PhD thesis
Heiðrikur Bergsson

Optimizing fish growth and animal welfare in aquaculture sea cages by profiling oxygen and water current

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Cover photo: APB5 automatic profiling buoy in an aquaculture sea cage. Photo by Heiðrikur Bergsson
Acknowledgments

During my Ph.D. I have received a great deal of support and assistance from both colleges, friends, and family.

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Heiðrikur Bergsson, 01-04-2021
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Preface

After finishing my M.Sc. degree in 2014, I was employed by the Danish Ministry of Food, Agriculture and Fisheries as a project manager working with Remote Electronic Monitoring (REM) and fisheries compliances. Even though the work allowed some interesting research, I was determined to return to the scientific field of fish physiology. Therefore, in 2015, during the summer holiday, I attended the five-week summer course *Fish swimming* at the Friday Harbor Laboratories, University of Washington, USA. During the stay, I became even more convinced that it was fish physiology I preferred to work with and began researching possibilities of doing a Ph.D. On arriving back in Denmark, my M.Sc. supervisor, John Fleng Steffensen, proposed that we apply for a project regarding optimization of Danish aquaculture from the Green Growth and Development program (GUDP). The project materialized with the help of seven other participants (2 universities, 6 companies), and in 2016 the grant for the GUDP FITFISH project was approved. In April 2017, I started my Ph.D. candidate position on the project at the Marine biological section in Elsinore.

To complete a Ph.D. at the University of Copenhagen, the student must pass course credits accumulating to approx. 30 ECTS (Table 1). Furthermore, the student is required to have a change of scientific environment. In 2019, I moved to the Faroe Islands on a three-month research stay at the aquaculture company Luna A/S. The main focus of the stay was to study swimming energetics and smoltification in Atlantic salmon (*Salmo salar*). As for the present dissertation, the stay has not yet yielded a manuscript.

Teaching is also a requirement to obtain a Ph.D. which I completed as a TA on the courses Marine Biology (M.Sc. program) in 2017-2019 and Fish Biology (M.Sc. program) 2018/2019 and 2019/2020, as well as co-supervised several of the fish physiology groups master students.

Additionally, from 2018-2019 I was the Ph.D.-employee group representative on the section council at the Marine Biological Section.

The present dissertation contains three manuscripts, one of which (MS1) has been submitted to the Elsevier journal, Aquaculture. During my Ph.D., multiple papers were published from my M.Sc. project and my work for the Danish government (Table 2). These papers are not included in the evaluation of this dissertation.
Table 1. Competed courses and meetings

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Table 2. Scientific publications during my Ph.D. that are not included in the dissertation.

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Included manuscripts

MS1 (Submitted to Aquaculture):
Postprandial somnolence: Does feeding affect swimming speed preferenda in Rainbow trout (Oncorhynchus mykiss)
Heiðrikur Bergsson, Steffan Kalsø, Morten Bo Søndergaard Svendsen, John Fleng Steffensen

MS2 (Manuscript):
Model of oxygen conditions within aquaculture sea cages
Heiðrikur Bergsson, Morten Bo Søndergaard Svendsen, John Fleng Steffensen

MS3 (Manuscript):
Aquaculture fish farming in an exposed area: Is oxygen a limiting parameter
Heiðrikur Bergsson, Morten Bo Søndergaard Svendsen, John Fleng Steffensen
Resumé of thesis work

In Danish marine aquaculture, production is limited by discharge, based on the amount of food administered. Additionally, feed not consumed by the fish, due to suboptimal conditions within sea cages, increases the impact on the surrounding environment and results in a financial loss for the fish farmer. If suboptimal environmental conditions, such as hypoxia, can be predicted before they occur, feed waste can be reduced, with the benefits of increasing production or decrease the environmental nutrient load.

The aim of this Ph.D. thesis was to create a model that can predict oxygen conditions within a sea cage based on fish physiology and the physical/chemical parameters that affect oxygen consumption of the fish and oxygen conditions within a sea cage. The model was created and tested using a three-step process that involved laboratory work, field measurement, and computer modeling. The laboratory work consisted of determining different aspects of fish physiology such as swimming energetics, swimming preferenda, and the oxygen demand due to digestion, all used in the model. The fieldwork was performed on an aquaculture site and consisted of measurements of chemical and physical parameters inside a sea cage during the farming seasons. The acquired parameters were also used in the model to get environmental limits of what can be expected in a sea cage. Finally, by combining field measurements with fish physiology determinations, a model was created that predicts oxygen conditions within an aquaculture sea cage. The measured physical and chemical parameters from within the sea cage were compared with the open and free marine data from the Copernicus Marine Service to estimate the suitability of modeled data on a local scale as an alternative to field measurements. With minor adjustments, the modeled data could be used for further analysis. In this case, the benefits of using modeled data are that Copernicus provides a six-day forecast of the parameters, and by combining forecast data and the oxygen prediction model, a six-day forecast of predicted oxygen condition within the sea cage is achieved.

The applicability of oxygen condition predictions inside aquaculture sea cages is vast since they can inform the fish farmer of potential decreases in oxygen conditions. Using the predictions, the farmers can plan feeding durations and quantity, especially if oxygen is predicted to decrease below the threshold for suboptimal digestion. The model can also be used as a tool for planning the location and maximum reasonable size of aquaculture cages. With an optimized feeding plan, the fish farmer can reduce feed waste, reduce impact on the local surrounding environment, decrease financial loss, and improve general fish welfare. Additionally, with decreased discharge, production can be increased.
I danske havbrug er produktion begrænset af næringssaltsudledning, som er beregnet ud fra mængden af foder der gives. Derudover påvirker foder, der ikke fordøjes eller spises af fisken på grund af suboptimale forhold i havbure, det omgivende miljø negativt og resulterer i et økonomisk tab for fiskeopdrætteren. Hvis suboptimale forhold, såsom hypoxi, i havbure kan forudsiges, kan foderspild reduceres, og give mulighed for en øget produktion og nedsat miljøbelastning.

Målet med denne ph.d.-afhandling var at lave en model, der kan forudsige iltkoncentrationer i et havbure baseret på fiskens fysiologi og de fysiske og kemiske parametre, der påvirker iltforbruget i fisk som samt iltkoncentrationen i havbure.


Med en optimeret foderingsplan kan fiskeopdrætteren reducere foderspild, mindske indvirkningen på det lokale havmiljø, mindske økonomiske tab samt forbedre den generelle fiskevelfærd. Endvidere, da udledningen er reduceret, kan produktionen øges.
Introduction

Jacques Cousteau once stated, “We must plant the sea and herd its animals using the sea as farmers instead of hunters. That is what civilization is all about – farming replacing hunting”. This statement has never been more accurate than today, with the demand for protein increasing with the global human population's continuous increase. Predictions of population growth are estimated to increase from 7.8 billion people today to 9.7 billion by 2050 (United Nations, World population prospects, 2019). To feed the growing population, food production must follow, and one of the primary protein resources will be from aquatic organisms. Since the 1990’s the total tonnage of captured fish has remained somewhat unchanged (Fig. 1). The reason is mainly due to the majority of fish stocks being maximally utilized. During this period, an alarming trend has occurred, with the percentage of underfished stocks, while the percentage of overfished stocks increases (FAO, 2020). If the trend continues, a collapse of multiple fisheries is inevitable. The other primary source of aquatic organism protein is aquaculture, which since the 1980s has increased production by a factor of 5.5 (Fig. 1).

![Figure 1](image)

Figure 1 Historical data of capture and aquaculture production. Capture production is based on landings, and total aquaculture production is based on aquatic organisms, including fish, molluscs, crustaceans, and aquatic plants (source: FAO).

The steep incline in production has made aquaculture the fastest growing food production industry in the World, and in 2014 the total aquaculture production increased above total catches. If the trends continue, the total production of cultured finfish will exceed catches in the near future. As of 2018, finfish aquaculture production constituted 51.2% of all aquaculture production, of which 44.6% were from fresh and brackish water production, mainly of different Carp species and Nile tilapia (*Oreochromis niloticus*). The residing 6.6% were from marine aquaculture, with the Atlantic salmon (*Salmo salar*) as the predominant species. Whereas Asia and especially China are the primary producers of freshwater finfish, marine finfish are primarily produced in Norway and Chile, with relatively large productions in Canada, UK, and the Faroe Islands (Iversen et al., 2020).
Danish aquaculture production mainly consists of finfish, but Blue mussel (*Mytilus edulis*) production has increased substantially during recent years (Fig. 2). The Rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) is still the predominant species, especially in freshwater production.

**Figure 2** Aquaculture production in Denmark. (source: FAO and Fishery statistics – Danish Fisheries Agency)

Being native to Northeast Asia and the Westcoast of North America, the Rainbow trout were imported to Europe around the 1880s. This was due to the species’ applicability in aquaculture, and as opposed to the native trout species in Europe, they are less affected by fluctuations in oxygen concentrations, temperature, and water quality (Dahlstrøm, 2006).

According to the Danish Fisheries statistics during the last decade (2009-2019), aquaculture production of Rainbow trout in Denmark has annually increased by 880t in land and sea-based production (Fig. 2). Even though the number of land-based farms decreased from 203 to 126 during this period, they still constituted 98% of the production increase. The increase in land-based production is due to the optimization of farms, changing from simple earth ponds to more advanced systems. Among the optimizations made are the implementations of recirculating aquaculture systems (RAS), which utilize treatment processes to remove waste from the water. The production is limited by governmental regulations on the amount of discharge allowed calculated from the administered feed (Skov et al., 2020); if waste is reduced, production can be increased.

These advances are not possible in sea-based aquaculture since the cages are open to the environment. Alternative solutions to increase sea-based production is to move farms offshore, where the increased nutrient load will not affect the local environment since it is diluted into the open water masses. Such a solution will be accompanied by multiple new challenges regarding the fish’s susceptibility to a harsher environment and the equipment’s durability (Hvas et al., 2020). For the production to increase at present aquaculture sites, nutrient discharge per kg produced fish will have to be reduced. One approach to reducing discharge from farms is by controlling the
timing of feeding and the amount of feed administered during any given situation. Fish farmers typically feed based on past experience and with the use of feeding charts based on temperature, species, type of feed, size of fish, and more to estimate the amount that should be administered.

Even though oxygen concentration/saturation thresholds for when feed is suboptimally utilized in Rainbow trout and Atlantic salmon are known at different temperatures (Pedersen, 1987; Remen et al., 2016), these values are not included in existing feeding charts. This is likely due to oxygen being a rapidly changing parameter, i.e., substantial fluctuations in oxygen concentrations can occur throughout the day and can be different from cage to cage. Therefore, to use the thresholds would require oxygen sensors inside every cage, continually measuring oxygen concentrations. Although preferable, this solution might not be practical since sensors have to be checked and cleaned regularly, and due to the harsh environment, they will likely also have to be replaced with regular intervals, causing an extra financial burden on the farmer. Another approach is to predict when oxygen concentrations/saturations will decrease below the thresholds and only measure during these periods to determine if predictions are correct. Using predictions, oxygen (and temperature) can essentially be included as a parameter in feeding charts. At present, there are no methods of predicting oxygen concentrations/saturation in aquaculture sea cages.

Therefore, the aim of the thesis is to create a model that predicts oxygen concentrations/saturations based on fish physiology and the chemical/physical parameters within the cage. In the following, the model will be referred to as the \textbf{O}$_2$ \textbf{a}quaculture \textbf{m}odel. Furthermore, the thesis determines if the free and open marine data from the EU Copernicus Marine Service is reliable on a local scale as an alternative to real-time field measurements. The Copernicus Marine Service provides historical temperature, salinity, current velocity, and flow direction data and a six-day forecast of these parameters. By applying the modeled Copernicus predictions to the O$_2$ aquaculture model and combining it with knowledge of the swimming energetics and swimming speed of the cultured fish as well as the cage dimensions and fish density, predictions of oxygen concentrations/saturations for up to six days can be calculated. These predictions are highly applicable in aquaculture settings since they allow farmers to pause feeding in advance, thereby decreasing feed waste and discharge into the surrounding environment and increasing relative fish growth. Finally, by pausing feeding, fish are more resilient to handling hypoxic events as their metabolism is not limited by increased oxygen consumption from digestion, thereby increasing animal welfare.
Theory

In order to create and test the $O_2$ aquaculture model, several important parameters had to be acquired. This section covers the theory behind the different fish physiological parameters used in the model.

Energetics in normoxia and hypoxia

When determining metabolic rates in fish, the most common approach is to measure the oxygen consumption ($\dot{M}O_2$) of the animal as a proxy. Although $\dot{M}O_2$ might not directly reflect the metabolic rate (Nelson, 2016), the precision and consistency of oxygen measurements make $\dot{M}O_2$ well suited for determination of effects of the studied parameters.

The cost of living, also referred to as the Standard Metabolic Rate (SMR) (Beamish, 1964; Beamish and Mookherjii, 1964), is often determined as the baseline $\dot{M}O_2$ for non-digesting fish at rest (Denis Chabot et al., 2016). Temperature has a profound effect on SMR in fishes, and with an increase of 10°C, a factorial increase in $\dot{M}O_2$ will range from 1.62 to 2.64 (Christensen et al., 2020; Clarke and Johnston, 1999; Killen et al., 2016; Schurmann and Steffensen, 1997; Tirsgaard et al., 2015). This factorial increase is referred to as the $Q_{10}$.

The effects of digesting food or postprandial effects will also cause an increase in $\dot{M}O_2$. This increase is referred to as Specific Dynamic Action (SDA) (Chabot et al., 2016; Jobling and Davies, 1980). Postprandial $\dot{M}O_2$ will initially increase until a peak is reached and gradually decrease back to SMR. The duration and magnitude of SDA depend mainly on temperature, feed size, and composition, as well as oxygen conditions (Frisk et al., 2013; Jordan and Steffensen, 2007; Tirsgaard et al., 2015). The latter two are limited by the digestive system of each species, being the bulk size of the meal or the composition of the feed, while the temperature affects the entire fish. The magnitude of the increase in $\dot{M}O_2$ can be given as a percentage of SMR and has been reported to range from 31-265% of SMR depending on species and temperature (Alsop and Wood, 1997; D. Chabot et al., 2016; Eliason et al., 2007; Eliason and Farrell, 2014; Jordan and Steffensen, 2007; Pang et al., 2011; Tirsgaard et al., 2015).

Swimming energetics refers to the energy used to propel the fish at a given swimming speed. As swimming speed increases, $\dot{M}O_2$ will increase exponentially until the fish reaches exhaustion. The speed at exhaustion is referred to as the critical swimming speed ($U_{crit}$). At this point, the Active Metabolic Rate (AMR) is determined, and everything in between SMR and MMR is referred to as the Routine Metabolic Rate (RMR) (Fry, 1971).
By dividing swimming speed with oxygen consumption, the **Cost of Transport** (COT) can be determined. COT is the amount of oxygen used at any given swimming speed per Body length (Bl\(^{-1}\)) swum. The swimming speed at the minimum COT, being the point at which the least amount of energy is used Bl\(^{-1}\), is referred to as the optimal swimming speed (U\(_{\text{opt}}\)). Therefore, from an energetic point of view, fish swimming at U\(_{\text{opt}}\) will be the most economical if required to swim from point A to B. It will, however, be cheaper not to swim at all. What eventually dictates the swimming speed of the fish is a combination of the energetics and the behavior of the fish itself. The speed at which the fish voluntarily choose to swim is referred to as the **Preferred swimming speed** (U\(_{\text{pref}}\)). In a study by Tudorache et al. (2011), the U\(_{\text{opt}}\) was found to equal U\(_{\text{pref}}\) in unfed Brook charr (Salvelinus fontinalis). During normal operations in aquaculture farms, fish are fed and will therefore have an increased \(\dot{\text{M}}\text{O}_2\) caused by SDA (D. Chabot et al., 2016; Jobling and Davies, 1980; Jordan and Steffensen, 2007; Tirsgaard et al., 2015). How SDA affects swimming preferenda in Rainbow trout was tested in the present thesis (Thesis-MS1).

Several difficulties arise when measuring \(\dot{\text{M}}\text{O}_2\) in swimming fish during SDA since the magnitude of SDA on \(\dot{\text{M}}\text{O}_2\) changes over time (D. Chabot et al., 2016; Jobling and Davies, 1980; Jordan and Steffensen, 2007; Tirsgaard et al., 2015). This is especially apparent when experiments run for several hours. Studies that have measured swimming energetics during SDA (Alsop and Wood, 1997; Dupont-Prinet et al., 2009; Pang et al., 2010; Thorarensen and Farrell, 2006) show that the increase in \(\dot{\text{M}}\text{O}_2\) caused by SDA persist throughout the swim protocol, even though the intestinal blood flow is progressively reduced with increased swimming speed (Dupont-Prinet et al., 2009; Thorarensen and Farrell, 2006). This suggests that SDA can be downregulated when fish are forced to swim to compensate for the swimming muscles' increased energy demand. Since the increase in \(\dot{\text{M}}\text{O}_2\) caused by SDA or a combination of SDA and increased swimming speed will persist during increasing swimming speeds, and as the Active Metabolic Rate (AMR) is unchanged, the **Critical Swimming Speed** (U\(_{\text{crit}}\)) is reduced.

