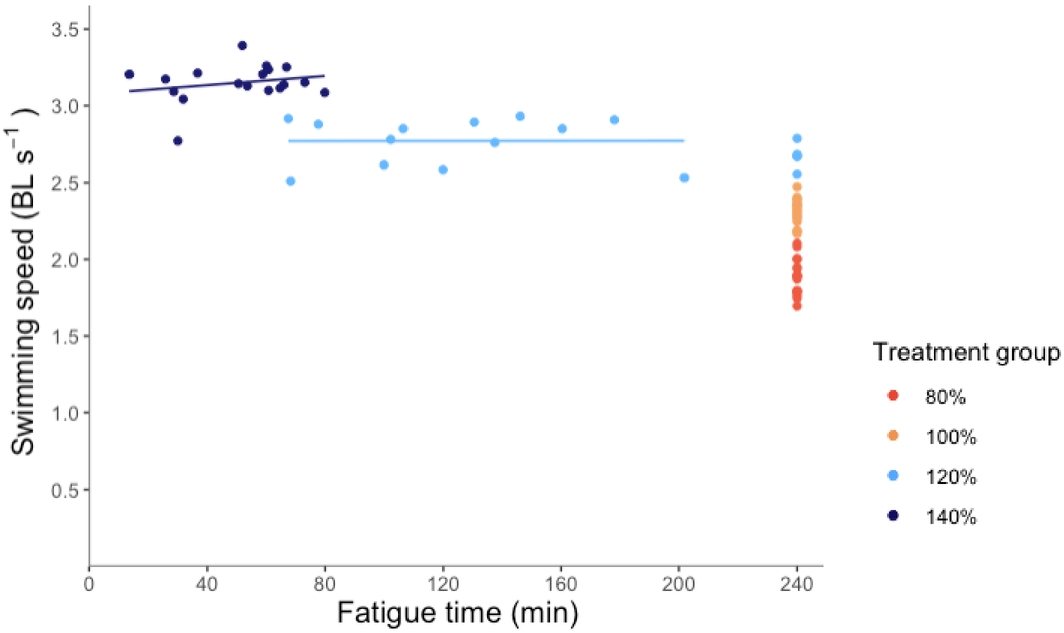
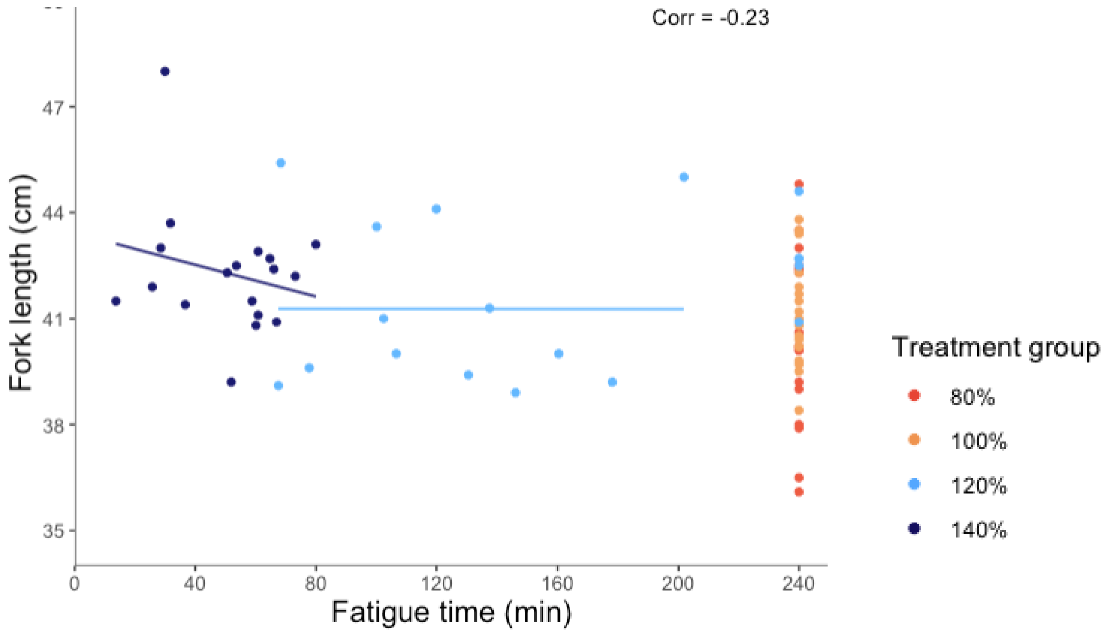


Figure 11. *Relative peak speeds experienced by each individual fish as a function of fatigued time in part A of the fluctuating current trials with different peak speeds. The lines for treatment groups 120% and 140%* U_{crit} *indicate linear regressions for the fish that fatigued. The data points at the 240 minutes mark represent fish that completed the four-hour test.*

A Pearson correlation test between fatigued time and L_f for the fatigued fish revealed a correlation coefficient of -0.229 (p-value = 0.208), indicating no significant correlation. The same test was also performed for fatigued time and K, resulting in a correlation coefficient of -0.373 (p-value = 0.035), a significant negative correlation (Fig. 12).

A



B

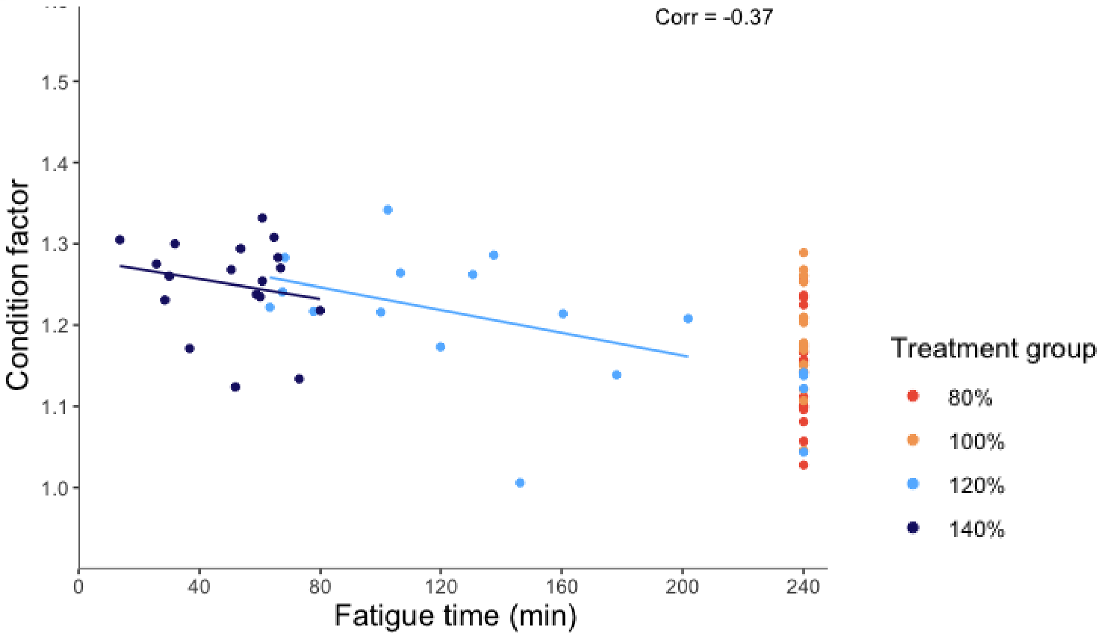


Figure 12. *The relationship between L_f (A) and K (B) versus fatigued time in part A of the experiment with different current peak speeds and the same wave period of 60 s. The lines for treatment groups with a peak speed of 120% and 140% indicate linear regressions of the fatigued fish. The data points at the 240 minutes mark represent fish that completed the four-hour test.*

3.4 Fluctuating water currents part B: Different wave periods

In addition to the wave period of 60 s, two other wave periods of 30 s and 120 s were tested at a peak speed of 120% U_{crit}. At 30 s, 17% of the fish completed the test (3 out of 18), and at 120 s, 11% completed it (2 out of 18). The mean fatigue time was 113 ± 11 minutes and 123 ± 10 minutes for the 30 s and 120 s wave period groups, respectively (Table 4).

Table 4. Test results of part B using different periodicities but the same peak current.

Treatment group (% of U_{crit})	Peak current speed (cm s^{-1})	Fish completed (%)	Fatigue time (of those that fatigues)	Wave period (s)
120	114	17	113 ± 11 minutes	30
120	114	22	119 ± 10 minutes	60
120	114	11	123 ± 10 minutes	120

There was no significant difference in fatigue time between the three treatment groups with the same peak speed but different wave periods of 30, 60, and 120 s (Kruskal–Wallis test, χ^2 = 0.816, p-value = 0.665) (Fig. 13).

