STUDYING BEHAVIOR OF CULTURED ATLANTIC SALMON (*SALMO SALAR*) USING 3D ACOUSTIC TELEMETRY IN NOVA SCOTIA, CANADA

by

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Dalhousie University is located in Mi'kma'ki,
the ancestral and unceded territory of the Mi'kmaq.
We are all Treaty people.

For my dad who never wanted me to work with sharks…

…there is still time.

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ABSTRACT

Climate change is altering ocean environments worldwide, requiring a better understanding of how these shifting will affect fish welfare in aquaculture farms. Storms have increased in strength and frequency, potentially affecting fish welfare. Furthermore, ocean temperature rise and increased occurrence of low oxygen events affect fish behavior, physiology, and health. Farms worldwide have begun to experiment with oxygen supplementation systems to counteract low oxygen from climate change. In this thesis, high resolution, high frequency acoustic tags were used to track movement of Atlantic salmon at two commercial fish farms in Nova Scotia, Canada. The positioning of 30 fish was recorded to characterize fish movement through four variables: depth, velocity, distance from center, and turning angle. A baseline of fish behavior was determined and the effects of temperature, dissolved oxygen, feeding, storms, and an oxygen supplementation system were studied. Overall, this thesis provides information on fish behavior leading to improved understanding of fish welfare in a changing climate.

LIST OF ABBREVIATIONS USED

DO	Dissolved Oxygen
GAIN	Green Aquaculture Intensification
HR2	Hydroacoustic Receiver 2
HPE	Horizontal Positioning Error
NSERC	National Science and Engineering Research Council of Canada
NSEW	North, South, East, West
OFI	Ocean Frontier Institute
OWI	Operational Welfare Indicators
RMSE	Root Mean Square Error
TMS	Tricaine mesylate

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CHAPTER 1 : GENERAL INTRODUCTION

Fish welfare is a complex concept with different definitions that ultimately fall into three main categories: 1) Feelings-based, 2) Function-based, and 3) Nature-based definitions (Huntingford *et al*., 2006). Feelings-based definitions require subjective mental states of fish requiring them to feel well and be free from pain or fear (Dawkins, 1998). Function-based definitions require fish to be in good health and not forced beyond their biological capacities (Segner *et al*., 2012). Nature-based definitions follow the guidelines of fish with good welfare will express their natural swimming behaviors (Huntingford *et al*., 2006).

Function-based definitions are often the most commonly used to understand welfare of farmed fish as it is the easiest to measure and observe (Huntingford *et al*., 2006). Ocean net pen aquaculture can affect fish welfare and health as a result of aquaculture practices or a changing environment (Føre *et al*., 2017). Some of these practices include high stocking density (or over stocking), and poor handling techniques. Environmental threats include storm events, increasing water temperatures, and decreasing dissolved oxygen levels. Determining the effects of these factors on fish health and welfare requires the understanding of both physiological and behavioral measurements (Ashley, 2007).

Many of the known physiological and behavioral indicators are linked with environmental changes. Water temperature, dissolved oxygen, light intensity, feed, and water currents are some of the most studied variables affecting physiology and behaviour of cultured salmon (Johansson *et al.*, 2006, 2009; Oldham *et al.*, 2018; Martins *et al.*, 2011). Deviations from optimal conditions can lead to physiological and behavioral changes to cope with suboptimal conditions, despite of which decreased welfare can emerge.

To counteract negative effects on fish health, farmers have begun to experiment with techniques to keep environmental conditions as close to optimal as possible. In the case of maintaining dissolve oxygen levels, these techniques involve installing aeration or oxygen supplementation systems on site to increase or maintain dissolved oxygen levels within cages. There are several oxygenation techniques, which are explained in chapter 3, but it essentially involves installing dissolved oxygen loggers and infrastructure that pumps either nanobubbles of oxygen or oxygenated water into the cage. This allows the oxygen levels to be monitored and maintained should levels drop too low leading to potential stress on the individuals. The relationship between behavior and welfare can be determined by understanding these abnormal behaviors as it links with stressors (Martins *et al.*, 2011; Damsgård *et al.*, 2019). Ongoing efforts emphasize improvement of fish welfare and optimization of farming procedures, which has led to an increase in environmental and fish technology on farms as compared to the start of aquaculture (Føre *et al*., 2018).