All Bony fishes regulate physiologically to maintain \(\dot{\text{M}}\text{O}_2\) during decreases in ambient oxygen (hypoxia) at least until ambient oxygen tensions decreases below their critical oxygen tension (pO\(_2\)\(_{\text{crit}}\)) (Grieshaber et al., 1988; Svendsen et al., 2019). The **Aerobic Scope** (AS), equal to the difference between SMR and AMR, is reduced with progressive hypoxia (Claireaux et al., 2000; Farrell and Richards, 2009; Fry, 1971; Jordan and Steffensen, 2007). The reduction is caused by a decrease in AMR in decreasing oxygen tensions, resulting in a decrease in maximum swimming speed (Domenici et al., 2013). In the present thesis, the minimum required oxygen tension (pO\(_2\)) for fish swimming at U\(_{\text{opt}}\) is determined (Thesis-MS2).
Materials and Methods

Experimental species
Rainbow trout (Fig. 3) being the main finfish species in Danish aquaculture as well as the species produced by the aquaculture companies participating in the GUDP FITFISH project, it was the obvious choice to use as the experimental species in the present thesis. The history of farming Rainbow trout goes back hundreds of years, and the species is today the most widely farmed species of trout. As for 2018, the Rainbow trout had the 15th highest yield of the world’s finfish aquaculture production (FAO, 2020). The high production is also reflected in the diversity at which Rainbow trout are farmed, ranging from small-sized aquaponics to large-scale sea cages.
In addition to being well suited for aquaculture production, the Rainbow trout have been used extensively in multiple fields of research such as carcinogenesis, toxicology, comparative immunology, disease ecology, nutrition, and physiology (Thorgaard et al., 2002). In relation to use in fish physiology, the species robustness to tolerate wide ranges of salinity, temperature, and fluctuations in water quality (Dahlstrøm, 2006; Hardy, 2002) makes them ideal for experiments.
In the present thesis, Rainbow trout were well suited for swimming experiments, requiring minor encouragement to start swimming in the swim tunnels (Thesis-MS1, MS2).

![Figure 3](image-url) The Rainbow trout (*Oncorhynchus mykiss*). Photo: Heiðrikur Bergsson.
Respirometry

All determinations of oxygen consumption ($\dot{M}O_2$) during swimming in normoxia were carried out using a 31.5 L and a 90 L Steffensen-type swim tunnel, using intermittent-flow respirometry (Bushnell et al., 1984; Steffensen, 1989; Steffensen et al., 1984; Svendsen et al., 2016) (Thesis-MS1, MS2). The determinations were further used to calculate the COT and $U_{opt}$. Using the 90L swim tunnel, $\dot{M}O_2$ measurements during hypoxia were determined using a combination of intermittent-flow respirometry and closed respirometry. The closed respirometry was chosen to simulate an aquaculture situation. The determinations were used to calculate the minimum partial pressure of oxygen needed to sustain $U_{opt}$ (Thesis-MS2).

To simulate an aquaculture cage in the laboratory, an open circular tank respirometer was used. Swimming was encouraged by creating a current within the tank using a 175 L/min submersible pump. Moreover, the fish used varied in size, as is common in aquaculture sea cages. The setup was used to determine the increase in $\dot{M}O_2$ caused by Standard Dynamic Action with and without currents (Thesis-MS2).

Preferred swimming speed

The method for determining preferred swimming speed ($U_{pref}$) is based on fish behavior and how the fish reacts to the stimuli surrounding the experimental setup. In the present thesis, $U_{pref}$ was determined in a novel setup consisting of a circular raceway with a range of water velocities and a video tracking system. Using video tracking, the current at the position which the fish primarily chose to position itself was used to calculate the preferred swimming speed (Thesis-MS1).

The benefit of using a raceway with a range of water velocities to determine swimming preferenda as opposed to a swim tunnel is that fish will not experience a constraint due to the swim section's size, as in a swim tunnel. In a swim tunnel, the fish is forced to swim at a preset flow and will not be able to select a preferred flow regime. In other words, their exploratory behavior gets limited (Tudorache et al., 2013).

Tailbeat frequency

In the present thesis, the tail beat frequency (TBF) at different swimming speeds was determined for Rainbow trout and found to be equal in fish swimming in a swim tunnel respirometer and a raceway (Thesis-MS1). Using this information, the initial idea was to analyze the captured video footage from within the sea cage to estimate tail beat frequency in the cage. By applying the TBF data to the “TBF vs. Swimming speed” equation (Thesis-MS1), an estimate of swimming speed, and hence $M_O2$ within the cage, could have been determined. Furthermore, each video from the sea cage had a timestamp, which could be used to find the current velocity at the time of the video.
and combine the different swimming speeds with current velocities. Throughout the 2020 farming season, 1964 videos were recorded, but due to limited power supply and internet bandwidth, video recordings were limited to 15 seconds sequences. The video analysis revealed that the short sequences (15 sec.) for each video were not enough to get stable counts of tail beats. The fact that fish are free to swim around in the cage meant that the duration a fish spent in frame was short at best, even with video being recorded with 25 frames per second. The experiment was therefore deemed unsuccessful. The video recordings did not go to waste since swimming trends revealed that the fish maintained a relatively high swimming speed even at low current velocities. This supports the idea that fish primarily swim at their optimal or preferred swimming speed (Davison and Herbert, 2013; McKenzie et al., 2021).

**Field measurements**

All field measurements (temperature, salinity, oxygen, current velocity) were acquired at an aquaculture site of the island of Musholm, which is located in the Great belt, in Denmark (Storebælt: DK) (55°28'47.0"N 11°03'23.5"E) during the farming seasons 2018, 2019, and 2020. The only season where all types of measurements were acquired was the 2020 season (Fig. 4). For each season, measurement equipment was added, modified, or moved, using the knowledge gained from the previous season. The changes are illustrated in Fig. 4 with the different data acquisition types and the duration they acquired data.

**The sea cage**

In the present thesis, all field measurements were acquired from the same sea cage (Thesis-MS3). The cage had a circumference of 160m and a depth of approximately 8 meters. The location of the cage was on the southwest-side of the farm (Fig. 5).
Figure 4 Durations for each type of measurement during the 2018-2020 farming seasons. The only season with a close-to-complete dataset was the 2020 season.

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<td>Red</td>
<td>Red</td>
<td>Red</td>
</tr>
</tbody>
</table>

Figure 5 The aquaculture farm site with the cage used in the present study (black square). The image is a modification of a snapshot retrieved from drone footage of the farm. The measuring buoy can be seen in the center of the cage (yellow) (https://youtu.be/5xiGE5VYjWE, 29-03-2021, content from user: Musholm Havbrug).
Farming season 2018

During the first farming season, the only measurement equipment deployed was an autonomous measuring buoy (APB5, SAIV A/S, Bergen, Norway), fixed in the middle of the sea cage. The buoy profiled the water column using a CTD (SD208, SAIV A/S, Bergen, Norway) with a built-in oxygen probe (RINKO III, JFE Advantech Co. Ltd., Nishinomiya, Hyogo, Japan). The CTD inside the buoy was winched down through the water column, acquiring temperature, salinity, and oxygen measurements for each of the top 5 meters. The cage’s total depth was 7-8 meters, but since water currents can deform the netting on the cage and move the CTD towards the side netting, a conservative max depth of 5 meters was chosen for the automatic profiling. This ensured that the CTD and the netting would never come into contact, which could have resulted in the net being torn, causing fish to escape the cage.

Since the CTD was autonomous and only connected to the buoy via a nylon rope, no data was transmitted during profiling. When the CTD returned to the surface, acquired data was transferred via radio signal to the buoy and transmitted via the internet to a webserver, resulting in close to real-time data. Data from the webserver was continually analyzed, plotted, and uploaded to the project’s webpage. For direct implementation of measurements, the farm managers and researchers received an email warning whenever oxygen decreased below 70% air saturation.

During the first season, the current velocity was measured using an ADCP current profiler (Aquadopp Z-Cell 600 kHz, Nortek, Rud, Norway) mounted on the buoy inside the cage. Since the profiler utilizes the doppler effect to measure current velocities, the density of fish that interfered with the sound beam caused disturbances in measurements. This effect is mainly caused by the fish’s swim bladder due to the difference in speed of sound that travels through gas and surrounding tissues and seawater.

The disturbances in measurements were not realized until the end of the season, and therefore, ADCP measurements from the first farming season were discarded. In general, the 2018 farming season, although not completely successful, gave some insights into best practices for achieving the wanted measurements.
Farming season 2019

Additional oxygen loggers (MiniDOT, Precision Measurement Engineering, Inc., California, USA) were acquired during the second farming season to compare oxygen concentrations inside and outside of the cage. The loggers were mounted at a depth of 4 meters, north and south of the cage.

After approximately one-third of the season had elapsed, the buoy malfunctioned and had to be brought to shore for repairs. Not all repairs could be mended at site, and therefore some internal components were shipped to the supplier in Norway for repairs. This resulted in no measurements being acquired from within the cage for several weeks. After the buoy was fixed and returned to the cage, only two weeks of measurements could be acquired before it again malfunctioned. The second malfunction was an easy fix since it only required replacing batteries in the CTD. The CTD is turned on and off by the buoy, and since the buoy malfunctioned during a profile, the CTD had been logging continually, almost draining the batteries.

While changing the CTD batteries, the MiniDOT oxygen loggers were retrieved and found to be covered with biofouling. This affected the oxygen measurements, which had decreased to close to zero approximately after one-third of the season had elapsed. After cleaning the loggers, they were again mounted on the same positions and left for the rest of the season.

To acquire sonar and video data from the cage, a custom system was built that was powered by the feeding barge. The system consisted of a watertight housing (Blue Robotics, California, USA) with an IP camera and Blue Robotics sonar, which was mounted in the middle of the cage. Sonar and video data were sent via cable to a control box mounted on the side of the cage. Power was supplied via a cable between the control box and the feeding barge. This system never acquired any data, as the cable between the control-box and feeding barge broke on the first day of the deployment. This deemed the system unusable.

All in all, the second farming season was misfortunate but taught us plenty of valuable lessons.
Farming season 2020

The last farming season of the project turned out to be a great success. Based on what we had learned in the previous seasons, errors and malfunctions were close to eliminated. The sonar/video setup was changed into one compact unit and mounted on the side of the buoy (Fig. 6). It was supplied with power from the buoy, and utilizing the buoy’s internet connection, sonar and video data were uploaded at regular intervals.

The oxygen loggers were fitted with copper plating surrounding the sensor to reduce biofouling. This was effective on the south-mounted sensor, but the north-mounted was again covered by biofouling, which resulted in limited data.

The knowledge gained in the first season resulted in the ADCP profiler being mounted on the feeding barge next to the cage. To autonomize data acquisition, the ADCP was connected to a Raspberry Pi 3 (RPi3, Raspberry Pi Foundation, Cambridge, UK) that analyzed and uploaded data every 15 min. The RPi3 was powered by a 12 volt, 20 AHr lithium battery pack, which was charged by a solar panel.

All data from the farming season 2020 are used in the third manuscript (Thesis-MS3) that tests the O₂ Aquaculture model.

---

**Figure 6** Sonar and camera setup in watertight housing based around a Raspberry Pi 3.
**O₂ Aquaculture model online user interface**

To make the O₂ aquaculture model as accessible as possible, a webapp was created containing all the functionalities mentioned in the present thesis (Thesis-MS2).

The webapp (Fig. 7) was created using a Flask webapp framework combined with HTML, CSS, JavaScript, and Python (Python Software Foundation) and can be accessed at http://o2aquaculturemodel.pythonanywhere.com.

The parameters used in the model and how they are used are listed below.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Primarily used to</th>
<th>Secondly used for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Calculate oxygen solubility</td>
<td>Determining which swim energetics are used</td>
</tr>
<tr>
<td>Salinity</td>
<td>Calculate oxygen solubility</td>
<td></td>
</tr>
<tr>
<td>Flow velocity</td>
<td>Replenishment rate of oxygen</td>
<td>Determine min. swim speed</td>
</tr>
<tr>
<td>SMR</td>
<td>Calculate MO₂ during swimming</td>
<td></td>
</tr>
<tr>
<td>Exponent (Swim)</td>
<td>Calculate MO₂ during swimming</td>
<td>Calculate optimal swim speed</td>
</tr>
<tr>
<td>Ucrit</td>
<td>Determine max. MO₂</td>
<td></td>
</tr>
<tr>
<td>SDA</td>
<td>Calculate MO₂ increase</td>
<td></td>
</tr>
<tr>
<td>Cage Circ.</td>
<td>Calculate cage volume</td>
<td></td>
</tr>
<tr>
<td>Cage Depth</td>
<td>Calculate cage volume</td>
<td></td>
</tr>
<tr>
<td>Number of fish</td>
<td>Calculate fish density</td>
<td></td>
</tr>
<tr>
<td>Avg. mass of fish</td>
<td>Calculate fish density</td>
<td></td>
</tr>
<tr>
<td>Avg. length of fish</td>
<td>Calculate swim speed in Bl/s</td>
<td>Calculate swim speed in Bl/s based on flow velocity (cm/s)</td>
</tr>
</tbody>
</table>
Variables and calculations
Fields can be populated using experimental data from this dropdown menu or you can use your own custom data. Whatever you choose, you can always change the parameters and submit the new values to update the plots.

1. Environmental variables
In this section, the environmental variables are added to the model.

<table>
<thead>
<tr>
<th>Changeable:</th>
<th>Temperature</th>
<th>Salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calculated:</th>
<th>Dissolved Oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;70% 70% - 40% 40% - 20% 20% - 0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Changeable:</th>
<th>Oxygen concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.39 mg O2/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calculated:</th>
<th>Oxygen threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.8 mg O2/mL</td>
</tr>
</tbody>
</table>

| Updates: | Plot B, C, D |

2. Swimming energetics
In this section the swimming energetics and critical swimming speed are added to calculate the optimal swimming speed in fed and unfed fish. Use the SDA field to add post-prandial M02. Results are plotted on the right.

<table>
<thead>
<tr>
<th>Changeable:</th>
<th>SWR</th>
<th>mg O2/kg*h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Changeable:</th>
<th>SDA</th>
<th>% of SWR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>41</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calculated:</th>
<th>Upt - fed</th>
<th>0.9</th>
<th>IL/h</th>
</tr>
</thead>
</table>

| Updates: | Plot A, B, C, D |

3. Cage dimensions
Here the cage dimensions are added in order to calculate the cage volume.

<table>
<thead>
<tr>
<th>Changeable:</th>
<th>Circumference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>160 m</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calculated:</th>
<th>Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50.9 m</td>
</tr>
</tbody>
</table>

| Updates: | Plot A, B, C, D |

4. Fish density
In this section the total number of fish in the cage as well as the avg mass and length are added, to calculate the total mass and density

<table>
<thead>
<tr>
<th>Changeable:</th>
<th>Number of fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>150 x 1000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Changeable:</th>
<th>Avg. Length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>42 cm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calculated:</th>
<th>Total mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3750 kg</td>
</tr>
</tbody>
</table>

| Updates: | Plot A, B, C, D |

5. Swimming and current information
In this section the swimming speed and current velocity are added for plot D.

<table>
<thead>
<tr>
<th>Changeable:</th>
<th>Water flow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 cm/s</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Changeable:</th>
<th>Water flow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.9 IL/h</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calculated:</th>
<th>Swimming speed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>378 cm/s</td>
</tr>
</tbody>
</table>

| Updates: | Plot D |

Figure 7 The O2 Aquaculture models online graphical user interface
Aims and objectives

The study aimed to create and test a model that can predict oxygen concentration within aquaculture sea cages based on fish physiology and the physical/chemical parameters within the cage. This was achieved using field measurements, experimental studies, and computer modeling, described in the present dissertation's three chapters.

Chapter 1
The chapter’s objectives were to determine if the preferred swimming speed was equal to the optimal swimming speed of Rainbow trout. Furthermore, the chapter also determines if a change occurs in swimming preferenda in Rainbow trout when fish are fed ad libitum.

Chapter 2
The objectives of the chapter were to create a model that predicts oxygen concentrations within aquaculture sea cages. The model was based on fish physiology combined with the physical and chemical parameters of the cage. Determinations of swimming energetics in normoxia and hypoxia and the effects of SDA are also included in this chapter.