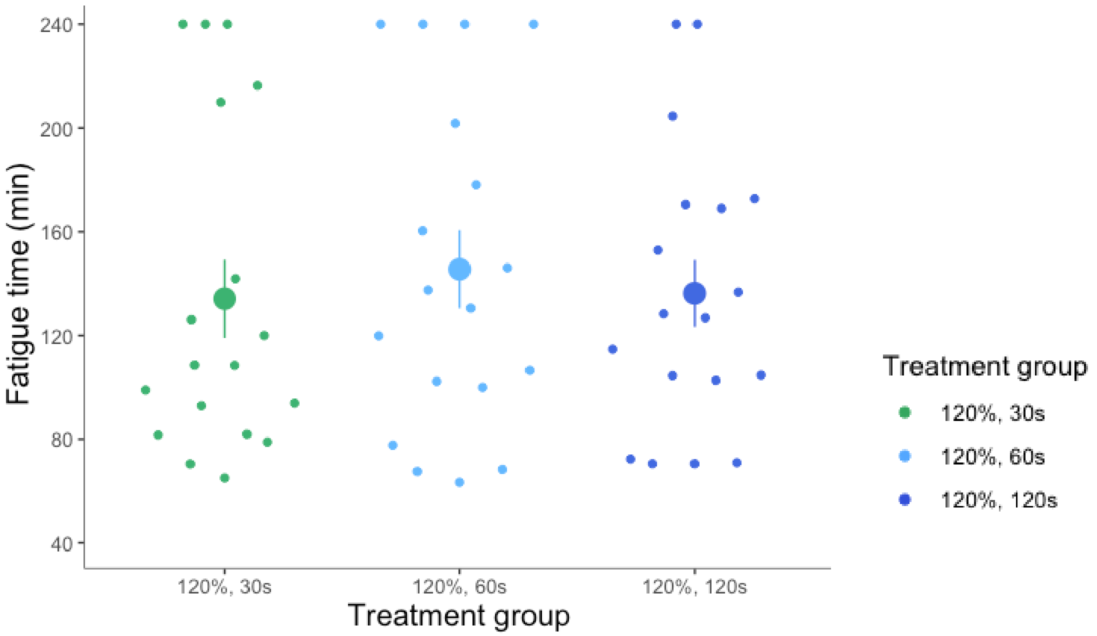


Figure 13. *The fatigue time for fish in part B of the study with different wave periods but the same current peak speed. The point ranges indicate mean ± s.e.m for each treatment group, and the data points at the 240 minutes mark indicate fish that completed the four-hour test.*

Fatigue time was unaffected by the relative swimming speed in the treatment groups with 30 s (Linear regression, $R^2 = 4.21*10^{-2}$, p-value = 0.463) and 120 s (Linear regression, $R^2 = 3.55*10^{-3}$, p-value = 0.827) wave period (Fig. 14).

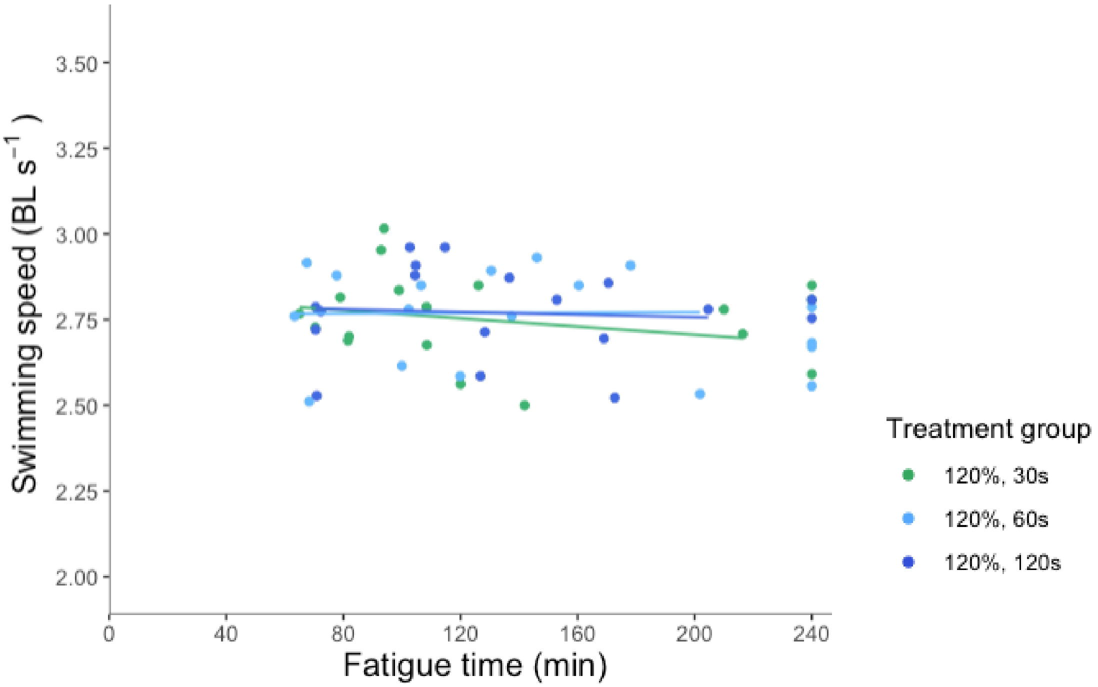
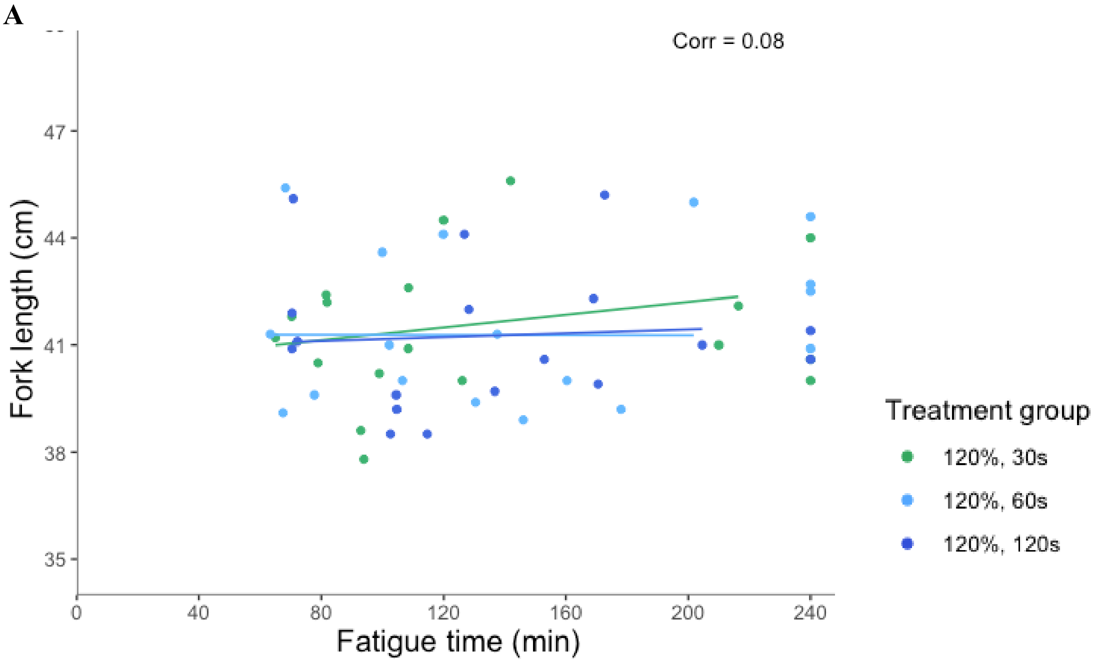


Figure 14. *Individual relative peak swimming speeds experienced in part B of the experiment as a function of fatigued time. The lines indicate linear regressions for the fatigued fish for the three treatment groups with different wave periods. The data points at the 240 minutes mark represent fish that completed the four-hour tests.*

A Pearson correlation test was conducted between fatigued time and L_f for the groups with different wave periods, which revealed a correlation coefficient of 0.080 (p-value = 0.60), indicating no significant correlation. Furthermore, there were no significant differences between fatigued time and K in the three treatment groups (Pearson, Coeff = -0.187, p-value = 0.219) (Fig. 15).

A



B

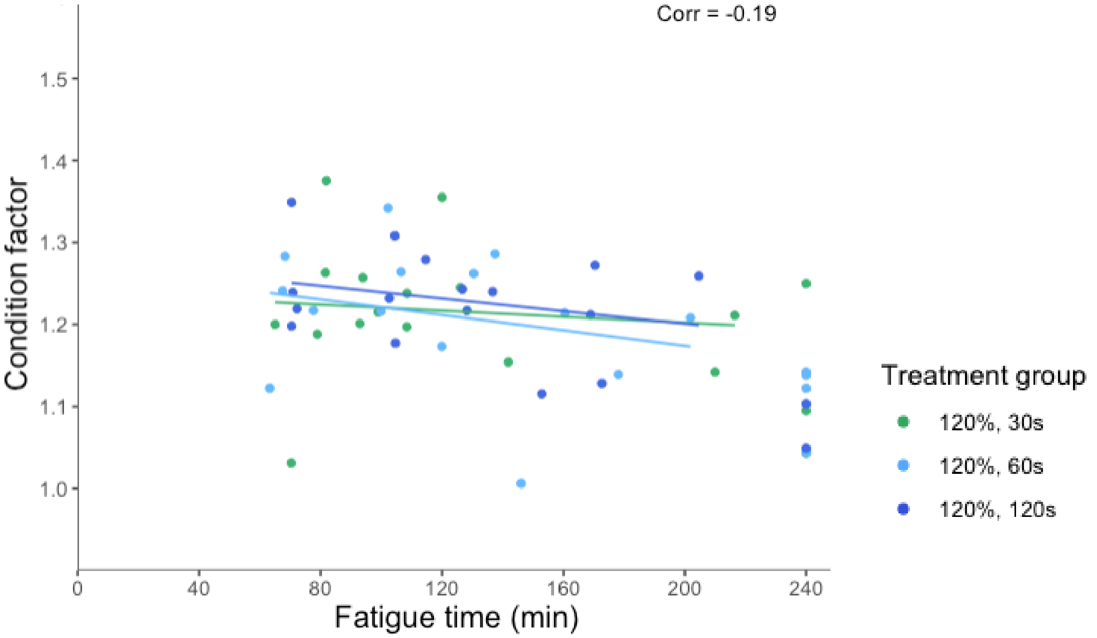


Figure 15. *The relationship between L_f (A) and K (B) versus fatigued time in part B of the experiment with different wave periods but the same current peak speed. The lines for treatment groups with a wave period of 30, 60, and 120 s indicate linear regressions of the fatigued fish. The data points at the 240 minutes mark represent fish that completed the four-hour test.*

4. Discussion

4.1 Discussion of material and method

4.1.1 Experimental design

The wave periods chosen for the trials were generally longer than we would have expected under natural circumstances (NauticalCampus, 2019). Shorter wave periods were desired to simulate more natural conditions and induce a higher frequency of accelerations for the fish, as it is conceivable that the acceleration would require some anaerobic effort, potentially resulting in earlier fatigue. However, this was not possible due to the motor's limitations. It was therefore decided to have a greater variance of 30 s between the three treatment groups with different wave periods to provide a gradual progression in wave durations, facilitating the assessment of potential effects of wave periods on salmons swimming performance. Using a smaller swim tunnel system with a reduced volume could have allowed for the replication of shorter waves. However, this would necessitate a decrease in the number of fish in each trial, resulting in the need for more trials to ensure the same statistical validity (see Chapter 4.1.2).

