Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Rachel Coffey

Impact of salinity on oxygen consumption, swim performance, and blood physiology of brown trout (*Salmo trutta*)

Master's thesis in Natural Resources Management - Biology
Supervisor: Bengt Finstad
Co-supervisor: Sindre Håvarstein Eldøy, Jan Grimsrud Davidsen, Anders Finstad

May 2024

NTNU
Norwegian University of Science and Technology

Rachel Coffey

Impact of salinity on oxygen consumption, swim performance, and blood physiology of brown trout (*Salmo trutta*)

Master's thesis in Natural Resources Management - Biology
Supervisor: Bengt Finstad
Co-supervisor: Sindre Håvarstein Eldøy, Jan Grimsrud Davidsen, Anders Finstad
May 2024

Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

NTNU
Norwegian University of Science and Technology

Acknowledgments

This master's thesis was written as the final work of my master's degree in Natural Resource Management in Biology.

I would like to extend a huge thanks to Bengt Finstad for sharing with me his invaluable mentorship and tips in blood physiology and laboratory techniques, which really enriched and improved my work. I'm also extremely grateful to Sindre Håvarstein Eldøy for his encouragement, constructive feedback, and continuous help with R analyses. His support and advice throughout the experimental period and statistical analyses of data have been incredibly valuable. I would also like to thank Jan Grimsrud Davidsen for his helpful guidance and kind words during the thesis drafting stage.

Additionally, I'm grateful for Thomas Weiseth at Lundamo hatchery for providing the fish and their rearing information for use in this master project. I'd like to say a big thank you to Dag Altin at Sealab for his helpful assistance with equipment setup, technical troubleshooting, and all-around support during my work there, and to Tora Bardal at Sealab, who aided with the cortisol analyses. Thanks also to Iurgi Salaverria at Sealab for kindly assisting with the initial preparation regarding the logical and technical aspects prior to starting this master project. Additionally, I extend my thanks to Daniel Morgenroth from the University of Gothenburg, Sweden, for his hands-on help and advice in conducting U_{Crit} swim trials during the summer months of 2023. The follow-up and continued motivation to my work from all my supervisors and supporting staff has been instrumental in shaping my thesis.

Finally, I want to thank my family, friends, and colleagues for their endless encouragement and kind words. Their unwavering support has been a huge source of motivation throughout this journey, for which I am extremely grateful.

Rachel Coffey

Trondheim, May 2024

Abstract

While the impact of salinity on euryhaline fish species has been well-documented, there remains a significant knowledge gap concerning the effects on hatchery-derived brown trout (*Salmo trutta*), particularly regarding their physiology and swim performance. To address this gap, 30 landlocked *S. trutta* individuals from Lundamo hatchery in Trøndelag were divided into 4 treatment groups, and individually tested via U_{Crit} trials within an inter-flow closed respirometry chamber in brackish water (9.5 – 15.0 ppt) and in full-strength seawater (31.3 – 34.6 ppt). The effect of salinity was measured on oxygen consumption (MO_2), and swim performance - quantified by total trial time (minutes) and maximum swim speed (m/s) - and blood biochemical markers, specifically osmolality, chloride, glucose, lactate, and cortisol. Significant differences in MO_2 responses to treatments post-trial were observed across brackish water and full-strength seawater treatment groups. Full-strength seawater groups did not exhibit reduced swimming performance during U_{Crit} trials compared to brackish water groups. Plasma osmolality and chloride levels showed no significant differences across the experimental timeline and treatment groups, suggesting that the trout adapted well to the altered salinity among the treatments. Glucose, lactate, and cortisol levels suggested that exercise-induced stress or exhaustion did not significantly contribute to the observed results, implying alternative stressors most likely related to handling and/or the inherent stress response to salinity changes. The results suggest a complex interplay of factors influencing the physiological responses of these hatchery-derived *S. trutta* individuals, warranting further exploration. More comprehensive studies are necessary to investigate the underlying factors driving salinity tolerance for *S. trutta* in both hatchery and wild conditions.

Sammendrag

Selv om virkningen av saltholdighet på euryhalin fiskearter er godt dokumentert, er det fortsatt et betydelig kunnskapshull om effekten på brunørret (*Salmo trutta*), spesielt når det gjelder deres fysiologi og svømmeytelse. I dette forsøket ble 30 landlåste *S. trutta*-individer fra Lundamo settefiskanlegg i Trøndelag delt inn i 4 behandlingsgrupper, og individuelt testet via U_{Crit}-forsøk i et inter-flow lukket respirometrikammer i brakkvann (9,5 – 15,0 ppt) og i sjøvann (31,3 – 34,6 ppt). Effekten av salinitet ble studert for oksygenforbruk (MO_2), og svømmeytelse - kvantifisert ved total prøvetid (minutter) og maksimal svømmehastighet (m/s) - samt biokjemiske markører i blod, spesielt osmolalitet, klorid, glukose, laktat og kortisol. Signifikante forskjeller i MO_2-respons ble observert på tvers av brakkvanns- og sjøvannsbehandlingsgrupper. Sjøvannsgrupper viste ikke redusert svømmeytelse under U_{Crit}-forsøk sammenlignet med brakkvannsgrupper. Blodplasmaosmolalitet og kloridnivåer viste ingen merkbare forskjeller på tvers av den eksperimentelle tidslinjen og/eller behandlingsgruppene, noe som tyder på at ørreten tilpasset seg godt til den endrede saltholdigheten blant behandlingene. Glukose-, laktat- og kortisolnivåer antydet at treningsindusert stress eller utmattelse ikke bidro signifikant til de observerte resultatene, noe som tyder på alternative stressfaktorer som mest sannsynlig er relatert til håndtering og/eller iboende stressrespons på endringer i saltholdighet. Resultatene antyder et komplekst samspill av faktorer som påvirker de fysiologiske responsene til *S. Trutta*. Mer omfattende studier er nødvendig for å undersøke de underliggende faktorene som driver saltholdighetstoleranse for *S. trutta* i både kultiverte og ville bestander.

Table of Contents

1.0 | Introduction

Brown trout *(Salmo trutta)* is a resilient species renowned for its adaptability to a variety of aquatic environments (Thorstad *et al.* 2016). *S.trutta* exhibit two main life history strategies: residency in freshwater – which are typically referred to as "brown trout", or anadromy – which are typically referred to as "sea trout". Anadromous species are those that spawn in freshwater and migrate to estuarine and coastal waters for the purpose of feeding and growth (Jonsson and Jonsson, 1989)(Figure 1). Anadromy is a life-history trait which has a plethora of trade-offs between improved opportunities for growth and development and a reduction in survival rates out at sea (Nevoux *et al*. 2019; Birnie-Gauvin *et al.* 2021). While the explanations of the variation in this life history of *S.trutta* have not been fully comprehended within the scientific literature, it is believed that the likelihood of *S.trutta* to migrate is somewhat dependent on genetic make-up, and somewhat dependent on phenotypic plasticity (Jonsson and Jonsson, 1993). Perhaps due to their migration tendencies, *S. trutta* have demonstrated exceptional adaptability across various salinity conditions. Extensive research has documented their ability to thrive in brackish water and seawater habitats, highlighting their ability to tolerate and even flourish in environments with fluctuating salinity levels (Jonsson and Jonsson, 1993; Thorstad *et al*. 2016).

Notably, behavioural studies such as those by Moore and Potter (1994), Moore *et al.* (1998), and Jensen and Rikardsen (2012) have revealed that individual *S. trutta* smolts required no prior acclimation when transitioning from freshwater to saltwater environments. Likewise, genetic studies have highlighted the role of local adaptation in enhancing their survival in specific habitats (Ferguson *et al.* 2019). These studies have emphasised their ability to reside in estuarine waters, where they experience significant salinity shifts within the tidal cycle, suggesting a high level of adaptability to various environments (Baglinière and Maisse, 1999; Jensen and Rikardsen, 2012).

However, habitat use and migration of *S. trutta* are most often confined to similar coastal zones and as such the species may be sensitive to negative impacts within these areas (Thorstad *et al*. 2016). Therefore, having an anadromous life history means that *S. trutta* can be affected by changes in both estuarine and marine ecosystems, including widespread habitat degradation, altered ecosystem productivity, salmon lice infestations, and climatic changes (Jonsson and Jonsson, 2009; Elliott and Elliott, 2010). Over recent decades, there has been a noticeable decline in *S. trutta* populations across several countries (ICES, 2013), a trend that is also evident in Norway (Thorstad *et al*. 2019).

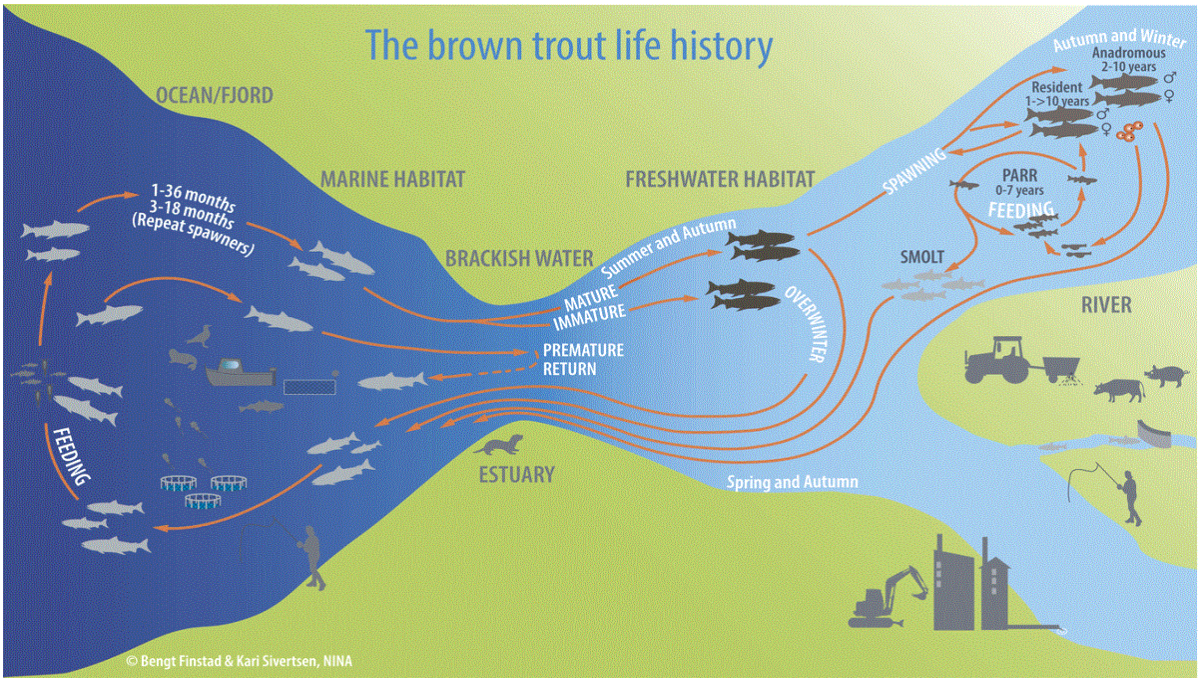


Figure 1. Life history of *S. trutta* (© Finstad and Sivertsen, NINA, 2022).

Hatcheries have long been paramount in supporting the management and conservation of various fish species, with hatchery-reared fish comprising significant portions of populations worldwide (Aprahamian *et al*. 2003; Paquet *et al*. 2011). In Norway, where fish populations have been affected by dam construction and hydropower development, hatchery smolts are annually introduced into select rivers to augment the sea-run segment of the population and facilitate marine feeding migrations before returning to spawn in freshwater habitats (Davidsen *et al*. 2014). Despite these efforts, these actions pose ecological challenges, potentially jeopardising the resilience and long-term viability of wild populations due to genetic introgression and the poor adaptability of farmed fish to wild conditions (Karlsson *et al*. 2016). For example, it has been shown that wild salmon generally survive better than their hatchery-reared counterparts (Skaala *et al.* 2019, Larocque and Fisk, 2020; Doogan *et al.* 2023). Notable physiological and behavioural differences between hatchery-reared and wild populations have been observed (Chittenden *et al.* 2010), raising concerns about the adaptability of hatchery-derived trout to varying salinity environments. Research findings have indicated that hatchery-reared *S. trutta* often exhibit inferior traits compared to their wild counterparts, displaying poorer performance attributed to the artificial hatchery environment and genetic factors (Fjellheim *et al*. 1995; Sundell, 1998; Brockmark and Johnsson, 2010). These disparities may have significant implications for population dynamics and resilience to environmental changes, particularly in the face of climate change. Nonetheless, it is suggested that wild *S. trutta* may

possess adaptations better suited to cope with fluctuating salinity conditions compared to hatchery-reared counterparts (Aarestrup *et al.* 2014), suggesting potential negative impacts from hatchery releases on wild fish populations in general.

Salinity, the measure of dissolved salt content in aquatic environments (Sivapriya *et al.* 2022), stands as a fundamental environmental parameter influencing the physiology and ecology of *S. trutta*. Variations in salinity levels directly impact the species' metabolic processes through osmotic stress, which can disrupt physiological equilibrium and essential biological functions (Kültz, 2015). As euryhaline species, wild *S. trutta* can adapt to a wide range of salinity levels, albeit with an energetic cost associated with residing in full-strength seawater or pure freshwater due to the need for active salt transport, a regulatory process known as osmoregulation. This regulatory mechanism is essential for maintaining internal osmotic and ionic balance, enabling *S. trutta* to cope with fluctuating salinity levels while supporting cellular and physiological functions (Tseng and Hwang, 2008).

Salinity tolerance in fish has also been extensively associated with variations in metabolic rate (Morgan and Iwama, 1991; Ern *et al.* 2014; Djiba *et al.* 2021). Metabolic rate stands as a cornerstone in the physiology of organisms as it describes the overall energy expenditure involved in maintaining biological processes (Nelson and Chabot, 2017). Oxygen consumption, often referred to as MO_2, acts as a fundamental proxy which is frequently used to indirectly measure metabolic rate. It quantifies the amount of oxygen utilised by an organism during aerobic respiration (Nelson, 2016; Lighton, 2018). In teleost fish, shifts in salinity can directly influence their oxygen consumption rates due to the energy-intensive processes of osmoregulation, with potential consequences for their standard metabolic rate (SMR). SMR, defined as the minimal metabolic rate of an organism in a resting, unstressed state, is substantially affected by osmoregulatory activities (Chabot and Farrell, 2016; Nelson and Chabot, 2017).

Salmonoids residing in brackish water normally demonstrate lower MO_2 values because the energetic requirements for salmonoids are minimised in brackish water, where the osmotic gradient between the blood and the surrounding environment is reduced (Morgan, 1998; Ern *et al*. 2014). Conversely, the opposite is true for moving into seawater, where more energy is needed and thus MO_2 rates must increase to facilitate the required osmoregulatory processes, leading to increased metabolic demands and osmoregulatory costs (Ern *et al*. 2014; Hvas *et al.* 2018). Often, osmoregulatory capability translates into an alternated physiological

performance. As metabolic rates fluctuate in response to changes in salinity, fish may experience corresponding adjustments in swim performance, including changes in endurance, speed, and agility (Christensen *et al.* 2019). As such, metabolic shifts can be expected to be closely intertwined with swim performance in salmonoids wherein salinity variations can influence locomotory abilities.

Blood biochemical markers such as osmolality, chloride, glucose, lactate, and cortisol are vital indicators of physiological status in fish, including *S. trutta*, and respond to changes in salinity levels which provide insights into the species' adaptive mechanisms. Both blood plasma osmolality and chloride levels have been documented to increase in fish in parallel with increases in salinity, indicating challenges in maintaining internal equilibrium (Martinez-Alvarez *et al*. 2005; Kültz, 2015; Christensen *et al*. 2019). Elevated cortisol levels, for instance, can indicate exposure to stressors such as changes in salinity and temperature fluctuations (Lemos *et al.* 2023).

Investigations on euryhaline fish under changing salinity conditions are scarce within the scientific literature (Seale and Breves, 2022) and studies on the effects of changing salinity levels on hatchery-reared *S. trutta* especially are almost non-existent. Addressing this gap in knowledge, the primary aim of this study was to quantify the energetic costs associated with osmoregulation in hatchery-reared *S. trutta* when residing in brackish water and in full-strength seawater. This may aid in determining how hatchery-reared *S. trutta* adapt metabolically to salinity changes and will increase the knowledge on the effects of salinity on *S. trutta* in both hatchery and wild conditions. MO_2, swim performance during critical swimming tests (U_{Crit} trials), and blood biochemical markers were investigated to achieve this aim, in which the following hypotheses were tested:

H1. MO_2 are significantly altered in response to salinity changes, showing lower values in brackish water groups compared to full-strength seawater groups both pre- and post-treatment, reflecting a metabolic adjustment to altered salinity conditions.

H2. Full-strength seawater groups exhibit reduced swimming performance during U_{Crit} trials compared to brackish water groups due to higher energy consumption and osmoregulatory costs in seawater.

H3. Blood plasma osmolality and blood plasma chloride levels exhibit distinct increases across the experimental stages in response to increasing salinity levels.

2.0 | Materials and Methods

Animal husbandry

This research was approved under the animal ethics permit FOTS id 26025 approved by Mattilsynet and abided to Norwegian procedures and legislations of animal usage in scientific research. All experiments were conducted using *S. trutta* sourced from Lundamo hatchery, Trøndelag. A total of 30 fish, approximately 35 cm in size and similar in body condition were selected for the trials. Mean weight was calculated to be 1.06 kg (range: 0.61 – 1.40 kg), and mean length was determined to be 38 cm (range: 42.08 – 44.00 cm). The hatchery-reared individuals, derived from landlocked populations, had previously only resided in freshwater water environments with no prior exposure to brackish water, full-strength seawater, or the marine environment.

The broodstock, landlocked trout, were sourced from Jonsvatnet in Trøndelag (63.3655°N 10.5820°E) in October 2019. They were spawned on the 15th of October, followed by the emergence of eye eggs on the 20th of December. Hatching occurred on the 20th of February 2020, and start feeding began on the 20th of April 2020, with 0.5 mm feed. The fish were then moved from Ler to Lundamo on the 22nd of May where they were fed 1.0-1.2 mm feed. This cycle of movement between Ler and Lundamo continued, with varying feed sizes (1.5 – 4 mm), until their delivery to NTNU Sealab in Trondheim on the 25th of March 2023. Mean water temperature varied between 4.9 °C in Ler to 13.2 °C in Lundamo during the production period and the fish were kept at a natural light regime after start feeding.

The fish were housed at Sealab for approximately five months, from March to August 2023. The fish were randomly selected and divided equally (n = 15) and randomly between two 2000 L holding tanks, designated as Tank 1 and Tank 2. Prior to measurements, all fish were acclimated to the new salinity conditions for a minimum of seven days. Both tanks contained brackish water (salinity ranging between approximately 9.50 – 15.01 ppt), and Tank 2 was adjusted to full-strength salinity (salinity ranging between approximately 31.30 – 34.65 ppt) after one week, following another week of acclimatisation. Both tanks had continuous flow-through and were manually flushed every two days to prevent waste buildup. Fish were fed commercial feed daily until satiation through automated feeding devices, with the pellet size adjusted from 2 mm to 4 mm after the first week and a half (Nutra Olympic, Skretting; www.skretting.com) and received 1% of their body mass per day each day. Both tanks were naturally maintained at a temperature range of 9.2 - 10.8 °C and the light-dark cycle followed

an approximate 16:8 pattern throughout the experiment. Water temperature (°C)**,** water salinity (ppt) and dissolved oxygen (mg/L and %) were recorded daily, both manually using a YSI ProSolo Optical Dissolved Oxygen and Conductivity Meter and also via automated loggers (model numbers 20607210 and 20607208, respectively), placed approximately halfway down the side of each tank. Continuous monitoring of feed size (mm), and quantity (grams) were also conducted. No mortalities occurred during the acclimation or experimental periods.

Swim respirometry and U_{Crit}

To measure metabolic rate in *S. trutta*, intermittent-flow respirometry was employed, a method commonly utilised in research (Clark *et al.* 2013; Hvas *et al.* 2019). This approach involves placing a fish in a gas-impermeable respirometry swim chamber equipped with an oxygen sensor and subjecting it to alternating 'closed' and 'flush' phases over a set duration. During this time, the decline in oxygen levels in the water surrounding the fish is measured which provides an indirect indication of aerobic metabolism (Nelson, 2016). Additionally, the swim chamber allows for conducting critical swimming tests, known as "U_{Crit}", to assess the impact of environmental stressors on swimming performance (Brett, 1964). The U_{Crit} trial involves incrementally increasing water velocity in pre-determined increments (typically 1 body length per second; (BL s^{-1})) until the fish exhibits signs of fatigue. This test has been frequently employed to evaluate the effects of salinity changes on swimming performance (Hammer, 1995; Plaut, 2001).