Chapter 3
The chapter’s objectives were to measure physical and chemical parameters within an aquaculture cage and determine if the O₂ aquaculture model (Thesis-MS2) was applicable in practice. Additionally, the measured parameters were compared to Copernicus Marine Service forecast data to determine if modeled environmental data can be used on a local scale.
Summary of the results

Manuscript 1

Title: Postprandial somnolence: Does feeding affect swimming speed preferenda in Rainbow trout (Oncorhynchus mykiss)

Aim:
- Determine if optimal swimming speed ($U_{\text{opt}}$) is equal to preferred swimming speed ($U_{\text{pref}}$) in Rainbow trout
- Determine if swimming preferenda changes when fish are fed ad libitum
- Determine if the relationship between tail beat frequency and swimming speed is different in swim tunnel experiments compared to raceway experiments

Methods: Swim tunnel respirometry, Raceway tracking of fish, Tail beat frequency

Results and Conclusions:
1. Optimal ($U_{\text{opt}}$) and preferred swimming speed ($U_{\text{pref}}$) were equal in Rainbow trout at 10°C.
2. There was no significant difference between $U_{\text{opt}}$ and $U_{\text{pref}}$ in fed and unfed Rainbow trout.
3. No significant difference was found between tail beat frequency in swim tunnel and raceway experiments when fish were swimming in the same flow velocity.
Manuscript 2

Title: Model of oxygen conditions within aquaculture sea cages

Aim:
- Determine swimming energetics at three different temperatures during normoxia and hypoxia in Rainbow trout.
- Determine optimal swimming speed ($U_{opt}$) at three different temperatures
- Determine Specific Dynamic Action (SDA) in Rainbow trout at 10℃ fed continuously
- Create a model to predict oxygen concentrations/saturations in an aquaculture sea cage based on fish physiology and chemical/physical parameters affecting the cage.

Methods: Swim tunnel respirometry, Open tank respirometry, Computer modeling

Results and Conclusions:
1. Swimming energetics in Rainbow trout was found at three different temperatures (10, 15, and 20℃), which resulted in $Q_{10}$ of 2 and with the highest aerobic scope measured at 15℃.
2. Optimal swimming speed was found to increase with temperature
3. In the present thesis, SDA for Rainbow trout was determined to be approximately 40% of SMR at 10℃. Additionally, SDA duration is prolonged when fish are forced to swim.
4. A model was created to predict oxygen concentrations/saturation within aquaculture sea cages, depending on fish density, ambient temperature and oxygen content, water flow through the cage, fish swimming speed, and fish cage diameter.
Title: Aquaculture fish farming in an exposed area: Is oxygen a limiting parameter

Aim:
- Determine ranges of physical and chemical parameters within a sea cage throughout a season.
- Determine if Copernicus Marine Service data can be used on a local scale
- Determine if oxygen is a limiting parameter in exposed aquaculture areas
- Determine swimming speed in a sea cage compared to current velocities

Methods: CTD, ADCP, Modeled data, Sonar, Camera

Results and Conclusions:

1. Diurnal fluctuations in measured parameters were apparent, especially in oxygen and current velocity
2. With minor adjustments, the Copernicus data can be used as a predictor of physical and chemical parameters within a cage.
3. Oxygen is not a limiting factor within aquaculture cages, with only 0.3% of measured oxygen concentrations below the suboptimal feed utilization threshold.
4. Although not quantified, swimming speed trends compared to currents revealed that fish swim relatively fast even in low current velocities.
5. Profile measurements visualized on heatmaps are shown in Fig. 8-10
Figure 8 Profiles from farming season 2018
Figure 9 Profiles from farming season 2019
Figure 10 Profiles from farming season 2020. Data from this season was used in manuscript 3
Concluding remarks and future perspectives

Being able to predict oxygen concentration/saturation within sea cages can help fish farmers determine when conditions in the cages are suboptimal for feed digestion. By reducing administered feed during such conditions, the fish farmer will reduce the amount of feed waste and thereby achieve a higher relative growth (feed conversion ratio). Increased fish welfare is also likely since fish will have a greater aerobic scope when not fed during hypoxic events, which will make them more resilient to the reduced ambient oxygen. Additionally, the impact on the surrounding area will be reduced, resulting in an environmentally healthier and more acceptable fish farm. In general, since discharge into the surrounding environment can be reduced, the production can be increased at the same environmental load. If nothing else, this should be an incentive to conduct future research into this area.

By making the model free of charge and available online, farmers, researchers, teachers, students, and general interest groups alike can apply their values or use the presets and get an increased knowledge on how oxygen concentration/saturation change when the energetics of the fish, as well as the physical and chemical parameters within the cage, are adjusted.

As for future perspectives, creating an application that automatically calculates the predicted oxygen concentrations based on the cultured fish's physiology in the sea cage and utilizing the Copernicus Marine Data forecast for the environmental parameters would be of great interest for fish farmers.

Finally, since the present O₂ aquaculture model is based on a model of schooling fish created by John Fleng Steffensen some time ago (+25 years), it would not be a great leap to convert the present online model into a model of fish schools. A model over oxygen conditions within fish schools could result in a greater understanding of how oxygen concentration/saturation changes through the schools.
References


MS1: Postprandial somnolence: Does feeding affect swimming speed preferenda in Rainbow trout (Oncorhynchus mykiss)

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c Copenhagen Academy for Medical Education and Simulation, Rigshospitalet, Capital Region of Denmark. DK-2100, Copenhagen, Denmark

Abstract

Intermediate swimming speeds have a positive effect on fish welfare in nature and aquaculture. During normal operations in aquaculture, fish are hardly ever fasted, and therefore, estimates for a suitable swimming speed should include measurements on fed fish. A circular raceway was used in the present study to determine the preferred swimming speed in fed and unfed fish. To compare preferred and energetically optimal swimming speed in unfed fish, a swim tunnel respirometer was used. Fasted rainbow trout showed no significant difference when swimming at preferred and optimal swimming speed (Upref fasted 1.01± 0.29 Bl s−1, Uopt fasted 0.89 ± 0.17 Bl s−1). Neither was there any significant difference in swimming speed when comparing fasted and fed Rainbow trout swimming at the preferred swimming speed (Upref fasted 1.01± 0.29 Bl s−1, Uopt fed 0.94 ± 0.15 Bl s−1). The application value of this knowledge is high in aquaculture settings since it can be used as a benchmark for flow velocity within tanks and cages.
1. Introduction

Swimming is an essential part of the daily life of fishes, with implications for ecology such as migration (Lennox et al., 2016), predator-prey interactions (Domenici, 2001; Domenici et al., 2011), and overall fitness (Castro et al., 2011). The environment affects life and thus swimming of fishes; examples include disease such as parasites (Palstra et al., 2007), but the physical and chemical environment also interacts with changing behavior and schooling (Domenici et al., 2000; Marras et al., 2015), turbidity and sediment negatively impacting physiological performance (Johansen and Jones, 2013) and lastly temperature (Johansen et al., 2015), salinity (Christensen et al., 2018), and flow (Tudorache et al., 2013), changing swimming characteristics.

Life in aquatic environments demands fish to balance environmental entities (Fry, 1958, 1947). For fishes, flow and swimming speed (Ware, 1975), salinity (Bœuf and Payan, 2001; Christensen et al., 2016; Claireaux and Lagardère, 1999; Dutil et al., 1997; McKenzie et al., 2001) and temperature (Eliason and Farrell, 2016; Fry, 1958, 1947), all possess regions of optimality for physiological performance. Presence in optimal regimes reflects itself in higher growth (Angilletta, 2004; Fry, 1947; Khan et al., 2014; Palstra et al., 2015), control of sexual maturation (Graziano et al., 2018; Palstra et al., 2010), and maximum swimming performance (Claireaux, 2006; Claireaux et al., 2000). These are essential factors for ecology, conservation, and aquaculture (McKenzie et al., 2016).

When faced with a stressor, fish inherently avoids where stress acts on them (Fry, 1958). This leads fish to locate themselves in regions where the balance of environmental entities acts with the least stress, being interpreted as preferred environments (Fry, 1947). The classic example is temperature, however, related to locomotion, spending energy on swimming, changes
physiological response to, e.g., hypoxia in the Adriatic sturgeon \((Acipenser naccarii)\) (McKenzie et al., 2007), enabling a regulatory response to hypoxia (McKenzie et al., 2007; Svendsen et al., 2019). Theoretical optimal swimming speed \((U_{opt})\), determined as the speed at least cost of transport by forced swimming in a swim tunnel respirometer and preferred swimming speed \((U_{pref})\) in an environment with differentiated flow regimes, are reported to be equal in European eel \((Anguilla anguilla)\) (Palstra et al., 2008) and Brook charr \((Salvelinus fontinalis)\) (Tudorache et al., 2011) as well as in Yellowtail kingfish \((Seriola lalandi)\) that grows optimally at this speed (Palstra et al., 2015). All examples represent situations where increased metabolic expenditure leads to improved organismal performance. Thus, quantifying swimming performance in fishes can lead to applicable information on performance measures (Brett, 1972; Fulton et al., 2013; Tudorache et al., 2013).

In the present study, we looked into the effect of ad libitum feeding on \(U_{pref}\) in Rainbow trout \((Oncorhynchus mykiss)\). It is not intuitive how increased metabolism relates to digestion, combined with swimming on an organismal level. Thus, the study is performed to clarify in which flow regimes satiated Rainbow trout thrive better. This information is valuable for basic understanding of fish ecology but especially relevant in aquaculture settings, where physiological optimal performing fish directly impacts the bottom line.

2. Material and methods

2.1 Collection and husbandry

Rainbow trout \((Oncorhynchus mykiss (Walbaum, 1792))\) with a standard length (Ls) of 27.5 ± 1.4 cm and body mass, 220.8 ± 41.6 g (average ± S.D) were obtained from a commercial fish trader (Fishlab, Viby J, Danmark). The fish were kept in a flow-through tank (approx. 600L) supplied with seawater \((O_2>95\%, 10°C, \text{Salinity 30})\) at the Marine Biological Laboratory, University of
Copenhagen, in Elsinore, Denmark. Pre-experimental feeding was maintained by the staff at Øresunds Aquarium, University of Copenhagen.

2.2 Study design

Fish were subjected to two experimental setups to determine $U_{\text{pref}}$ and $U_{\text{opt}}$. A volitional swimming test was used to determine $U_{\text{pref}}$ (Fig. 1) – see Preferred Swimming Speed, whereas $U_{\text{opt}}$ was determined by swim-tunnel respirometry – See Optimal Swimming Speed. Fish were divided into two groups: group 1 consisting of moderately fed fish (48h fasting before experimentation, to ensure no apparent Specific Dynamic Action, SDA) and group 2 consisting of fish fed ad libitum, with no fasting before commencing trials. Group 1 was used to determine $U_{\text{opt}}$ and $U_{\text{pref}}$, while group 2 was only used to determine $U_{\text{pref}}$.

2.3 Preferred swimming speed ($U_{\text{pref}}$)

The experimental setup consisted of two circular tanks, a larger tank with an inner diameter of 150cm, a water level of 9.3cm, and a smaller perforated inner tank with an outer diameter of 56 cm. The smaller tank was fixed in the center of the larger tank to reduce the area of low water speed and ensure a circular flow (Fig. 1). The space between the two tanks constituted the swimming section (width 47cm). Water flow was generated using a submersible pump (5000 l h$^{-1}$) located in the center of the smaller tank connected to a nozzle, fixed to the outer tank's wall (See Fig. 1). The water speed in the swimming section of the experimental setup was measured using a flow probe (Vane Wheel FA ZS25 Hoentzsch, Weiblingen, Germany) at three different diameters in a circular pattern at 8 points with a 45-degree angle between each measurement. Water speeds gradually decreased from 42 to 13.5 cm s$^{-1}$ from the outside of the swim section towards the center, respectively (See Fig. 1).

A video camera mounted above the experimental setup was used to record video footage of the swimming section and the swimming fish. The experiment was recorded at 25Hz the following
18-20 h period after introduction to the tank. The resulting video footage was analyzed using Id
tracker (Pérez-Escudero et al., 2014) (www.idtracker.es) for fish tracking and MATLAB 2013a to
analyze the tracked paths.

The preferred swimming speed was determined using the most frequent position obtained from a
2D histogram of the tracked coordinates and translated to swimming speed equal to modelled flow
at that position.

2.4 Optimal swimming speed ($U_{opt}$)

After the preferred swimming trial, fish from group 1 were moved to the setup used for determining
swimming energetics. These swimming trials were conducted in a 31.5 l Steffensen-type swim
tunnel, using intermittent-flow respirometry (Bushnell et al., 1984; Steffensen, 1989; M. B. S.
Svendsen et al., 2016). Oxygen saturation was measured using a fiber-optic oxygen meter (PreSens
Fibox 3, Regensburg, Germany) connected to the control software Autoresp (Loligo systems). A
video camera was mounted approximately 0.4 m above the swim-tunnel to record video footage
of the fish in the swim tunnel.

The fish were introduced to the swim tunnel at a water flow of 0.5 Bl s$^{-1}$, allowing 12-16 h,
acclimation to the new environment, and reaching a baseline oxygen consumption ($\dot{M}O_2$)
(Svendsen et al., 2019). During the acclimation period, $\dot{M}O_2$ was measured to ensure that the
baseline was reached. The $\dot{M}O_2$ determinations were completed at 11 min intervals with measuring
loops consisting of 240s flush, 60s wait periods, and measurement periods of 360s. In general, a
steep decline in $\dot{M}O_2$ was seen in the first 2-5 h after introduction to the swim tunnel, leaving
sufficient time for the baseline determination.

Following the acclimation period, oxygen consumption rate was measured with gradually
increasing swimming speeds by 0.5 Bl s$^{-1}$ increments every three measurement cycles until the fish
were fatigued. Measurements were executed at 0.5, 1.0, 1.5, 2.0 and 2.5 Bl s$^{-1}$ for each fish. All
determinations of $\dot{M}O_2$ measurements with an $r < 0.75$ were excluded from further analysis (Svendsen et al., 2016b).

$$y = a \cdot \exp(b \cdot U)$$  \hspace{1cm} \text{Eq. 1}$$

An exponential function, equation 1, was fitted to data for each fish ($y$: $\dot{M}O_2$, $x$: swim speed), and optimal swimming speed was determined by the tangent ($y=ax$) to that fit. By mathematically solving the two equations, $U_{opt}$ was determined using equation 2 discussed in Tudorache et al. (2013)

$$U_{opt} = b^{-1}$$  \hspace{1cm} \text{Eq. 2}$$

The cost of transport was determined by dividing $\dot{M}O_2$ with the swimming speed, enabling oxygen cost per body length swum. Plotting the exponential regression through the values for COT against swimming speed, $U_{opt}$ could be confirmed as the lowest cost of transport ($COT_{min}$). Maximum Metabolic Rate (MMR) was determined from the forced swimming data as the highest measured $\dot{M}O_2$ value.

2.5 Tail beat frequency

The tail beat frequency was determined to verify if the fish were swimming in similar fashions in both setups. We wanted to assess if the fish were drag-riding in the preferred swim test, thus having a different stride-length and tail beat frequency at the expected swim speed.

Usually, the tail beat frequency is counted by reviewing video footage from the respective trials and established by a fish's duration to perform ten tail beats and repeated five times. In the present study, the tail beat frequency in the $U_{opt}$ trials was determined using automated custom Python (Van Rossum and Drake Jr, 1995) software with OpenCV (Bradski and Kaehler, 2008) for image analysis and YoloV3 (Redmon and Farhadi, 2018) for object detection. The software was able to automatically detect the tail (x,y coordinates) for each frame. Using the coordinates and the timestamp for each frame, the raw tailbeat pattern was plotted. By applying a high pass filter, the
entire fish’s movement within the swim tunnel was removed, and amplitude over time was plotted. The duration between each peak was calculated, and the average tailbeat frequency could be determined.

Using this method enabled an increased amount of tail beats for analysis to determine the average tailbeat for each swimming speed (manual, approx. 50 vs. 600+ tail beats automatically per video), thereby giving a more realistic result and reducing the manual workload and error.

The same approach was modified to work with the $U_{pref}$ trials, but it was deemed imprecise due to the distance between the camera and fish. Therefore, the tail beats were counted using the manual method, i.e., the duration for ten tail beats. This was repeated five times at the preferred region in the $U_{pref}$ trials. The stride length was calculated using equation 3 (Svendsen et al., 2016; Wardle, 1975).

$$U = f_t \cdot l_s \Leftrightarrow l_s = \frac{U}{f_t}$$  \text{Eq. 3}

Where $U$ is the average swimming speed in BL s$^{-1}$, $f_t$ the average tailbeat frequency in Hz, and $l_s$ the stride length in BL tailbeat$^{-1}$.