There was a significant difference between the first group tested, the U_{crit} trials, and the last treatment group, 140% U_{crit}, for all three size parameters, which could have given an incorrect U_{crit} estimate for the bigger fish in the last treatment group. However, the U_{crit} group was the first group to be tested, while approximately five weeks passed between the first and final treatment group, meaning that some size disparities were to be expected. To account for variations in U_{crit} between fish stocks as well as other environmental or biological variables, the subsequent fluctuating current trials were standardized to a percentage of the U_{crit}, making the U_{crit} represent a current water exposure that will cause imminent fatigue regardless of context. Furthermore, the effect of solid blocking, which occurs when an object causes an increase in flow around itself because of the blocking of flow through a portion of the fixed tunnel cross-section to satisfy the needs of continuity, was not corrected for in any trial. This was because the size of the tested fish relative to the large cross-sectional area in the swim chamber did not exceed 10% in any of the trials since the fish were evenly distributed across the swim section and hardly overlapped while swimming (Bell & Terhune, 1970; Plaut, 2001).

4.1.2 Number of fish used in the trials

Conducting multiple experimental trials in swim tunnels is time-consuming, typically allowing only one daily trial (Hvas & Oppedal, 2017c). Moreover, like the majority of biological experiments, it is constrained by limited resources and experimental space. Consequently, six fish per trial were chosen in the swimming performance protocols, both in the U_{crit} and the fluctuating water current. This was so that several fish could be tested simultaneously to obtain a greater number of individual measurements within each treatment group for robust statistical validity and simultaneously limit the time used in the laboratory. Furthermore, the chosen number of fish corresponds to a relative stocking density of approximately 10 kg m^{-3}, which is comparable to commercial aquaculture farms (Turnbull et al., 2005). The limited number of individuals included in the study may have hindered the potential benefits derived from schooling behavior observed in commercial farms (Johansson et al., 2020, Herskin & Steffensen, 1998). However, Hvas and Oppedal (2019b) found no improvement of U_{crit} in salmon when comparing groups and individuals swimming in the same swim tunnel used in the present study. Furthermore, if the number of fish had been increased in each trial, a potential concern would be the need to account for solid blocking. An increased number could also introduce the risk of obtaining an inaccurate measure of fatigue time or encountering other potential human errors. This situation could arise if multiple fish simultaneously reached the state of fatigue, requiring the simultaneous removal of several fish from the swim tunnel. The number of fish tested per trial was therefore chosen as the most appropriate compromise based on above considerations.

4.1.3 Potential for additional physiological measurements

This experiment focused on quantifying swimming capacities of Atlantic salmon subjected to novel water current regimes. Other studies on the swimming physiology of fish sometimes utilize additional measurements such as sampling to measure various hematological parameters and oxygen uptake rates to assess metabolic rates and CoT. Owing to the aim of this study, together with methodological and practical considerations, it was decided not to use those methods in the present study, as explained in more detail below.

Blood samples

In other studies, involving swim tunnels, blood sampling of fish is sometimes done immediately after the fish fatigue to provide a snapshot of stress and acid-base levels which then can be compared to the baseline status of fish that did not undergo a swim test as well as status after a period of recovery, provided the fish were kept alive after the swim trial. In the present study, this was often not feasible in many of the trials since several fish completed the four-hour tests. Due to the large volume of the swim tunnel (1905 liters), standard sedation drugs for salmon, such as isoeugenol, benzocaine, or tricaine mesylate (FHI, 2022), were considered impractical because it would have required large quantities of the chosen drug in each trial. Additionally, extracting sedated fish in the frontal part of the swim chamber would have been challenging, potentially prolonging their exposure to the drug, which may have induced more stress on the salmon (Zahl et al., 2010). Therefore, the fish that completed the test were not sedated, and since sampling blood from only a portion of the fish would have compromised the validity of the results, blood sampling was excluded from the present study.

Oxygen uptake rate measurements

In respirometry studies on fish, the rate of oxygen uptake (MO_2) is used as an indirect measurement of the aerobic metabolic rate, determined by monitoring the reduction of oxygen levels in the water within a sealed chamber over time. A key underlying assumption in this method is that trials are performed under steady-state conditions such that the measured rate of change in oxygen is proportional to the energy production in the mitochondria of the fish (Nelson, 2016). Respirometry techniques can be combined with swim tunnel setups so that MO_2 is measured while fish are swimming to infer energetic efforts across defined activity levels (e.g., Hvas & Oppedal, 2019b). In respirometry protocols, when assessing swimming performance, the measuring is typically conducted for 10-20 minutes at a constant current speed before the system is flushed to reestablish the oxygen levels to obtain a robust trace of the linear decline in ambient oxygen (Lee et al., 2003: Hvas et al., 2017a, 2018b). As such, it was impractical to carry out these measurements when the water flow was alternating between high and low current speeds that moreover would violate the assumption of steady-state conditions as the fish constantly were changing their activity levels. Theoretically, it may have provided an average MO_2 for the tested fish over a number of cycles with fluctuating currents, but it would have been challenging to interpret the results with regards to identifying transitions from aerobic to anaerobic swimming and how long the fish swam anaerobically. Furthermore, in practice, small air bubbles emerged within the system when the motor accelerated the current speed to 120% U_{crit} (114 cm s^{-1}) or higher, and these observed air bubbles would have made it impossible to measure MO_2 reliably. Hence, owing to the alternating flow regime and the issues with air bubbles, MO_2 was not measured in the present study.

4.1.4 Statistical analyzes

The choice of statistical analyses in this study was based on the characteristics of the data. A one-way ANOVA was chosen to investigate significant differences in the size parameters between all treatment groups and between fatigue time and the different treatment groups in part A of the fluctuating water current trials. Prior to the analysis, the fish distribution within each size parameter was assessed using density plots and Shapiro-Wilk tests, revealing an approximately normal distribution. However, when examining the effect of different wave periods on fatigue time in part B, the data did not meet the assumption of normal distribution, even after applying a log transformation. Hence, a Kruskal-Wallis test, which accommodates nonparametric data, was determined as more appropriate for this analysis. As in most biological studies, the p-value chosen as the significant level for all conducted analyses was > 0.05. However, 0.05 is only a well-established cutoff, and results with higher p-values can still provide important biological relevance (Fay & Gerow, 2018).

4.2 Discussion of results – Swimming performance of Atlantic salmon in fluctuating water currents

4.2.1 The critical swimming speed

The critical swimming speeds (U_{crit}) measured in the present study were generally similar to previous work on Atlantic salmon post-smolts of comparable sizes and temperatures when using the same swim tunnel setup (Remen et al., 2016; Hvas et al., 2017a, 2017c, 2020, 2021a) (summarized in Table 5). However, some differences between studies are expected since the U_{crit} will vary due to the different sizes, temperatures, acclimation history, health status, and other unaccounted variations between experimental works (Remen et al., 2016; Hvas et al., 2017a).

Table 5. *An overview of obtained U_{crit} values of Atlantic salmon from previous studies using the same swim tunnel system.*

Average L_f (cm)	Average W (g)	Temperature (°C)	U_{crit} (cm s^{-1})	U_{crit} (BL s^{-1})	Reference
41.1 ± 0.2	*837 ± 13.6*	*9*	*94.5 ± 1.6*	*2.4 ± 0.05*	*The present study*
-	289 ± 9	14	90.9 ± 1.2	-	Remen et al., 2016
37 ± 0.5	491 ± 19	8	84.7 ± 0.4	2.30 ± 0.8	Hvas et al., 2017a
43 ± 0.6	849 ± 36	13	97.2 ± 1.6	2.27 ± 0.04	Hvas et al., 2017b
42 ± 0.4	949 ± 34	12	115.3 ± 1.2	2.74 ± 0.05	Hvas et al., 2020
38 ± 0.4	667 ± 23	12	107.3 ± 1.2	2.80 ± 0.04	Hvas et al., 2021a

4.2.2 The anaerobic burden of swimming above 80% critical swimming speed

In the present study, all tested Atlantic salmon post-smolts were able to complete the four-hour tests in fluctuating water currents with peak speeds of 80% and 100% U_{crit} that required the fish in the 100% group to swim at their U_{crit} for short durations. The threshold of aerobic sustained swimming in Atlantic salmon is approximately 80% of the U_{crit}, meaning that swimming is solely powered by the red slow muscle fibers when swimming below this speed (Beddow & McKinley, 1999; Hvas & Oppedal, 2017c; Hvas et al., 2021a). When the current speed exceeds 80% U_{crit}, swimming will be powered by a combination of slow red aerobic and fast white anaerobic muscles (Kiessling et al., 2006). Anaerobic swimming efforts represent a non-steady state that causes significant physiological changes such as an accumulation of lactate, elevated stress hormones levels, and osmoregulatory disturbance in the blood (Kieffer, 2000; Davison, 1997). As such, if the fish are forced to swim above their aerobic speed limit for prolonged periods, they will eventually become fatigued (Hvas et al., 2020). This has previously been tested when Atlantic salmon post-smolts were forced to swim at constant current, where the salmon could endure for four hours at 80% of the obtained U_{crit} (97.2 ± 1.6 cm s^{-1}) while maintaining a steady oxygen uptake rate that suggested swimming remained aerobic. However, only 2 out of 24 fish completed the test at 100% U_{crit} (Hvas et al., 2017a). The 80% U_{crit} threshold for aerobic swimming was corroborated in a more recent study, where Atlantic salmon were able to endure 72 hours of constant swimming at this level. At the same time, increasingly higher speeds predictably caused fatigue significantly sooner (Hvas et al., 2021a). Moreover, the 80% U_{crit} threshold for sustained swimming also appears consistent across temperature and fasting periods (Hvas, 2022).