For the purpose of this project, swim trials were conducted in a sealed intermittent-flow swim-tunnel respirometer (Loligo Systems; www.loligosystems.com); as described in Cai *et al.* (2013) and Hvas *et al.* (2018, 2019). The respirometer (65.5 cm × 19.5 cm × 18.0 cm) was submerged in a 90 L tank (148.0 cm × 68.0 cm × 38.0 cm)(Figure 2) with a tube volume of 0.255 L which featured a rectangular working section and was equipped with temperature and oxygen sensors. Flow was manually generated by a motor-driven propeller controlled by a frequency inverter outside of the swim chamber (MOVITRAC® LTE-B+, 200-240VAC; 50/60 Hz, Sew Eurodrive, USA). Both the sensors and the flush pump were connected to a computer programme (AutoResp™ v2, Loligo Systems, Denmark)**.**

A constant flow of either brackish water or seawater, at a rate of 420 L/hour, was supplied to the chamber via plastic tubes, manually switched depending on the planned salinity for each trial. Water flow was maintained at a temperature range of 9.5 – 10.5 °C and was directed

through the external section of the chamber to ensure stable temperatures. To minimise visual disturbance and fish stress, the swim-tunnel respirometer was partially covered with black plastic bags and a large tarp. Additionally, a bright lamp was positioned at the top of the swim tunnel to motivate fish to hold position in the shaded front part of the swim chamber.

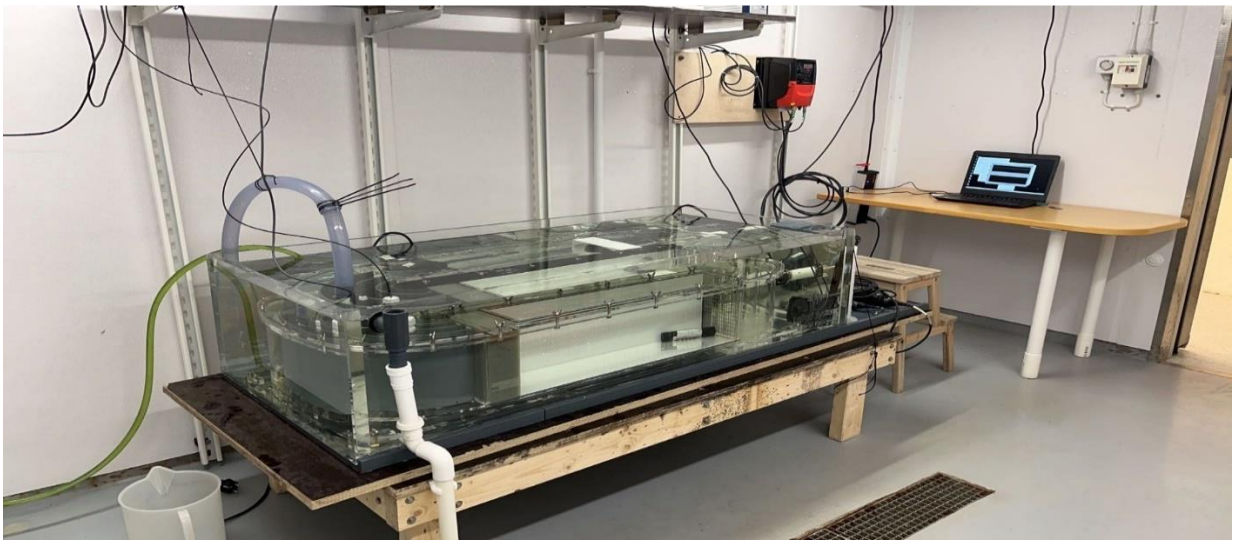


Figure 2. Sealed intermittent-flow swim-tunnel respirometer. *Photo: Rachel Coffey*.

To begin the experimental trials, individual fish in the holding tanks were chosen at random. Each fish was sedated using Bezoak solution (3.8 ml in 15 L of water matching the salinity of the holding tank; Figure 3). Before placement in the swim chamber, a blood sample (0.9 ml) and body measurements (weight (kg), total length (cm), fork length (cm), height (cm), width (cm), circumference (cm)) were taken. Following transfer to the respirometer, each fish underwent an 18-hour acclimation period to recover from handling (Figure 3), followed by 1 hour of pre-treatment tracking. During these periods, activity measurements were recorded at a swim speed of 0.5 BL/s, which was later converted to meters per second (m/s) for standardised unit baseline calculation using a formula derived from Fulton *et al.* (2007)(see "Appendix 1").

After the 18-hour acclimation period and 1-hour pre-treatment tracking, the trial commenced by transitioning the water flow from brackish water to saltwater or vice versa, depending on the experimental group. Each trial lasted for approximately 4 hours - depending on the willingness of each fish to swim during the U_{Crit} trials - with constant measures, following a

pre-determined protocol of a 3-minute flush, 2-minute wait, and 10-minute measure cycles, totalling 15-minute intervals during both acclimatisation and the trials. The U_{Crit} trials began during the last hour of each experiment, coinciding with the start of a new 15-minute loop in the AutoResp™ software. During these trials, the swimming speed increased by 0.5 BL/s (approximately 0.22 m/s) for every cycle while the system was in flush mode. The primary measurement investigated in these trials was "endurance," or the time-to-fatigue, defined as the duration a fish maintained its position in flowing water (Cano-Barbacil *et al.* 2020). Swimming activity of each fish was observed, tracked, and timed until it could no longer maintain its position and ceased swimming, resting against the back fence of the swim chamber. The U_{Crit} protocol included step heights of 0.5 BL/s per increment (up to 2.5 BL/s) and step lengths or intervals of 15 minutes. Solid blocking correction was applied to correct for increased water velocity in the swim chamber from the fish. The fractional error (%) was then calculated automatically within the AutoResp™ software using the formula from Bell and Terhune (1970) to adjust the swim speed for each fish, ensuring accurate measurement during the trials.

Following the conclusion of each U_{Crit} trial, the fish was gently netted and carefully removed from the swim tunnel. Subsequently, humane euthanasia was administered through an overdose of Benzoak solution (5 ml/L in 10 L water matching the current salinity used for the U_{Crit} trial). A final blood sample (0.9 ml) was then collected and analysed in the laboratory using the same methods as described for the pre-trial assessment.

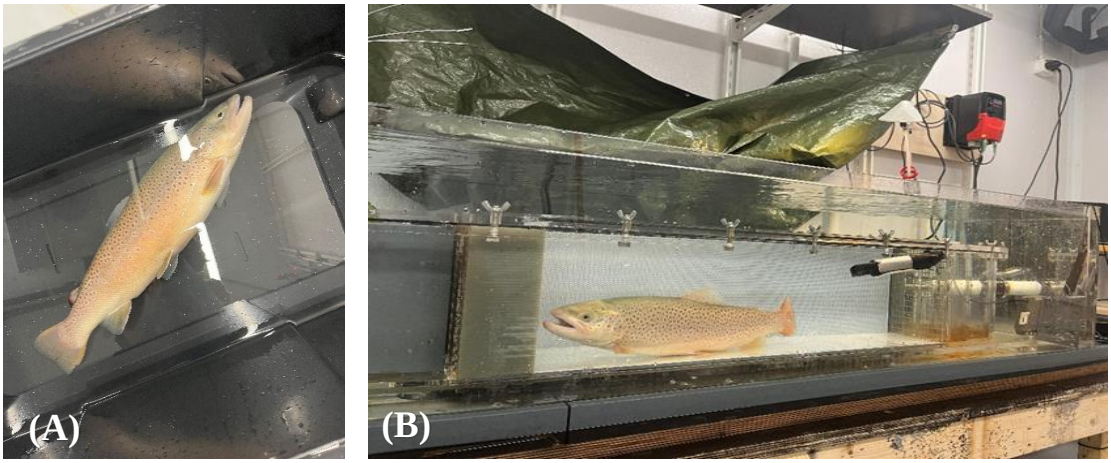


Figure 3. Lab images of fish sedation and transfer to swim chamber. **(A)** Fish 21 sedated in a sedation solution (Bezoak; 3.8 ml in 15 L of water) prior to blood sampling and the measurement period. **(B)** Fish 28 pictured after the transfer to the swim chamber section of the swim-tunnel respirometer. *Photos: Rachel Coffey*.

Background respiration

To account for bacterial respiration in the main swim tunnel, a trial period was completed before and after the entire experimental period, when the tunnel was empty. Each measurement period was approximately 16 hours in total. The lowest MO_2 value (mgO2/kg/hr) was noted for all trials and plotted in a scatter plot. This scatter plot was then visually inspected for correlations. To further investigate and confirm if the background respiration was significant, a linear regression analysis was also performed to determine if the level of background respiration was significant. As the swim tunnel was regularly flushed between trials, background respiration was not detected and therefore had negligible influences on the reported values (see "Appendix 1")**.**

Blood sampling

Blood samples were collected from each individual both before and after the trial. Methods for blood sampling were the same for both pre- and post- trial and were as follows: BD Microlance 0,6*25 mm needle heads were used for each fish due to their higher body fat percentage. Needles were prepared with 1 drop of Heparine 5000 IE/ml LEO to prevent clotting of blood before each trial. 0.9 ml of blood was drawn, and samples were immediately placed on wet ice and transported to the lab where it was transferred into a 1.5 ml conical centrifuge tube using a 200 µl pipette. Glucose (mmol/L) and lactate (mmol/L) levels were then measured using the "Contour Next" and "XPER Technology L1" meters, respectively. The remaining blood was centrifuged (Hettich EBA model 35) at a pre-set 80% speed for 3 minutes to separate red blood cells, platelets, and plasma. A minimum of 0.3 ml of plasma was transferred into 0.6 ml Eppendorf microtubes using a clean pipette technique. In total, 60 samples were collected (30 pre-trial and 30 post-trial). The microtubes were then frozen at -18 °C. At the end of the experimental period, plasma samples were analysed for osmolarity (mOsm/kg), chloride (mmol/L), and cortisol (nmol/L).

Both osmolality and chloride samples were analysed from the blood plasma in unison. To ensure the validity of results and to ensure caution with testing because of the finite nature of the plasma, samples were analysed in sets of five, followed by taking a new standard test. Five blood plasma samples were randomly chosen from the storage bag in the freezer in the lab prior to each analysis. These randomly selected samples were placed in an Eppendorf freezer stand on wet ice to remain cool. Samples were warmed by hand first for a few minutes, then placed

in a vortex mixer for a few seconds until sufficiently melted (Classic Advanced Vortex Mixer, VELP SCIENTIFICA). Osmolality was measured first using an osmometer (Fiske^{R} Micro-Osmometer Model 210), followed by chloride using a chloride analyser (Chloride Analyser, Model 926 S, Sherwood) (Figure 4). Procedures followed the sampling protocol kindly provided by Bengt Finstad, NTNU (February 2022). Standard tests using both osmolality and chloride standard solutions were taken as baselines for the tests before starting on the actual blood plasma samples. Normal values were observed with these standard tests if a bit higher than expected. Plasma samples were tested twice for validation, as results were higher than expected, with the second test results considered accurate and taken as the actual final value. A new standard test was carried out for both parameters after every fifth sample to ensure that measurements were consistently correct.

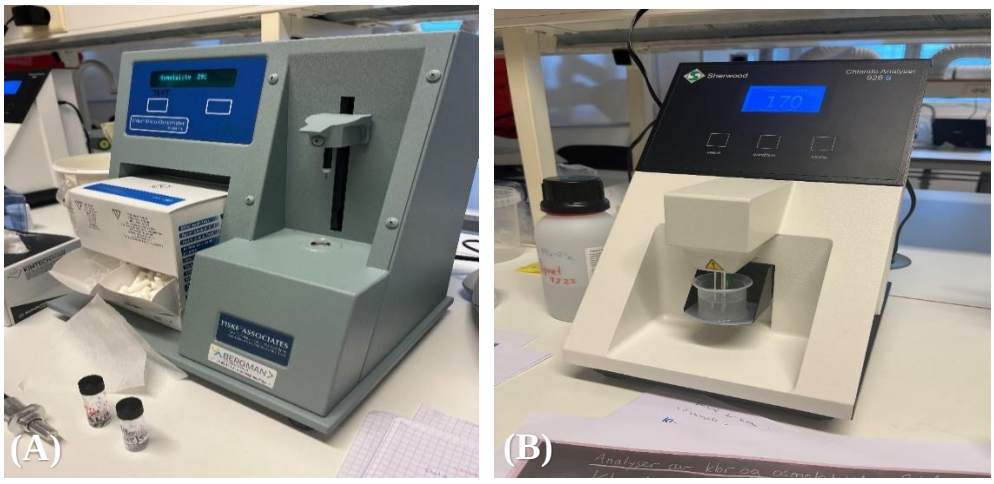


Figure 4. Lab images of analyzers used in blood plasma analysis. **(A)** Osmolality analyzer (Fiske^{R} Micro-Osmometer Model 210) which was used to analyze the osmolality (mOsm/kg). **(B)** chloride analyzer (Model 926 S, Sherwood) which was used to analyse the chloride (nmol/L) of all 60 blood plasma samples. *Photos: Rachel Coffey*.

Cortisol was then tested using the ELISA DEH3388 Procedure using microplate wells (following the steps provided by the Demeditec Diagnostics user manual, www.demeditec.com). The optical density of each well was determined at 450 nm and the wells were read within 15 minutes of finishing each test using the linked SkanIt Software programme provided by Thermo Fischer Science (version 7.0.2, 2023). Cortisol values were automatically calculated using this programme and recorded in an Excel file, with concentrations measured in ng/ml. Additionally, standard curve graphs were generated for each

well, as detailed in the "Appendix 1." Samples 21 and 23 were re-tested with a second plate due to large deviations observed between the initial results and replicates within their respective wells. For the same reason, sample numbers 2, 4, 6, 8, 9, 10, 14, 17, 18, 19, 40, 50, and 56 were later re-tested using another test kit, using 4 replicates for accurate results to resolve this, and a mean value calculated from these 4 replicates was taken for the final values for these specific sample numbers.

Removal of individual fish

All individual fish MO_2 data were visually inspected first via line plots using their raw MO_2 values. Fish that revealed high time tracks of MO_2 indicated that these individuals were not well-acclimatised to the swim chamber after the acclimation period and were therefore omitted from further analysis. Out of the 30 fish tested throughout this experimental period, 13 fish were then removed from the dataset, including: Fish 1, 3, 5, 6, 8, 9, 10, 11, 14, 15, 16, 18, and 25. Fish 11 and Fish 25 were also removed due to partial loss of data because of technical errors with the AutoResp^{TM} software. 17 fish, which, based on their MO_2 values, appeared to be sufficiently calm before the trials, remained in the dataset (treatment groups BWBW n = 4; BWSW n = 5; SWBW n = 3; SWSW n = 5). Experimental timelines for these 17 remaining individual fish are illustrated in the “Appendix 1” section.

Statistical analysis

All statistical analyses and plotting of data were conducted in the R environment within RStudio (R version 4.3.1, 2023; RStudio version 2023.12.1+402). To test for normality, data was visually assessed first via histogram, as well as completion of Shapiro-Wilks tests at the significant level of $P \leq 0.05$. In all cases, data was found to be non-normally distributed, hence non-parametric statistical tests were chosen with a significant level of $P \leq 0.05$.

Prior to analysis, the MO_2 values were standardised in R. Standardisation allowed values to be adjusted to a common scale or baseline, making it easier to compare the relative effects of the different treatments. Baseline MO_2 values for each fish were calculated using the pre-treatment stage data and merged with a summary table. To establish a standardised baseline, minimum MO_2 values from the pre-treatment stage were chosen as they better reflect the metabolic state before experimental interventions. This approach minimised the impact of extreme outliers and aligned with the metabolic conditions closer to the present study's parameters. By using this

standardised baseline, potential variations induced by stress during the acclimation period were effectively mitigated and values were now close enough to SMR values (Chabot *et al.* 2016). Subsequent analysis focused on measuring the relative change in MO_2 and other variables post-treatment. Thus, this approach enabled one-way testing to assess the degree of change in these variables rather than focusing solely on absolute values. It accounted for variations induced by stress during acclimation, ensuring a more accurate representation of baseline metabolic activity.

Total time (endurance) during the swim challenges and maximum swim speed were chosen to quantify swim performance as they are commonly used variables within the scientific literature to measure swim performance in studies involving fish locomotion and physiology (Ojanguren and Brana, 2003; Farrell, 2008). Regarding osmolality and chloride levels, these variables were specifically chosen to assess osmoregulatory abilities in *S. trutta* due to their critical roles in maintaining internal fluid balance and ion regulation.

Statistical differences in standardised MO_2 response values, swim performance (total trial time and maximum swim speed), osmolality, and chloride levels between treatment groups pre- and post-treatment were assessed using Kruskal-Wallis tests. These tests focused on within-group changes, evaluating if each treatment group individually exhibited significant differences between pre- and post-trial stages. The “kruskal.test” function in R's base package was used for comparisons between non-normally distributed groups. In the case of the MO_2 test, “experimental stage” was used as an explanatory variable and in all other variable tests, “treatment” was used as the explanatory variable. MO_2, total trial time, maximum swim speed, osmolality, and chloride were all used as response variables in their respective tests. Post-hoc analyses were conducted using the Dunn's test “dunn.test” function in R, including the Bonferroni method "=bonferroni" function to control for the familywise error rate. The Dunn’s test output provided all possible pairwise comparisons (i.e., six possible comparisons in the case of the four treatment groups). The adjusted p-values were used to determine whether there were statistically significant differences between these treatment groups.

3.0 | Results

Environmental parameters

The two loggers measuring temperature, salinity, and dissolved oxygen (DO) placed approximately halfway down the side of the two holding tanks produced a temperature (°C), salinity (ppt) and dissolved oxygen (mg/L) profile for Holding Tanks 1 and 2 throughout the experimental period, as shown in Figure 5. Throughout the experimental period, water temperature ranged from 9.1 to 10.5 °C in Holding Tank 1, with the lowest temperature of 9.1 °C occurring on multiple days in early June, and the highest temperature of 10.5 °C observed on the 30th of July (Figure 5). Salinity levels were more pronounced in early May, with variations ranging from 9.5 - 17.0 ppt. After this period, salinity began to stabilise, and the rest of the experimental period showed relatively consistent levels, fluctuating between approximately 9.5 - 15.0 ppt. DO levels in Tank 1 also fluctuated during this period, with the lowest DO value (9.27 mg/L) observed on the 26th of June and the highest value (10.92 mg/L) observed on the 3rd of June.

After fully transitioning to full-strength seawater, Holding Tank 2 exhibited similar temperature variations, ranging from 9.2 to 10.8 °C throughout the experimental period after the initial adjustment in salinity. The lowest temperature (9.2 °C) occurred on multiple dates in June, while the highest temperature (10.8 °C) was observed on the 30th of July. Salinity levels in Tank 2 fluctuated between 31.3 - 34.6 ppt with the lowest value (31.3 ppt) occurring on the 17th of June and the highest value (34.6 ppt) occurring on the 24th of June. DO levels in Tank 2 mirrored the temperature pattern, with the lowest value (7.89 mg/L) observed on the 26th of June and the highest value (10.8 mg/L) observed on the 30th of July.

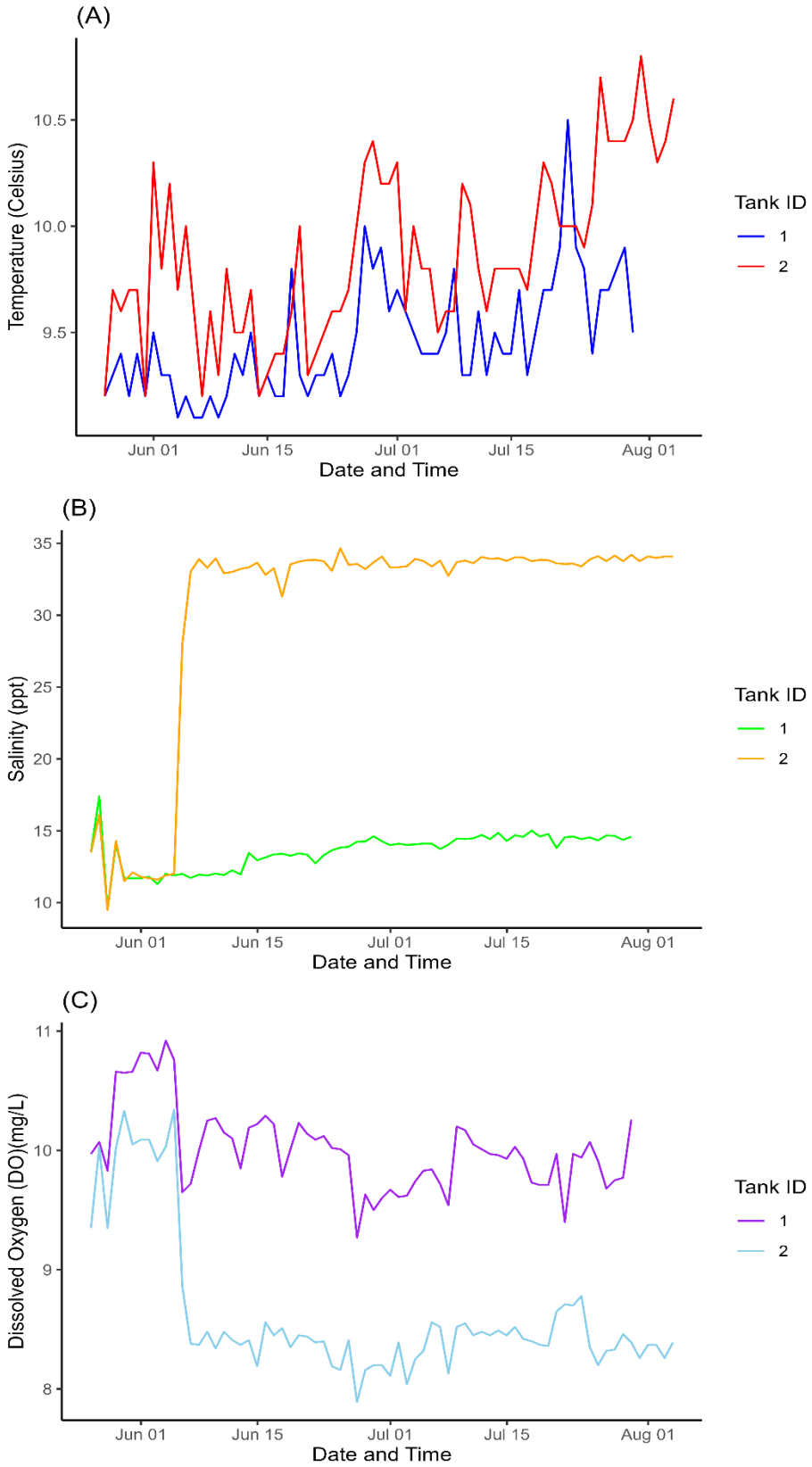


Figure 5. Plots of holding tanks displaying data from the start of the acclimatisation period to the end of the entire experimental period, from 25.05.2023 – 04.08.2023. **(A)** Temperature (in Celsius) time series of Holding Tanks 1 and 2. **(B)** Salinity (in ppt) time series of Holding Tanks 1 and 2. **(C)** Dissolved oxygen (DO; in mg/L) time series of Holding Tanks 1 and 2.