For some species, such as Atlantic salmon (*Salmo salar*), it is difficult to relate actions independent of its triggers, meaning it is difficult to understand behavioral responses separate from the potential cause. So as to study these behavioral changes, two experiments were designed (chapters 2 & 3). A method of using fish positioning to infer fish behavior which then can advise on fish welfare were used throughout the thesis. In chapter 2, the effects of environmental conditions on fish swimming and behavior were analyzed, using the following fish variables: depth, swimming speed, horizontal distance, and turning angle. Fish positioning data used to calculate these variables were collected via acoustic tags over a 5-month period. Environmental conditions, as well as aquaculture practices, were studied to assess events that might cause changes in swimming behavior such as rapid change in temperature and DO, feeding periods, and storms.

In chapter 3, the effects of an oxygen supplementation system, and associated changing environmental conditions, on fish behavior and positioning were investigated. Acoustic tags were used to record fish positioning over a 5-month period and the same fish variables were calculated to characterize fish movement and behavior as in Chapter 2. An oxygen supplementation experiment operated for approximately one month during the study period to keep DO within net pens at practical thresholds (no lower than 6.0 mgL^{-1}), allowing behavior to be investigated during and after the project.

By studying fish movement and behavior at two different locations under different environmental conditions, a baseline behavior of individuals can be established for Nova Scotia cultured salmon. The two datasets offer information on fish positioning and behavior that can help characterize general movement patterns during normal conditions, and any deviations during stressful conditions. For example, understanding diel patterns on a cold day versus a warm day, and how this could change feeding habits. The methods deployed in this study can be applied to sites worldwide, but each geographical location will have different environmental conditions. Therefore, the outcomes of this study cannot be applied elsewhere without considering the specific environmental conditions. The ultimate goal of this study is to help farmers identify and improve fish welfare by employing a function-based definition of welfare.

CHAPTER 2 : DETERMINING THE EFFECTS OF ENVIRONMENTAL EVENTS ON CULTURED ATLANTIC SALMON BEHAVIOR USING 3-DIMENSIONAL ACOUSTIC TELEMETRY

2.1. Abstract

The health and welfare of farmed fish are highly dependent on environmental conditions. Under suboptimal conditions, the negative impact on welfare can cause changes in fish behavior. Acoustic tags can provide high resolution and high frequency data to monitor fish positioning within the cage, which can be used to infer swimming behavior. In this study, implanted acoustic tags were used to monitor the three-dimensional positioning of Atlantic salmon (*Salmo salar*) at a commercial farm in Nova Scotia, Canada. The one-month study period allowed the characterization of background behavior and changes in behavior in relation to different environmental conditions, namely, water characteristics in terms of dissolved oxygen and temperature caused by the fall overturn, storm conditions, and feeding activity. The three-dimensional position of 15 fish was recorded using high temporal resolution (3 s). Fish movement was characterized by calculating four fish variables: distance from the center of the cage [m], depth [m], velocity [ms^{-1}], and turning angle [°]. The population swam in a counterclockwise swimming direction around 4 ± 2 m depth at an average speed of 0.61 ± 0.38 ms^{-1}. After the fall overturn, the population moved significantly towards cage center while decreasing velocity, and non-significant differences in depth and turning angle were observed. During feeding periods, a significant increase in depth and velocity, as well as a reduction in turning angle were observed. The storm event did not cause any significant change in the four fish variables. While some of the behavioral changes were difficult to assess with respect to causation, the high resolution, high frequency data provide unique detailed positioning information to further our understanding of the swimming behavior of farmed fish.

2.2. Introduction

Farmed Atlantic salmon are subject to a variety of environmental stressors, including diseases, marine heatwaves, harmful algal blooms, and anoxic events. For example, the high stocking density in net pens can lead to ideal conditions for bacterial infections and diseases (Sindermann, 1984). Beyond disease and parasites, temperature and water quality can be highly variable in net pens due to vertical stratification and diffusion-advection of water masses due to tides and winds. Since fish are highly mobile, their ability to change positioning within the net pen can be critical in stress avoidance. Accordingly, fish movement can provide insights on behavior, defined as a 'representation of a reaction to the environment as a fish perceives it' (*sensu* Martins *et al*., 2011).