While there have been some studies on the impact of waves on sea cages (Huang et al., 2008; Kristiansen et al., 2015; Chu et al., 2020), only a limited number have explored how alternating water current (i.e., waves) affects the swimming capacity of Atlantic salmon or any fish species in general. For the fish to maintain its position in the water when exposed to waves, it must follow the movement of the water and accelerate in the active phase and decelerate in the passive phase. It is conceivable that Atlantic salmon would experience a bigger strain since the acceleration was thought to require some anaerobic efforts, leading to earlier fatigue than in the sustained swimming trials. However, the fish in the 80% and 100% U_{crit} treatment groups exhibited impressive endurance in the present study and completed trials with absolute swimming speeds of 76 and 95 cm s^{-1}, respectively (relative swimming speeds of 1.9 and 2.3 BL s^{-1}).

A study conducted by Hinch and Bratty (2011) on adult sockeye salmon (*Oncorhynchus nerka*) migrating through a demanding river passage may be used to explain why the Atlantic salmon were able to endure the trial at 100% U_{crit}. They found that fish exhibited different tactics, resulting in successful and unsuccessful migrants. Fish that successfully passed the area never exceeded their U_{crit} (2.3 BL s^{-1}) for more than three minutes, while unsuccessful migrants were found to exceed their U_{crit} for more extended periods (each longer than 10 minutes). The successful migrants also had relatively shorter residencies and used the environment more efficiently, conserving their energy to transit the passage (Hinch & Bratty, 2011). In the present study, the fish in the 100% U_{crit} treatment group were forced to swim at their U_{crit} briefly before swimming at a lower current speed, and it was observed that some of the fish, especially in the latter part of the trial, rested at the bottom of the swim tunnel, while others swam calmly throughout the low-intensity interval. It can therefore be inferred that the tested fish, despite briefly exceeding swimming speed over 80% U_{crit}, did not rely heavily on anaerobic metabolism and were able to conserve energy at the low current interval, resulting in the ability to complete the four-hour trials even with peaks of 100% U_{crit}. However, at a current peak speed of 120% U_{crit}, most individuals could not complete the trial, and at 140% U_{crit}, all fish became fatigued within 1.5 hours. Based on the findings, the accumulated burden eventually became too severe when peak currents reached 120% and 140% U_{crit} and caused fatigue, effectively defining a time-dependent tolerance limit for maximum peak current regimes.

4.2.3 Swimming performance with different wave periods but with the same peak speed

The present study found that the fatigue time of Atlantic salmon post-smolts was unaffected by the different wave periods of 30, 60, and 120 s at a peak speed of 120% U_{crit} over a four-hour period. When Johannesen et al. (2020) observed the effect of wave periods on Atlantic salmon at an exposed farm on the Faroe Islands, they found indications of biological significance that were related to the distribution of fish vertically and horizontally in the water column and in relation to the cage wall at night and daytime. Furthermore, Dam (2015), which studied Atlantic salmon at the same exposed Faroe Island location, was unable to draw any conclusions regarding how wave periods affected the swimming performance or group behavior. Despite varying wave periods, no significant difference in average swimming speed was observed during the high wave period in that study (Dam, 2015). Other studies of how Atlantic salmon swimming capacity, or farmed fish species in general, are affected by different wave periods are hard to come by. A study by Marcoux and Korsmeyer (2019) examined the impact of oscillatory movements in a wave motion respirometer on wild coral reef fish. Decreasing wave periods increased metabolic rates and net costs of swimming for fish with locomotion most similar to salmon, suggesting that wave periods may affect energy consumption during swimming. However, the same fish showed no signs of exhaustion or difficulty maintaining their position (Marcoux & Korsmayer, 2019). The results from the present study imply that different wave periods had no significant impact on fatigue time in salmon, thereby providing valuable insights regarding offshore aquaculture. Nevertheless, due to the absence of literature focusing specifically on the effect of wave periods on farmed Atlantic salmon, further research, both in the laboratory and field, will be necessary to provide a more comprehensive and holistic understanding of the effects.

4.2.4 Threshold values to fluctuating water currents

Up until now, the welfare indicators used as guidelines for biological limits of farming Atlantic salmon at offshore aquaculture sites are based on U_{crit} and sub-U_{crit} values, such as the sustained swimming limit, minimum CoT, and behavioral group patterns in sea cages (Hvas et al., 2020). When only aerobic muscles are used to power swimming in fish, it can be maintained indefinitely in theory. However, the fish could still become fatigued, like a marathon runner who "hits the wall," even though the running (or swimming) is entirely aerobic owing to the depletion of energy stores (Kiessling et al., 2006; Beamish, 1978; Hvas et al., 2020). Because of this, Hvas et al. (2020) argue that the threshold values for chronic current conditions lasting much longer (days or weeks) should be lower, even though post-smolt salmon have shown impressive results in sustained swim trials (Hvas et al., 2021a). Additionally, despite the fish's ability to sustain aerobic swimming for extended durations, the growth potential may be reduced if they constantly use most of their energy on constant swimming (Farrell et al., 1991; Solstorm et al., 2015). Therefore, even though Atlantic salmon can tolerate wave action with peak currents of 100% U_{crit} for short durations, it is essential to note that constant speeds above preferred swimming speeds or optimal cruising speeds may result in behavioral restrictions and reduced growth (Solstorm et al., 2015; Hvas et al., 2020).

Based on the results from the present study, the strongest median current speed measured at the three recommended offshore farming areas in Norway is found to be within the tolerance limits of the tested Atlantic salmon (Appendix A, Table 6). However, it is important to note that extreme weather events, such as storms and hurricanes, likely will result in current and wave speeds exceeding the strongest current measured in these areas (IntraFish, 2017; Hvas et al., 2020). The results of the present study primarily represent an upper threshold value that is ≈20% higher than for chronic water currents exposures during brief periods of extreme conditions, to ensure that the fish do not experience exhaustion with death as the worst-case scenario.

4.2.5 Fork length and how it affects fatigue time.

The fork length (L_f) of fish in the 120% treatment groups ranged from 37.8 to 45.6 cm; in the 140% group, it ranged from 39.2 to 48 cm. This represented a difference in swimming speed of 3.01 to 2.50 BL s^{-1} and 3.39 to 2.77 BL s^{-1} for the 120% and 140% groups, respectively, which could explain the variation of endured time of individual fish at the different current peak speeds. When looking at swimming speed, the relative scale is more sensitive to size differences, while the absolute scale is less sensitive and appears approximately constant within a limited size range, such as those assessed in the present study. This means that, in general, smaller fish attain higher relative swimming speeds, while larger fish swim faster in absolute scales (Brett, 1964; Remen et al., 2016; Hvas, 2018a). Therefore, a significant negative correlation between swimming speed in BL s^{-1} and fatigue time was expected, indicating that bigger fish could endure longer. However, when looking at the treatment groups exposed to a fluctuating water current, there was no significant correlation between L_f and the fatigue time of individual fish. A more robust pattern where bigger fish endures a higher water current emerges when treatment groups exhibit a greater size disparity (Remen et al., 2016; Oldham et al., 2019). Therefore, it is likely that the variation in L_f in each treatment group was not big enough to cause any significant difference in fatigue time.

4.2.6 The effect of condition factor

All fish in the present study had a condition factor above 1.1, which, based on Stien et al. (2013) review of selected welfare indicators, indicates that the salmon were in good health. The condition factor for farmed Atlantic salmon usually falls within the range of 0.9 - 1.3, where below 0.9 indicate an emaciated fish (Stien et al., 2013; Noble et al., 2018). It could therefore be hypothesized that fish with a higher condition factor would perform better in the fluctuating water current trials. However, based on the results, there was a significant negative correlation between the condition factor and fatigue time for the fish in the 120% and 140% U_{crit} treatment groups, indicating that the slightly leaner fish performed better. In a previous swim tunnel study, the condition factor was concluded to be a poor indicator of individual variance in swimming performance when varying between 0.96 and 1.07 (Hvas & Oppedal, 2017c). Moreover, a recent study by Hvas et al. (2021b) also showed that Atlantic salmon, which were fasted for up to four weeks, did not exhibit a significant difference in their critical swimming speed, despite a gradual decrease in condition factor from 1.03 to 0.89. However, a study by Wilson et al. (2021) on non-fed wild sockeye salmon smolts assessed swimming performance by subjecting the fish to weekly swim challenges over six weeks, where the fish decreased in condition from 0.72 when sampled to 0.57 in week six. Concomitantly with the decrease in condition, swim performance decreased throughout the study, with only 12% of the fish completing the swim test in week six compared to 83% in week one, and the authors concluded that the continuous decline in the condition factor was a reliable indicator of swimming performance. It is therefore more likely that the condition factor would have influenced swimming performance if it was below 0.9 or decreased significantly over more extended periods. Given that the condition factors of the tested fish in the present study were well within healthy ranges for Atlantic salmon, it can be assumed that the small variation in condition had a minimal effect on the findings.