Observational notes from U_{Crit} trial results

Throughout all active U_{Crit} trials, it was visually observed that all fish, regardless of salinity origin or salinity treatment, needed a lot of encouragement to swim. Frequent breaks were observed with all fish where a few seconds were taken at least twice during each U_{Crit} trial. During each break, the fish would rest (i.e., became inactive and ceased swimming against the current) where its belly would rest on the bottom of the swim chamber as they were not able to regulate their swim bladder being confined within the chamber. The water flow was increased very briefly by circa 3 seconds when this happened to encourage swimming behaviour, and then reset back to the pre-calculated swim speed for the individual fish.

H1. MO_2 values across the salinity treatment groups

MO_2 response values from the treatments varied across all salinity treatment groups, notably the SWSW and BWSW groups which showed a positive 16.37 mgO2/kg/hr and 11.97 mgO2/kg/hr difference in the post-treatment stage compared to the pre-treatment stage; respectively. This was a 25.29% and 14.48% increase in MO_2 response values post-treatment; respectively, after exposure to the saltwater treatment. In contrast, the brackish water treatment groups [BWBW and SWBW groups] displayed negative MO_2 differences in responses within the post-treatment stage, demonstrating a decrease of 18.14 mgO2/kg/hr and 1.34 mgO2/kg/hr unit values, respectively (approximately a 16.05% and a 2.07% decrease in MO_2 response values in the post-treatment stage) (Figure 6). However, the largest total mean value observed was in the BWBW group [M = 162.33 mgO2/kg/hr; SD ± 79.46 mgO2/kg/hr] and lowest mean was observed in the BWSW group [M = 106.46 mgO2/kg/hr; SD ± 61.95 mgO2/kg/hr]. Ten outliers were identified in the standardised dataset with at least one outlier found in all treatment groups. Outliers ranged from the lowest [0.27 mgO2/kg/hr] in the BWBW group to the highest [3.45 mgO2/kg/hr] in the BWSW group, with the largest number of outliers found in the BWBW group which contained 4 outliers in total.

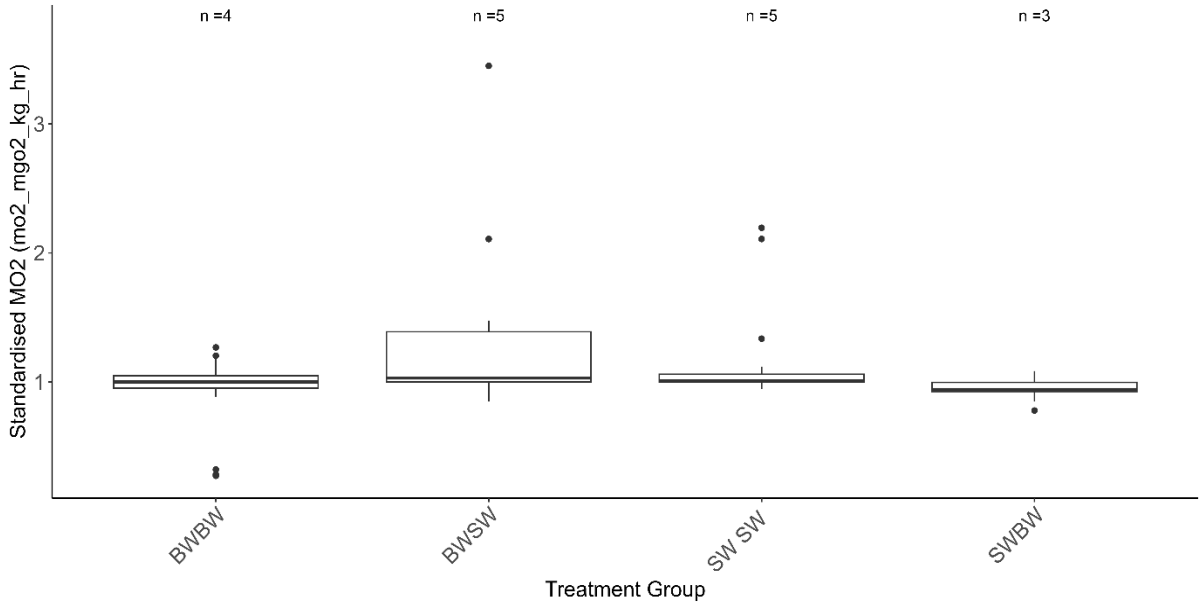


Figure 6. Effects of post-treatment on standardised MO_2 levels (mgO2/kg/hr). These were taken as the lowest MO_2 noted during acclimation time before the pre-treatment stage began, among the treatment groups. The y-axis represents the standardised MO_2 response values (mgO2/kg/hr), while the x-axis depicts the treatment groups.

A statistically significant difference was observed in standardised MO_2 responses pre- and post-treatment between the four salinity groups (Kruskal-Wallis rank sum test; H (3) = 12.71, P = 0.005)). Therefore, Hypothesis 1 (H1), stating that: "MO_2 values significantly alter in response to salinity changes, showing lower values in brackish water groups compared to full-strength seawater groups both pre- and post-treatment, reflecting a metabolic adjustment to altered salinity conditions" could be accepted.

A post-hoc Dunn's test with Bonferroni correction identified significant differences in post-treatment MO_2 responses between the SWSW and SWBW ($p < 0.05$, adjusted p-value = 0.001*) as well as SWBW and SWSW ($p < 0.05$, adjusted p-value = 0.001*) groups. However, no significant differences were found between BWBW and BWSW (adjusted p-value = 0.26) or BWBW and SWSW (adjusted p-value = 1.00) groups.

H2. Impact of salinity changes on swim performance across treatment groups

Total trial time varied between groups, yet the groups initially exposed to seawater in the holding tanks revealed the highest timings in total trial duration. The SWSW treatment group demonstrated the highest duration in mean total trial time [M = 47.80 minutes; SD ± 1.32 minutes], while BWSW treatment group demonstrated the lowest total trial time [M = 29.69

minutes; SD ± 11.92 minutes] out of all groups (Figure 7; (A)). Likewise, the SWSW group showed the highest variation in total trial time values while the BWSW had the lowest variation. Two outliers were noted within the dataset, one each within the BWSW and SWSW groups, respectively (49 minutes and 50 minutes outliers in the BWSW and SWSW groups; respectively).

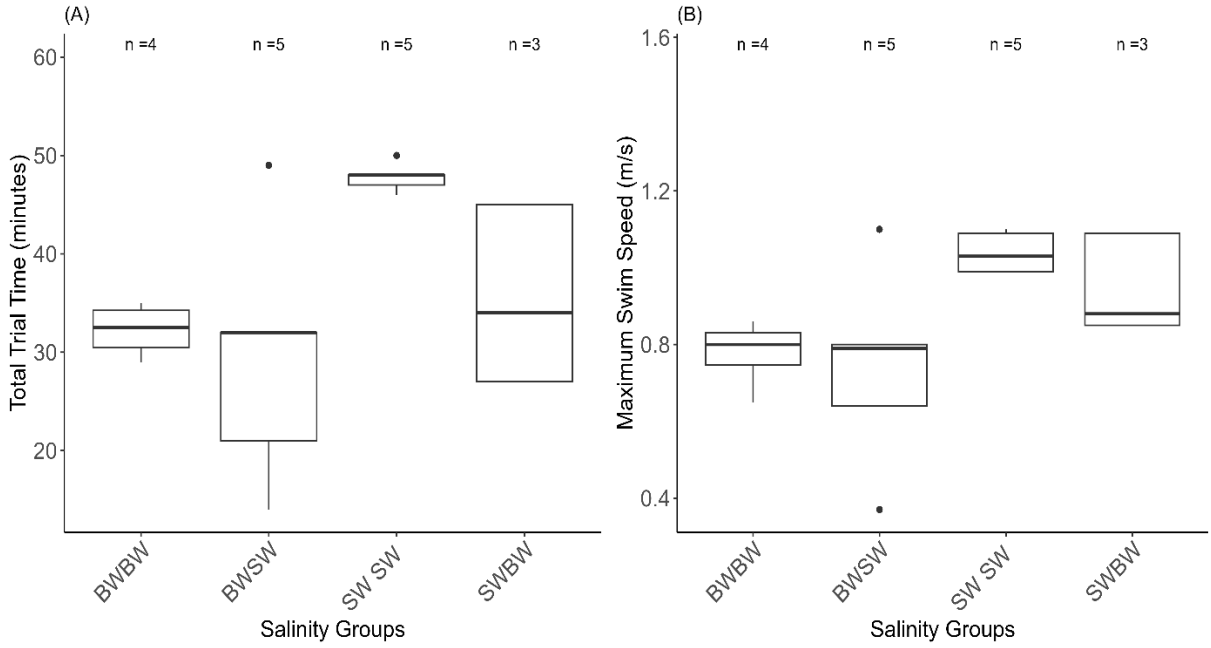


Figure 7. Boxplots depicting total trial time (in minutes) and maximum swim speed (in m/s) achieved at the conclusion of the U_{Crit} trials across treatment groups. **(A).** Total trial time across all groups. The y-axis represents the total duration of the U_{Crit} swim trial (minutes), while the x-axis depicts the treatment groups. **(B).** Maximum swim speed across all groups. The y-axis represents the final or maximum swim speed values (m/s), while the x-axis depicts the treatment groups.

The SWSW treatment group had the highest mean maximum swim speed out of all the groups [M = 1.04 m/s; SD ± 0.05 m/s], while the BWSW treatment group demonstrated the lowest maximum swim speed [M = 0.74 m/s; SD ± 0.24 m/s]. The BWSW had the highest variation in maximum swim speed while BWBW had the lowest. Two outliers were noted within the dataset (0.37 m/s and 1.10 m/s), both within the BWSW group (Figure 7; (B)).

There were statistically significant differences observed among the four salinity treatment groups with regards to their swim performance during the U_{Crit} trials (total trial time and maximum swim speed)(Kruskal-Wallis rank sum test; totaltime; H (3) = 40.05, P = 1.04e-08;

and; msfinishspeed; H (3) = 39.51, P = 1.35e-08)), which indicated that there was an impact of salinity treatments on swimming abilities.

A post-hoc Dunn's test with Bonferroni correction was conducted following the Kruskal-Wallis results, which revealed selective differences in swimming performance among the four groups. The SWSW group showed significantly higher total trial time compared to others (SWSW vs. BWBW: $p < 0.05$, adjusted p-value = 0.000*; SWSW vs. BWSW: $p < 0.05$, adjusted p-value = 0.000*; SWBW vs. SWSW: $p < 0.05$, adjusted p-value = 0.001*), and no significant differences were found between BWBW, BWSW, and SWBW groups (BWBW vs. BWSW: $p > 0.05$, adjusted p-value = 1.000; BWBW vs. SWBW: $p > 0.05$, adjusted p-value = 1.000; BWSW vs. SWBW: $p > 0.05$, adjusted p-value = 1.000).

The post-hoc analysis of final swim speed demonstrated a significant difference between the SWSW and BWBW groups ($p < 0.05$, adjusted p-value = 0.000*), as well as between the SWSW and BWSW groups ($p < 0.05$, adjusted p-value = 0.000*). Significant differences were observed between the SWBW and BWBW groups ($p < 0.05$, adjusted p-value = 0.007*) and between the SWBW and BWSW groups ($p < 0.05$, adjusted p-value = 0.009*). However, no significant differences were found between the BWSW and BWBW groups ($p > 0.05$, adjusted p-value = 1.000) or between the SWBW and SWSW groups ($p > 0.05$, adjusted p-value = 0.330).

Consequently, Hypothesis 2 (H2) stating that: "Full-strength seawater groups exhibit reduced swimming performance during U_{Crit} trials compared to brackish water groups due to higher energy consumption and osmoregulatory costs in seawater" was not fully supported and was therefore rejected.

A highly significant, positive correlation was observed between fish length (in cm) and both total trial time (p-value = 6.32e-07; Adjusted R-squared = 0.008) and final swim speed (p-value = < 2.2e-16; Adjusted R-squared = 0.18) (Figure 8).

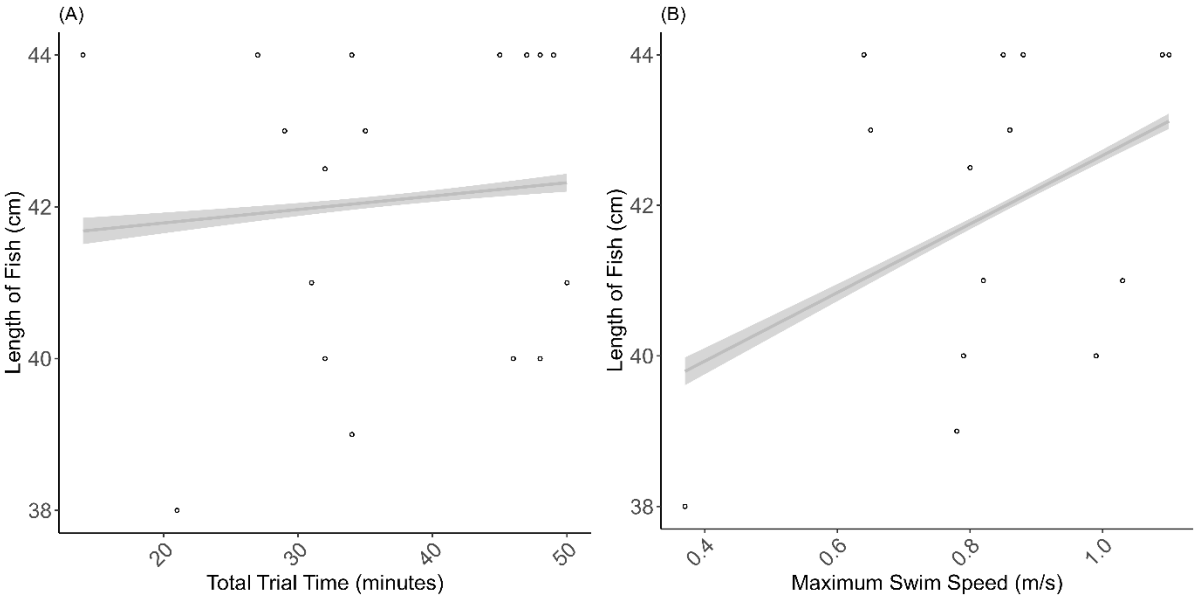


Figure 8. Scatter plots for length of fish (cm), total trial time (minutes), and maximum swim speed (m/s). **(A)** Comparison between total U_{Crit} trial time and length of each fish. **(B)** Comparison between maximum swim speed and length of each fish. In both graphs, fish length appeared to show a generally positive correlation to total trial time and maximum swim speed.

H3. Blood plasma parameters (osmolality (mOsmol/kg) and chloride (mmol/L)), glucose (mmol/l), lactate (mmol/l), and cortisol (ng/ml)

Osmolality values demonstrated an overall increase across all treatment groups post-trial. The SWSW treatment group demonstrated the highest mean osmolality values in total across the entire experimental period [M = 341.20 mOsmol/kg; SD ± 17.37 mOsmol/kg] while BWBW treatment group demonstrated the lowest mean osmolality values in total across the entire experimental period [M = 322.38 mOsmol/kg; SD ± 16.61 mOsmol/kg] (Figure 9; (A)). Likewise, the SWBW treatment group had the highest variation in osmolality post-trial, while SWSW treatment group had the lowest post-trial. One osmolality outlier was identified [290 mOsmol/kg], noted in the post-trial stage of the BWSW treatment group.

Chloride levels varied across treatment groups, with increases observed post-trial for BWSW, SWSW, and BWBW groups (Figure 9; (B)). The largest post-trial increase was noted in the BWSW group (5.00 mmol/L, approximately 3.77% higher than pre-trial levels). Slight increases were also seen in the SWSW and BWBW groups (1.20 mmol/L and 0.25 mmol/L, approximately 0.86% and 0.19% higher, respectively), while the SWBW group experienced a decrease of approximately 8.33 mmol/L (5.62%) post-trial. The highest mean chloride value throughout the entire experimental period was in the SWBW group [M = 144.17 mmol/L; SD ± 6.18 mmol/L], and the lowest was in the BWBW group [M = 134.63 mmol/L; SD ± 11.43

mmol/L]. Six outliers were identified, with the highest [156 mmol/L] found in the pre-trial stage of the BWBW group and the lowest [126 mmol/L] found in the post-trial stage of the BWSW group.

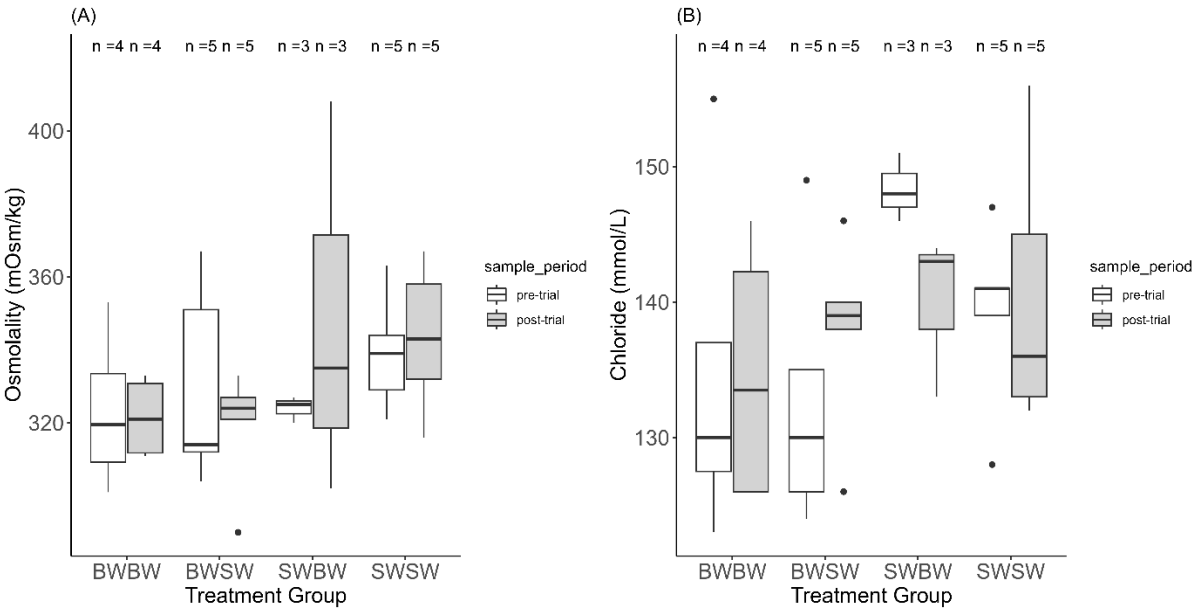


Figure 9. Osmolality (mOsmol/kg) and chloride (mmol/L) measured pre- and post-trial amongst the different treatment groups. **(A)** Osmolality comparison (pre-trial vs. post-trial) by treatment group. (**B)** Chloride comparison (pre-trial vs. post-trial) by treatment group.

The Kruskal-Wallis rank sum tests results indicated no statistically significant difference in osmolality (H (7) = 6.53, P = 0.47)) or chloride (H (7) = 13.18, P = 0.07)) among the four salinity groups during the sampling period. Therefore, Hypothesis 3 (H3), stating that "Blood plasma osmolality and blood plasma chloride levels exhibit distinct increases across the experimental stages in response to increasing salinity levels" was rejected. For this reason, it was not deemed necessary to perform a post-hoc test.

Increases in glucose were consistently observed across all treatment groups after each trial (Figure 10; (A)). The SWSW group exhibited the highest increase, with a post-trial rise of 2.56 mmol/L (approximately 92.09% higher than pre-trial levels). The BWSW group showed an increase of approximately 1.80 mmol/L post-trial (approximately 72.58% higher), while the BWBW group had a post-trial increase of 1.78 mmol/L (approximately 67.62% higher). The SWBW group displayed the lowest post-trial increase, with a rise of 1.30 mmol/L (approximately 44.94% higher). The highest total mean glucose value throughout the experimental period was observed in the SWSW group [M = 4.06 mmol/L; SD ± 1.62 mmol/L],

while the lowest was noted in the BWSW group [M = 3.38 mmol/L; SD ± 1.71 mmol/L]. Two outliers [1.90 mmol/L and 7.80 mmol/L] were found in the glucose dataset, both within the pre-trial and post-trial stages of the BWSW group, respectively.