For each fish in group 1, using the determined preferred swimming speed, the corresponding tailbeat frequency and stride length from the swim tunnel experiments were calculated using linear regressions fitted swimming values.

2.6 Statistics

Statistical comparison of the data for group 1 swimming at $U_{opt}$ and $U_{pref}$ were compared using a Dependent T-test. Thereafter a comparison between groups 1 and 2 $U_{pref}$ data was compared using an Independent T-test.

Assumptions for the paired and independent T-tests were checked using the Shapiro-Wilks test to test for normality and the Levene’s test to check for equality of variance, respectively.
To check if the fish were drag-riding in the $U_{\text{pref}}$ setup, stride-length and tail beat frequency for each fish in group 1 from both setups were compared using dependent t-tests and the Shapiro-Wilks test to test for normality.

The statistical level of significance was set at $\alpha=0.05$.

3. Results

3.1 Preferred swimming speed ($U_{\text{pref}}$)

After introduction to the circular raceway, the fish spent 5-30 mins exploring the new environment before settling at a preferred position. The fish remained at the same position throughout most of the measuring period (approximately 20h). $U_{\text{pref}}$ was determined for all fasting and ad libitum fed fish used in the experiment, averaging $1.01 \pm 0.29$ Bl s$^{-1}$ and $0.94 \pm 0.15$ Bl s$^{-1}$ (average $\pm$ S.D.), respectively. The test for equality of variances between $U_{\text{pref}}$ fasting and ad libitum fed fish groups was not significant (Levene's, $F = 4.056$, $p = 0.06$). This allowed for a T-test comparison assuming equal variance. The comparison of means showed no significant difference between the two groups (Independent T-test, $t(21) = 1.077$, $p = 0.29$). In other words, there was no significant difference in the preferred swimming speed with or without food (Fig. 3).

3.2 Optimal swimming speed ($U_{\text{opt}}$)

Due to the increased oxygen consumption caused by feeding (SDA), only group 1, the fasting group, was used to determine $U_{\text{opt}}$. The average $U_{\text{opt}}$ was $0.89 \pm 0.17$ Bl s$^{-1}$ (average $\pm$ S.D.), and the average standard and maximum metabolic rate were $43.4 \pm 6.1$ and $462.5 \pm 91.2$ mg O$_2$ kg$^{-1}$ h$^{-1}$ (average $\pm$ S.D.), respectively. This gives an absolute aerobic scope (AS) of $419.0 \pm 92.4$ mg O$_2$ kg$^{-1}$ h$^{-1}$ (average $\pm$ S.D.), which is a factorial scope of $9.9 \pm 2.8$. The critical swimming speed was $2.1 \pm 0.2$ Bl s$^{-1}$ (average $\pm$ S.D.).

For comparison reasons, a theoretical $U_{\text{opt}}$ fed group was created using the unfed group's swimming data and adding theoretical SDA-induced oxygen consumption (SDA = 40% of SMR) at all
swimming speeds. This resulted in an optimal swimming speed of 1.04 ± 0.23 (average ± S.D.)
and a reduction in AS to 401.1±93.0 mg O₂ kg⁻¹ h⁻¹(average ± S.D.), which is a factor of 6.7±2.0.

An in-depth explanation of the theoretical SDA line is described in the discussion section.

Equality of variance analysis between the U_{opt} fasting group and the theoretical fed group was not
significant (Levene's, F = 2.462, p = 0.13). Further analysis showed no significant difference
between the measured U_{opt} data and the theoretical U_{opt} fed data (Independent T-test, t(24) = -
1.898, p = 0.07).

3.3 Tail beat frequency and stride length

Linear regression was fitted through the tail beat frequency (TBF) and swimming speed data in
the group 1 (fasted fish) U_{opt} experiments. The fitted line (TBF = 1.12 • Swimming speed + 1.08)
had an R² = 0.98. The average TBF and stride length (SL) for fish swimming at 0.5 and 2.5 Bl s⁻¹
was 1.6±0.2, and 3.7±0.4 tail beats s⁻¹, and 0.31±0.04 and 0.68±0.07 Bl tail beat⁻¹, respectively.

For each of the measured U_{pref} TBF, the corresponding swimming speed was used to find the
equivalent TBF at U_{opt} using the equation from the fitted regression line and compared statistically.
The same procedure was used for the SL, and the fitted regression line (SL = 0.17 • Swimming
speed + 0.27) had an R²=0.93 (Fig. 4).

The TBF and SL data from group 1 (fasted fish), U_{pref} and U_{opt} were normally distributed (Shapiro-
Wilk: W(13) = 0.88, p = 0.08), W(13) = 0.88, p = 0.07) and there was no significant difference
between the two (Dependent T-Test, f(12) = 1.35, p = 0.20, f(12) = 1.57, p = 0.14).

Since no significant difference could be found between tailbeat frequency nor stride length in the
two setups, drag-riding could be excluded as a factor.

3.4 Compared swimming experiments

The U_{opt} and U_{pref} data for group 1 (fasted fish) was normally distributed (Shapiro-Wilk: W(13) =
0.948, p = 0.572) and no significant difference could be found between the two datasets
Dependent T-Test, \( t(12) = 0.99, p = 0.342 \). There was no significant difference between the optimal and preferred swimming speed in fasting fish (Fig. 3).

The measured \( U_{\text{pref}} \) fed fish group and calculated \( U_{\text{opt}} \) fed fish group showed homoscedasticity (Levene's, \( F = 3.196, p = 0.09 \)), and no significant difference was found between them (Independent T-test, \( t(21) = 1.70, p = 0.11 \)).

4. Discussion

This study aimed to determine if a difference between fed and unfed fish was present in preferred swimming speed (\( U_{\text{pref}} \)) compared to the optimal swimming speed (\( U_{\text{opt}} \)) and to evaluate future utilizations for aquaculture operations.

4.1 New setup - Same principle

In a study by Tudorache et al., (2011), the preferred swimming speed was obtained using a tilted elongated raceway with changing flow velocities from one end to the other. A somewhat similar method was used in the present study, but instead of an elongated raceway, a circular flow doughnut-shaped raceway was used. The raceway in Tudorache et al., (2011) produced a flow velocity ranging from 10 to 110 cm s\(^{-1}\) with linear flow, whereas velocities in the present study ranged from 13.5 to 42 cm s\(^{-1}\) with circular flow. Although a smaller range, the fish still had a choice-range from approx. 0.5 to 1.5 Bl s\(^{-1}\). Optimal swimming speed in Rainbow trout previously has been found within this range (Bushnell et al., 1984; Gerry and Ellerby, 2014; Teulier et al., 2013).

The authors are aware of the apparent issue with determining swimming performance in a non-linear flow setup since the fish will be exposed to a relative side-current instead of a head-on current. Although not optimal for general practice, most modern aquaculture facilities exclusively apply circular tanks, and hence all production fish will experience a non-linear flow. The experienced side-current will be reduced with the ratio between fish size and tank diameter.
One of the benefits of using a circular tank for these types of behavioral experiments is that fish can choose to go with the flow without hitting a back-grid. Although not directly beneficial for the experimental outcome, given the experiment's longevity, it is likely that this behavior will subside, and the fish will start to swim. The lack of restrictions within the setup reduces stress and therefore optimizes results.

4.2 Preferred swimming speed ($U_{\text{pref}}$)

Intuitively, one would expect the behavior of fish fed ad libitum to be less exploratory than fasted fish. Behaviorally, fed fish do not need to forage for food, and on an energetic level, their aerobic scope will be decreased (Jobling and Davies, 1980). A greater aerobic scope equals more excess energy, which could result in a more active behavior, but not necessarily a different preferred swimming speed. This was also the case in the present study (Fig. 3) since we found no significant difference in preferred swimming speed in fed and unfed fish.

4.3 Optimal swimming speed ($U_{\text{opt}}$)

In metabolic energetics, postprandial effects or specific dynamic action (SDA) causes an increase in oxygen consumption ($\dot{MO}_2$) (Chabot et al., 2016; Jobling and Davies, 1980). Postprandial $\dot{MO}_2$ will initially increase until a peak is reached and gradually decrease back to the Standard Metabolic Rate (SMR). Several difficulties arise when trying to measure $\dot{MO}_2$ in swimming fish during SDA since the magnitude of SDA on $\dot{MO}_2$ is dynamic and changes over time (Chabot et al., 2016). This is especially apparent for lengthy experiments and is the main reason why we chose not to conduct $U_{\text{opt}}$ experiments on ad libitum fed fish (Group 2). The $\dot{MO}_2$ increase caused by SDA will persist throughout a swimming protocol (Alsop and Wood, 1997; Eliason and Farrell, 2016; Pang et al., 2011, 2010; Thorarensen and Farrell, 2006). Theoretical exponential lines were fitted to all measured swimming data, resulting in an average exponential line ($\dot{MO}_2_{\text{SDA}}$) above the average measured $\dot{MO}_2$ line (Fig. 2). Using the calculated data, the average $U_{\text{opt}}$ was calculated and
compared to the unfed Rainbow trout's optimal swimming speed. Even though there was a 0.15 Bl s\(^{-1}\) difference between calculated and measured \(U_{\text{opt}}\), no significant difference was found.

### 4.4 Trail beat frequency and stride length

The common quantifiable denominator between the two experiments (\(U_{\text{opt}}\) and \(U_{\text{pref}}\)) was the tail beat frequency and thereby, the stride length. Statistical analysis showed no significant difference between tail beat frequency and stride length in the two experiments. Therefore, it could be concluded that fish were swimming similarly in the \(U_{\text{pref}}\) and \(U_{\text{opt}}\) experiments, hence the fish were not drag-riding (Fig. 4).

### 4.5 Limitations

When experiments are conducted on fed fish, the duration of experiments will always pose an issue since the increase in oxygen consumption caused by SDA will change over time (Chabot et al., 2016; Jobling and Davies, 1980). It is, therefore, uncertain if \(U_{\text{pref}}\) in the fed fish could have changed during the experiment (20h).

Since the \(U_{\text{pref}}\) determination method is solely based on the fish's behavior, the researcher will have less control over the experiment than in the \(U_{\text{opt}}\) experiments. This was also the case with one fish in the present study. The fish utilized an area within the tank where drag-riding was possible. The drag-riding was discovered in the post-experimental analysis of the video footage, and the fish was excluded from further analysis.

### 4.6 Perspectives

The benefit of using a raceway instead of a swimming respirometer to evaluate swimming preferenda is that fish are uninhibited and free to choose a preferred flow regime rather than a forced one. Therefore, the method simulates natural conditions to a greater extent. In the present study, \(U_{\text{pref}}\) and \(U_{\text{opt}}\) were the same in unfed and fed fish. This has a high implication value for aquaculture since, under normal operations, fish in aquaculture will never be fasted.
Many modern land-based aquaculture facilities use circular to close-to circular tanks. The main reason is the circular flow which, not being obstructed by sharp corners, ensures equal water exchange in the entire tank. The equal water exchange contributes with even oxygen distribution, resulting in a uniform environment for the fish (Timmons et al., 1998). As in the present study, the circular flow creates a velocity gradient from the outer edge towards the center. Since fish in rearing tanks have a natural size variation, the gradient can be utilized to give all fish sizes the option to swim at $U_{\text{pref}}$. Velocity can be adjusted as fish grow to sustain the preferred swimming speed.

A similar approach can be used in ocean-based aquaculture, but since direct control of flow velocity is not an option, a different method must be used. A study by Winthereig-Rasmussen et al., (2016) showed that cage positioning has a similar effect. As the current moves through the cages, the flow velocity is reduced. Knowing the average flow velocity in an area, fish can be size sorted, placing the largest fish in the outer cages with the highest flow. If possible, as fish grow, cages can be moved towards more exposed areas, keeping the fish swimming at the preferred swimming speed.

Future research in this subject could include experiments to determine if $U_{\text{opt}}$ and $U_{\text{pref}}$ in fed fish are equal at different temperatures. Increased temperature results in an increased optimal swimming speed in Rainbow trout (Claireaux, 2006). However, it is uncertain if the preferred swimming speed will increase accordingly.

Furthermore, similar studies should be conducted on essential aquaculture species since fish swimming at preferred or optimal swimming speeds will show reduced aggression, higher quality meat, improved growth, and overall optimize fish welfare in aquaculture (Palstra and Planas, 2011).
References


Tudorache, C., O’Keefe, R.A., Benfey, T.J., 2011. Optimal swimming speeds reflect preferred


Figure captions

**Figure 1** Preferred swimming speed setup: A: Heatmap depicting the calculated flow in the setup used to determine $U_{\text{pref}}$. Measured velocity values are given as open circles (o). The flow velocities were calculated by extrapolating between points, hence the missing values in proximity to the walls. B: Image of the experimental setup consisting of a submerged perforated inner tank containing a pump (1) connected to a nozzle (2). Since the image is captured during an experiment, a fish can be seen in the tank (3).

**Figure 2** Overview of experimental results. A) The average oxygen consumption during swimming ($\dot{M}_{\text{O}_2\text{,U}}$) on the primary axis and the average cost of transport (COT) on the secondary axis. B) Horizontal bar charts showing optimal swimming speed in unfed and fed fish. $U_{\text{opt}}$ for fed fish data was calculated from the $U_{\text{opt}}$ unfed data. C) Horizontal bar charts showing preferred swimming speeds in fed and unfed fish.

**Figure 3** Bar charts depicting average optimal and preferred swimming speed in fed and unfed rainbow trout. The $U_{\text{opt}}$ Ad Libitum data is calculated based on swimming experiments from $U_{\text{opt}}$ Control. There is no significant difference between any of the groups.

**Figure 4** Tail beat and stride length measurements: A) Gray crosses (x) depict tail beat frequency at all swimming speeds in the $U_{\text{opt}}$ experiment with a regression line fitted through the points. Black dots (•) are tail beat frequency from the $U_{\text{pref}}$ experiments. B) Stride length. Legends are the same as in A).
Figure 1

A) 

B)
Figure 2

![Graph showing swimming speed vs. \( \dot{MO}_2 \) and COT for different conditions.](image)

- A) Graph of \( \dot{MO}_2 \) and COT against swimming speed.
- B) Box plots for \( U_{OPT} \) (Control) and \( U_{OPT} \) (Ad Libitum) — Theoretical.
- C) Box plots for \( U_{PREF} \) (Control) and \( U_{PREF} \) (Ad Libitum).
Figure 3

Swimming speed (BL s⁻¹)

$U_{OPT}$ (Control)  $U_{OPT}$ (Ad Libitum) – T  $U_{PREF}$ (Control)  $U_{PREF}$ (Ad Libitum)

n.s.
Figure 4

(A) Frequency (TB s$^{-1}$)

\[ y = 1.12x + 1.08, R^2 = 0.98 \]

(B) Stride length (BL TB$^{-1}$)

\[ y = 0.17x + 0.27, R^2 = 0.93 \]
MS2: Model of oxygen conditions within aquaculture sea cages

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Abstract

To ensure optimal food intake, growth, and general fish health in aquaculture sea cages, interactions between drivers that affect oxygen conditions need to be understood. The main drivers are oxygen consumption and water exchange, caused by flow through the cage. Swimming energetics in Rainbow trout (Oncorhynchus mykiss (Walbaum, 1792) in normoxia and hypoxia at 10, 15, and 20℃ were determined as well as postprandial effects at 10℃. Using the determinations, a conceptual model of oxygen conditions within sea cages was created. By applying the model to a case study, results show that with a temperature increase of 10℃, oxygen concentration will decrease six times faster. To maintain optimal oxygen concentration within the cage, the flow velocity must be a factor of 5.8 higher.

The model is highly relevant for offshore aquaculture sea cages since the model results can explain why and when suboptimal conditions occur. The model also can be used to estimate the suitability of new aquaculture sites.

Key Words: Aquaculture, Model, Swimming energetics, SDA, Hypoxia
1. Introduction

Oxygen availability is a critical parameter in ensuring food intake, optimal growth, and cultured fish health (Brandt et al., 2009; Chabot and Claireaux, 2008; Foss et al., 2002; Nelson, 2016). The primary drivers for oxygen availability within aquaculture sea cages can be divided into slow and fast-changing parameters, i.e., variations occurring seasonally to daily or hourly to min\(^{-1}\). In the present study, the slow-changing parameters are temperature, salinity, and atmospheric pressure, directly affecting oxygen solubility (Svendsen et al., 2016). The fast-changing parameters are the current velocity and oxygen consumption (\(\text{ṀO}_2\)) of the fish. The balance between these two fast-changing parameters is essential in retaining suitable oxygen conditions in the cages since the \(\text{ṀO}_2\) depletes oxygen while the current velocity replenishes it.