4.3 The method: testing effects of large ocean waves in a small laboratory vs. natural conditions

One of the study's aims was to develop a method to test fish in fluctuating water currents using a computer system that automatically could alternate motor output between peak and minimum speeds in a large swim tunnel setup, where peak speeds and periodicity of current fluctuations could be manipulated to test the tolerance limits of the fish systematically. Waves with 30, 60, and 120 s periods were created, with 30 s as the shortest wave period. However, it is important to recognize that wave periods in natural environments typically exhibit more significant variability and irregularity, ranging from seconds to a few tens of seconds within the same time frame (Wright et al., 1999; NauticalCampus, 2019), which differ from the simulated uniform waves. At the exposed farms in the Faroe Islands, the longest reported wave periods ranged from 14–20 s and were described as more complex because of the varying durations (Dam, 2015; Johansson et al., 2020). However, the farm site was shallow and relatively close to the coast, and more extended wave periods are expected in deeper and more open waters where there is less influence from the seabed and the coast (Albretsen et al., 2019). Depending on the specific locations of offshore farms, wave periods more similar to those tested in the present study may occur. However, further research in natural conditions is necessary to understand the complexities of wave patter and the effects on Atlantic salmon's welfare and swimming performance at offshore locations.

The laboratory waves had minor variations in peak speeds but a consistent wave period, and the environment inside the swim tunnel was controlled and stable, ensuring constant temperature and oxygen levels. In contrast, the waves at offshore sites are anticipated to have waves with not only varying periods but also varying wave speeds and heights (Wright et al., 1999; NauticalCampus, 2019), as documented in Dam (2015) and Johansson et al. (2020) studies. However, stable temperature levels have been identified as potential advantages of offshore aquaculture (Bjelland et al., 2015; Hvas et al., 2020), and at the exposed farm in the Faroe Islands, little to no differences in temperatures were reported during the observation periods (Dam, 2015; Johansson et al., 2020). However, despite oxygen also being acknowledged as a potential advantage in offshore farms, reduced water exchange associated with low currents may increase the risk of hypoxia because of larger constructions, a condition proven to negatively impact the swimming performance of Atlantic salmon (Oldham et al., 2019). It is important to note that these duration variances and differences in environmental conditions, which are challenging to replicate accurately in a laboratory experiment, may impact the applicability of the findings in the present study in an actual real-world scenario.

While testing fish in controlled laboratory conditions may not accurately reflect a natural offshore environment, the developed method and findings still provide valuable insights into how fish reacts to waves. Based on the findings of Hinch and Bratty (2011), the authors discuss the possibility that swimming performance tested and established in a laboratory by fish swimming in a constant water current until they fatigue may underestimate the fish's performance when the water flow alternates between fast and slow speeds in a field situation. The results of the present study, where the fish could endure a higher percentage of U_{crit} with an alternating current speed than with a constant for the same amount of time, corroborate this assumption.

4.4 Implications for fish welfare in offshore aquaculture

4.4.1 Differences in group behavior

The present study observed some collisions between the fish in the swim trials, and the collisions were more frequent when the fish approached fatigue and got dragged back simultaneously at the rear of the tunnel. In a commercial sheltered aquaculture farm, a single cage can contain up to 200,000 fish (Akvakulturdriftsforskriften, 2008 §47a), and in bigger offshore farms, this number is expected to be much higher (Hvas et al., 2020). Individual fish usually swim in the same direction forming a circular school pattern (Juell, 1995), and if the current speed increases, the schooling structure will gradually change from circular to standing against the current to optimize energy consumption (Johansson et al., 2020, Herskin & Steffensen, 1998). When standing against the current, the Atlantic salmon may improve their performance by reducing the CoT (Hvas et al., 2017b), and studies have reported that the tail beat frequency of other schooling fish species was lower for fish swimming at the group's rear end (Fields, 1990; Herskin & Steffensen, 1998). Regarding waves, it is essential to know whether the fish are able to maintain sufficient behavioral control and prevent collisions with other fish and the surroundings, particularly when faced with powerful oscillatory water movements with high peak speeds. Collision risks may also increase at high stocking densities due to potential reductions in cage volume caused by waves and stronger currents (Klebert et al., 2015, Hvas et al., 2020). This necessitates more robust offshore constructions capable of withstanding current exposures without collapsing to smaller volumes, thus impacting the group behavior of fish. The impacts of waves become an even bigger concern as the fish approaches fatigue, resulting in unacceptable welfare conditions (Hvas et al., 2020), highlighting the importance of defining upper threshold values for offshore farming.

4.4.2 An adaptive species

Atlantic salmon is generally a flexible and adaptive species when exposed to environmental changes (Hvas et al., 2020), which also was observed during the trials where there were chaotic swimming and more frequent collisions between the fish in the beginning compared to the latter part of the trials (except when approaching fatigue). Previous studies have shown that Atlantic salmon quickly adapt and adjust their individual swimming behavior and group behavior for optimal performance when exposed to a sudden environmental change, such as documented in the earlier mentioned exposed sites (Johansson et al., 2020; Dam, 2015), training regimes (Castro et al., 2011), and in submerged cages (Korsøen et al., 2012). This suggests that Atlantic salmon may require a period of acclimation to adapt to the new environmental conditions following their transition to an offshore farm. During this adjustment phase, initial chaotic swimming patterns and spatial distribution within the sea cage may be observed. However, the fish is anticipated to adapt to the novel environments quickly, provided the predefined threshold values are not exceeded.

Given that previous studies within the area have mainly focused on growth performance, critical swimming capacity, or sustained swimming capacities at constant current speeds (Jobling et al., 1993; Castro et al., 2011; Solstorm et al., 2015; Remen et al., 2016, Hvas et al., 2017c, 2021a, 2021b), the findings of the present study represent novel and important contributions to the ongoing work toward establishing welfare guidelines for acceptable conditions at offshore locations where bigger waves and stronger currents are expected.

4.5 Future perspectives

The present study shows that Atlantic salmon can endure higher peak speeds with fluctuating currents compared to constant peak current speeds. However, the swim trials only lasted for four hours, and it is essential to acknowledge that peak currents and waves at an offshore location may persist for several hours or even days during storms (Hvas et al., 2020). Moreover, it will be essential to consider the impact of lower temperatures, as stormy events and stronger currents are more likely to occur during the winter season. This is especially important for Atlantic salmon as their swimming capacity has been documented to decrease significantly at very low temperatures (Hvas et al., 2017a). Further studies should therefore seek to investigate how long-term exposure to both wave-induced conditions and the more complex wave dynamics in field situations with natural settings impacts the behavior and welfare of farmed Atlantic salmon.

5. Conclusion

In the present study, two main research questions were examined:

The alternation between high and low current speeds did not result in earlier fatigue times for Atlantic salmon when compared to swimming limits for critical and sustained swimming capacities in trials with constant current speed. In fact, Atlantic salmon endured more than four hours of fluctuating currents with peaks corresponding to the critical swimming speed. To consistently cause fatigue, peak currents of 120% and 140% of the critical swimming speed were required.

Question 1: *Will fluctuating water currents affect the tolerance limits of Atlantic salmon and result in earlier fatigue compared to a constant current speed?*

H_{01}: Tolerance limits to fluctuating water currents will be the same as constant current exposure of similar peaks, **is rejected**.

H_{A1}: Tolerance limits to fluctuating water currents will differ from constant current exposure of similar peaks, **is accepted**.

When testing the swimming capacities of Atlantic salmon exposed to three different wave periods but at the same peak current speed, no significant differences in fatigue time were found. This suggested that more frequent acceleration efforts to the same peak speed were not associated with increased anaerobic swimming efforts.

Question 2*: Will different wave periods significantly affect fatigue time?*

H_{02}: There will be a significant difference in fatigue time regarding wave periods, **is rejected**.

H_{A2}: There will not be a significant difference in fatigue time regarding wave periods, **is accepted**.

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Appendix A

Impact assessment for offshore aquaculture off the coast of Norway

The three areas the Norwegian Directory of Fisheries have recommended for public overall impact assessment are “Norskerenna sør” located 30 - 40 nautical miles (nm) off the coast of Rogaland, “Frøyabanken nord”, located 30 - 70 nm off the coast of Trøndelag, and “Trænabanken”, located 20 - 79 nm off the coast of Salten (Fig. 16) (Fiskeridirektoratet, 2022).

Figure 16. *The locations of the three recommended areas for offshore aquaculture in Norway. Obtained from Fiskeridirektoratet (2022)*

Based on the evidence base and present circumstances of the three areas recommended for offshore Aquaculture, the median of the strongest current speed measured was 0.57 m s^{-1}, a velocity well below the tested speeds examined during the fluctuating water current trials, and the highest median of average current speeds was measured at 0.26 (Norskerenna sør). The expected key figures for the three areas in listed in Table 6. (Fiskeridirektoratet, 2022).