Decreases in lactate were consistently observed across all treatment groups post-trial (Figure 10; (B)). The SWBW group exhibited the highest unit decrease, with a decrease of 1.40 units (approximately 45.65% lower than pre-trial levels). Following this, the SWSW group showed a decrease of 0.92 mmol/L post-trial (a decrease of approximately 39.32%). The BWBW group experienced a decrease of 0.75 mmol/L post-trial (approximately 32.61% lower than pre-trial levels), while the BWSW group demonstrated the lowest decrease of all groups, with a decrease of 0.54 mmol/L (approximately 26.73% lower than pre-trial levels). Yet from Figure 10 (B), mean lactate concentrations showed an increase in the post-trial stage for the SWSW group. There is also great variability within the concentrations for both pre-trial and post-trial periods. The SWBW group exhibited the highest total mean lactate value across the experimental period [M = 2.41 mmol/L; SD ± 1.34 mmol/L], while the BWSW group had the lowest [M = 1.75 mmol/L; SD ± 0.41 mmol/L]. Two outliers [1.40 mmol/L and 6.30 mmol/L] were identified for lactate within the dataset, both occurring in the pre-trial stages of the BWSW and SWSW groups, respectively.

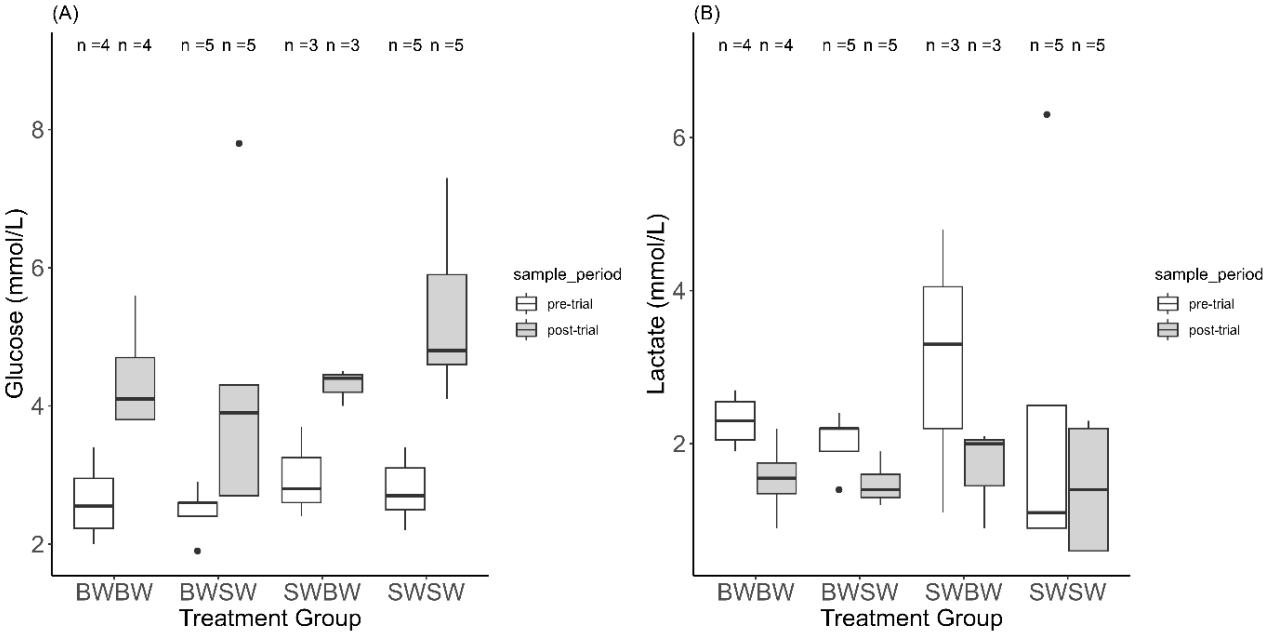


Figure 10. Boxplots displaying glucose (mmol/L) and lactate (mmol/L) values pre-trial and post-trial across treatment groups. The y-axis of both graphs displays glucose and lactate on their respective panels, while the x-axis on both graphs displays the treatment groups. **(A)** Glucose levels pre-trial and post-trial. **(B)** Lactate levels pre-trial and post-trial.

Increases in cortisol were observed across all treatment groups after each trial (Figure 11). The highest mean unit cortisol increase post-trial was recorded in the SWBW group, with a 326.27 ng/ml group mean value increase (approximately a 490.98% increase). Conversely, the BWSW group showed the lowest unit increase post-trial, with a 241.49 ng/ml group mean value increase (approximately a 941.83% increase from pre-trial). The highest total mean cortisol value throughout the experimental period was observed in the SWBW group [M = 228.17 ng/ml; SD ± 143.18 ng/ml], while the lowest was observed in the BWSW group [M = 131.18 ng/ml; SD ± 157.07 ng/ml]. Pre-trial means exhibited low variation, while larger variation was observed in all groups post-trial, with the SWSW group showing the largest variation and the SWBW group showing the smallest. No cortisol outliers were identified within the dataset.

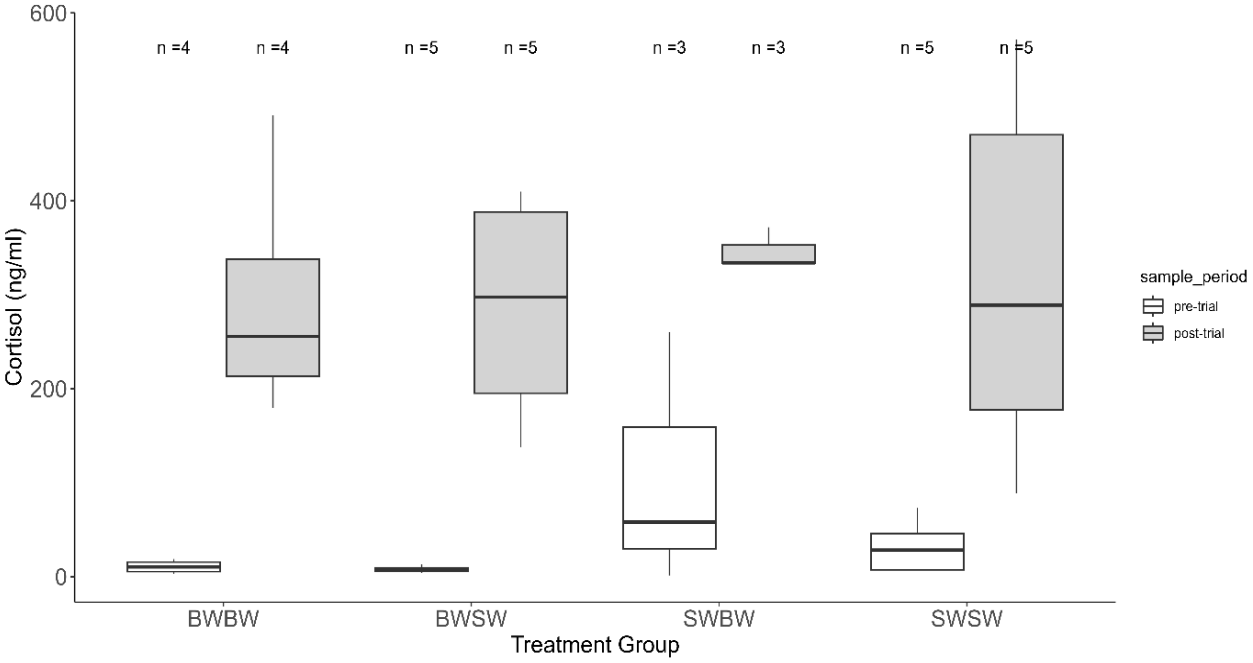


Figure 11. Cortisol (ng/ml) comparative analysis pre-trial vs. post-trial grouped by salinity treatment. The x-axis depicts the treatment groups, while the y-axis represents cortisol levels.

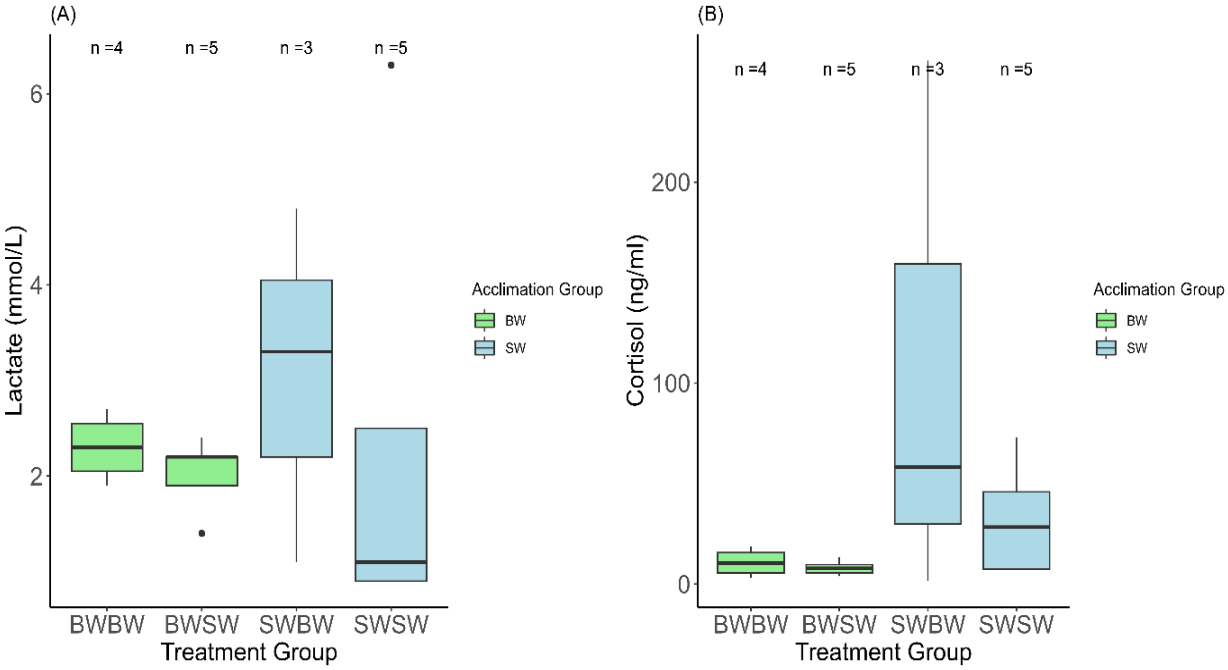


Figure 12. Boxplots displaying pre-trial lactate (mmol/L) and pre-trial cortisol (ng/ml) values across BW- and SW- acclimated groups. The y-axis of both graphs displays glucose and lactate on their respective panels, while the x-axis on both graphs displays the treatment groups. Acclimation groups are colour-coded. **(A)** Lactate levels pre-trial. **(B)** Cortisol levels pre-trial.

4.0 | Discussion

Salinity is a crucial environmental factor influencing *S. trutta* individuals, impacting their metabolic activity, behaviour, and distribution (Kültz, 2015). The present study aimed to enhance the understanding of hatchery-reared *S. truttas'* osmoregulatory mechanisms in response to changing salinity conditions, and the findings highlighted their adaptive abilities. The hatchery-reared individuals under investigation were a landlocked species, having previously resided exclusively in freshwater. As such, these trout have evolved in environments devoid of exposure to brackish water and full-strength seawater, contrasting with their wild anadromous counterparts. Yet the inherent plasticity in the genetic make-up of *S. trutta* allows them to navigate transitions between differing salinity environments with relative ease. Initially unfamiliar with full-strength seawater, the hatchery-reared fish adapted surprisingly well to the new, higher salinity. Transitioning to brackish water and full-strength seawater seemingly posed no issues, and the fish seemed to thrive in both settings. The noticeable yet not drastic increase in MO_2 rates after exposure to seawater for the first time reflected their successful adaptation. However, significant results in the MO_2 response to salinity change suggested increased metabolic demands despite the fishes ability to handle the transition effectively. Full-strength seawater groups did not exhibit reduced swimming performance during U_{Crit} trials compared to brackish water groups. Plasma osmolality and chloride levels showed no significant differences across the experimental timeline and treatment groups.

H1. MO_2 in response to salinity changes

MO_2 values were significantly altered in response to salinity changes, indicated a significant difference in MO_2 response rates between pre- and post-treatment stages among the four salinity groups. Response values in standardised MO_2 were generally higher in the saltwater treatment groups than in brackish water treatment groups post-treatment, notably the SWSW and BWSW groups, both of which showed a positive increase in the post-treatment stage, suggesting a metabolic adjustment to altered salinity conditions via a higher energy consumption within seawater. This interpretation aligns with several established studies such as those by Gracia-López *et al.* (2006), and Tseng and Hwang (2008), demonstrating variations in fish metabolic activity in different salinity environments. Overall, the research has suggested that fish exposed to higher salinity levels may experience increased metabolic demands due to the energy-intensive process of osmoregulation required to maintain internal ion balance,

which results in elevated MO_2 rates compared to those in lower salinity environments (Ern *et al*. 2014).

However, numerous studies contradict these results, with their findings showing a significant increase in MO_2 responses after brackish water treatments for the specific wild fish species studied. This was the case for tilapia (*Oreochromis mossambicus*)(Iwama *et al.* 1997), bat rays (*Myliobatis californica*)(Meloni *et al.* 2002), and Atlantic salmon (*Salmo salar*)(Seddiki *et al*. 1996; Hvas *et al*. 2018) amongst other species, with Hvas *et al*. (2018) also showing that U_{Crit} was unaffected by salinity treatment with Atlantic salmon. Other authors found no change in oxygen consumption within the range of normal salinities irrespective of the salinity the fish species was acclimated to (Morgan, 1998; Plaut, 2000); and argue that MO_2 rates do not necessarily reflect osmoregulatory costs. However, it has been hypothesised that it would be energetically beneficial for a fish to inhabit salinity levels proximate to its internal osmolality, thus nearing the isosmotic point believed to reduce osmoregulatory expenditures, as suggested by previous studies (Boeuf and Payan, 2001; Urbina and Glover, 2015; Shaughnessy *et al.* 2022). Consequently, the outcomes of this present study, notably the observed decrease in energy expenditure in brackish water, support this hypothesis. Thus, the observed response of hatchery-derived *S. trutta* to changing salinity conditions in terms of MO_2 adaptability holds significant implications within the broader context of their physiology, especially considering their landlocked status and complete lack of exposure to the marine environment, as despite this, they have demonstrated a high tolerance to full-strength salinity. While previous studies have suggested that wild *S. trutta* may possess superior adaptability and fitness (Brockmark and Johnsson, 2010) to fluctuating environmental conditions like temperature (Leik *et al.* 2021) compared to their hatchery-reared counterparts, the present study's findings challenge this notion. The results indicate that these landlocked hatchery-derived *S. trutta*, unaccustomed to full-strength seawater, exhibited comparable MO_2 response patterns to wild fish populations when faced with changes in salinity levels. This suggests that hatchery-reared *S. trutta* individuals are not inherently disadvantaged in their ability to acclimate to salinity variations in terms of metabolism, as was previously thought.

H2. Impact of salinity changes on swim performance

Swimming is a vital and energetically demanding behaviour integral to the life history of *S. trutta*. The findings indicate that *S. trutta* acclimated to full-strength seawater did not exhibit

noticeable impairments in swim performance, contrary to expectations if ion balance issues were present. However, during the U_{Crit} trials it was visually observed that all fish, regardless of salinity origin or salinity treatment, were unmotivated to swim in general, and an overall low motivation to swim may have masked subtle differences in swim performance among treatment groups. However, the results suggested a difference in swim performance (in this case, quantified via total trial time (minutes) and maximum swim speed (m/s)) within the two different salinities across the four treatment groups. Total trial time varied between groups regardless of salinity treatment, yet despite the hypothesised increase in osmoregulatory demands directly related to increasing salinity levels, the groups initially acclimated to SW in the holding tanks [SWSW, SWBW] revealed the highest timings. The SWSW treatment group had the highest mean maximum swim speed post-trial out of all the groups while the BWSW treatment group demonstrated the lowest. This unexpected outcome contradicts the assumption that *S. trutta* would have to undergo greater energy expenditure and therefore a poorer swim performance in full-strength seawater.

However, the temporal aspect of the salinity challenge within this study is significant, considering the long-term implications of salinity acclimatisation in the holding tanks for the ionic balance of these *S. trutta* individuals. While the brief exposure during the swim chamber may not have sufficiently altered the trouts' ionic balance, it could potentially indicate a stress response to the salinity change (Kültz, 2015).

While there were differences observed between the groups in terms of swimming performance, the post-hoc test indicated that these differences were selective and not consistent across all comparisons. Additionally, the analysis suggested that the impact of salinity on swimming performance may not be straightforward, as certain groups exhibited reduced performance while others did not. Specifically, the SWSW group displayed a significant difference in total U_{Crit} trial time compared to the other salinity groups, while the BWBW, BWSW, and SWBW groups did not. Likewise, no significant differences were found between the BWSW and BWBW groups, or between the SWBW and SWSW groups. Thus, while the results generally support the notion that salinity changes in general affect swimming performance, there are nuances that need to be considered. For instance, the lack of significant differences between SWBW and SWSW groups in maximum swim speed, combined with the presence of outliers in both groups, suggests that the impact of full-strength seawater on swim performance might not be as distinct as initially hypothesised and indicates a complex relationship between salinity and swimming performance. However, it is important to interpret these findings cautiously due

to the potential influence of human bias in assessing individual fish exhaustion, particularly in the SWSW trials, which should be avoided in the future.

Only a select few studies supported the results of the present study. Plaut (2000) measured critical swimming speed in the killifish (*Aphanius dispar*) acclimated to a wide range of salinities, from freshwater to 400% seawater (140 ppt). At extremely high salinities (>200% seawater), critical swimming speed was drastically reduced. Similar results were found by Swanson (1998) for milkfish (*Chanos chanos*) at various salinity levels, with both studies suggesting a potential constraint on osmoregulatory abilities and swimming activity. However, it seems that stress-related factors, indicated by elevated cortisol levels, could have contributed to these outcomes, which may be similar for the present study.

In contrast, several more studies contradicted these results. For instance, Fang *et al*. (2019) found that coho salmon (*Oncorhynchus kisutch*) reared at 2.5, 5, 10 or 30 ppt salinities showed no effect of salinity on swimming performance. Similar results were observed in a salinity study on juvenile shortnose sturgeon (*Acipenser brevirostrum*) by Penny *et al.* (2019). Likewise, a study on shiner perch (*Cymatogaster aggregate)* by Christensen *et al.* (2018) found that critical swimming speed (in BL s^{-1}) was not significantly different between acclimated to near-isosmotic, brackish water than hyperosmotic, saltwater. One line of reasoning for these present results could be that regardless of the added energetic cost of salinity, the cost of osmoregulation may have appeared to be minimal in respect to the energetic demands of swimming, and consequently had no impact on the maximal swimming performance of these fish. Since the individuals appeared to be adequately pre-acclimatised from the holding tanks, the cost of osmoregulation seemed to play a minor role in the overall swim performance. Consequently, the U_{Crit} results did not distinctly indicate an optimal salinity level for locomotory advantages. This reinforces the notion of S. *trutta*'s physiological flexibility to thrive in diverse environmental conditions and effectively cope with osmotic challenges across varying salinity levels.

There was a highly significant, positive correlation observed between fish length (cm) and both total trial time and maximum swim speed. Despite the well-documented association between seawater adaptation and larger body size (Bjerknes *et al*. 1992; Devlin *et al.* 2000; Eldøy *et al.* 2021; Jensen *et al*. 2022), since all individuals measured around the same size this reasoning was disregarded here as it was not relevant to the present study.

Taking salinity out of the equation, variations in individual fish responses, species-specific reactions, and hatchery-rearing environments, all likely contributed to the observed swimming performance abilities of *S. trutta* in this study. Furthermore, the hatchery-derived nature of these fish, unaccustomed to natural currents, may have contributed to their lower total trial time and critical swim speed in general, noted in their lack of motivation to swim during trials and quickness to fatigue, regardless of treatment or origin of salinity, which aligns with patterns observed in other hatchery-derived salmonids. For example, Pedersen *et al*. (2008) noted that hatchery-reared *S. trutta* smolts demonstrated significant stamina compared to Atlantic salmon smolts, suggesting both individual and species-specific variations. Ralph *et al*. (2012) further emphasised the role of heritage and rearing environment in shaping swimming performance in hatchery-derived individuals of rainbow trout (*Oncorhynchus mykiss*) and *S. trutta*. Numerous studies have unequivocally established that inter-individual variation is a common phenomenon (Plaut, 2001), which perhaps highlights that the variability in the swimming capacity of fish transcends the singular impact of salinity. As such, future research should focus on understanding individual and species-specific factors affecting swimming performance in hatchery-reared fish.

H3: Impact of salinity change on blood biochemical markers

Plasma osmolality and plasma chloride levels showed no significant difference across the experimental timeline, despite distinct patterns emerging in metabolic rate and swim performance in response to varying salinity conditions within the four treatment groups. While the results revealed fluctuations in values pre- and post-treatment, a statistically significant result for both biomarkers were perhaps not reached due to a low sample size. Despite this, these results suggest that neither long-term exposure to salinity in the holding tanks nor short-term salinity exposure within the swim chamber seemed to impede osmolality or chloride, further confirming that the *S. trutta* individuals were well-adapted to handle the change in salinity.