The possible range of swimming speeds narrows with increased current flow. During high water flow, the only option for the fish is to swim against the current, while at low water flow, the fish can regulate swimming at an intermediate speed, such as the preferred swimming speed (\(U_{\text{pref}}\)) (Tudorache et al., 2011). As \(\text{ṀO}_2\) increases exponentially with increasing swimming speed (Brett, 1964), even slight increments in swimming speed during low current velocities will profoundly affect oxygen availability within the cages.

Besides causing a decrease in oxygen solubility, increased temperature will also increase \(\text{ṀO}_2\). The increase can be quantified using the factorial increase in \(\text{ṀO}_2\) with a temperature increase of 10°C (\(Q_{10}\)), ranging from 1.62 to 2.64 in fishes (Christensen et al., 2020; Clarke and Johnston, 1999; Killen et al., 2016; Schurmann and Steffensen, 1997; Tirsgaard et al., 2015).

Increases in \(\text{ṀO}_2\) are not solely caused by external factors but also by internal factors such as digestion. Digestion or postprandial effects will increase the \(\text{ṀO}_2\) for several hours after feeding depending on temperature, portion size, and species (Jordan and Steffensen, 2007; Pang et al., 2010; Tirsgaard et al., 2015). The increase is referred to as Standard Dynamic Action (SDA) (Chabot et al., 2016; Jobling and Davies, 1980).
In the present study, we suggest that feeding can be optimized by modeling and understanding oxygen availability within sea cages. If oxygen concentration decreases below a threshold, the feed is suboptimally utilized, and the energetic value of feed consumed is reduced (Pedersen, 1987; Remen et al., 2016). Continual decrease in oxygen concentration within the cage will cause loss of appetite (Claireaux et al., 2000; Wang et al., 2009).

A conceptual model was made to understand how fish and the environment affect oxygen availability within sea cages. With this understanding, feed waste from cages can be optimized. In Danish aquaculture, production in sea cages is limited by the discharge from aquaculture farms (Skov et al., 2020); hence if the discharge is reduced, production can be increased.

2. Materials and methods

All fish experiments agreed with the EU Directive 2010/63/EU for animal experiments and performed with permission from the Danish Animal Experiments Inspectorate (License number: 2018-15-0201-01466).

2.1 Experimental fish

Rainbow trout (Oncorhynchus mykiss (Walbaum, 1792) were chosen as the model species since it is the only species farmed in Danish sea cage aquaculture (Danish Fisheries Agency statistics). The fish were obtained from a land-based aquaculture farm (Sten Kjær ApS, Brørup, Denmark). Due to the relatively large fish size (1510.8 ± 479.7 g) and limited space in the laboratory, fish were obtained on three separate occasions, approximately 30 fish in each batch.

Fish were kept in two flow-through holding tanks, with 20 fish in a 1300 L circular tank and ten fish in a square 700L tank, both connected to the laboratory system water. The system provided fully aerated (95% > air saturation dissolved oxygen (air sat. DO)), recirculated, filtered seawater at 10°C, and salinity of approx. 30. The light: dark regime was 12:12 h. Pressurized air was bubbled through the water column to ensure that CO₂ did not exceed the recommended levels (Fivelstad et al., 2015).
2.2 Acclimation

All experiments were conducted in seawater with a salinity of 30. Since the fish had been reared in freshwater, acclimation to the new salinity was allowed before experiments could be performed. The acclimation was set to a minimum of 4 weeks since the state of smoltification was unknown. Smoltified salmonids can effectively acclimate to new salinities within days (McCormick and Saunders, 1987; Morgan and Iwama, 1998). Initially, the fish would not eat, and therefore feeding was postponed for two weeks. After that, fish were fed 8mm commercial trout pellets daily (BioMar A/S, Brande, Denmark). When the initial acclimation period was over, the fish were tagged using sterile 8 mm PIT tags (ADEQID microchip tags, Eickemeyer Aps, Vojens, Denmark). PIT tags do not affect swimming performance in rainbow trout (Newby et al., 2007). After tagging, the fish were size sorted for the experiments and returned to the two holding tanks. Since the experiments required the fish to be acclimated to different temperatures, after salinity acclimation, batch two and three were acclimated to 15 and 20°C for a minimum of four weeks (Bouchard, 2003), respectively. During this acclimation period, all fish were monitored for general health, especially for infections at the PIT tag puncture wound.

For consistency, the 10°C batch was also kept for four weeks before experiments could commence. Three 500W heaters (Aqua Medic titanium heater, TH-500) were mounted vertically in the holding tank and controlled by a programmable thermoregulator (PR5714, PR Electronics, Rønde, Denmark) programmed to heat one °C above the benchmark temperature (15 and 20°C). To ensure cooling and water exchange, a second thermoregulator (PR5714, PR Electronics, Rønde, Denmark), programmed to turn on when the benchmark temperature was reached, controlled two pumps (56L/min, EHEIM GmbH, Deizisau, Germany), placed in an adjacent tank and connected to the setup. The adjoining tank did not contain fish and was continuously supplied with system water. Using this method, the temperature could be maintained at 15.0 and 20.0±0.2°C while ensuring suitable water exchange.
2.3 Study design

In the present study, swimming energetics in normoxia and hypoxia were determined using ten replicates at three different temperatures. Carryover effects on Standard Metabolic Rate (SMR) between the experiments were tested using a crossover protocol, i.e., five fish exposed to normoxia than hypoxia, and five fish in a reversed protocol for each temperature.

SDA experiments were conducted on 12 fish simultaneously while swimming or at rest. To reduce external disturbance during experiments, all setups were covered by dark fabric and remotely monitored via video cameras. Finally, acquired data was used to create a model of oxygen availability within an aquaculture sea cage.

2.3.1 Swimming energetics - Normoxia

Swimming energetics and critical swimming speed were determined using a swim tunnel respirometer (90L Steffensen type) with a swim section of 20x20x70 cm (h,w,l). The oxygen was measured using a fiber-optic oxygen meter (Fibox 3, Precision Sensing GmbH, Regensburg, Germany) without temperature compensation. The oxygen meter was recalibrated between each temperature change. Oxygen consumption was calculated using data acquisition software (AutoResp, Loligo Systems, Tjele, Denmark) which controlled the flush, wait, and measuring periods (600s, 180s, and 720s), resulting in one measurement every 25 min. Flow in the respirometer was generated using a propeller connected to an electric motor (AC-motor, DRS71, SEW Eurodrive), controlled by a motor controller (Movitrac MCLTE, B0004-101-1-20, SEW Eurodrive). Calibration of flow velocity used to calculate swimming speed (Bl s⁻¹) was measured using a flow probe (Vane Wheel, Höentzsch, Weiblingen, Germany). Using the measured flow and the mV signal, a regression line was fitted through the points resulting in the flow calibration equation.

To ensure minimum temperature fluctuations in the swim tunnel, the same setup used in the holding tank was applied. Experimental temperatures were 10, 15, and 20°C.
Postprandial effects on oxygen consumption were reduced by fasting the fish for a minimum of 24 hours before introduction to the respirometer (Jobling and Davies, 1980). During the first 24h in the respirometer, the fish were swimming at 0.4 Bl·s⁻¹. Initially, $\dot{MO}_2$ measurements showed a steep decline, followed by a plateau. The swimming protocol had one determination per increment with increments of 0.3 Bl·s⁻¹ until the fish fatigued.

The critical swimming speed ($U_{crit}$) was determined as the point at which a fish would continuously rest on the swim section’s back-grid after three consecutive attempts at encouraging swimming by shortly decreasing flow velocity. When $U_{crit}$ was reached, the experiment was terminated. Bacterial respiration and accumulation of waste products were reduced by emptying and cleaning the setup between each experiment. To check for bacterial background respiration, oxygen measurements in the swim tunnel without fish were conducted on multiple occasions and always negligible.

2.3.2 Swimming energetics - Hypoxia

The swimming energetics in hypoxia was conducted in the same setup as the swimming energetics in normoxia.

Hypoxia was achieved by utilizing the standard self-inducing hypoxia method letting the fish decrease the water’s ambient oxygen tension (Rogers et al., 2016). As opposed to bubbling with nitrogen, self-induced hypoxia to reduce oxygen tension will cause waste products to accumulate in the setup. The waste products may affect the experimental outcome and simulate what happens in a sea-cage with slow-moving water (limited exchange)(Regan and Richards, 2017; Snyder et al., 2016).

Hypoxia experiments were initiated by changing the flush and wait periods to one second after an initial 24h acclimation in the swim tunnel. To determine the minimum oxygen tension required for swimming at different swimming speeds, experiments were completed at 0.4 and 1.0 Bl·s⁻¹. The critical oxygen tensions ($P_{crit}$) at 0.4 and 1.0 Bl·s⁻¹ were determined as the pO₂ when the fish stopped swimming and rested on the swim section’s back grid. At this point, the flush and wait
were changed back to pre-experimental settings. After 24 hours, the second hypoxia trial was initiated by increasing the swimming velocity to 1.0 Bl·s⁻¹ and using the same protocol as in the first trial.

2.3.3 SDA experiments

The respirometer consisted of a circular tank (diameter 150 cm, water depth 80 cm). Water outflow occurred in the center of the tank through a riser tube (diameter 10 cm). The riser tube was covered by a PVC pipe (outer diameter 20 cm) with a perforated bottom edge to ensure water and waste removal from the bottom of the tank. The center riser tube and PVC pipe also ensured a circular flow (Fig. 3). The total volume of water was 1342 L.

The water was kept at a temperature of 10±0.2°C and salinity of 30. Flow in the tank was generated using a submersible pump (Power Craft, 175 L/min) mounted at a depth of 15 cm, slightly tilted downwards at a shallow angle to the tank wall. Flow in the respirometer was measured using a flow probe (Vane wheel, Höentzsch, Weiblingen, Germany) at three different depths. For each depth, four transects with six measurements in each were noted (Fig. 3). The flow velocity ranged from 10 to 50 cm·s⁻¹, which corresponded to an average swimming speed of 0.19 to 0.94 Bl·s⁻¹, respectively.

Open-tank respirometry is affected by the gas-exchange at the tank’s surface, and the exchange should therefore be quantified. Quantification was achieved by conducting experiments in the tank with and without flow. By turning off the water supply and bubbling nitrogen through the water column, the oxygen tension was reduced to approximately 50% air sat. DO. Using a four-channel Optical Oxygen Meter (FireStingO2, PyroScience GmbH, Aachen, Germany), the oxygen content was measured and logged until equilibrium between water and air was reached. No fish were in the tank during these experiments.

Respirometry measurements were calculated using the freeware AquaResp (www.aquaresp.com, last accessed: 06-03-2021). AquaResp was also used to control Flush, Wait, and Measuring
durations (F:900s, W:120s, M:1380s) using a USB-Switch C mechanical relay (Cleware GmbH, Germany). Two pumps (56L/min, EHEIM GmbH, Deizisau, Germany) connected to the respirometer tank were placed in an adjacent tank. The adjacent tank was continually supplied with system water and did not contain any fish. During the flushing period, the relay also opened a solenoid valve that controlled airflow to air stones ensuring degassing of CO\textsubscript{2} in the respirometer.

The combined mass of all fish was 23.7 kg, giving a fish density of 17.7 kg/m\textsuperscript{3}. In aquaculture cages, fish size variation is common, and 12 fish ranging from 781g to 3074g with an average mass of 1976.4 ± 667.9 g (average ± SD) were chosen for the experiment.

The hypoxia experiments aimed at estimating the magnitude of SDA at rest and during swimming. Since \(\dot{M}O_2\) during SDA changes over time (Chabot et al., 2016; Jobling and Davies, 1980; Jordan and Steffensen, 2007), two methods were used. The first method measured SDA’s effects with and without current on fish fed 2% of total body mass (TBM) once (three replications). The second method was again with and without current, and fish were fed 1% of TBM each day (one replication). The constant feeding method (method 2) will cause the \(\dot{M}O_2\) to reach equilibrium and plateau over time, resulting in stable \(\dot{M}O_2\) measurements that can be used to determine the magnitude of SDA. Method 1 data were used to create a theoretical plateau that could be compared to method 2 data.

Feeding was administered using an automatic fish feeder (XClear, Son en Breugel, The Netherlands), which was programmed to dispense the predefined ration over an hour. The extended feeding duration prevented pellets from accumulating on the bottom and ensured that all pellets were consumed.

2.4 Theory/calculations

Applying the measured parameters from the present study into a model required data analysis. The data was analyzed using python 3.8.5 with data analysis packages. The model was likewise created using python 3.8.5.
2.4.1 Swimming energetics - Normoxia

Swimming energetics (x: Swimming speed; y: \( \dot{M}O_2 \)) during normoxia were fitted to the following exponential function.

\[
y = a \cdot e^{b \cdot x}\quad \text{Eq. 1}
\]

The optimal swimming speed (\( U_{\text{opt}} \)) is determined as the tangent to the fitted regression and is expressed using the following equation (Tudorache et al., 2013).

\[
U_{\text{opt}} = b^{-1}\quad \text{Eq. 2}
\]

Where \( b \) is the resulting exponent from the fitted swimming equation (Eq. 1).

The critical swimming speed (\( U_{\text{crit}} \)) was calculated using the equation from Brett (1964).

\[
U_{\text{crit}} = u_i + \left( \frac{t_i \cdot u_{ii}}{t_{ii}} \right)\quad \text{Eq. 3}
\]

Where \( u_i \) is the swimming speed maintained throughout a completed increment (Bl\cdot s^{-1}), \( t_i \) is the duration spent swimming at the incomplete increment (min), \( t_{ii} \) is the duration of the whole incomplete increment (min), and \( u_{ii} \) is the velocity increase between increments (Bl\cdot s^{-1}).

The optimal swimming speed (\( U_{\text{opt}} \)) is the speed at which the cost of swimming one body length is the lowest and can be illustrated as the lowest Cost of Transport (COT). COT is calculating by dividing \( \dot{M}O_2 \) with the swimming speed.

To compare \( \dot{M}O_2 \) at different temperatures in the model, the considerable variation in fish mass had to be mass adjusted. The SMR and the Maximum Metabolic Rate (MMR) were body mass adjusted using the method described in Steffensen et al. (1994) and Frisk et al. (2012). The chosen target body mass was 1.5 kg, close to the average for all fish (1452.7 ± 490.1 g).

\[
\dot{M}O_2(1.5\text{kg}) = \dot{M}O_2 \cdot \left( \frac{M}{1.5} \right)^{(1-A)}\quad \text{Eq. 4}
\]
\[ \text{Ṁ}_\text{O}_2 (1.5\text{kg}) \] is the adjusted oxygen consumption rate when fish mass is 1.5 kg, \( \text{Ṁ}_\text{O}_2 \) is the measured oxygen consumption (mg O2 kg\(^{-1}\) h\(^{-1}\)), \( M \) is the body mass, and \( A \) is the mass exponent. The mass exponent chosen in the present study was 0.89, which Jerde et al. (2019) found suitable for Rainbow trout.

Finally, the factorial Aerobic Scope (AS) is calculated by dividing MMR with SMR.

### 2.4.2 Swimming energetics - Hypoxia

Data from the hypoxia experiments (swimming at 0.4 and 1.0 Bl\(\cdot\)s\(^{-1}\) and MMR) were fitted to the Hill equation (Hill, 1910). The Hill equation is a sigmoid function and is expressed as follows:

\[
\text{metabolic scope}_{\text{Hypoxia}} = \frac{\text{MMR}}{1 + \left(\frac{\text{pO}_2}{b}\right)^c} \quad \text{Eq. 5}
\]

Where MMR is the Maximum Metabolic Rate, \( b \) is the \( P_{50} \), \( c \) is the Hill coefficient, and \( x \) is the partial pressure of oxygen (pO\(_2\)). Applying the \( \text{Ṁ}_\text{O}_2 \) during \( U_{\text{opt}} \), the Hill equation can estimate the minimum pO\(_2\) required to sustain \( U_{\text{opt}} \). Constants used in the Hill equation for 10, 15, and 20°C were \( P_{50} \): 6.50, 6.04, and 6.24 and Hill coefficient: 3.50, 5.51, and 5.70, respectively.

### 2.4.3 SDA experiments

To quantify the SDA, the baseline \( \text{Ṁ}_\text{O}_2 \) of the fish, when no feed was administered, was acquired. This was achieved by measuring oxygen consumption for 48 h before feeding. Since the fish were free to swim around during the experiment in both methods (with and without current), the acquired baseline for \( \text{Ṁ}_\text{O}_2 \) is referred to as Routine Metabolic Rate (RMR). Large variations in \( \text{Ṁ}_\text{O}_2 \) were measured during the daytime in all experiments, but during nighttime, the measurements, without current, stabilized. Therefore, the RMR baseline in experiments with no current was determined by all night measurements’ median value. For consistency, the same approach was used for experiments with current. To isolate the increased oxygen consumption caused by SDA (\( \text{Ṁ}_\text{O}_2 \text{SDA} \)), the RMR was subtracted. To compare SDA methods, the \( \text{Ṁ}_\text{O}_2 \text{SDA} \) for
method one (2% TBM once) experiments was reduced by 50% to simulate fish being fed 1 % of TBM. By repeatedly cumulating the halved $\text{MO}_2\text{SDA}$ with an offset of 24 hours for the duration of the method two experiments (1% SDA), a theoretical SDA constant feed value was calculated.