***Table 6**. Key figures for the three recommended areas, "Norskerenna sør" (A), "Frøyabanken nord" (B), and "Trænabanken" (C), for offshore aquaculture in Norway. The tables are obtained from Fiskeridirektoratet (2022)*

A

"Norskerenna sør"	
Total area (km^2)	485
Distance from baseline (nm)	30-40
Sea depth	270-290
Median of strongest current speed ($m\ s^{-1}$)	0,57
Median of average current speed ($m\ s^{-1}$)	0,26
Median of lowest temperature measurements (°C)	4,90
Median of highest temperature measurements (°C)	15,90
Median of highest significant wave height (m)	4,46
Median of average significant wave height (m)	1,78

B

"Frøyabanken nord"	
Total area (km^2)	2327
Distance from baseline (nm)	30-70
Sea depth	300-375
Median of strongest speed velocity ($m\ s^{-1}$)	0,21
Median of lowest temperature measurements (°C)	6,80
Median of highest temperature measurements (°C)	13,90
Median of highest significant wave height (m)	5,14
Median of average significant wave height (m)	2,12

C

"Trænabanken"	
Total area (km^2)	4698
Distance from baseline (nm)	20-79
Sea depth	240-430
Median of strongest current speed ($m\ s^{-1}$)	0,45
Median of average current speed ($m\ s^{-1}$)	0,21
Median of lowest temperature measurements (°C)	6,33
Median of highest temperature measurements (°C)	13,54
Median of highest significant wave height (m)	5,08
Median of average significant wave height (m)	2,13

Appendix B

Method development

Preparation

Before commencing the fluctuating water current trials, it was imperative to conduct initial testing of the swim tunnel system after installing the Programmable Logic Controller (PLC) (set up is shown in Fig. 17). A testing phase was therefore necessary as prior studies had yet to be conducted using the swim tunnel system after the PLC implementation. All tests were done without any fish in the swim tunnel system.

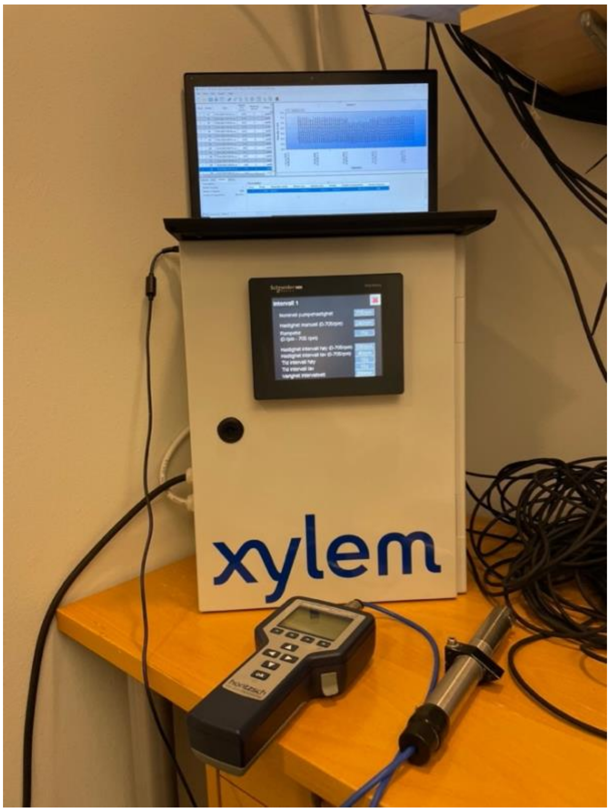

Figure 17. *The set-up of the PLC with the handheld flow meter and associated flow sensor underneath.*

The PLC offered several programmable options, including the following (Fig. 18):

- Manual speed (rounds per minute, RPM) (*Norwegian: "Hastighet manuell"),*
- Ramp time (s) (*Norwegian: "Rampetid"),*
- The speed of the high-intensity interval (RPM) (*Norwegian: "Hastighet intervall høy"),*
- The speed of the low-intensity interval (RPM) (*Norwegian: "Hastighet interval lav"),*
- Time on the high-intensity interval (s) (*Norwegian: "Tid intervall høy"),*
- Time on the low-intensity interval (s) (*Norwegian: "Tid intervall lav"),*
- Duration of sets of intervals (min) (*Norwegian: "Varighet intervallsett")*

Figure 18. *The screen of the PLC with the programmable options. The "Nominell pumpehastighet" (translated to Nominal pump speed) was the motors max pump speed.*

Manual speed

The Manual speed could be set to a specific RPM ranging from 0 to 705 RPM, making the speed of the water current in the swim tunnel constant if the Manual speed option was chosen. This option was used during the overnight acclimation of fish transferred to the swim tunnel, during the U_{crit} trials, and during the warm-up before the fluctuation swim, as all these activities necessitated a consistent current speed. To determine which RPM value corresponded to the speed experienced in the tunnel, the handheld flow meter (Höntzsch Flow Measuring Technology) was used. The flow sensor was attached to the inside of the swim tunnel, with the flow meter taking measurements every other second for five minutes at one specific pump speed. The data was then uploaded from the flow meter to an associated computer program (Software UCOM for Configuring Höntzsch Transducers), and the speed the RPM value equaled was determined based on the mean current speed of each test, as shown in Table 7.

Table 7. *The different tested pump speeds (RPM) and the corresponding mean current speed experienced in the swim tunnel (cm s^{-1}).*

Pump speed (RPM)	Water Current speed (cm s^{-1})
50	7
100	16
150	26
200	36
250	46
300	57
350	67
400	78
450	89
500	101
600	125
650	136

A regression analysis was then performed in Microsoft Excel (version 16.73) to calculate which RPM value corresponded to the speed planned for the experiments (Fig. 19).

Regression of calibration data

Regression statistics	
Multiple R	0,999315399
R-square	0,998631268
Adjusted R-square	0,998506837
Standard error	7,524343776
Observations	13

Variance analysis

	fg	*SK*	*GK*	*F*	*Significance-F*
Regression	1	454377,2268	454377,227	8025,63302	4,18206E-17
Residuals	11	622,7732419	56,6157493		
Total	12	455000			

	Coefficients	*Standard error*	*t-state*	*P-value*
Point of interest (POI)	32,20534889	4,115691604	7,82501509	8,0559E-06
Flow meter (m s^{-1})	460,8288304	5,143988547	89,5858974	4,1821E-17

Figure 19. *The regression analysis of the calibration data in Microsoft Excel.*

The following formula was used to find the needed RPM value:

$$RPM = POI + (speed * Fm),$$

where RPM is rounds per minute, POI is the Point of interest coefficient, speed is the wanted speed, and Fm is the Flow meter coefficient.

To confirm that the values from the regression analysis indicated the correct speed experienced in the swim tunnel, new tests with the flow meter were completed resulting in a final calibration.

Ramp time

The ramp time was the motor's acceleration time, i.e., indicating the duration required to reach the wanted higher or lower water current speeds. The standard acceleration time was programmed to range from 0 to 705 RPM. Therefore, the ramp time needed to be calculated to fit the chosen time interval since the RPM values of the low- and high-intensity intervals never were 0 and 705, respectively.

The formula to calculate the ramp was calculated as follows:

$$a_t = \frac{r}{705} * (RPM_{high} - RPM_{low}),$$

where a_t is the amount of time needed to attain the wanted speed (s), r is acceleration time (s), RPM_{high} is the RPM value for the high-speed interval, and RPM_{low} is the RPM value for the low-speed interval.

The speed of the high- and low-intensity intervals

The selection of high- and low-intensity interval speeds were based on the desired percentage of the mean Ucrit obtained from the Ucrit trials for the fish group. Consequently, the RPM values required for different speeds had to be determined before testing each treatment group. The calibration results presented in Table 7 served as a reference. Still, due to the fluctuating nature of the water current between high and low intensity, additional tests were conducted to ensure the attainment of the required speeds, particularly for the low-intensity interval. Similar to the Manual speed calibration method, multiple tests with various RPM values were performed on both the high- and low-intensity intervals using the flow meter. These tests enabled the determination of the RPM values corresponding to the desired speed for all treatment groups (Fig. 20). To enhance accuracy, the tests were extended to eight minutes, providing an increased number of wave cycles.

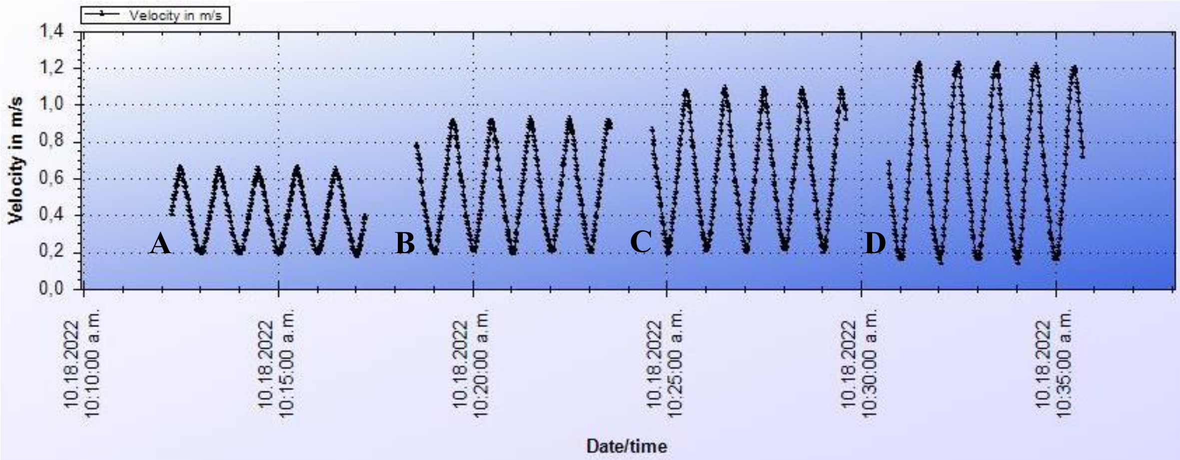

Figure 20. *Testing of different current speeds based on percentages of the U_{crit}, showing current peak speeds of 80 (A), 100 (B), 120 (C), and 140% (D) of U_{crit}. All with a lower speed of 20% of Ucrit.*

Time on the high- and low-intensity intervals

In addition to the RPM value and corresponding speed, the duration of an interval (equivalent to the wave period) had to be considered. Especially how short the intervals could be was interesting because it was unsure if the motor was able to accelerate the water current to the highest planned peak speed within the given timeframe and at the same time be enough seconds for the water current to decrease to the low pace before a new acceleration. Therefore, further tests with the flow meter were conducted (measurements every other second for eight minutes), varying both the RPM and the duration of the high and the low intervals to examine the limits of the simulated wave period (Fig. 21). The result suggested that the shortest wave period was 30 seconds (15 seconds at the high and 15 seconds at the low intensity).