Numerous studies have reported that trout presented with varying salinity levels undergo hyperosmotic stress which causes significant changes in blood plasma markers such as osmolality and chloride (Finstad *et al.* 1988; Al-Jandal *et al.* 2011, Toorchi *et al*. 2012). Contrary to established literature, the results of the present study revealed no statistically significant differences in osmolality or chloride levels among the four treatment groups during

both pre-trial and post-trial periods. This stands in contrast to studies like Ureke *et al*. (2010), which demonstrated poorer adaptation and lower survival rates in *S. trutta* individuals compared to Atlantic salmon and hybrids after seawater exposure. These unexpected findings indicate that the *S. trutta* individuals tested in this experiment may not have experienced hyperosmotic stress despite the salinity changes. These results further confirm a high level of pre-acclimatisation, emphasising the well-developed salinity tolerance of the individuals from their previous holding tank environments. The absence of mortality rates further supports the notion that all individuals quickly adapted to the new salinity conditions. The unexpected ease with which the fish handled the treatments raises questions about the potential factors influencing their stress or exhaustion levels.

Elevations in glucose levels were consistently noted across all treatment groups after each trial, with post-trial mean values exhibiting a higher elevation than values in the pre-trial stage. Notably, the SWSW group displayed the largest increase in glucose, while the BWSW group showed the least. Yet lactate levels displayed an unexpected pattern by consistently decreasing across all treatment groups after each trial - a finding that challenges prevailing literature and implies that the fish did not attain a state of proper physiological fatigue or reach critical exhaustion levels by the conclusion of the U_{Crit} trials. Further analysis revealed that the SWBW group experienced the most notable reduction in lactate, while the BWSW group exhibited the smallest decrease.

These decreases in lactate after salinity exposure are in line with results from other parallel studies, such as those by Sangiao-Alvarellos *et al*. (2003) which demonstrated that lactate levels in gilt-head bream (*Sparus aurata)* decreased in parallel with the increase in salinity, and Tsui and Chen (2012) who found no significant differences in lactate levels in grouper fish (*Epinephelus malabaricus*) after exposure to various salinity levels. Nevertheless, the SWSW group demonstrated an increase in mean lactate levels in the post-trial stage. These general decreases conflict with findings on lactate levels increasing in studies from Nelson *et al*. (1996) on Atlantic cod (*Gadus morhua*) populations, and a parallel study by Hvas *et al.* (2018) on Atlantic salmon (*Salmo salar*), which proposed that higher post-fatigue lactate in seawater suggests less efficient exercise recovery compared to lower salinities. Perhaps, then, these decreases in lactate within this present study indicated that in general, *S. trutta* have developed sufficient exercise recovery abilities.

Elevations in cortisol levels were consistently observed across all treatment groups following each trial. The link between stress and hormonal responses, particularly the surge in plasma cortisol, is well-documented in *S. trutta* as an osmoregulatory response to varying salinity conditions (Pickering, 1982). Cortisol, as demonstrated by Krumschnabel and Lackner (1993) and Plaut (1998), provides glucose to fulfil the energy requirements needed for osmoregulation in seawater and influences further rises in plasma glucose. These findings are also consistent with the conclusions drawn by Sarkheil *et al.* (2017) in their study on Caspian brown trout smolts. Thus, results of this present study confirm that the heightened cortisol and glucose levels may be linked to their roles in facilitating the salinity acclimation of hatchery-derived *S. trutta*.

Contrary to the general trend observed in salmonoid studies, where swimming to exhaustion results in elevated plasma glucose, lactate, and cortisol levels across all salinity groups (Wood, 1991; Carbonara *et al.* 2012; Hvas *et al.* 2018), the results of this study deviate from this pattern. The absence of consistent increases in these parameters post-exhaustion, as well as the highest mean cortisol level increase post-trial being in the SWBW group, suggests that salinity stress, exercise-induced stress, or exhaustion did not contribute significantly to the observed results. Instead, it implies that the stress experienced by *S. trutta* was likely attributed to other factors, potentially arising from the handling process and the inherent stress response to the salinity changes. However, considering the findings from the pre-treatment plot (Figure 12), which aimed to minimise these confounding factors, it seems that salinity acclimation had some impact on the physiological responses of fish. Specifically, the observed differences in pre-treatment lactate and cortisol levels between the BW- and SW-acclimated groups suggest that these variations reflect the effect of the salinity conditions. The higher variation in lactate levels in the SW-acclimated groups compared to the BW-acclimated groups suggests that salinity acclimation may have influenced the production of lactate. Additionally, the consistent elevation and variation in cortisol levels across both SW-acclimated groups compared to the BW-acclimated groups implies a stress response due to the higher salinity conditions, which indicates a potential effect of salinity stress on the production of cortisol. Therefore, while handling, U_{Crit} trials, and the inherent stress response likely played a role, the salinity treatment also impacted the physiological responses of the fish. As such, future studies should further investigate the stress response of *S. trutta* to salinity changes, while considering the role of handling processes and inherent stress responses in influencing blood biomarker levels.

Furthermore, in future studies, extending the acclimatisation period for the *S. trutta* individuals is recommended to ensure better adjustment to experimental conditions, leading to reduced stress responses and more representative MO_2 values. Additionally, greater attention should be given to sample size considerations. Despite initially having a sufficient sample size of 30 individuals in total and an equal number within each group, the number of fish per treatment group decreased after data cleaning, potentially affecting the statistical power to accurately detect the effects of salinity treatments. Therefore, future studies would benefit from larger sample sizes to enhance the robustness of their findings and improve the general reliability of conclusions regarding the effects of salinity treatments on fish physiology.

Conclusion

In conclusion, the present study highlights significant knowledge gaps regarding the impact of salinity changes on landlocked, hatchery-reared *S. trutta* physiology and swim performance. Through respirometry methods, U_{Crit} trials, and blood sampling, significant differences in oxygen consumption were observed across brackish water (9.5 – 15.0 ppt) and in full-strength seawater (31.3 – 34.6 ppt) treatment groups. Full-strength seawater groups did not exhibit reduced swimming performance during U_{Crit} trials compared to brackish water groups. Plasma osmolality and chloride levels showed no significant differences across the experimental timeline and treatment groups, suggesting that the trout adapted well to the altered salinity treatments. Glucose, lactate, and cortisol levels suggested that exercise-induced stress or exhaustion did not significantly contribute to observed results, implying alternative stressors likely related to handling and/or the inherent stress response to salinity changes. These findings challenge conventional expectations within existing scientific literature and highlight the adaptive osmoregulatory mechanisms in hatchery-reared *S. trutta*. Moving forward, further comprehensive studies are warranted to investigate the underlying factors driving salinity tolerance for both hatchery-reared and wild *S. trutta* populations.

References

Aarestrup, K., Baktoft, H., Koed, A., del Villar-Guerra, D. and Thorstad, E.B., 2014. Comparison of the riverine and early marine migration behaviour and survival of wild and hatchery-reared sea trout *Salmo trutta* smolts. *Marine Ecology Progress Series*, *496*, pp.197-206.

Al-Jandal, N.J. and Wilson, R.W., 2011. A comparison of osmoregulatory responses in plasma and tissues of rainbow trout (*Oncorhynchus mykiss*) following acute salinity challenges. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *159*(2), pp.175-181.

Aprahamian, M.W., Smith, K.M., McGinnity, P., McKelvey, S. and Taylor, J., 2003. Restocking of salmonids—opportunities and limitations. *Fisheries Research*, *62*(2), pp.211-227.

Baglinière, J.-L., and Maisse, G., 1999. *Biology and ecology of the brown and sea trout.* Chichester, UK: Springer Praxis Publishing, Chichester.

Bell, W.H., and Terhune, L.D.B., 1970. *Water tunnel design for fisheries research*. Fisheries Research Board of Canada, Biological Station. 195, 1-69.

Birnie-Gauvin, K., Bordeleau, X., Cooke, S.J., Davidsen, J.G., Eldøy, S.H., Eliason, E.J., Moore, A. and Aarestrup, K., 2021. Life-history strategies in salmonids: The role of physiology and its consequences. *Biological Reviews*, *96*(5), pp.2304-2320.

Bjerknes, V., Duston, J., Knox, D. and Harmon, P., 1992. Importance of body size for acclimation of underyearling Atlantic salmon parr (*Salmo salar L.*) to seawater. *Aquaculture*, *104*(3-4), pp.357-366.

Bœuf, G. and Payan, P., 2001. How should salinity influence fish growth?, *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *130*(4), pp.411-423.

Brett, J.R., 1964. The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Board of Canada*, *21*(5), pp.1183-1226.

Brockmark, S. and Johnsson, J.I., 2010. Reduced hatchery-rearing density increases social dominance, post-release growth, and survival in brown trout (*Salmo trutta*). *Canadian Journal of Fisheries and Aquatic Sciences*, *67*(2), pp.288-295.

Cai L, Taupier R, Johnson D, Tu Z, Liu G, Huang, Y, 2013. Swimming capability and swimming behavior of juvenile *Acipenser schrenckii. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology.* 319:149−155.

Cano-Barbacil, C., Radinger, J., Argudo, M., Rubio-Gracia, F., Vila-Gispert, A., and García-Berthou, E., 2020. Key factors explaining critical swimming speed in freshwater fish: a review and statistical analysis for Iberian species. *Scientific Reports*, *10*(1), p.18947.

Carbonara, P., Corsi, I., Focardi, S., Lembo, G., Rochira, S., Scolamacchia, M., Teresa Spedicato, M. and Scott McKinley, R., 2010. The effects of stress induced by cortisol administration on the repeatability of swimming performance tests in the European sea bass (*Dicentrarchus labrax L.). Marine and Freshwater Behaviour and Physiology*, *43*(4), pp.283-296.

Chabot, D., Steffensen, J.F. and Farrell, A.P., 2016. The determination of standard metabolic rate in fishes. *Journal of Fish Biology*, *88*(1), pp.81-121.

Chittenden, C.M., Biagi, C.A., Davidsen, J.G., Davidsen, A.G., Kondo, H., McKnight, A., Pedersen, O.P., Raven, P.A., Rikardsen, A.H., Shrimpton, J.M. and Zuehlke, B., 2010.

Genetic versus rearing-environment effects on phenotype: hatchery and natural rearing effects on hatchery-and wild-born coho salmon. *PLoS One*, *5*(8), p.e12261.

Christensen, E.A., Illing, B., Iversen, N.S., Johansen, J.L., Domenici, P. and Steffensen, J.F., 2018. Effects of salinity on swimming performance and oxygen consumption rate of shiner perch (*Cymatogaster aggregate*). *Journal of Experimental Marine Biology and Ecology*, *504*, pp.32-37.

Christensen, E.A., Stieglitz, J.D., Grosell, M. and Steffensen, J.F., 2019. Intra-specific difference in the effect of salinity on physiological performance in European perch (*Perca fluviatilis*) and its ecological importance for fish in estuaries. *Biology*, *8*(4), p.89

Clark, T.D., Sandblom, E. and Jutfelt, F., 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *Journal of Experimental Biology*, *216*(15), pp.2771-2782.

Davidsen, J.G., Daverdin, M., Arnekleiv, J.V., Rønning, L., Sjursen, A.D. and Koksvik, J.I., 2014. Riverine and near coastal migration performance of hatchery brown trout (*Salmo trutta*). *Journal of Fish Biology*, *85*(3), pp.586-596.

Demeditec Diagnostics user manual, 2023, *ELISA DEH3388 Procedure for Cortisol*, Available at: https://www.demeditec.com/en/products/cortisol-elisa-deh3388/ifu-deh3388-cortisol-elisa-ce-3-02-22-m.pdf, (Accessed: 21/11/2023).

Devlin, R.H., Swanson, P., Clarke, W.C., Plisetskaya, E., Dickhoff, W., Moriyama, S., Yesaki, T.Y. and Hew, C.L., 2000. Seawater adaptability and hormone levels in growth-enhanced transgenic coho salmon, *Oncorhynchus kisutch*. *Aquaculture*, *191*(4), pp.367-385.

Djiba, P.K., Zhang, J., Xu, Y., Zhang, P., Zhou, J., Zhang, Y. and Luo, Y., 2021. Correlation between metabolic rate and salinity tolerance and metabolic response to salinity in grass carp (*Ctenopharyngodon idella*). *Animals*, *11*(12), p.3445.

Doogan, A., Cotter, D., Bond, N., Ó'Maoiléidigh, N. and Brophy, D., 2023. Partitioning survival during early marine migration of wild and hatchery-reared Atlantic salmon (*Salmo salar L.)* smolts using acoustic telemetry. *Animal Biotelemetry*, *11*(1), p.39.

Eldøy, S.H., Bordeleau, X., Lawrence, M.J., Thorstad, E.B., Finstad, A.G., Whoriskey, F.G., Crossin, G.T., Cooke, S.J., Aarestrup, K., Rønning, L., and Sjursen, A.D., 2021. The effects of nutritional state, sex, and body size on the marine migration behaviour of sea trout. *Marine Ecology Progress Series*, *665*, pp.185-200.

Elliott, J. and Elliott, J.A., 2010. Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: predicting the effects of climate change. *Journal of Fish Biology*, *77*(8), pp.1793-1817.

Ern, R., Huong, D.T.T., Cong, N.V., Bayley, M. and Wang, T., 2014. Effect of salinity on oxygen consumption in fishes: a review. *Journal of Fish Biology*, *84*(4), pp.1210-1220.

Fang, Y., Chan, V.K., Hines, C.W., Stiller, K.T., Richards, J.G. and Brauner, C.J., 2019. The effects of salinity and photoperiod on aerobic scope, hypoxia tolerance and swimming performance of coho salmon (*Oncorhynchus kisutch*) reared in recirculating aquaculture systems. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *231*, pp.82-90.

Farrell, A.P., 2008. Comparisons of swimming performance in rainbow trout using constant acceleration and critical swimming speed tests. *Journal of Fish Biology*, *72*(3), pp.693-710.

Ferguson, A., Reed, T.E., Cross, T.F., McGinnity, P. and Prodöhl, P.A., 2019. Anadromy, potamodromy and residency in brown trout *Salmo trutta*: the role of genes and the environment. *Journal of Fish Biology*, *95*(3), pp.692-718.

Finstad, B., Staurnes, M. and Reite, O.B., 1988. Effect of low temperature on sea-water tolerance in rainbow trout, *Salmo gairdneri*. *Aquaculture*, *72*(3-4), pp.319-328.

Finstad, B., and Sivertsen, K., 2022. *The brown trout and sea trout life history* [Figure 1]. Adapted from NINA (Norwegian Institute for Nature Research). Retrieved from https://nina.no/Portals/NINA/Bilder%20og%20dokumenter/brosjyre%20vatnevassdraget%20engelsk_web.pdf, (Accessed on 03/01/2023).

Finstad, B., February 2022, Norges teknisk-naturvitenskapelige universitet (NTNU), *Klorid og osmolalitet lab*, Available at: Klorid og osmolalitet Lab.pdf, (Accessed on 09/10/2023).

Fjellheim, A., Raddum, G.G., and Barlaup, B.T., 1995. Dispersal, growth, and mortality of brown trout (*Salmo trutta L.*) stocked in a regulated west Norwegian river. *Regulated Rivers: Research and Management*, *10*(2-4), pp.137-145.

Fulton, C.J., 2007. Swimming speed performance in coral reef fishes: field validations reveal distinct functional groups. *Coral Reefs*, *26*(2), pp.217-228.

Gracia-López, V., Rosas-Vázquez, C. and Brito-Perez, R., 2006. Effects of salinity on physiological conditions in juvenile common snook *Centropomus undecimalis. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *145*(3), pp.340-345.

Hammer, C. 1995. Fatigue and exercise tests with fish. *Comparative Biochemistry and Physiology 112* (1): 1–20.

Hvas, M., Nilsen, T.O. and Oppedal, F., 2018. Oxygen uptake and osmotic balance of Atlantic salmon in relation to exercise and salinity acclimation. *Frontiers in Marine Science*, *5*, p.368.

Hvas, M. and Oppedal, F., 2019. Influence of experimental set-up and methodology for measurements of metabolic rates and critical swimming speed in Atlantic salmon (*Salmo salar). Journal of Fish Biology*, *95*(3), pp.893-902.

ICES (2013). *Report of the Workshop on Sea Trout (WKTRUTTA),* 12–14 November 2013, ICES Headquarters, Copenhagen, Denmark. p. 280.

Iwama, G.K., Takemura, A. and Takano, K., 1997. Oxygen consumption rates of tilapia in fresh water, sea water, and hypersaline sea water. *Journal of Fish Biology*, *51*(5), pp.886-894.

Jensen, J. L. A., & Rikardsen, A. H., 2012. Archival tags reveal that Arctic charr *Salvelinus alpinus* and brown trout *Salmo trutta* can use estuarine and marine waters during winter. *Journal of Fish Biology*, 81(2), 735–749.

Jensen, A.J., Diserud, O.H., Finstad, B., Fiske, P. and Thorstad, E.B., 2022. Early-season brown trout (*Salmo trutta*) migrants grow and survive better at sea. *Journal of Fish Biology*, *100*(6), pp.1419-1431.

Jonsson, B. and Jonsson, N., 1993. Partial migration: niche shift versus sexual maturation in fishes. *Reviews in Fish Biology and Fisheries*, *3*, pp.348-365.

Jonsson, N. and Jonsson, B., 1998. Body composition and energy allocation in life-history stages of brown trout. *Journal of Fish Biology*, *53*(6), pp.1306-1316.

Jonsson, B. and Jonsson, N., 2009. Migratory timing, marine survival, and growth of anadromous brown trout *Salmo trutta* in the River Imsa, Norway. *Journal of Fish Biology*, *74*(3), pp.621-638.

Karlsson, S., Diserud, O.H., Fiske, P., Hindar, K. and Handling editor: W. Stewart Grant, 2016. Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations. *ICES Journal of Marine Science*, *73*(10), pp.2488-2498.

Krumschnabel, G. and Lackner, R., 1993. Stress responses in rainbow trout (*Oncorhynchus mykiss alevins*). *Comparative Biochemistry and Physiology Part A: Physiology*, *104*(4), pp.777-783.

Kültz, D., 2015. Physiological mechanisms used by fish to cope with salinity stress. *The Journal of Experimental Biology*, *218*(12), pp.1907-1914.

Larocque, S.M., Johnson, T.B. and Fisk, A.T., 2020. Survival and migration patterns of naturally and hatchery-reared Atlantic salmon (*Salmo salar*) smolts in a Lake Ontario tributary using acoustic telemetry. *Freshwater Biology*, *65*(5), pp.835-848.

Lejk, A.M., Smoliński, S., Radtke, G. and Martyniak, A., 2021. Higher growth variability and stronger responses to temperature changes in wild than hatchery-reared sea trout (*Salmo trutta L.). Ecology and Evolution*, *11*(15), pp.10207-10224.

Lemos, L.S., Angarica, L.M., Hauser-Davis, R.A. and Quinete, N., 2023. Cortisol as a stress indicator in fish: sampling methods, analytical techniques, and organic pollutant exposure assessments. *International Journal of Environmental Research and Public Health*, *20*(13), p.6237.

Lighton, J.R., 2018. *Measuring metabolic rates: a manual for scientists*. Oxford University Press.

Martínez-Álvarez, R.M., Morales, A.E. and Sanz, A., 2005. Antioxidant defenses in fish: biotic and abiotic factors. *Reviews in Fish Biology and Fisheries*, *15*, pp.75-88.

Meloni, C.J., Cech Jr, J.J. and Katzman, S.M., 2002. Effect of brackish salinities on oxygen consumption of bat rays (*Myliobatis californica*). *Copeia*, *2002*(2), pp.462-465.

Moore, A., Ives, M., Scott, M. and Bamber, S., 1998. The migratory behaviour of wild sea trout (*Salmo trutta* L.) smolts in the estuary of the River Conwy, North Wales. *Aquaculture*, *168*(1-4), pp.57-68.

Moore, A., and Potter, E.C.E., 1994. The movement of wild sea trout, *Salmo trutta* L., smolts through a river estuary. *Fisheries Management and Ecology*, *1*(1), pp.1-14.

Morgan, J.D., and Iwama, G.K., 1991. Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (*Oncorhynchus mykiss*) and fall chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences*, *48*(11), pp.2083-2094.

Morgan, J.D., 1998. *Energetic aspects of osmoregulation in fish,* (Doctoral dissertation, University of British Columbia).

Nelson, J.A., Tang, Y. and Boutilier, R.G., 1996. The effects of salinity change on the exercise performance of two Atlantic cod (*Gadus morhua*) populations inhabiting different environments. *Journal of Experimental Biology*, *199*(6), pp.1295-1309.

Nelson, J.A., 2016. Oxygen consumption rate v. rate of energy utilization of fishes: a comparison and brief history of the two measurements. *Journal of Fish Biology*, *88*(1), pp.10-25.

Nelson, J.A. and Chabot, D., 2017. 'Energy consumption: metabolism (general)'. In *Reference Module in Life Sciences.* Elsevier.

Nevoux, M., Finstad, B., Davidsen, J.G., Finlay, R., Josset, Q., Poole, R., Höjesjö, J., Aarestrup, K., Persson, L., Tolvanen, O. and Jonsson, B., 2019. Environmental influences on life history strategies in partially anadromous brown trout (*Salmo trutta*, Salmonidae). *Fish and Fisheries*, *20(6)*, pp.1051-1082.