2.4.4 The model

The model consists of three main calculation series, which describe the oxygen conditions within an aquaculture cage during changes in biological and environmental factors. All calculation series are calculated for fed and unfed fish.

The oxygen solubility is calculated as described in Svendsen et al. (2016). The sizes of circular cages in aquaculture are usually referenced using the circumference of the cages. Therefore, the cage volume is calculated using circumference and depth as in the following equation:

$$V = \pi \cdot \left(\frac{C}{2\cdot\pi}\right)^2 \cdot d$$ \hspace{1cm} Eq. 6

Where $V$ is the volume, $C$ is the circumference, and $d$ is the depth of the cage. The equation is a combination of the equation for the volume of a cylinder and radius from the circumference. Using the cage volume, the fish density is calculated using the following equation:

$$\rho = \frac{Q \cdot m}{V}$$ \hspace{1cm} Eq. 7

Where $\rho$ is the fish density, $Q$ is the total number of fish, $m$ is the average mass of the fish, and $V$ is the cage volume.

The first calculation series (CS1) derives the time for oxygen concentration to reach anoxia from fish at rest and swimming at $U_{\text{opt}}$ and with no water flow through the cage. The series also calculates the duration to oxygen depletion when fish are fed, i.e., $\text{MO}_2$ during SDA.

The average oxygen consumption for resting fish and fish swimming at $U_{\text{opt}}$ when fed and unfed was subtracted from the ambient oxygen concentration over time. This can be expressed using the following equation:
\[ y_{nf} = [O_2]_{cage} - \dot{M}O_2 \cdot \rho \cdot t \quad \text{Eq. 8} \]

Where \( y_{nf} \) is oxygen concentration at time \( t \) anywhere in the cage with no flow and \([O_2]_{cage}\) is the oxygen concentration in the cage, \( \dot{M}O_2 \) is the oxygen consumption, \( \rho \) is the fish density within the cage, and \( t \) is the time. Since fish cannot sustain swimming at \( U_{\text{opt}} \) below the critical oxygen concentration, found using the Hill equation (Eq. 4), the model calculates a lower swimming speed and subsequently the \( \dot{M}O_2 \). The updated \( \dot{M}O_2 \) is the result of the Hill equation using the oxygen concentration in the cage.

The second calculation series (CS2) calculates the oxygen concentration distribution through the cage, given a preset current velocity and swimming speed in fed and unfed fish. This is achieved using the following equations:

\[ y_x = [O_2]_{cage} - (\dot{M}O_{2(\text{sec})} \cdot \rho \cdot v^{-1}) \cdot D \quad \text{Eq. 9} \]

Where \( y_x \) is the oxygen concentration at distance \( x \) from the upstream edge of the cage and \([O_2]_{cage}\) is the oxygen concentration in the cage, \( \dot{M}O_{2(\text{sec})} \) is the oxygen consumption in seconds, \( \rho \) is the fish density, \( v^{-1} \) is the time for the current to move 1 unit, and \( D \) is the diameter of the cage. As in the first series, a limit is added to the swimming velocity if oxygen concentration decreases below the minimum requirement for swimming at \( U_{\text{opt}} \).

The third calculation series (CS3) calculates the oxygen concentration when current velocity and swimming speed are the same and when swimming speed is at \( U_{\text{opt}} \) until current velocity exceeds \( U_{\text{opt}} \). To compare \( \dot{M}O_2 \) at different swimming speeds with the corresponding current velocities, the units have to be unified, i.e., convert swimming speed from \( \text{Bl s}^{-1} \) to \( \text{cm s}^{-1} \). The oxygen concentration is calculated using the following equation:

\[ y_{wvss} = \dot{M}O_2 U \cdot \rho \cdot v^{-1} \cdot v \quad \text{Eq. 10} \]

Where \( y_{wvss} \) is the oxygen concentration during a given current velocity and swimming speed and \( \dot{M}O_2 U \) is the oxygen consumption during swimming, \( \rho \) is the fish density, \( v^{-1} \) is the time for the
current to move 1 unit, and \( v \) is the current velocity (unit s\(^{-1}\)). If swimming speed and current velocity are the same, \( \dot{M}O_2U \) is directly correlated with the velocity, and if fish are swimming at \( U_{opt} \), \( \dot{M}O_2U \) is unchanged until the current velocity equals or exceeds \( U_{opt} \).

During hypoxia, feed is suboptimally utilized, and thresholds for the onset of the decrease are reported for Rainbow trout (\textit{Oncorhynchus mykiss}) at 15\(^\circ\)C to be at 6.0 mg O\(_2\) l\(^{-1}\) (Sal: 0, approx. 68.9\% air sat. DO) (Pedersen, 1987). Similar values were found for Atlantic salmon (\textit{Salmo salar}) at 11, 15, 19\(^\circ\)C with limits at 53±1, 66±3 and 76±4 \% air sat. DO, respectively (Remen et al., 2016). The proximity of the two studies’ results suggests that air sat. DO thresholds are similar for these two species of salmonids. Fitting the values from both studies to a linear regression resulted in the following equation.

\[
\text{Threshold (% air sat. DO)} = 2.875 \cdot T + 22.85 \quad \text{Eq. 11}
\]

Where \( T \) is the temperature.

2.5 Case study using the model

The case study was based on a standard to large aquaculture sea cage during tidal change with low currents and at three different temperatures (10, 15, and 20\(^\circ\)C) with a salinity of 30 and an atmospheric pressure of 1 atm. The mass-adjusted swimming energetic values used at 10, 15, and 20\(^\circ\)C were SMR of 54.8, 60.5, 99.8 mg O\(_2\) kg\(^{-1}\) h\(^{-1}\) with corresponding exponents at 1.114, 0.909, 0.646, respectively. The average body mass was 1.5 kg with a length of 44.1 cm. Using a circumference of 160 m and a depth of 8 m, the cage volume was 16297.5 m\(^3\) and a diameter of 50.9 m. The fish density within the cage was 25 kg/m\(^3\), and SDA was estimated to be 41\% of SMR.

For the second calculation series in the model, the current velocity was 5 cm/s, and the swimming energetics were based on fed fish swimming at \( U_{opt} \).
All statistical analyses were performed using SPSS version 27.

To check for possible carryover effects in SMR during swimming energetics in normoxia, an Independent T-test was used on all temperatures. A test on means between temperatures was performed on the SMR, MMR, and \( U_{\text{opt}} \) data from the normoxia experiments.

The means from the \( P_{\text{crit}} \) at 0.4, 1.0 Bl·s\(^{-1}\) experiments and the minimum required \( PO_2 \) for swimming at \( U_{\text{opt}} \) in the hypoxia experiments between temperatures were tested.

The statistical level of significance was set at \( \alpha=0.05 \) for all statistical tests.

### 3. Results

#### 3.1 Swimming energetics - Normoxia

Results from the normoxia swimming trails are illustrated in Fig. 1 and values reported in Table 1. SMR was determined by extrapolating the fitted swimming equation to a swimming speed of 0 Bl·s\(^{-1}\). Since there was a considerable mass difference between the fish used in each temperature, a mass size correction was applied (Eq. 5). The body mass adjusted values are used in the model. The \( Q_{10} \) was 2.0 and 1.8 in the measured and adjusted SMR, respectively. The aerobic scope was highest at 15°C.

#### 3.2 Swimming energetics - Hypoxia

Results from hypoxia trails are shown in Fig. 1 and Table 2. While the fish were swimming at 0.4 Bl·s\(^{-1}\), the critical oxygen tension showed an increasing trend with temperature. This was also the case with fish swimming at 1.0 Bl·s\(^{-1}\) from 10 to 20°C. Using the Hill equation (Eq. 4), the minimum required oxygen tension for fish swimming at \( U_{\text{opt}} \) was determined for each temperature. At 15°C, the average minimum requirement is lower than the calculated requirements for 10 and 20°C.
3.3 SDA experiments

Experiments to quantify gas exchange at the surface ran for 8 hours and showed no difference in oxygen tension in experiments without currents. The oxygen was measured in the middle of the water column, and although gas exchange occurs at the surface, the absence of current to mix water vertically resulted in no changes being measured. With current, oxygen was replenished at a changing rate directly affected by the ambient oxygen tension. Minimum ambient oxygen in the SDA experiments was approx. 80% air sat. DO. Therefore, the replenishment rate was calculated from 80 to 100% with increments of 5% points. Assuming the decrease in oxygen during experiments is linear, the SDA experiment’s measuring period (1380 s) was divided into four equal groups of 345 sec. During ambient oxygen tensions at 80-85%, 85-90%, 90-95%, and 95-100%, the replenishment rate was 0.6, 0.5, 0.3 and 0.2% points, respectively. Although there is an effect on the measurements, since the effect is the same for all experiments with current, the change is disregarded.

Visual representation of the results from the SDA experiments are shown in Fig 2.

The experiment with continually feed fish resulted in $\bar{\dot{M}}O_2_{\text{SDA}}$ of 41.3 and 90.1% of RMR, with and without current, respectively. Whereas the calculated theoretical $\dot{M}O_2_{\text{SDA}}$ from fish fed once was 51.1 and 119.3% of RMR, with and without current, respectively. Even though the percentage of $\bar{\dot{M}}O_2_{\text{SDA}}$ was higher with current, the duration before $\bar{\dot{M}}O_2_{\text{SDA}}$ reached baseline RMR after feed was around 24 h longer than experiments without current, indicating that a considerable part is due to increase spontaneous oxygen consumption.

3.4 Case study based on model

Results from the case study are illustrated in Fig 4 and Table 3.

The model showed that in a cage with no current and fed fish swimming at $U_{\text{opt}}$ (CS1), the oxygen content reaches the air sat. DO threshold six times faster with a temperature increase of 10°C. With an increase of 10°C, the oxygen concentration at the downstream edge of the cage with a current
velocity of 5 cm/s and a fish density of 25 kg·m$^{-3}$ (CS2) decreased by 39%. The model also illustrated that if fish at the downstream edge of the cage are to utilize feed optimally at 20°C, the cage diameter should not exceed 29.4 m. Finally, with a temperature increase of 10°C, the minimum flow through the cage to sustain optimal feed utilization is increased by 7.2 cm/s (CS3).

3.5 Statistics

3.5.1 Carryover effects on SMR

SMR data in 10, 15 and 20°C showed homoscedasticity (Levene’s, F = (0.01, 0.08, 0.93), p = (0.93, 0.79, 0.36)), respectively. There were no statistical significant differences between SMR means as determined by independent T-tests for 10, 15 and 20°C were (t(8) = (-1.34, 0.11, -0.30), p = (0.22, 0.92, 0.78), respectively. Hence, no carryover effect was present.

3.5.2 Normoxia experiments

Data for the SMR, MMR and optimal swimming speed ($U_{opt}$) at 10, 15 and 20°C were normally distributed (Shapiro-Wilk: SMR: W(28) = 0.98, p = 0.88; MMR: W(28) = 0.99, p = 0.97; $U_{opt}$: W(28) = 0.96, p = 0.36) and showed equal variance (Levene’s test, SMR: F(2,25) = 1.77, p = 0.19; MMR: F(2,25) = 2.08, p = 0.15; $U_{opt}$: F(2,25) = 0.8, p = 0.46). There was a significant difference between SMR, MMR and $U_{opt}$ means (one-way ANOVA, SMR: (F(2, 25) = 27.7, p < 0.01); MMR: (F(2, 25) = 4.51, p = 0.02); $U_{opt}$: (F(2, 25) = 21.8, p < 0.01)). The Tukey HSD test showed that statistically there was no difference in the means of SMR and $U_{opt}$ at 10 and 15°C. For MMR, the Tukey HSD test showed no significant difference between means at 15 and 20°C

3.5.3 Hypoxia experiments

In the hypoxia experiments, $P_{crit}$ at 0.4 and 1.0 Bl·s$^{-1}$ at 10, 15 and 20°C were both normally distributed (Shapiro-Wilk test, 0.4 Bl·s$^{-1}$: W(28) = 0.95, p = 0.17; 1.0 Bl·s$^{-1}$: W(28) = 0.96, p = 0.43), but only the 1.0 Bl·s$^{-1}$ showed equal variance (Levene’s test, 0.4 Bl·s$^{-1}$: F(2,25) = 4.78, p = 0.02; 1.0 Bl·s$^{-1}$: F(2, 25) = 2.0, p = 0.16). Using the Brown-Forsythe test the 0.4 Bl·s$^{-1}$ ($F$ =
(2,14.21), p = 0.054) showed no significant difference between means. This was also the case with
1.0 Bl·s⁻¹ data (One-way ANOVA, F(2, 25) = 2.7, p = 0.09).

The difference between the minimum required PO₂ for swimming at Uopt at different temperatures
was not normally distributed (Shapiro-Wilk: W(28) = 0.83, p < 0.01), and therefore the
nonparametric Kruskal-Wallis test was used (X²(2) = 16.1, p < 0.01) and showed there to be a
significant difference between groups. A Post Hoc test showed a statistical difference between all
group means except at 10 and 15°C.

4. Discussion

4.1 Swimming energetics – Normoxia

The standard metabolic rate increased with temperature from 10 to 20 °C, resulting in a Q₁₀ of 2.0
and 1.8 in the absolute and body mass adjusted values, respectively. Although a lower Q₁₀ was
found for adjusted SMR, it is still within the range for fishes 1.62 to 2.64 (Christensen et al., 2020;
Clarke and Johnston, 1999; Killen et al., 2016; Schurmann and Steffensen, 1997; Tirsgaard et al.,
2015). Significant increases were seen in SMR between 15 to 20°C and between 10 to 15°C in
MMR. This reflects the AS, which was greatest at 15°C. This is in accordance with the optimum
temperature (T₀pt)(Fry, 1947) in Rainbow trout, which at normoxia is approx. 15.8-17°C (Chen et
al., 2015; Jobling, 1981; Schurmann et al., 1991). At the T₀pt, the aerobic scope (AS) is highest,
i.e., the fish will have the highest surplus energy and best energy efficiency.

The optimal swimming speed increased significantly between 15 and 20°C. An increase in U₀pt
with increasing temperature is also reported in a study by Claireaux et al. (2006), where U₀pt in sea
bass (Dicentrarchus labrax) increased with temperature. The same was reported by Hoar and
Randall (1979) in Sockeye salmon (Oncorhynchus nerka).
4.2 Swimming energetics – Hypoxia

The mean critical oxygen tension for swimming at 0.4 and 1.0 Bl·s⁻¹ was not significantly different between temperatures. The same pattern was not seen at 1.0 Bl·s⁻¹, where the critical oxygen tension was unchanged or even decreased from 10°C to 15°C. This might be explained by the increase in AS in proximity to T_{opt}, resulting in increased hypoxia resilience.

With decreasing oxygen concentration, the appetite of the fish is reduced (Claireaux et al., 2000; Wang et al., 2009)

4.3 SDA experiments

The measured and theoretical SDA with and without flow resulted in similar percentages, and although the theoretical was higher on average, the calculated percentages were within the measured SDA standard error (Fig. 2). The reason behind measuring SDA during swimming was that the fish would be occupied by swimming, and therefore, spontaneous activity in form of aggression would be reduced (Adams et al., 1995; Christiansen and Jobling, 1990; Tudorache et al., 2013), resulting in more stable measurements. SDA measurements are essentially made even more complicated by adding another factor such as swimming. Therefore, the authors suggested that simplicity will yield better results, i.e., calculate a theoretical SDA plateau from resting fish fed once or use the method of continuous feeding to obtain the best results.