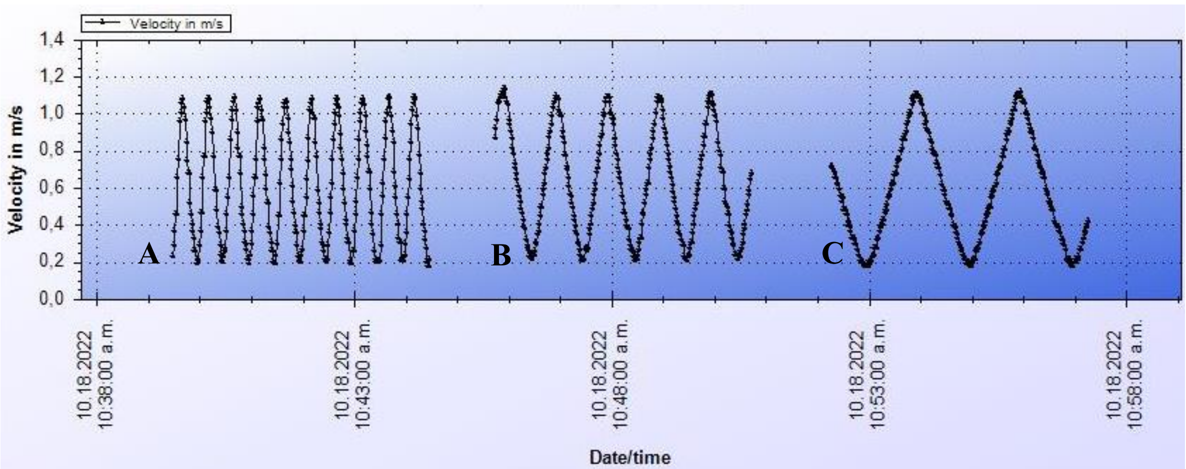

Figure 21. *Testing of different wave periods, showing 30 (A), 60 (B), and 120 s (C) at the same current speeds.*

Duration of sets of intervals

The duration of the sets of intervals was the total time the water currents were programmed to alternate between high and low current speeds. So, to prevent the system from shutting down before the completion of the fluctuating water current, the value was consistently set to 999 min.

Appendix C

1. Testing of size parameters for all groups tested

1.1 One-way ANOVA with TukeyHSD test

A

```
> anova_mass <- aov(mass ~ treatment_group, data = total)
> summary(anova_mass)
                 Df  Sum Sq Mean Sq F value   Pr(>F)    
treatment_group   6  594129   99022   5.135 0.000101 ***
Residuals       118 2275299   19282                     
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

B

```
> anova_lenght <- aov(fork_length ~ treatment_group, data = total)
> summary(anova_lenght)
                 Df Sum Sq Mean Sq F value Pr(>F)  
treatment_group   6   66.8  11.135   2.734  0.016 *
Residuals       118  480.6   4.073                 
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

C

```
> anova_k <- aov(condition_factor ~ treatment_group, data = total)
> summary(anova_k)
                 Df Sum Sq  Mean Sq F value   Pr(>F)    
treatment_group   6 0.1800 0.029992   5.387 5.98e-05 ***
Residuals       118 0.6569 0.005567                     
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

***Figure 22**. The outputs of the one-way ANOVAs for the mass (A), fork length (B), and condition factor (C) for all treatment groups in the present study. The output shows significant differences for all three size parameters.*

Table 8. *The outputs of the TukeyHSD for the mass (A), fork length (B), and condition factor (C) for all treatment groups.*

A

Fit: aov(formula = mass ~ treatment_group, data = total)

	diff	lwr	upr	p	Significance (p<0.05)
100% - 80%	63.722222	-75.13048	202.574928	0.8131047	Not significant
120%, 30s - 80%	96.888889	-41.96382	235.741594	0.3635172	Not significant
120%, 60s - 80%	100.444444	-38.40826	239.297150	0.3197433	Not significant
120%, 120s - 80%	85.722222	-53.13048	224.574928	0.5161217	Not significant
140% - 80%	184.055556	45.20285	322.908261	0.0022696	Significant
Ucrit - 80%	-48.052288	-188.93215	92.827572	0.9477034	Not significant
120%, 30s - 100%	33.166667	-105.68604	172.019372	0.9913529	Not significant
120%, 60s - 100%	36.722222	-102.13048	175.574928	0.9852164	Not significant
120%, 120s - 100%	22.000000	-116.85271	160.852706	0.9991108	Not significant
140% - 100%	120.333333	-18.51937	259.186039	0.1354842	Not significant
Ucrit - 100%	-111.774510	-252.65437	29.105350	0.2163595	Not significant
120%, 60s - 120%, 30s	3.555556	-135.29715	142.408261	1.0000000	Not significant
120%, 120s - 120%, 30s	-11.166667	-150.01937	127.686039	0.9999830	Not significant
140% - 120%, 30s	87.166667	-51.68604	226.019372	0.4954890	Not significant
Ucrit - 120%,30s	-144.941176	-285.82104	-4.061317	0.0394046	Significant
120%, 120s - 120%, 60s	-14.722222	-153.57493	124.130483	0.9999132	Not significant
140% - 120%, 60s	83.611111	-55.24159	222.463817	0.5464922	Not significant
Ucrit - 120%, 60s	-148.496732	-289.37659	-7.616872	0.0317909	Significant
140% - 120%, 120s	98.333333	-40.51937	237.186039	0.3453847	Not significant
Ucrit - 120%, 120s	-133.774510	-274.65437	7.105350	0.0744253	Not significant
Ucrit - 140%	-232.107843	-372.98770	-91.227984	0.0000521	Significant

B

Fit: aov(formula = fork_length ~ treatment_group, data = total)

	diff	lwr	upr	p	Significance (p<0.05)
100% - 80%	0.6111111	-1.4068624	2.6290846	0.9705783	Not significant
120%, 30s - 80%	0.9555556	-1.0624180	2.9735291	0.7897572	Not significant
120%, 60s - 80%	1.1000000	-0.9179735	3.1179735	0.6599307	Not significant
120%, 120s - 80%	0.7111111	-1.3068624	2.7290846	0.9391154	Not significant
140% - 80%	1.7944444	-0.2235291	3.8124180	0.1158869	Not significant
Ucrit - 80%	-0.6830065	-2.7304411	1.3644280	0.9529206	Not significant
120%, 30s - 100%	0.3444444	-1.6735291	2.3624180	0.9986430	Not significant
120%, 60s - 100%	0.4888889	-1.5290846	2.5068624	0.9906758	Not significant
120%, 120s - 100%	0.1000000	-1.9179735	2.1179735	0.9999990	Not significant
140% - 100%	1.1833333	-0.8346402	3.2013068	0.5780809	Not significant
Ucrit - 100%	-1.2941176	-3.3415522	0.7533169	0.4870012	Not significant
120%, 60s - 120%, 30s	0.1444444	-1.8735291	2.1624180	0.9999915	Not significant
120%, 120s - 120%, 30s	-0.2444444	-2.2624180	1.7735291	0.9998110	Not significant
140% - 120%, 30s	0.8388889	-1.1790846	2.8568624	0.8740318	Not significant
Ucrit - 120%,30s	-1.6385621	-3.6859966	0.4088724	0.2075548	Not significant
120%, 120s - 120%, 60s	-0.3888889	-2.4068624	1.6290846	0.9973195	Not significant
140% - 120%, 60s	0.6944444	-1.3235291	2.7124180	0.9454777	Not significant
Ucrit - 120%, 60s	-1.7830065	-3.8304411	0.2644280	0.1316405	Not significant
140% - 120%, 120s	1.0833333	-0.9346402	3.1013068	0.6759085	Not significant
Ucrit - 120%, 120s	-1.3941176	-3.4415522	0.6533169	0.3938643	Not significant
Ucrit - 140%	-2.4774510	-4.5248855	-0.4300164	0.0074803	Significant

C

Fit: aov(formula = condition_factor ~ treatment_group, data = total)

	diff	lwr	upr	p	Significance (p<0.05)
100% - 80%	0.043444444	-0.031165876	0.118054765	0.5863175	Not significant
120%, 30s - 80%	0.059777778	-0.014832543	0.134388099	0.2064247	Not significant
120%, 60s - 80%	0.046388889	-0.028221432	0.120999210	0.5073997	Not significant
120%, 120s - 80%	0.069722222	-0.004888099	0.144332543	0.0834223	Not significant
140% - 80%	0.106444444	0.031834124	0.181054765	0.0007414	Significant
Ucrit - 80%	-0.014673203	-0.090372783	0.061026378	0.9972316	Not significant
120%, 30s - 100%	0.016333333	-0.058276988	0.090943654	0.9945947	Not significant
120%, 60s - 100%	0.002944444	-0.071665876	0.077554765	0.9999998	Not significant
120%, 120s - 100%	0.026277778	-0.048332543	0.100888099	0.9392659	Not significant
140% - 100%	0.063000000	-0.011610321	0.137610321	0.1571566	Not significant
Ucrit - 100%	-0.058117647	-0.133817227	0.017581933	0.2513243	Not significant
120%, 60s - 120%, 30s	-0.013388889	-0.087999210	0.061221432	0.9982006	Not significant
120%, 120s - 120%, 30s	0.009944444	-0.064665876	0.084554765	0.9996707	Not significant
140% - 120%, 30s	0.046666667	-0.027943654	0.121276988	0.5000204	Not significant
Ucrit - 120%,30s	-0.074450980	-0.150150561	0.001248600	0.0570992	Not significant
120%, 120s - 120%, 60s	0.023333333	-0.051276988	0.097943654	0.9655240	Not significant
140% - 120%, 60s	0.060055556	-0.014554765	0.134665876	0.2017940	Not significant
Ucrit - 120%, 60s	-0.061062092	-0.136761672	0.014637489	0.1996865	Not significant
140% - 120%, 120s	0.036722222	-0.037888099	0.111332543	0.7581354	Not significant
Ucrit - 120%, 120s	-0.084395425	-0.160095005	-0.008695845	0.0185218	Significant
Ucrit - 140%	-0.121117647	-0.196817227	-0.045418067	0.0000944	Significant

2. Critical swimming speed trials

2.1 Pearson correlation tests

A