Ojanguren, A.F. and Brana, F., 2003. Effects of size and morphology on swimming performance in juvenile brown trout (*Salmo trutta L.). Ecology of Freshwater Fish*, *12*(4), pp.241-246.

Paquet, P.J., Flagg, T., Appleby, A., Barr, J., Blankenship, L., Campton, D., Delarm, M., Evelyn, T., Fast, D., Gislason, J. and Kline, P., 2011. Hatcheries, conservation, and sustainable fisheries—achieving multiple goals: results of the Hatchery Scientific Review Group's Columbia River basin review. *Fisheries*, *36*(11), pp.547-561.

Pedersen, L.F., Koed, A. and Malte, H., 2008. Swimming performance of wild and F1-hatchery-reared Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) smolts. *Ecology of Freshwater Fish*, *17*(3), pp.425-431.

Penny, F.M. and Kieffer, J.D., 2019. Lack of change in swimming capacity (U_{Crit}) following acute salinity exposure in juvenile shortnose sturgeon (*Acipenser brevirostrum). Fish Physiology and Biochemistry*, *45*, pp.1167-1175.

Pickering, A.D., Pottinger, T.G. and Christie, P., 1982. Recovery of the brown trout, *Salmo trutta L.*, from acute handling stress: a time-course study. *Journal of Fish Biology*, *20*(2), pp.229-244.

Plaut, I., 1998. Comparison of salinity tolerance and osmoregulation in two closely related species of blennies from different habitats. *Fish Physiology and Biochemistry*, *19*(2), pp.181-188.

Plaut, I., 2000. Resting metabolic rate, critical swimming speed, and routine activity of the euryhaline cyprinodontid, *Aphanius dispar*, acclimated to a wide range of salinities. *Physiological and Biochemical Zoology*, *73*(5), pp.590-596.

Plaut, I. 2001. Critical swimming speed: Its ecological relevance. *Comparative Biochemistry and Physiology 131, (1):* 41–50.

Ralph, A.L., Berli, B.I., Burkhardt-Holm, P. and Tierney, K.B., 2012. Variability in swimming performance and underlying physiology in rainbow trout (*Oncorhynchus mykiss)* and brown trout (*Salmo trutta*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *163*(3-4), pp.350-356.

Sangiao-Alvarellos, S., Laiz-Carrion, R., Guzmán, J.M., Martín del Río, M.P., Miguez, J.M., Mancera, J.M. and Soengas, J.L., 2003. Acclimation of *S. aurata* to various salinities alters energy metabolism of osmoregulatory and nonosmoregulatory organs. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *285*(4), pp.R897-R907.

Sarkheil, M., Sorki, M.P. and Raefipour, H., 2017. Effects of acclimation to seawater salinity on some blood parameters in wild Caspian brown trout, *Salmo trutta caspius. Comparative Clinical Pathology*, *26*, pp.1315-1318.

Seale, A.P. and Breves, J.P., 2022. Endocrine and osmoregulatory responses to tidally changing salinities in fishes. *General and Comparative Endocrinology*, *326*, p.114071.).

Seddiki, H., Boeuf, G., Maxime, V. and Peyraud, C., 1996. Effects of growth hormone treatment on oxygen consumption and sea water adaptability in Atlantic salmon parr and pre-smolts. *Aquaculture*, *148*(1), pp.49-62.

Shaughnessy, C.A., Balfry, S.K., and Bystriansky, J.S., 2022. The isosmotic point as critical salinity limit for growth and osmoregulation, but not survival, in the wolf eel *Anarrhichthys ocellatus*. *Fish Physiology and Biochemistry*, *48*(2), pp.471-480.

Sivapriya, V., Radhakrishnan, K., and Hussain, S.M., 2022. Benthos and its interaction with marine and estuarine ecosystem. In: *Ecology and Biodiversity of Benthos* (pp. 315-336). Elsevier.

Skaala, Ø., Besnier, F., Borgstrøm, R., Barlaup, B., Sørvik, A.G., Normann, E., Østebø, B.I., Hansen, M.M., and Glover, K.A., 2019. An extensive common-garden study with domesticated and wild Atlantic salmon in the wild reveals impact on smolt production and shifts in fitness traits. *Evolutionary Applications*, *12*(5), pp.1001-1016.

Sundell, K., Dellefors, C., and Björnsson, B.T., 1998. Wild and hatchery-reared brown trout, *Salmo trutta*, differ in smolt related characteristics during parr–smolt transformation. *Aquaculture*, *167*(1-2), pp.53-65.

Swanson, C., 1998. Interactive effects of salinity on metabolic rate, activity, growth, and osmoregulation in the euryhaline milkfish (*Chanos chanos*). *Journal of Experimental Biology*, *201*(24), pp.3355-3366.

Thorstad, E.B., Todd, C.D., Uglem, I., Bjørn, P.A., Gargan, P.G., Vollset, K.W., Halttunen, E., Kålås, S., Berg, M. and Finstad, B., 2016. Marine life of the sea trout. *Marine Biology*, *163*, pp.1-19.

Thorstad, E.B., and Forseth, T., 2019. Classification of the state of 430 Norwegian sea trout populations, *Status of wild Atlantic salmon in Norway 2019*, Trondheim: Norwegian Scientific Advisory Committee for Atlantic Salmon. Temarapport no. 7, 1- 150. [Translated from Norwegian version "*Status for norske laksebestander i 2019*"]. Available at: https://www.vitenskapsradet.no/Portals/vitenskapsradet/Pdf/Status%20of%20wild%20Atlantic%20salmon%20in%20Norway.pdf, (Accessed: 12/02/2024).

Toorchi, M., Bani, A. and Alizadehsabet, H., 2012. Effects of salinity on osmoregulation and plasma cortisol levels of juvenile Caspian trout, *Salmo trutta caspius* Kessler, 1877. *Journal of Applied Ichthyology*, *28*(1), pp.130-134.

Tseng, Y.C. and Hwang, P.P., 2008. Some insights into energy metabolism for osmoregulation in fish. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, *148*(4), pp.419-429.

Tsui, W.C., Chen, J.C., and Cheng, S.Y., 2012. The effects of a sudden salinity change on cortisol, glucose, lactate, and osmolality levels in grouper (*Epinephelus malabaricus*). *Fish Physiology and Biochemistry*, *38*, pp.1323-1329.

Urbina, M.A., and Glover, C.N., 2015. Effect of salinity on osmoregulation, metabolism and nitrogen excretion in the amphidromous fish, inanga (*Galaxias maculatus*). *Journal of Experimental Marine Biology and Ecology*, *473*, pp.7-15.

Urke, H.A., Koksvik, J., Arnekleiv, J.V., Hindar, K., Kroglund, F. and Kristensen, T., 2010. Seawater tolerance in Atlantic salmon, *Salmo salar L*., brown trout, *Salmo trutta L*., and *S. salar*× *S. trutta hybrids* smolt. *Fish Physiology and Biochemistry*, *36*, pp.845-853.

Wood, C.M., 1991. Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. *Journal of Experimental Biology*, *160*(1), pp.285-308.

Appendix 1. Figures and Tables

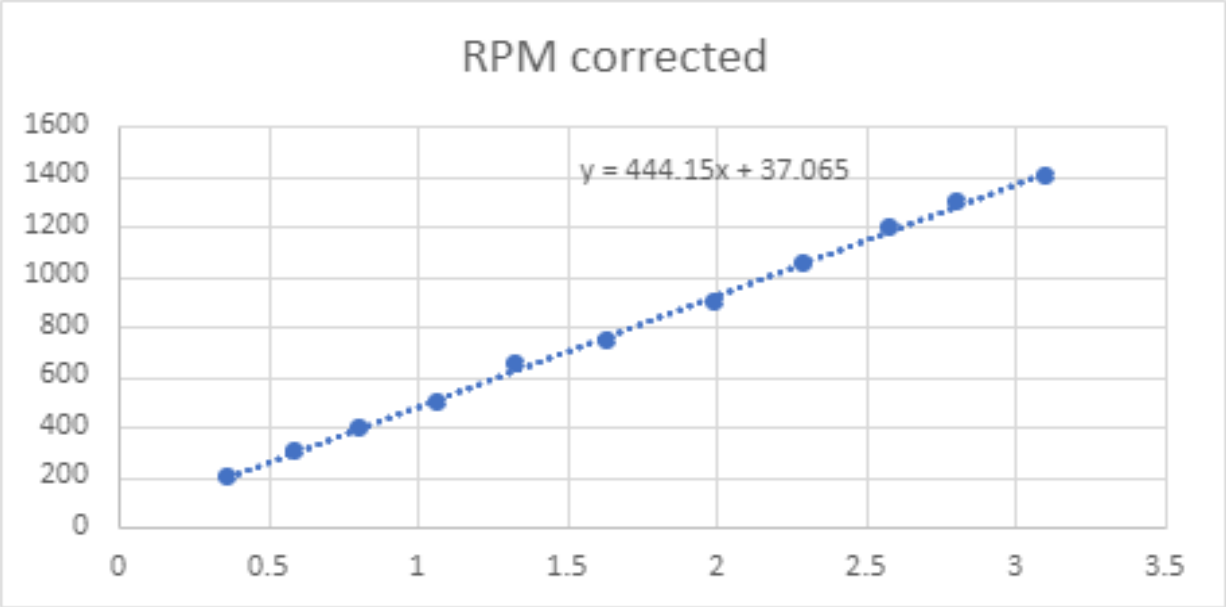


Figure A1. Plot calculator based off Fulton's (2007) paper, used to convert RPM to m/s.

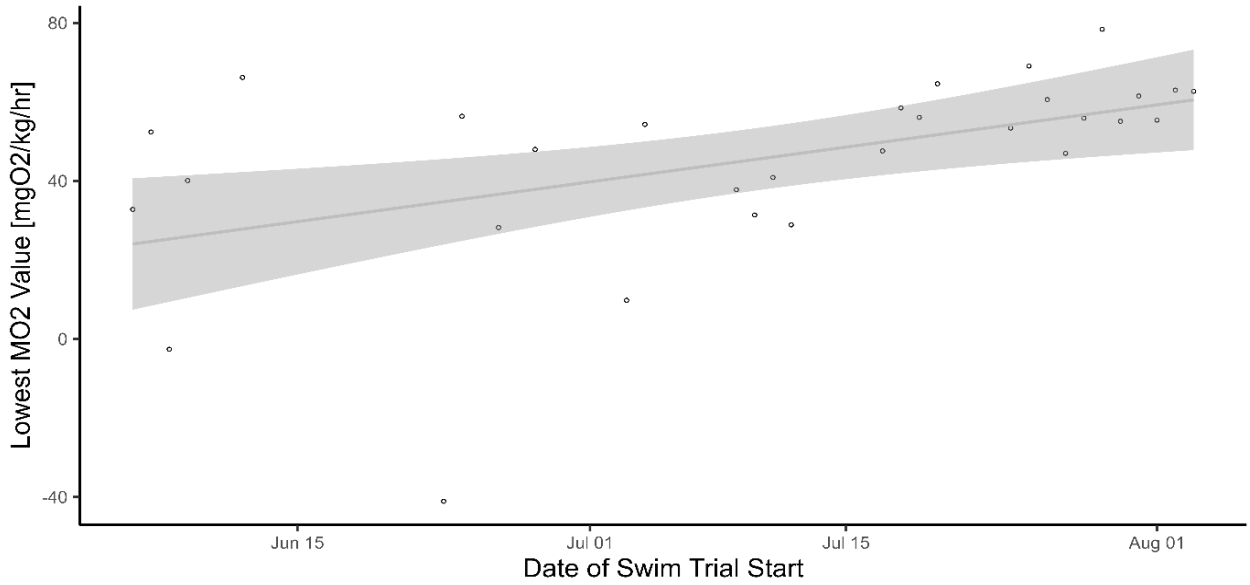


Figure A2. Lowest MO_2 within the swim chamber noted during all fish trials. The lower MO_2 values (mgO2/kg/hr) of each swim trial were weakly positively correlated but insignificant (p-value = 0.51; Adjusted R-squared = 0.22) based on the linear regression analysis results, indicating that background respiration was not influencing respirometry measurements.

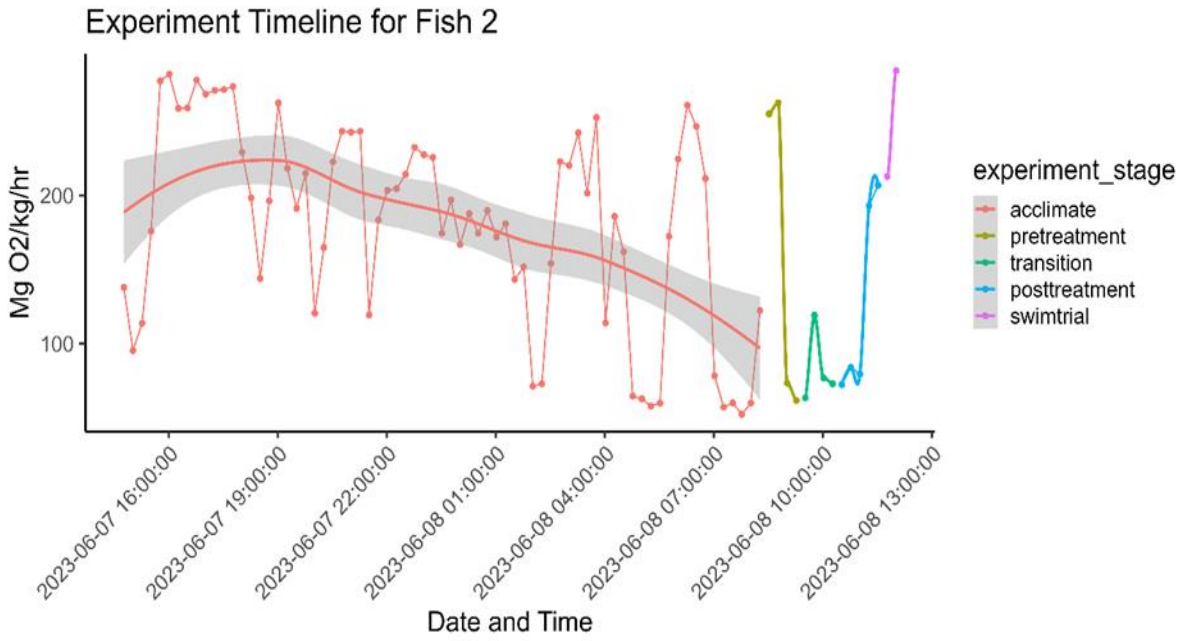


Figure A3. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 2 (BWSW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 2 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

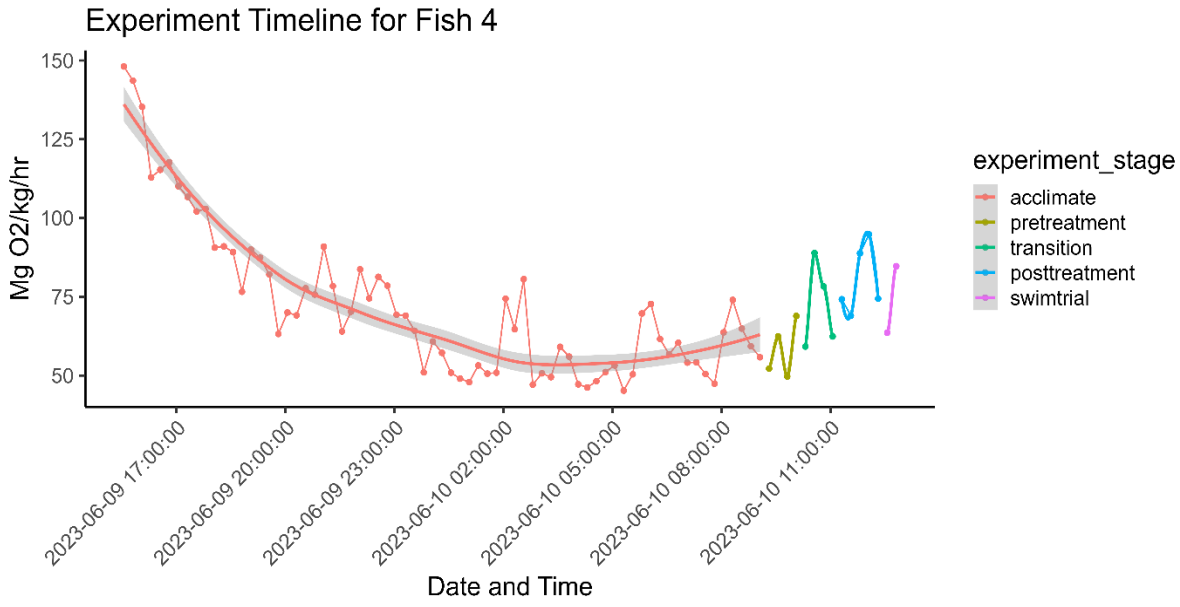


Figure A4. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 4 (BWSW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 4 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

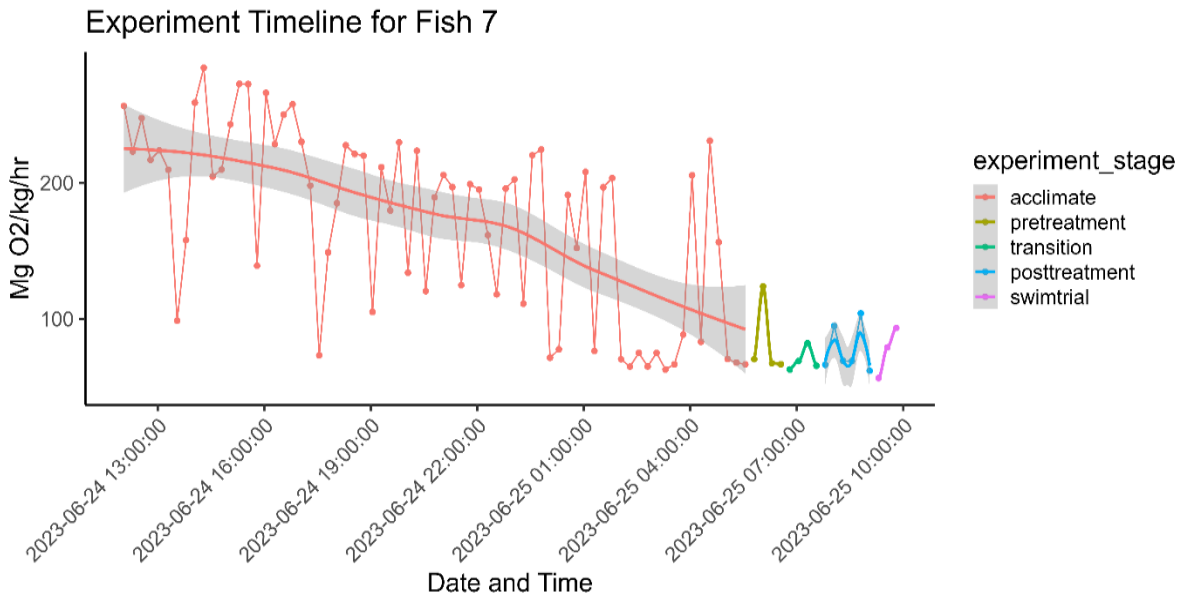


Figure A5. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 7 (SWBW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 7 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

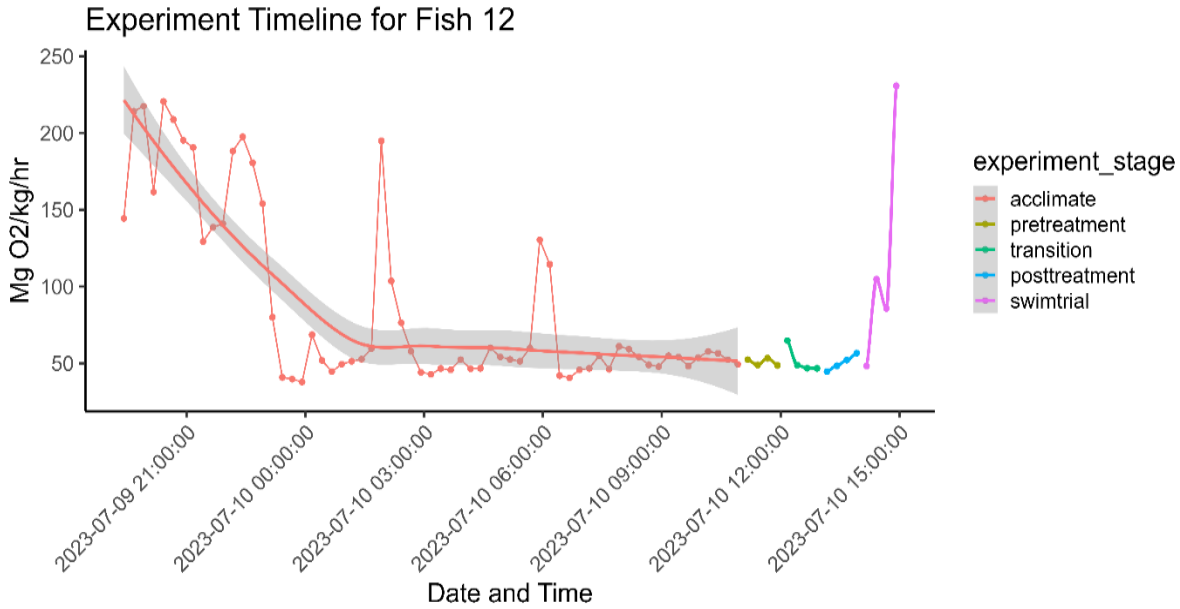


Figure A6. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 12 (SWBW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 12 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