The SDA experiments also showed that a postprandial effect in Rainbow trout would persist for around 108h at 10°C and fed 2% of body mass after feeding is halted. A study by Eliason and Farrell (2014) found the duration of SDA in Rainbow trout fed 1% of body mass at 10-11.5°C to be around 50h. With a 50% reduction in feed rations, the SDA duration is roughly halved. The experiments also showed that SDA duration would be even longer if fish are forced to swim in holding tanks.
Models are valuable tools for researchers to explain aspects of nature that are difficult to grasp, and the field of aquaculture research is no different. Models for growth performance (Føre et al., 2016) and behavior (Føre et al., 2013, 2009) have been created for Atlantic salmon (Salmo salar) in sea cages, but to the author’s knowledge, no models have been developed that explain oxygen availability within sea cages. The distribution of oxygen concentrations within sea cages has been reported to fluctuate vertically (Solstorm et al., 2018) and being affected by cage sizes (Oldham et al., 2018) and fish densities (Johansson et al., 2006). Our model was created to assess the limits for fish density and cage sizes based on environmental and biological parameters. The case study using the model showed that with increasing temperatures and fish preferring to swim at a preferred swimming speed, the likelihood of the oxygen concentration reaching suboptimal conditions is high. If periods of hypoxic conditions occur within the cage, the lower oxygen content will have a limiting effect on aerobic swimming since the AS and MMR are reduced (Domenici et al., 2013). Oxygen concentration below the DO threshold (Pedersen, 1987; Remen et al., 2016) for feed utilization will also cause digestion to be suboptimal, and if the decrease continues, appetite will be reduced (Claireaux et al., 2000; Wang et al., 2009). This will cause a financial loss for the fish farmer and increase the harmful effects of farming on the surrounding environment due to feed sinking out of the cage. Many of the challenges proposed in the present study can be overcome if the current velocity is maintained above the flow threshold found in CS3 or if durations of low water flow are short (CS1). These challenges are especially apparent with high temperatures. One option is to move farming into more exposed areas. This approach will also ensure better water exchange in the cages and disperse discharge from the farms into the open ocean and will therefore reduce effects on the surrounding environment.
A graphical user interface for the model is available on the following webpage:

http://o2aquaculturemodel.pythonanywhere.com/

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Conflicts of Interest: The authors declare no conflict of interest

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**Abbreviations**

SMR - Standard Metabolic Rate

RMR - Routine Metabolic Rate

MMR - Maximum Metabolic Rate

MO₂ - Oxygen Consumption Rate

Uₖₘₗₜ - Critical swimming speed

Uₜₜₗₚₜ - Optimal swimming speed

Uₚromosome - Preferred swimming speed

Air sat. DO - Air saturated dissolved oxygen

AS - Aerobic Scope

Tₜₜₗₚₜ - Optimum temperature
References


Christiansen, J.S., Jobling, M., 1990. The behaviour and the relationship between food intake and


behaviour of Atlantic salmon (Salmo salar L.) in commercial-size aquaculture net pens: Model details and validation through full-scale experiments. Aquaculture 464, 268–278. https://doi.org/10.1016/j.aquaculture.2016.06.045


Tables

Table 1 Results from swimming energetics in normoxia (Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>10°C</th>
<th>15°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of fish (n)</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Fish mass (g)</td>
<td>1997.0 ± 338.7</td>
<td>1373.3 ± 333.4</td>
<td>1075.0 ± 124.0</td>
</tr>
<tr>
<td>Total length (cm)</td>
<td>51.1 ± 1.7</td>
<td>46.5 ± 2.5</td>
<td>45.1 ± 1.4</td>
</tr>
<tr>
<td>SMR (mg O₂ kg⁻¹ h⁻¹)</td>
<td>53.1 ± 10.7</td>
<td>60.5 ± 20.0</td>
<td>104.6 ± 10.4</td>
</tr>
<tr>
<td>SMR(1.5kg) - Body mass adj.</td>
<td>54.8 ± 12.3</td>
<td>60.1 ± 21.8</td>
<td>100.7 ± 10.4</td>
</tr>
<tr>
<td>MMR (mg O₂ kg⁻¹ h⁻¹)</td>
<td>298.2 ± 50.2</td>
<td>414.9 ± 100.5</td>
<td>393.1 ± 102.3</td>
</tr>
<tr>
<td>MMR(U_{opt}) - Body mass adj.</td>
<td>305.4 ± 47.3</td>
<td>389.3 ± 95.5</td>
<td>380.6 ± 91.8</td>
</tr>
<tr>
<td>Aerobic scope</td>
<td>5.9 ± 2.0</td>
<td>7.8 ± 5.0</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td>U_{crit} (Bl s⁻¹)</td>
<td>1.6 ± 0.2</td>
<td>2.1 ± 0.3</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>U_{crit} (cm s⁻¹)</td>
<td>78.8 ± 10.2</td>
<td>94.7 ± 9.1</td>
<td>87.9 ± 12.8</td>
</tr>
<tr>
<td>U_{opt} (Bl s⁻¹)</td>
<td>0.90 ± 0.17</td>
<td>1.10 ± 0.27</td>
<td>1.62 ± 0.26</td>
</tr>
<tr>
<td>U_{opt} (cm s⁻¹)</td>
<td>45.5 ± 9.4</td>
<td>51.2 ± 13.0</td>
<td>72.7 ± 11.1</td>
</tr>
</tbody>
</table>

Table 2 Results from swimming energetics in hypoxia (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>10°C</th>
<th>15°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_{crit} @ 0.4 Bl s⁻¹</td>
<td>4.49 ± 0.56</td>
<td>5.12 ± 1.31</td>
<td>5.48 ± 1.27</td>
</tr>
<tr>
<td>P_{crit} @ 1.0 Bl s⁻¹</td>
<td>5.79 ± 0.97</td>
<td>5.43 ± 0.57</td>
<td>6.33 ± 0.67</td>
</tr>
<tr>
<td>P_{crit} @ U_{opt} (min req)</td>
<td>6.23 ± 0.51</td>
<td>5.74 ± 0.37</td>
<td>7.80 ± 0.89</td>
</tr>
</tbody>
</table>
Table 3 Results from the case study using the model. CS refers to the Calculation Series discussed in 2.4.4 The model. CS1 is oxygen decrease over time with no current, CS2 is oxygen decrease through the cage with flow velocity at 5 cm/s, and CS3 is oxygen concentration affected by flow and swimming simultaneously. All results are based on fed fish swimming at $U_{opt}$.

<table>
<thead>
<tr>
<th></th>
<th>10°C</th>
<th>15°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen solubility (mg O$_2$ l$^{-1}$)</td>
<td>9.39</td>
<td>8.44</td>
<td>7.63</td>
</tr>
<tr>
<td>Air sat. DO threshold (mg O$_2$ l$^{-1}$)</td>
<td>4.86</td>
<td>5.57</td>
<td>6.13</td>
</tr>
<tr>
<td>CS1: Air sat. DO threshold (min)</td>
<td>59</td>
<td>33</td>
<td>10</td>
</tr>
<tr>
<td>CS1: 70% Air sat. DO (min)</td>
<td>37</td>
<td>29</td>
<td>15</td>
</tr>
<tr>
<td>CS2: Downstream edge (mg O$_2$ l$^{-1}$)</td>
<td>8.1</td>
<td>6.9</td>
<td>5.0</td>
</tr>
<tr>
<td>CS2: Air sat. DO threshold $\text{max cage (m)}$</td>
<td>177.7</td>
<td>98.1</td>
<td>29.4</td>
</tr>
<tr>
<td>CS3: Air sat. DO threshold (flow: cm s$^{-1}$)</td>
<td>1.5</td>
<td>2.8</td>
<td>8.7</td>
</tr>
</tbody>
</table>
Figure captions

Figure 1 Swiming energetics in normoxia and hypoxia. Plots A, B, and C illustrate swimming energetics (-) with SDA (shaded area) from the normoxia experiments at 10, 15, and 20°C, respectively. Cost of transport (COT) is also illustrated on each plot with corresponding horizontal bars showing the average optimal swimming speed. Plots D, E, and F show MO2 measurements from the hypoxia experiments, at 10, 15, and 20°C, respectively. Swimming energetics in hypoxia at 0.4 and 1.0 Bl s⁻¹ and MMR are fitted to the Hill equation.

Figure 2 SDA experiments: Plot A. and B. show the SDA results after fish were fed 2% of body mass with and without current. Plot C. and D. show results of SDA experiments while fish are being fed 1% of body mass each day until cutoff. A cumulated SDA based on values from Plot A. and B. is illustrated in plots C. and D.

Figure 3 Heatmaps of flow velocity at three different depths. The black arrow illustrates where the pump was mounted and in which direction it was pumping. The nozzle of the pump was located at a depth of 15 cm and tilted slightly downwards. The pump generated a flow ranging from 10 to 50 cm s⁻¹.

Figure 4 Results from the case study using the model. The three columns show temperature change (10, 15, and 20°C). On each plot, the DO threshold for suboptimal feed utilization is illustrated as horizontal lines (Dotdashed). The first row is the duration to oxygen depletion (CS1) in fed and unfed fish at rest or swimming at Uopt. When oxygen concentration decreases below the minimum requirement for swimming at Uopt, the fish will decrease swimming speed and thereby oxygen consumption (@Uopt limit). The second row of plots illustrates oxygen concentration through the cage in fed and unfed fish, swimming at Uopt with a current velocity of 5 cm/s (CS2). The final row of plots illustrates oxygen concentration when fed and unfed fish are swimming at the same
speed as the current velocity and when fish are swimming at $U_{opt}$ until swimming speed matches with current velocity, then swimming speed, and current velocity are the same (CS3).
Figure 1
Figure 2
Figure 3

Depth: 15cm

Depth: 35cm

Depth: 70cm

Flow velocity (cm$^{-1}$)

10

15

20

25

30

35

40

45

50
Figure 4
MS3: Aquaculture fish farming in an exposed area: Is oxygen a limiting parameter

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Abstract

One of the benefits of moving aquaculture production from protected to more exposed areas, is an increase in water flow and thereby a greater water exchange within the cages. The increased water exchange should ensure adequate oxygen availability, but since these areas may be affected by tidal changes, periods of reduced or even no current will occur. During these periods, fish respiration will cause a decrease in oxygen in sea cages, but it is unknown if oxygen reaches suboptimal levels. To test if suboptimal oxygen levels occur within sea cages in relatively exposed areas, the water column within an aquaculture sea cage was monitored using a profiling CTD. Furthermore, current velocities were acquired from the site using an ADCP (Acoustic Doppler Current Profiler). All field measurements were fitted to a physiology-based model, which determines oxygen concentrations based on physical and chemical parameters. In addition, field measurements were compared to free and open Copernicus Marine Service data, to determine if modeled data can be used on a local scale.

Results showed that even in relatively exposed areas, fluctuations in oxygen concentration within sea cages occurred. Decreases were apparent during slow-moving water, caused by tidal change, and the magnitude of oxygen decrease was closely related to the duration of slow-moving water.
In the present study, 0.3% of oxygen measurements in the sea cage decreased below the oxygen concentration threshold for suboptimal utilization of feed. Therefore, the study concludes that oxygen availability is not an issue in relatively exposed studied area, but may be in large size cages and with higher fish densities.

Key Words: Aquaculture, CTD, Copernicus Marine Service, Rainbow trout

1. Introduction

Fluctuations in oxygen concentrations inside aquaculture sea cages located in protected areas are well documented (Burt et al., 2012; Johansson et al., 2007, 2006; Oldham et al., 2018; Oppedal et al., 2007; Solstorm et al., 2018). Fluctuations are mainly caused by fish respiration and low current velocities, limiting water exchange within the cages. Increased flow through the cages can be achieved by moving production to more exposed areas or even offshore (Hvas et al., 2020). Oxygen availability is normally assumed not to be an issue when farming in exposed areas, but these areas are still affected by tidal changes and slack water. Slack water is the relative short period when the tidal stream reverses and there is no flow. Although the duration of slack water is brief, before and after slack water the flow velocity is reduced, and water exchange from the ambient to the cages is limited.

The limited water exchange will affect oxygen concentration, especially in cages with higher fish densities and thereby higher oxygen consumption. The magnitude of the decrease in oxygen concentration depends on the flow velocity and duration of reduced flow. The oxygen threshold for when fish suboptimally utilized feed increases with temperature (Pedersen, 1987; Remen et al., 2016), i.e., during warmer periods, an even relatively minor decreases in oxygen concentration will affect food digestion.

In the present study, the aim was to measure oxygen concentration, salinity, temperature, flow velocity, and direction inside of an aquaculture sea cage located in a relatively exposed area. There is limited understanding of oxygen concentrations within Danish aquaculture sea cages since
regular practice is to measure oxygen from the feeding barge next to the cages. The data gathered should provide an in-depth understanding of the physical and chemical parameters at work inside a sea cage throughout the season.

In a study by Bergsson et al. (Thesis-MS2) a model was created that predicts oxygen concentration based on environmental and biological variables. The measured parameters from the present study are fitted to the model to explain oxygen fluctuations within the cage.

Furthermore, the study evaluates if the free and open data from Copernicus Marine Service is reliable on a local scale, by comparing it to measured field data. If reliable, it can be used as a proxy for aquaculture sites, resulting in a greater understanding of the suitability of present aquaculture locations without onsite measurements. The application can also be used to assist in the determination of new locations suitable for aquaculture.

2. Material and methods

2.1 Study location

One of the most exposed aquaculture sites in Danish waters is located in the Great Belt (DK: Storebælt) off the west coast of the island of Musholm (55°28'47.0"N 11°03'23.5"E). The Great Belt is the main water passages between the Baltic Sea and the Atlantic Ocean.

The site consisted of 12 circular cages positioned north to south and measurements in the present study were acquired from the southernmost of these cages during the farming season 2020 (May to September).

2.2 Sea cage

The sea cage used in the present study was a circular cage with a circumference of 160 m and a depth of 8 meters, resulting in a volume of 16297.5 m$^3$. The cage was stocked with Rainbow trout ($Oncorhynchus mykiss$ (Walbaum, 1792)), and the fish density normally increased from approx. 8 to 25 kg/m$^3$ throughout the season. Due to logistics, measuring equipment was deployed three
weeks after stocking the cages. To minimize interference with the harvest, equipment was retrieved three weeks before harvest. Assuming an exponential fish growth in monitored the cage, measurements were acquired with fish densities ranging from 9 to 21 kg/m$^3$.

2.3 Equipment and field measurements
An autonomous measuring buoy (APB5, SAIV A/S, Bergen, Norway), fixed in the middle of the sea cage, was used to acquire environmental data. The buoy profiled the water column using a CTD (SD208, SAIV A/S, Bergen, Norway) with a built-in oxygen probe (RINKO III, JFE Advantech Co. Ltd., Nishinomiya, Hyogo, Japan). Flow velocity and direction were acquired using an ADCP current profiler (Aquadopp Z-Cell 600 kHz, Nortek, Rud, Norway) mounted on the feeding barge next to the cage. To compare oxygen concentration inside and outside the cage, O$_2$-loggers (MiniDOT, Precision Measurement Engineering, Inc., California, USA) were suspended from mooring lines adjacent to the cage at depths of 4 meters up and downstream (north and south).
Visual and sonar data from within the cage were acquired using a custom solution, based on a Raspberry Pi 3 (RPi3, Raspberry Pi Foundation, Cambridge, UK) fitted into a 3” watertight enclosure and connected to a Ping Sonar (Blue Robotics, California, USA) with a 30$^\circ$ beam. Video footage was captured using a 5MP Wide Angle camera (OV5647, Jectse) mounted inside the enclosure. The setup was powered by and mounted on the side of the APB5 buoy.

Data acquisition frequency ranged from 1 to 30 min depending on the equipment, and all data, except the MiniDOT O$_2$-loggers, was continuously uploaded to a custom private cloud via 4G network.

2.4 Models
An alternative method of acquiring environmental data is via the E.U. Copernicus Marine Service, providing free and open marine data. In the present study, the "BALTICSEA_ANALYSISFORECAST_PHY_003_006" dataset was used (Spatial resolution: 2x2 km). Since the aquaculture site was located between grid points, a new point was created by
extrapolating values in relation to the site's location using neighbouring grid cells (Fig. 1). The values used from the Copernicus dataset were temperature, salinity, flow velocity, and direction from the measuring period (Jun-Aug 2020).

Variances in oxygen concentration within the cage were compared to the model created in a study by Bergsson et al. (Thesis-MS2). The model estimates oxygen concentrations within aquaculture sea cages based on changes in environmental and biological parameters. One of the model outputs used in the present study is estimates of oxygen concentration based on flow through the cage. The model also considers postprandial effects or Specific Dynamic Action (SDA), i.e., the increase in oxygen consumption when fish are fed (Chabot et al., 2016; Jobling and Davies, 1980; Jordan and Steffensen, 2007; Tirsgaard et al., 2015). During normal operations in aquaculture farms, the fish are fed, and therefore, the model for fed fish is appropriate to use in the present study.

2.5 Theory/calculations

The analysis was performed using python 3.8.5 (Python Software Foundation (Van Rossum and Drake Jr, 1995), available at www.python.org) with data analysis packages (Numpy (Harris et al., 2020), Pandas (McKinney, 2011) and SciPy (Virtanen et al., 2020)) to compare the measured parameters with the predicted model data.