```
> cor.test(ucrit$ucrit_cm,ucrit$fork_length,
+                  method = "pearson")

        Pearson's product-moment correlation

data:  ucrit$ucrit_cm and ucrit$fork_length
t = 0.086483, df = 15, p-value = 0.9322
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.4632917  0.4976294
sample estimates:
      cor
0.0223242
```

B

```
> cor.test(ucrit$ucrit_BL, ucrit$fork_length,
+                  method = "pearson")

        Pearson's product-moment correlation

data:  ucrit$ucrit_BL and ucrit$fork_length
t = -2.959, df = 15, p-value = 0.009753
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.8420387 -0.1785593
sample estimates:
       cor
-0.6071005
```

Figure 23. *Pearson correlation test between absolute U_{crit} (cm s^{-1}) (A) and relative U_{crit} (BL s^{-1}) (B) and fork length shows a significant negative correlation between the relative swimming speed and condition factor.*

3. Fluctuating water currents part A: Different peak speeds

3.1 One-way ANOVA with TukeyHSD test

```
> anova_peak <- aov(fatigue_minutes ~ treatment_group,data = fluctuating_percent)
> summary(anova_peak)
                Df Sum Sq Mean Sq F value Pr(>F)    
treatment_group  3 443022  147674   132.4 <2e-16 ***
Residuals       68  75856    1116                   
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Figure 24. *The output of the one-way ANOVA with a TukeyHSD test for the treatment groups in Part A of the fluctuating water current trials with different peak speeds. The output shows significant differences between all treatment groups except for the 80% and 100%* U_{crit} *where all fish in both groups completed the test.*

Table 9*. The outputs of the TukeyHSD for fatigue time and the different treatment groups.*

Fit: aov(formula = fork_length ~ treatment_group, data = total)

	diff	lwr	upr	p	Significance (p<0.05)
120% - 100%	-9.445000e+01	-123.77172	-65.12828	0	Significant
140% - 100%	-1.892389e+02	-218.56061	-159.91717	0	Significant
80% - 100%	-8.526513e-14	-29.32172	29.32172	1	Not significant
140% - 120%	-9.478889e+01	-124.11061	-65.46717	0	Significant
80% - 120%	9.445000e+01	65.12828	123.77172	0	Significant
80% - 140%	1.892389e+02	159.91717	218.56061	0	Significant

3.2 Linear regression

A

```
lm(formula = max_speed_BL ~ fatigue_minutes, data = filter(fluc_percent_w,
    treatment_group == "120%"))

Residuals:
     Min       1Q   Median       3Q      Max 
-0.25687 -0.11784  0.04526  0.12018  0.16083 

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)    
(Intercept)     2.766e+00  1.253e-01   22.07 4.41e-11 ***
fatigue_minutes 2.955e-05  9.978e-04    0.03    0.977    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.1549 on 12 degrees of freedom
Multiple R-squared:  7.307e-05,	Adjusted R-squared:  -0.08325 
F-statistic: 0.0008769 on 1 and 12 DF,  p-value: 0.9769
```

B

```
lm(formula = max_speed_BL ~ fatigue_minutes, data = filter(fluc_percent_w,
    treatment_group == "140%"))

Residuals:
     Min       1Q   Median       3Q      Max
-0.34831 -0.05134 -0.01521  0.07564  0.24085

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)      3.07431    0.08837  34.787   <2e-16 ***
fatigue_minutes  0.00150    0.00164   0.914    0.374
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1258 on 16 degrees of freedom
Multiple R-squared:  0.04967,	Adjusted R-squared:  -0.009728
F-statistic: 0.8362 on 1 and 16 DF,  p-value: 0.3741
```

Figure 25. *The output of the linear regressions for fatigued fish in the 120% U_{crit} (A) and 140% U_{crit} (B) treatment group in Part A.*

3.3 Pearson correlation tests

A

```
> cor.test(fluc_percent_w$fork_length, fluc_percent_w$fatigue_minutes,
+                 method = "pearson")

	Pearson's product-moment correlation

data:  fluc_percent_w$fork_length and fluc_percent_w$fatigue_minutes
t = -1.2864, df = 30, p-value = 0.2081
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.5347113  0.1304450
sample estimates:
       cor
-0.2286492
```

B

```
> cor.test(fluc_percent_w$condition_factor, fluc_percent_w$fatigue_minutes,
+                 method = "pearson")

	Pearson's product-moment correlation

data:  fluc_percent_w$condition_factor and fluc_percent_w$fatigue_minutes
t = -2.204, df = 30, p-value = 0.03533
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.63884579 -0.02830132
sample estimates:
       cor
-0.3733113
```

Figure 26. *Pearson correlation test between absolute U_{crit} (cm s^{-1}) (A) and relative U_{crit} (BL s^{-1}) and fork length (A) and condition factor (B), showing a significant negative correlation between fatigue time and condition factor in part A.*

4. Fluctuating water currents part B: Different wave periods

4.1 Kruskal-Wallis test

```
> kruskal.test(fatigue_minutes ~ treatment_group, data = subset_wave_w)

        Kruskal-Wallis rank sum test

data:  fatigue_minutes by treatment_group
Kruskal-Wallis chi-squared = 0.8163, df = 2, p-value = 0.6649
```

Figure 27. *The Kruskal-Wallis test indicated no significant differences between fatigue time and the treatment groups with different wave periods of 30, 60, and 120 s with a peak speed of 120%* U_{crit} *in part B of the fluctuating water currents trials.*

4.2 Linear regression

A

```
lm(formula = max_speed_BL ~ fatigue_minutes, data = filter(subset_wave_w,
    treatment_group == "120%, 30s"))

Residuals:
     Min       1Q   Median       3Q      Max
-0.24061 -0.07988  0.01168  0.07488  0.24685

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)      2.8249814  0.0954816  29.587  2.6e-13 ***
fatigue_minutes -0.0005946  0.0007868  -0.756    0.463
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.1349 on 13 degrees of freedom
Multiple R-squared:  0.04208,   Adjusted R-squared:  -0.03161
F-statistic: 0.5711 on 1 and 13 DF,  p-value: 0.4633
```

B

```
lm(formula = max_speed_BL ~ fatigue_minutes, data = filter(subset_wave_w,
    treatment_group == "120%, 120s"))

Residuals:
     Min       1Q   Median       3Q      Max 
-0.25429 -0.06303  0.01431  0.10281  0.18732 

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)    
(Intercept)      2.7962136  0.1142345  24.478 6.84e-13 ***
fatigue_minutes -0.0001964  0.0008799  -0.223    0.827    
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1434 on 14 degrees of freedom
Multiple R-squared:  0.003547,	Adjusted R-squared:  -0.06763 
F-statistic: 0.04984 on 1 and 14 DF,  p-value: 0.8266
```

Figure 28. *The output of the linear regressions for fatigued fish in the treatment groups with different wave periods of 30 s (A) and 120 s (B) with a peak speed of 120%* U_{crit} *showed no significant differences (p-values > 0.05) in part B. The linear regression for the treatment group with a 60 s wave period is given in 2—critical swimming speed trials.*

4.3 Pearson correlation tests

A

```
> cor.test(subset_wave_w$fork_length, subset_wave_w$fatigue_minutes,
+                 method = "pearson")

	Pearson's product-moment correlation

data:  subset_wave_w$fork_length and subset_wave_w$fatigue_minutes
t = 0.52844, df = 43, p-value = 0.5999
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.2183569  0.3652475
sample estimates:
       cor 
0.08032547 
```

B

```
> cor.test(subset_wave_w$condition_factor, subset_wave_w$fatigue_minutes,
+                 method = "pearson")

        Pearson's product-moment correlation

data:  subset_wave_w$condition_factor and subset_wave_w$fatigue_minutes
t = -1.2477, df = 43, p-value = 0.2189
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.4554578  0.1128106
sample estimates:
       cor
-0.1869127
```

Figure 29. *The Pearson correlation test between fatigue time and fork length (A) and condition factor (B) for the fluctuating water current trials in part B shows no significant correlation.*