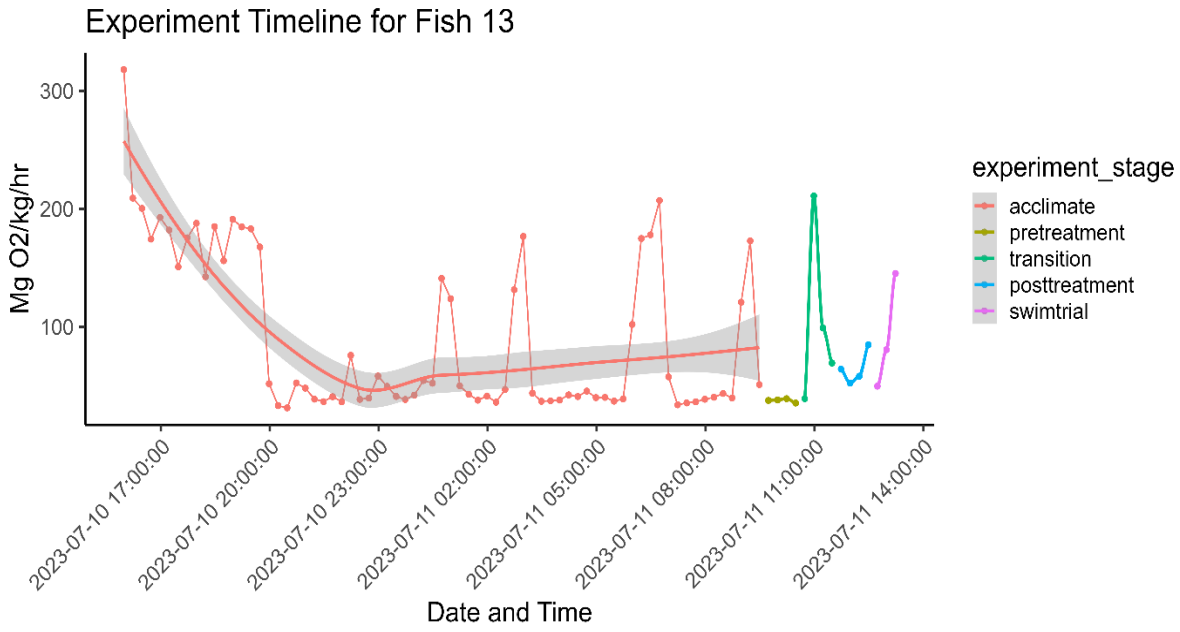


Figure A7. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 13 (BWSW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 13 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

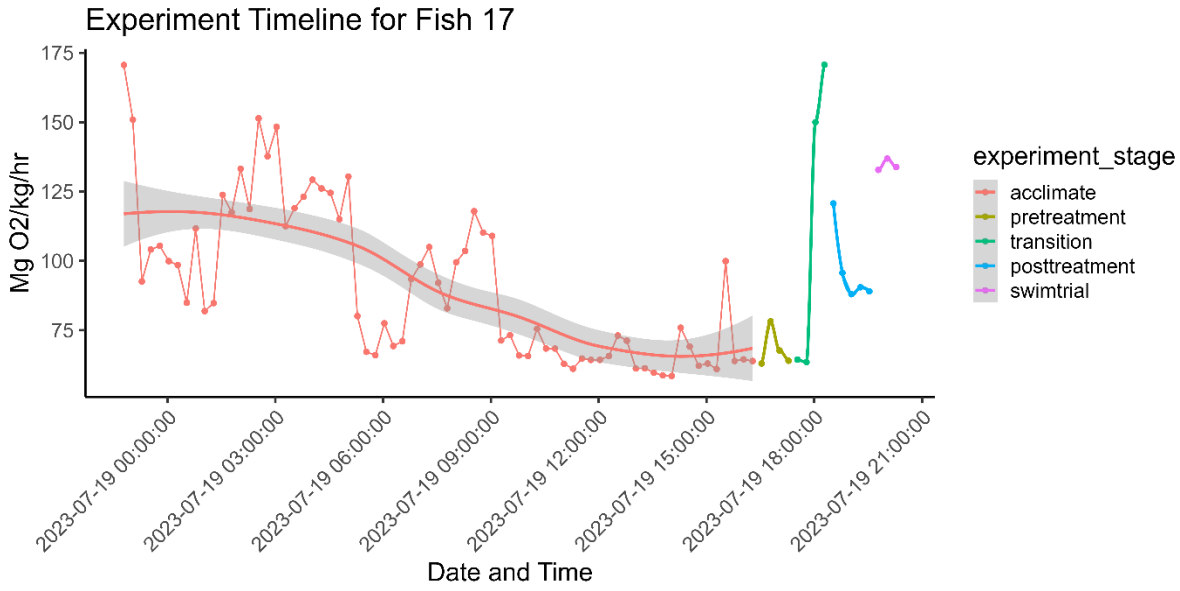


Figure A8. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 17 (BWSW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 17 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

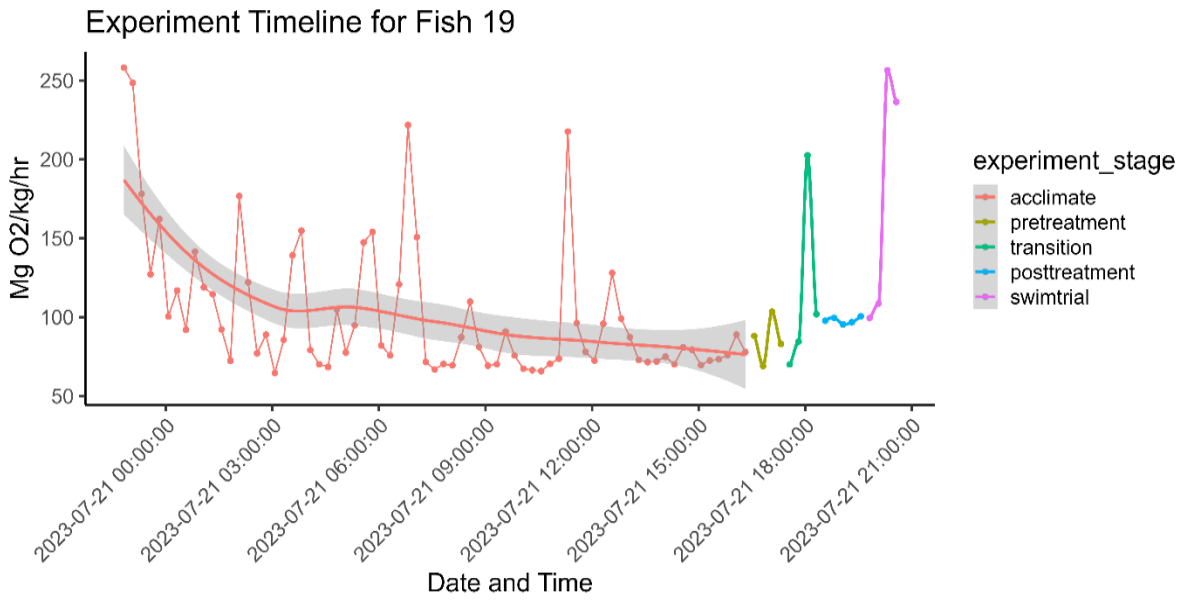


Figure A9. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 19 (BWSW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 19 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

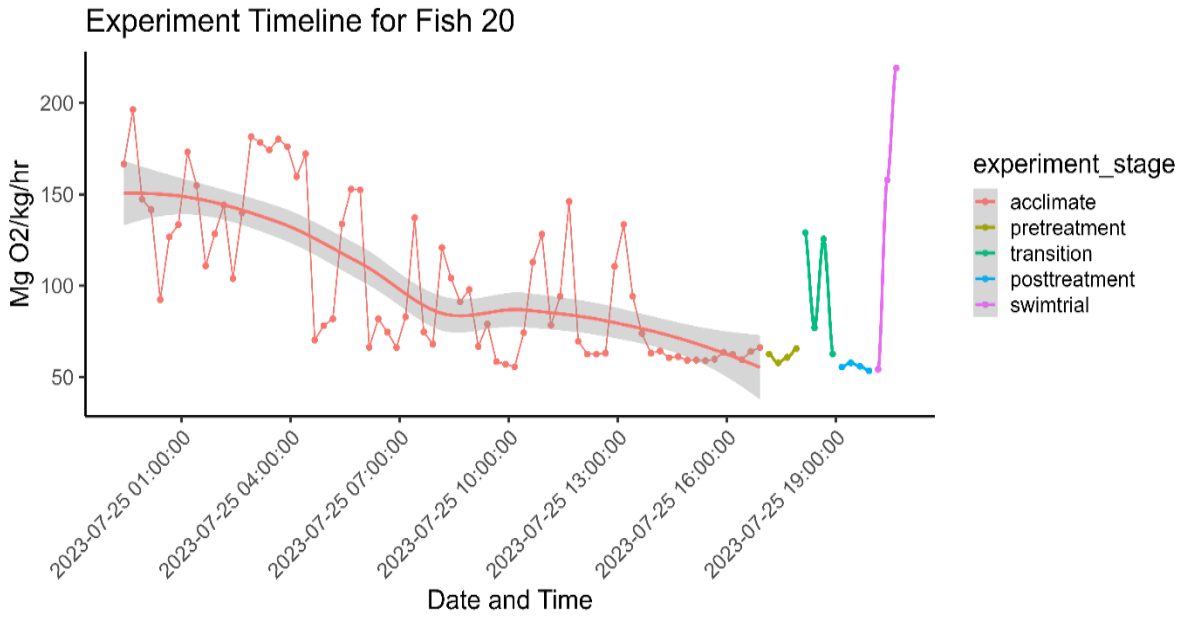


Figure A10. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 20 (SWBW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 20 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

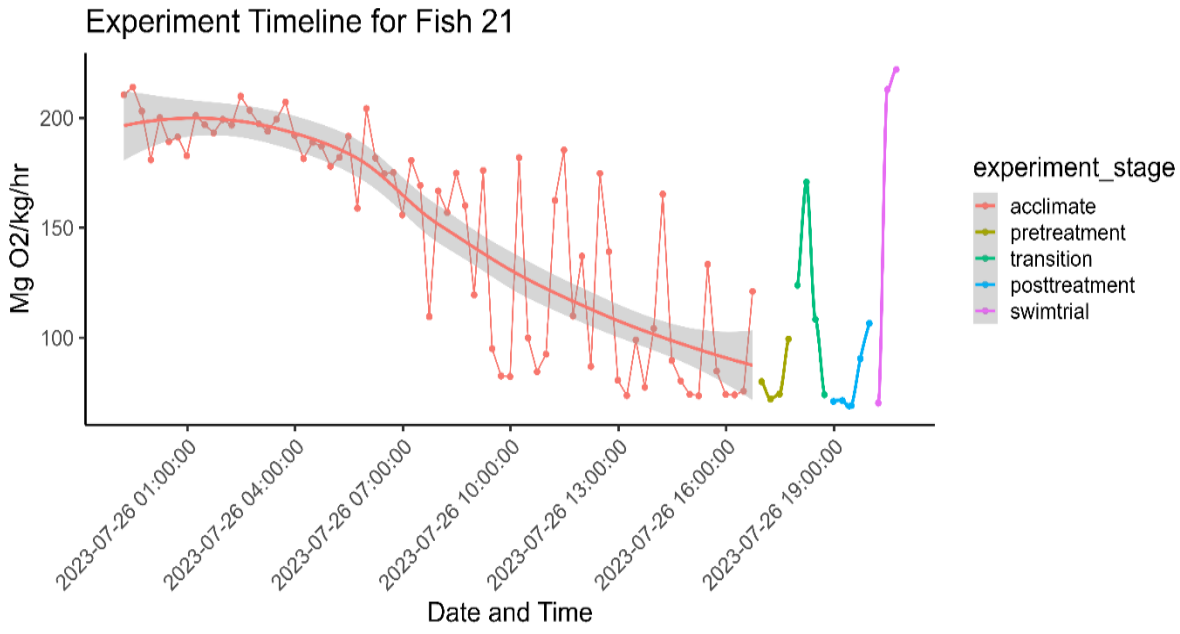


Figure A11. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 21 (BWBW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 21 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

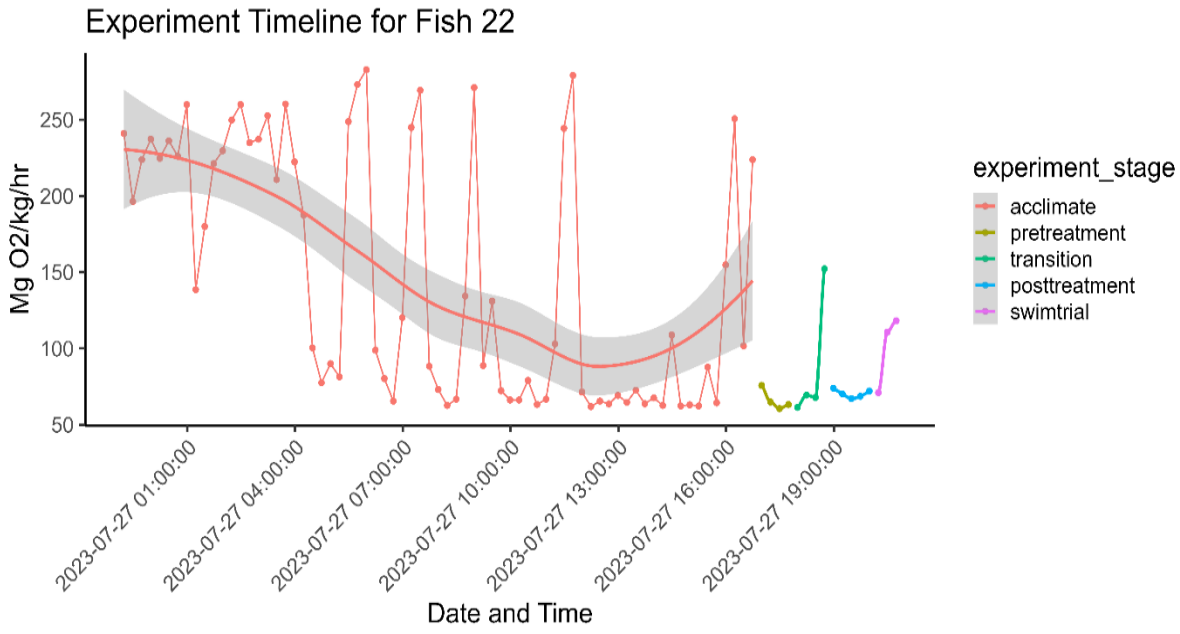


Figure A12. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 22 (BWBW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 22 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

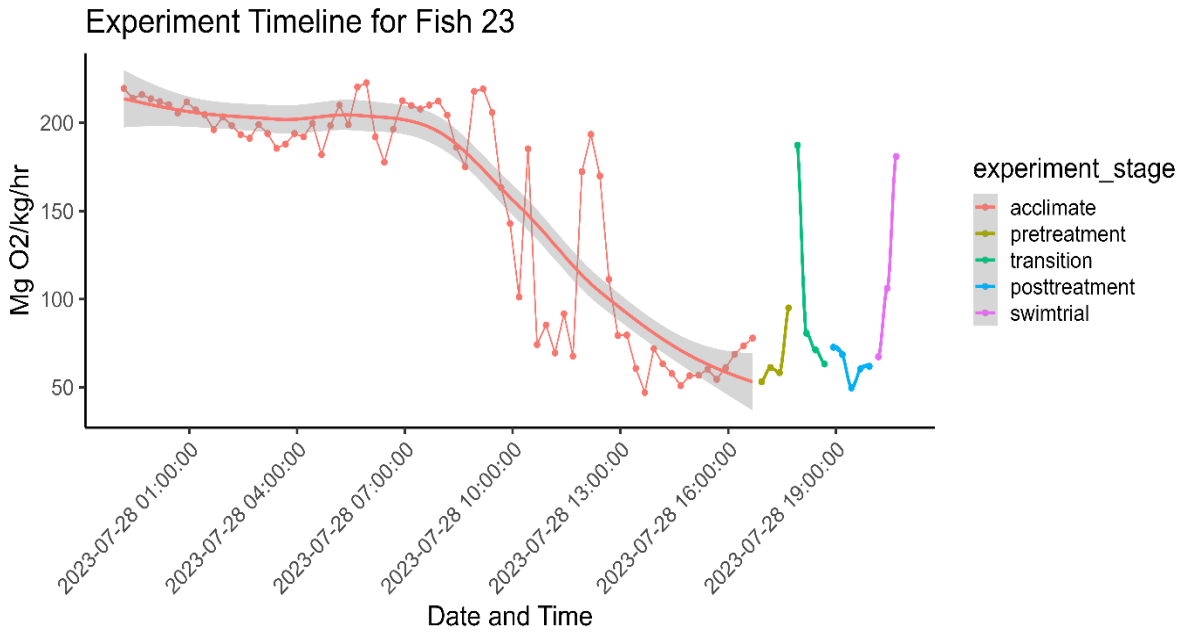


Figure A13. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 23 (BWBW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 23 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

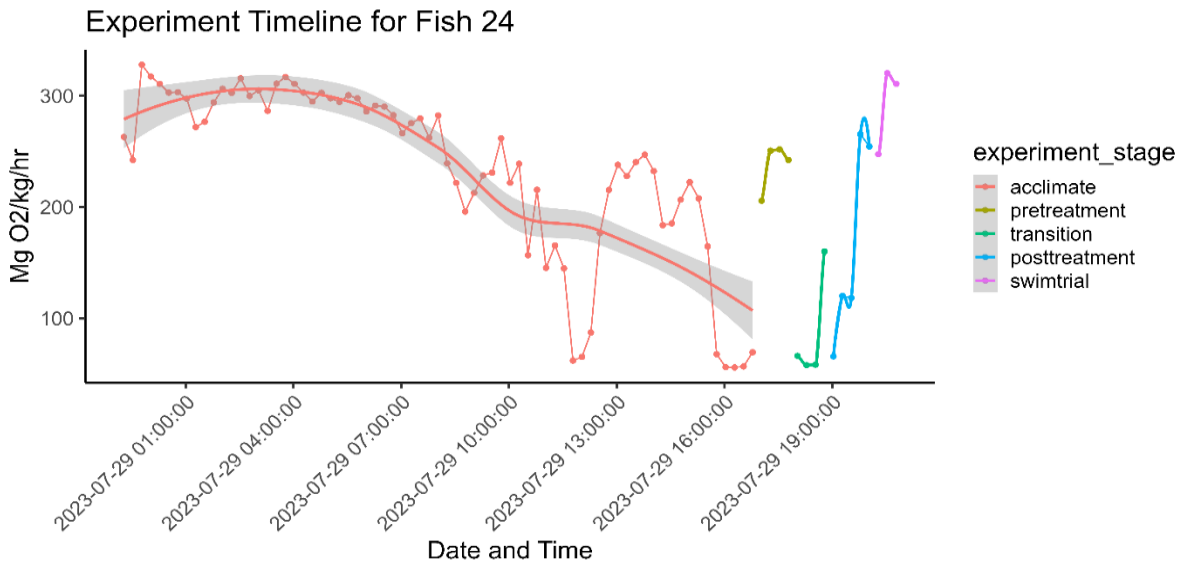


Figure A14. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 24 (BWBW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 24 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

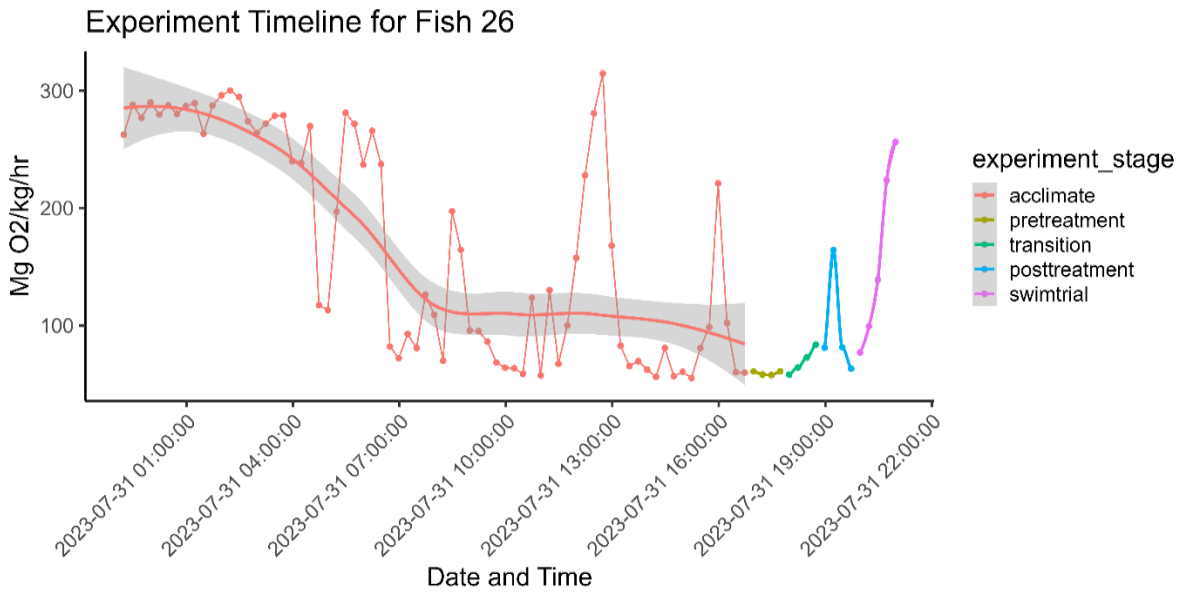


Figure A15. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 26 (SWSW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 26 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