2.5.1 Measured data

As water flows through cage netting, the velocity decreases (Winthereig-Rasmussen et al., 2016). In a review by Klebert et al. (2013), decreases in flow velocity without fish are noted to range between 15 to 20%. The decrease percentage will vary depending on the type of netting, biofouling, and fish density in the cage (Klebert et al., 2013). Since the current profiler was mounted on the feeding barge next to the cage, and the effects on flow velocity caused by fish densities are unknown, the measured flow velocity was reduced by 20% to simulate flow within the cage in accordance with Klebert et al. (2013). It was unfortunately impossible to use the ADCP in the cage due to high density of fish in the cage. Thresholds for slow water flow were chosen as
2.5 and 5.0 cm/s. To determine the duration for slow-moving water, the simulated flow velocity time-series was used. The duration was determined as the delta time from when flow decreased below the threshold to when the flow increased above the threshold again. To find the average duration for flow below 2.5 and 5.0 cm/s, Poisson distributions were fitted to the data.

The aim of the sonar data was to determine fish distribution within the cage. Signals from the sonar were averaged for each meter. Since the sonar beam angle is 30°, the covered area will increase with depth. Although the coverage will be greater with depth, the signal from a single fish will also be weaker due to the relative fish size with distance to sonar. Therefore, a relative sonar signal was calculated, based on the signal cone volume at signal depth and relative size of fish at that distance. This can be expressed using the following equations.

\[
SD_{rel} = \frac{SD_{\%}}{SB_{vol}/\left(\frac{18665}{D-1}\right)}
\]  

Eq. 1

Where \(SD_{rel}\) is the relative Sonar Data when using a 30° sonar beam at a given depth, \(SD_{\%}\) is the percentage of the total Sonar Data at depth D, \(SB_{vol}\) is the volume of the oblique (0-1m) or truncated Sonar Beam cone, and D is the depth in meters.

2.5.2 Measured and modeled data

The Copernicus data have an hourly resolution, and in order to compare it to the measured data, which had an acquisition rate of 30 min, the measured data were averaged for each hour.

In order to compare measured oxygen concentrations with the physiology-based \(O_2\) aquaculture model from Bergsson et al. (Thesis-MS2), swimming energetics for the same study are used. The swimming energetics are for three different temperatures. To fit measured data to the model, the data were grouped into the following three temperature groups: 10°C group (10±2.5°C), 15°C group (15±2.5°C), and 20°C group (20±2.5°C).
For each temperature group, a corresponding fish density, based on the time of the season when each temperature had the highest representation, was used. The fish density was used in the model to predict total oxygen consumption.

To quantify the fit between predicted and measured data, the $R^2$ was calculated for each temperature against modeled data with and without SDA. Since the predicted data is nonlinear, the following equation was used:

$$R^2 = 1 - \left( \frac{SS_e}{SS_t} \right)$$  Eq. 2

Where $SS_e$ is the sum of squares of the residuals and $SS_t$ is the sum of squares of the difference between measured and the average of measured data.

2.6 Statistics

All statistical analyses were performed using IBM SPSS Statistics, version 27.

For each of the compared datasets, the normality of data was checked using Shapiro-Wilk tests.

To compare CTD and ADCP data to Copernicus data, the correlation between data was checked using the Spearman Correlation Coefficient. Thereafter the Wilcoxon Signed-Ranks Test was used for each comparison. The same statistical tests were used to compare oxygen concentration inside and outside the cage.

The statistical level of significance was set at $\alpha=0.05$ for all statistical tests.

3. Results

3.1 Field measurements and Copernicus data

Averages of measured and modeled data are listed in Table 1 and visualized in Fig. 1 and 2.

Although both data types show the same trends, the measured data had a higher variance in directions (Fig.1). The measured and modeled flow velocity showed a significant positive correlation, but were still significantly different. The average duration for simulated flow velocities
within the cage below 2.5 and 5.0 cm/s were 7.3±5.6 min and 14.2±9.2 min, respectively and the longest durations for the two thresholds were 31.3 and 38.7 min (Fig. 3).

The measured and modeled temperature and salinity averages were both significantly different although there was a strong significant positive correlation (Fig. 2).

On retrieval of the O₂-loggers, the sensor on the logger mounted on the north side of the cage was covered by biofouling, and analysis of the data revealed only three weeks of usable data. Therefore, only data for the O₂-logger mounted on the south side of the cage was used for further analysis.

The majority of oxygen concentration measurements below 80% were found with currents moving in a westerly direction for both inside and outside of the cage, hence during tidal change.

As expected, the raw sonar signals were strongest near the bottom of the cage, since the beam covered a greater area with depth, although it should be mentioned that single fish closer to the sonar will result in a stronger signal, since they are relatively larger than fish near the bottom.

3.2 O₂ aquaculture model

The 10, 15, and 20°C groups contained 149, 2063, and 1456 measurements of oxygen concentrations from within the cage, respectively. The goodness of fit ($R^2$) between the model and measurements were 0.71 and 0.73 (10°C), 0.78 and 0.69 (15°C) and 0.34 and -0.62 (20°C) unfed and fed (SDA) (Fig. 4).

3.3 Statistics

Measured and modeled flow velocity, temperature and salinity data were not normally distributed (Shapiro-Wilk: Flow velocity: $W(3486) = 0.89$, $p < 0.01$; Temperature: $W(3378) = 0.99$, $p < 0.01$; Salinity: $W(3378) = 0.91$, $p < 0.01$).

Since data was not normally distributed, nonparametric correlations tests were used to check for correlation significance. There were strong positive significant correlations between measured and modeled temperature and salinity data (Spearman's correlation coefficient, Temperature: $r_s(1687) = 0.96$, $p < 0.01$, Salinity: $r_s(1687) = 0.92$, $p < 0.01$). The correlation between measured and
modeled flow velocity data was also significant with a positive correlation (Spearman's correlation coefficient, $r_s(1741) = 0.20$, $p < 0.01$).

Furthermore, all comparisons between measured and modeled data were significantly different (Wilcoxon signed-rank test, Flow velocity: $Z = -10.56$, $p < 0.01$, Temperature: $Z = -31.29$, $p < 0.01$, Salinity: $Z = -34.48$, $p < 0.01$).

The oxygen concentration between the inside and outside of the cage showed a significant positive correlation (Spearman's correlation coefficient, $r_s = 0.34$). Moreover, a significant difference was found between the two datasets (Wilcoxon signed-rank test, $Z = -3.28$, $p < 0.01$).

4. Discussion

In general, the measured and modeled (Copernicus) data showed the same trends, especially temperature and salinity (Fig. 1). The high correlation value but significant difference in averages between measured and model temperature and salinity indicates that there is an even offset throughout the data, likely caused by differences in measuring equipment and/or calibrations. By subtracting the average difference between measured and modeled data (Temperature: 0.46℃, Salinity: 1.91) and running the statistical analysis again, neither the measured or modeled temperature and salinity were significantly different. The measured and modeled flow had a lower positive correlation and even with the average difference subtracted (Flow: 0.034 m/s), the data was still significantly different. There are several possible reasons for this outcome. One could be that measured data has a higher resolution as opposed to modeled data. This is particularly apparent in the flow directions (Fig. 1). Another reason could be the location of the ADCP, which was mounted on the side of the feeding barge. The barge was located in between cages and it is therefore possible that the cages affected both the current direction and velocity with water being shunted around the cages.

The prevailing currents in both measured and modeled data were north-northwest and south-southeast. This is as expected since there is landmass on the east and west side of the location (Fig.
1. As with flow direction, the flow velocities were also highest in these two directions and related to the tide.

4.1 Field measurements

The optimal temperature ($T_{opt}$) for Rainbow trout ranges from 14.3 to 18.6 °C (Chen et al., 2015; Jobling, 1981; McMahon et al., 2008; Schurmann et al., 1991) and with critical thermal minima ($CT_{min}$) at approx. 0.0°C (Currie et al., 1998) and maxima ($CT_{max}$) between 26.5 and 29.8 °C (Alabaster and Welcomme, 1962; Currie et al., 1998). During the present study, temperatures ranged from 11.5 – 21.5 °C, with 76.7% of measurements within the optimal range for Rainbow trout.

Having an anadromous lifecycle, Rainbow trout will tolerate salinity ranges from 0-35 (Molony, 2001). During the present study, salinity ranged from 10.1 to 19.3, which is well within the tolerance range. More importantly, the salinity never decreased below 9 (iso-osmotic) (Delompré et al., 2019; Morgan and Iwama, 1991). Hence fish were not required to switch between osmoregulation states.

The optimal swimming speed ($U_{opt}$), the speed where cost of transport is the lowest, of unfed Rainbow trout at 10, 15 and 20°C is 0.9, 1.1, and 1.6 Bl/s, respectively (Thesis-MS1, Thesis-MS2). Assuming the average swimming speed was equal to the simulated flow within the cage and considering that fish are growing throughout the season and that optimal swimming speed increases with temperature (Thesis-MS2, Claireaux et al. (2006), Hoar and Randall (1979)), the maximum forced (by current) swimming speed never exceeded $U_{opt}$, i.e., the fish were not forced to swim faster than $U_{opt}$.

Video footage from the sea cage showed that Rainbow trout are always swimming, but at which speed is still unknown. In a study by Tudorache et al. (2011) the preferred swimming speed of Brook charr ($Salvelinus fontinalis$) was found to equal the optimal swimming speed. Likewise, in a study by Bergsson et al. (Submitted, Thesis-MS1), the same was found for unfed as well as fed...
Rainbow trout at 10°C. It is therefore likely that fish in aquaculture sea cages swim at or close to their optimal swimming speed.

The oxygen threshold for when fish suboptimally utilize feed increases with temperature (Pedersen, 1987; Remen et al., 2016). In Bergsson et al. (Thesis-MS2), the data from Pedersen (1987) and Remen et al. (2016) was fitted by linear regression. By applying the resulting equation to the measured temperature data, the threshold of suboptimal utilization of feed was determined. Subtracting the threshold from measured oxygen concentrations showed that throughout the season, only 0.3% of oxygen concentration measurements were below the threshold. These results reflect well with the measured durations of slow-moving water (Fig. 3), which on average were 14.2±9.2 and 7.3±5.6 min below 5.0 and 2.5 cm/s, respectively. The durations of slow-moving water are not long enough to cause a severe decrease in oxygen concentration but higher fish densities or larger diameter cages with the same fish density might be problematic.

The sonar data estimates of fish density showed that fish are somewhat evenly distributed, with the majority of fish located between 3-7 meters through the season. Using the correction (Eq.1), the fish distribution was highest in the top 3 meters (Table 2). This illustrates the issues with using a top-down sonar as opposed to a bottom-up sonar, which can be mounted on the bottom, several meters from the bottom of the cage. For the top-down method to be applicable, the cone volume for each measurement must be greater than 1-2 m³, to reduce the bias of fish swimming close to the sonar. This is also apparent in Table 2 in the corrected data. If the top 2 meters are excluded there is a somewhat even distribution according to the relative sonar signal ranging from 7.7 to 10.4. The bottom meter (7-8m) contains the sink-ring and the cone netting (bottom net) and it is therefore not likely to have the same fish density as the rest of the cage.
4.2 \( \text{O}_2 \) Aquaculture model

The goodness of fit \((R^2)\) for the 10 and 15°C groups was above 70 for the unfed regression and approximately 70 for the fed regression. Hence, 70% of the variation in oxygen concentration can be explained by the change in water current. The 10°C group only contained 149 oxygen measurements, and therefore the \( R^2 \) is not as reliable as the 15°C group with 2063 oxygen measurements.

The 20°C group had a low \( R^2 \) for the unfed regression (0.34) and a negative \( R^2 \) for the fed regression even though the group contained 1456 oxygen measurements. A probable reason for the low \( R^2 \) can be found in the ambient oxygen tension. Since data from the 20°C group was acquired during summertime, more sunlight can be expected, which in turn increases photosynthesis, causing oxygen supersaturation. Considering only physiological factors and respiration physiology of fish, the \( \text{O}_2 \) aquaculture model does not take photosynthesis into account. This is likely the reason for the poorer fit.

4.4 Perspectives

The present study showed that the duration of slow-moving water is short and therefore oxygen concentrations rarely decrease below thresholds for suboptimal feed utilization. This is also the case during warmer months, even though the oxygen consumption increases and oxygen solubility and hence concentration is reduced.

Future research in oxygen concentration within aquaculture cages could include a more in-depth analysis of the fish’s swimming speed, as at least Rainbow trout seem to be swimming at a relatively high speed even in low current velocities.

The usability of Copernicus Marine Service data as an alternative to measurements in the field is viable, although local measurements result in higher data resolutions. One of the true benefits of using Copernicus data is the forecast feature, which gives estimates of parameters six days in advance. By combining Copernicus data (flow velocity, temperature and salinity) with the \( \text{O}_2 \)
aquaculture model, estimates of expected decreases in oxygen concentration can help fish farmers reduce feeding when not optimal for the fish, hence also reduce feed waste for the benefit of the environment, and the overall optimization of production.

Acknowledgments

The authors would like to express our sincere gratitude to the aquaculture staff at Musholm, which helped with the deployment and retrieval of equipment, diving for sensors and general willingness to help with the project. The present study has been conducted using E.U. Copernicus Marine Service Information.

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**Table 1** Overview of measured and modeled parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured</td>
<td>Modeled</td>
</tr>
<tr>
<td>Velocity (cm/s)</td>
<td>2.3 – 73.8</td>
<td>1.1 – 59.6</td>
</tr>
<tr>
<td></td>
<td>14.0 ± 8.3</td>
<td>17.2 ± 10.9</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>11.5 – 21.5</td>
<td>11.4 – 20.9</td>
</tr>
<tr>
<td></td>
<td>16.7 ± 2.0</td>
<td>16.5 ± 2.4</td>
</tr>
<tr>
<td>Salinity</td>
<td>10.1 – 19.3</td>
<td>9.3 – 17.6</td>
</tr>
<tr>
<td></td>
<td>14.2 ± 2.5</td>
<td>12.2 ± 2.4</td>
</tr>
</tbody>
</table>

|                     | Inside      | Outside      |
| Oxygen (mg O₂ l⁻¹)  | 6.0 – 9.8   | 6.3 – 12.0    |
|                     | 8.6 ± 0.6   | 9.4 ± 0.6     |
| Oxygen (% air sat)  | 65.4 – 108.2| 65.4 – 124.2  |
|                     | 97.2 ± 5.6  | 97.1 ± 4.6    |

**Table 2** Sonar data.

<table>
<thead>
<tr>
<th>Depth m</th>
<th>Sonar signal % ± SD</th>
<th>Beam m³</th>
<th>Rel. sonar signal Eq. 1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>5.5 ± 7.0</td>
<td>0.075</td>
<td>37.0</td>
</tr>
<tr>
<td>1-2</td>
<td>8.5 ± 10.6</td>
<td>0.53</td>
<td>16.3</td>
</tr>
<tr>
<td>2-3</td>
<td>9.8 ± 11.0</td>
<td>1.43</td>
<td>10.4</td>
</tr>
<tr>
<td>3-4</td>
<td>12.6 ± 10.4</td>
<td>2.78</td>
<td>9.1</td>
</tr>
<tr>
<td>4-5</td>
<td>15.1 ± 11.0</td>
<td>4.59</td>
<td>8.3</td>
</tr>
<tr>
<td>5-6</td>
<td>19.4 ± 13.4</td>
<td>6.84</td>
<td>8.6</td>
</tr>
<tr>
<td>6-7</td>
<td>20.8 ± 15.5</td>
<td>9.55</td>
<td>7.7</td>
</tr>
<tr>
<td>7-8</td>
<td>8.0 ± 10.7</td>
<td>12.71</td>
<td>2.6</td>
</tr>
</tbody>
</table>
Figure captions

**Figure 1** Map of the area around the aquaculture site with superimposed wind rose plots of measured and modeled current velocity and direction. Measured data were acquired using an ADCP mounted on the feeding barge at the site.

**Figure 2** Field measurements and model data. A. Measured flow velocity of the site compared to modeled data from the Copernicus Marine Service. A. Frequency distribution of measured and modeled flow data and simulated flow within the cage. B and C. Measured and modeled temperature and salinity. B and C. Frequency distribution of temperature and salinity. D. Measured oxygen concentration inside and outside of the cage. D. Frequency distribution of measured and modeled oxygen concentration. E. Measured and predicted Oxygen concentration. The predicted oxygen concentration is based on fish swimming at the same speed as the current and fish swimming at $U_{\text{opt}}$ until current velocity exceeds $U_{\text{op}}$, then same speed as the current.

**Figure 3** The frequency distribution of the duration, current velocity remained below 2.5 and 5.0 cm/s.

**Figure 4** Results from the comparison between measured data and the $O_2$ aquaculture model used in the present study.
Figure 1
Figure 3

Simulated flow in cage
- Duration < 5 cm/s
- Duration < 2.5 cm/s
- Mean ± SD: 14.2 ± 9.2 min
- Mean ± SD: 7.3 ± 5.6 min