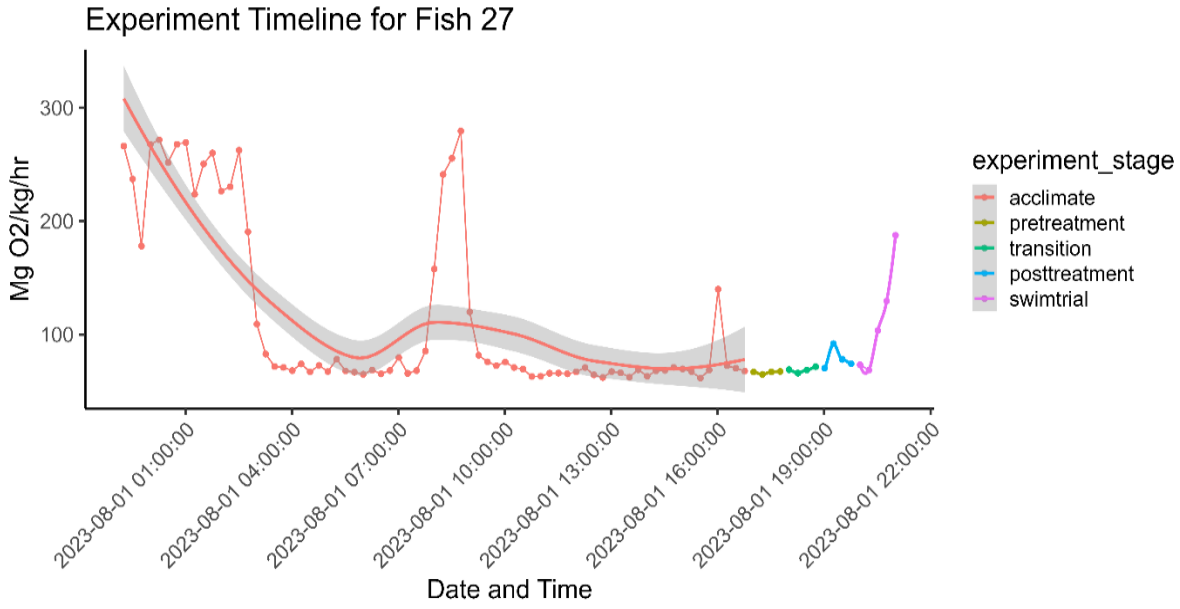


Figure A16. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 27 (SWSW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 27 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

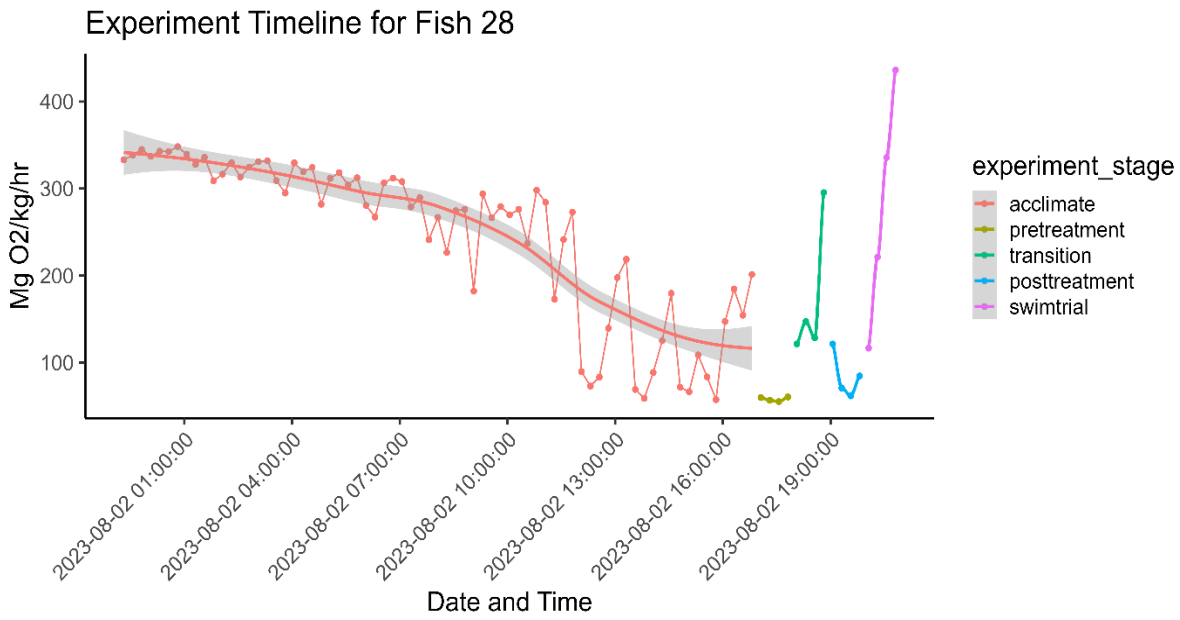


Figure A17. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 28 (SWSW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 28 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

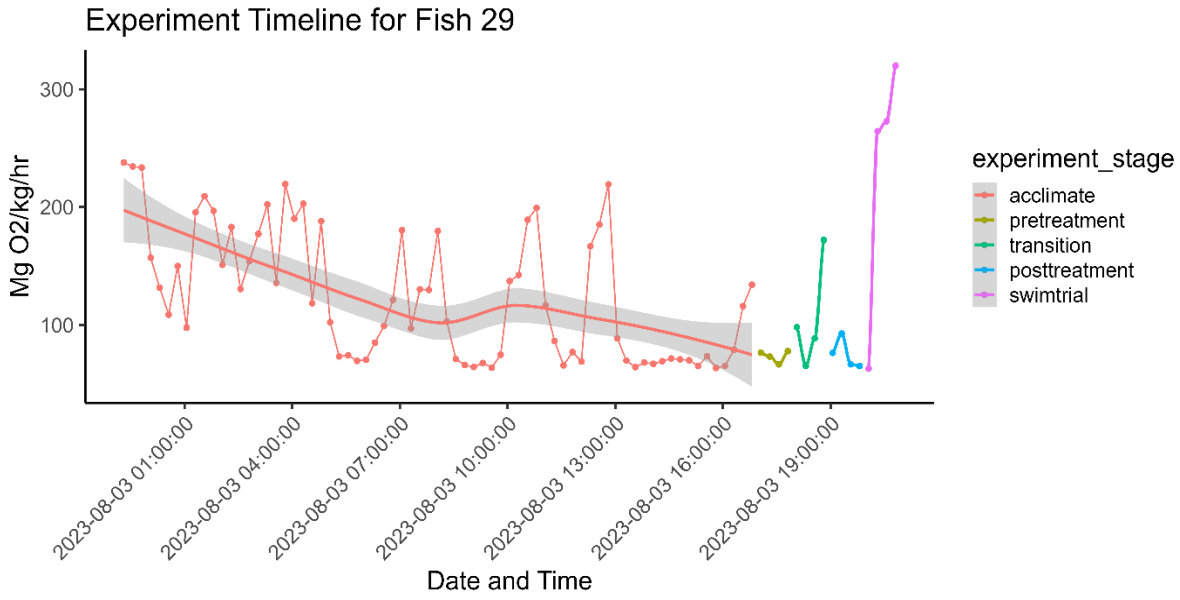


Figure A18. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 29 (SWSW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 29 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

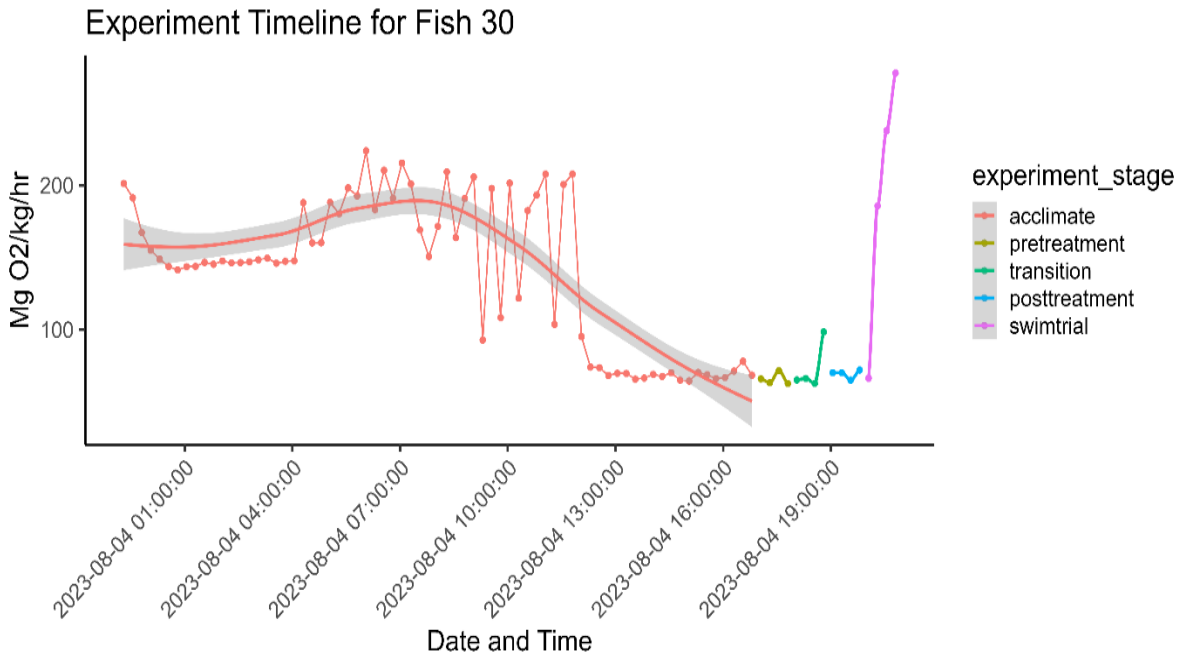


Figure A19. Line graph displaying MO_2 trends (mgO2/kg/hr) for Fish 30 (SWSW treatment group) throughout the entire experimental timeline. Experimental stages are colour-coded and MO_2 trends for Fish 30 throughout the acclimation, pre-treatment, transition, post-treatment and swim trial stages are observed from the left to the right of the graph.

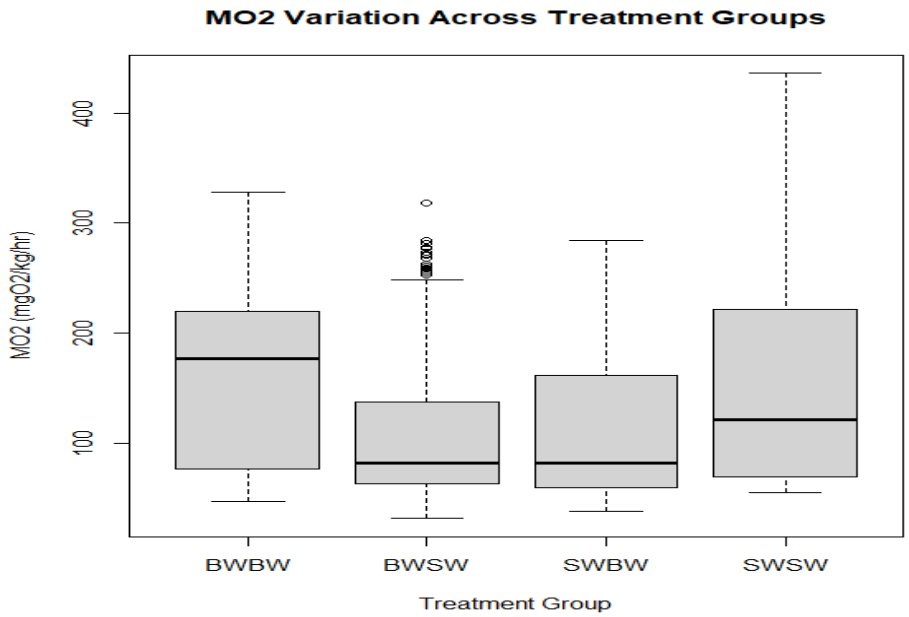


Figure A20. Variation in post-treatment MO_2 (mgO2/kg/hr). This plot displays the real, observed, non-standardised, raw values.

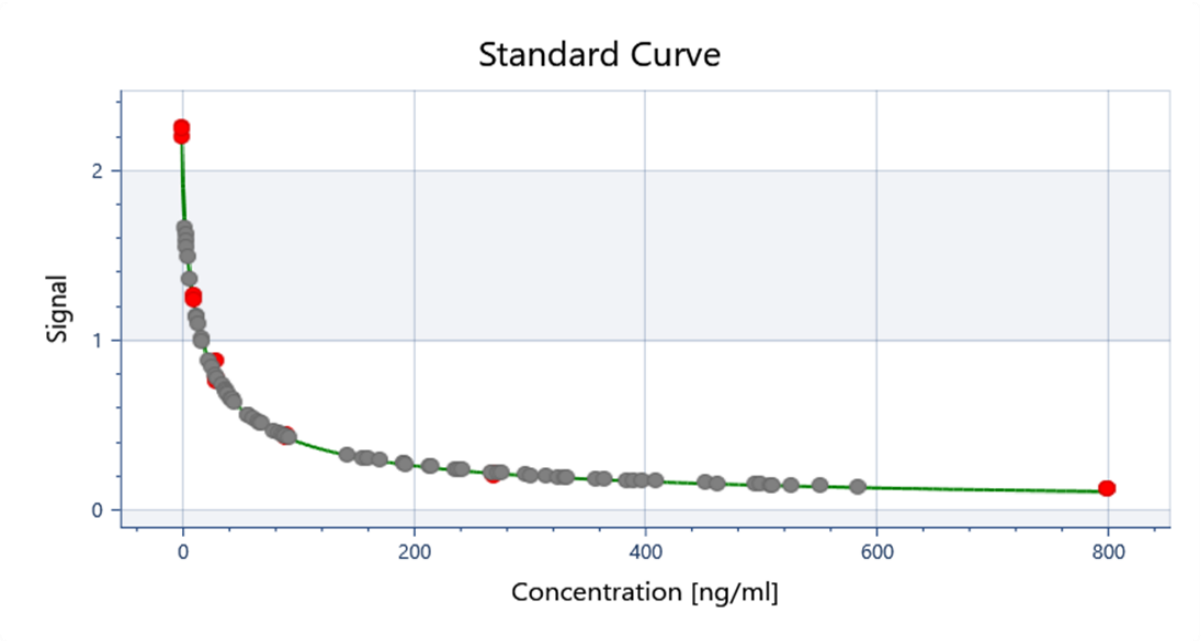


Figure A21. Cortisol ELISA Graph – Plate 1 (samples 1-35), showing standards, calibrators, controls, and samples in duplicates in ng/ml. The obtained signal of the standards (y-axis, linear) was plotted against their concentration (x-axis, logarithmic). A 4 Parameter Logistics model (4PL) was used when graphing to provide a good fit.

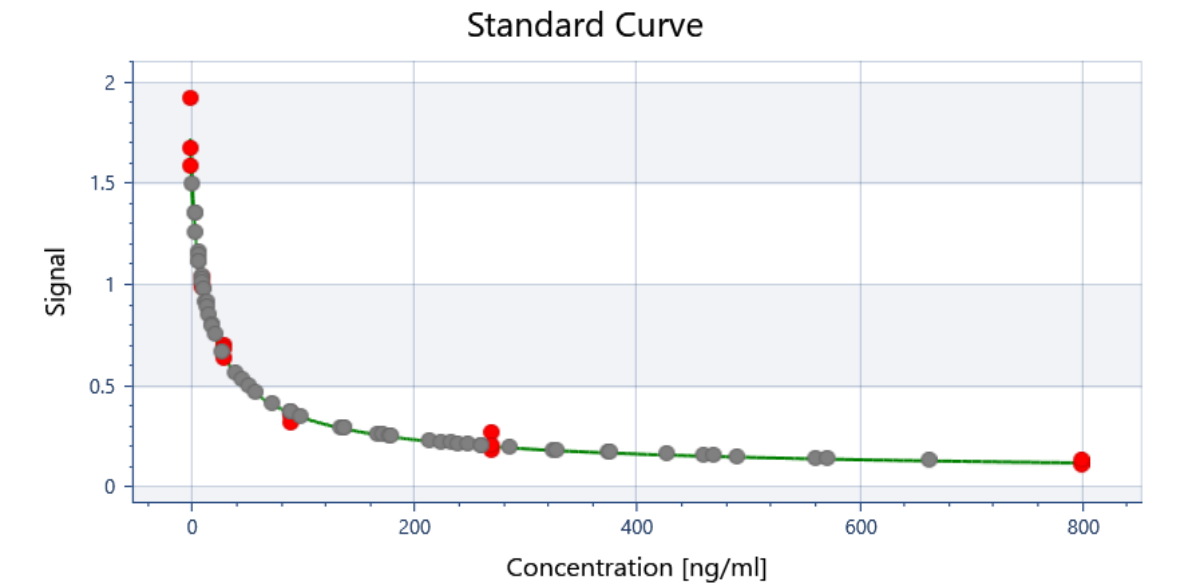
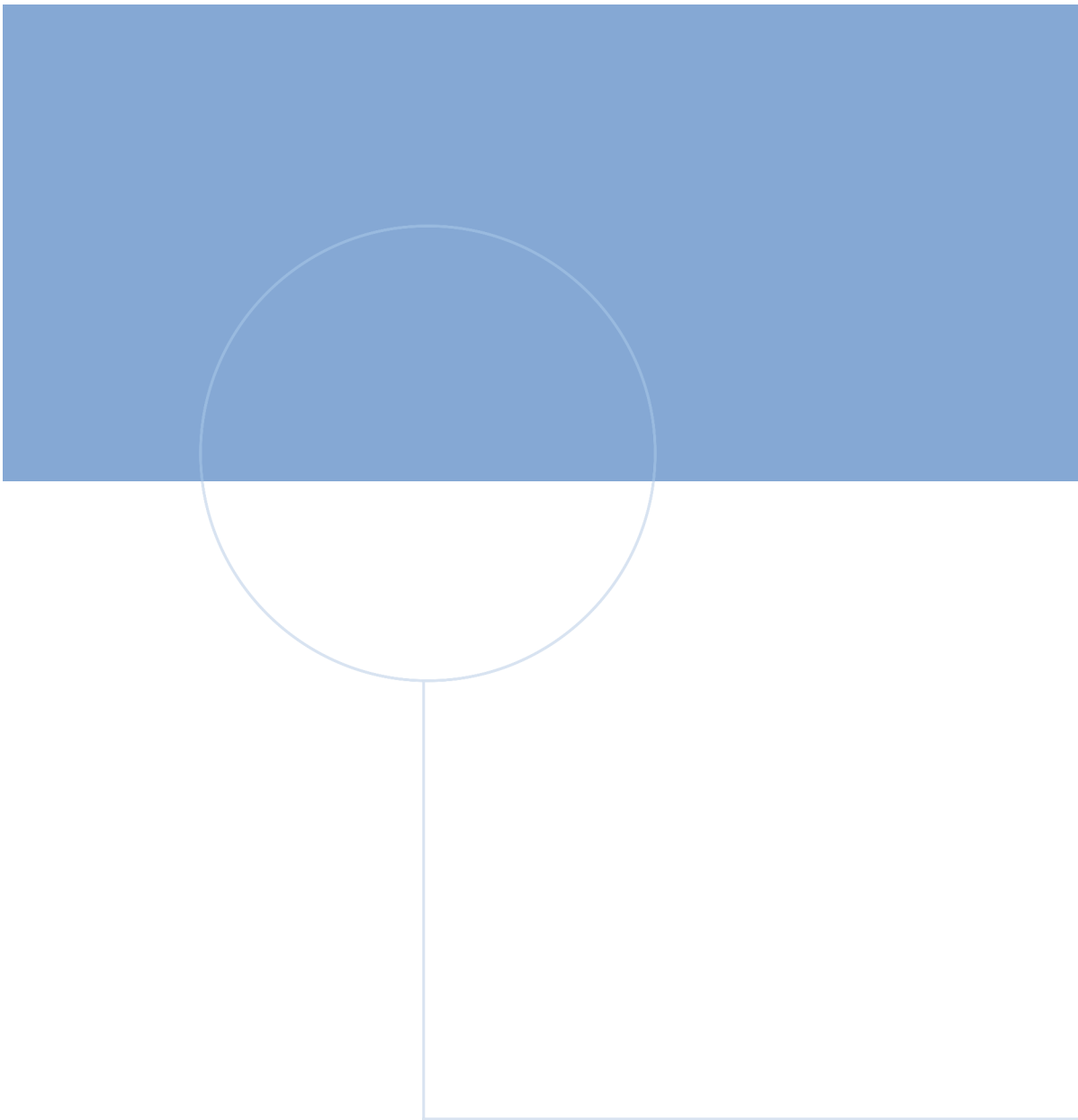


Figure A22. Cortisol ELISA Graph – Plate 2 (samples 36-60, 21, 23), showing standards, calibrators, controls, and samples in duplicates in ng/ml. The obtained signal of the standards (y-axis, linear) was plotted against their concentration (x-axis, logarithmic). A 4 Parameter Logistics model (4PL) was used when graphing to provide a good fit.



NTNU

Norwegian University of
Science and Technology