Water temperature has long been studied with respect to fish behavior (Ogilvie & Anderson, 1965; Javaid & Anderson, 1967; Elliott & Elliott, 2010; Oppedal *et al*., 2011). For example, Atlantic salmon (*Salmo salar*) are known to be temperature sensitive, and when a gradient is present, e.g. a thermocline, the fish will distribute themselves according to preferred temperature ranges (Coutant, 1977; Jobling, 1981). Water temperatures below 8°C led to active behavioral thermoregulation via vertical migration (Johansson *et al*., 2006; 2009). The upper preferred temperature range for Atlantic salmon is 16 – 18°C, with avoidance behavior occurring above the threshold (Oppedal *et al*., 2011). As warming climate has led to an increase in marine heatwaves, which are defined as an extended period of abnormally high sea surface temperature (Oliver *et al*., 2018), cultured salmon will be increasingly exposed to temperature stress. While warming waters can trigger thermal stress on farmed salmon, cold winter temperatures can also lead to significant mortality events, i.e., super chill (Hargrave *et al*., 2005).

Dissolved oxygen (DO) also has a major effect on the growth and performance of cultured fish. For example, at 15°C, Atlantic salmon require a dissolved oxygen level above 6.0 mgL^{-1} before their feed intake is reduced, while below 4.0 mgL^{-1} salmon are forced to switch to anaerobic metabolism (Burt *et al*., 2012; Dempster *et al*., 2016; Remen *et al*., 2012; Oldham *et al*., 2018). Some short-term effects of low DO are a decrease in swimming speed, indicative of conservation of energy, and a reduction in feeding activity (Martins *et* *al*., 2011, Oldham *et al*., 2018). Physiological effects of long-term low DO and elevated temperatures are decreased appetite and growth, and ultimately, increased mortality (Johansson *et al*., 2006; Remen *et al*., 2012, 2016; Gamperl *et al*., 2020). As with unfavorable temperatures, salmon can avoid low oxygen conditions via vertical migration within net pens, but other factors such as fish density, have been reported to override the effect of DO (Johansson *et al*., 2006; Oldham *et al*., 2018; Stehfest *et al*., 2017). Due to continued ocean warming, solubility of dissolved oxygen will decrease leading to more hypoxic events (Keeling *et a*l., 2009; IPCC, 2018).

Environmental conditions that lead to behavioral changes may be relevant in the context of farmed fish welfare, to understand welfare in a 'function-based' perspective changes in biological functioning in response to physiological stress are indicated by behavior (Martins *et al*., 2011). Increasing use of technology on fish farms has led to a greater capability in collecting data on environmental conditions as well as fish behavior. For example, real-time oxygen and temperature sensors are commonly deployed in salmon farming (e.g., Burke *et al*., 2021). Observations of fish behavior are generally of two types: visual and acoustic. For example, feed cameras are routinely used to monitor satiation and regulate feed input. A sophisticated analysis of size, biomass, and disease status have been derived from these types of video (e.g., Williams *et al*., 2006; Pinkiewicz *et al*., 2011). Acoustic observations generally involve echo-sounding of net pen populations or individual fish via implanted tags, providing information about their positioning and movement (e.g., Cooke *et al*., 2005; Cubitt *et al*., 2003; Broell *et al*., 2013; Thorstad *et al*., 2013; Macaulay *et al*., 2021). Acoustic fish tracking provides an individual-based, objective sampling of position allowing for high spatial and temporal resolution (Føre *et al*., 2017). Although previous studies have evaluated the feasibility of acoustic telemetry to assess individual fish movement (e.g., Juell & Westerberg, 1993; Cubitt *et al*., 2003; Clark *et al*., 2010; Broell *et al*., 2013; Thorstad *et al*., 2013; Kupilik & Petersen, 2014; Føre *et al*., 2017) not much attention has been paid to applying acoustic telemetry in aquaculture compared to ecological studies (e.g., Childs *et al*., 2008; Crossin *et al*., 2017; Haulsee *et al*., 2018; Kraus *et al*., 2018; Rothermel *et al.,* 2020).

Acoustic telemetry techniques have been used in aquaculture for chinook salmon (e.g.,Cubitt *et al*., 2003), Atlantic salmon (e.g.,Føre *et al*., 2011, 2017), Atlantic cod (e.g., Rillahan *et al*., 2011; Ward *et al*., 2012), and gilthead seabream (e.g.,Muñoz *et al.,* 2020; Palstra *et al*., 2021). These studies have focused on monitoring fish positioning and behavior in commercial sea pen settings. The accuracy and reliability of a three-dimensional system was tested on chinook salmon (Cubitt *et al*, 2003). Further, acoustic telemetry has been used to infer behavior, including vertical positioning and diel swimming patterns in farmed fish (e.g., Føre *et al*., 2011, 2017; Rillahan *et al*., 2011; Ward *et al*., 2012; Muñoz *et al*., 2020; Palstra *et al*., 2021). Individual fish tags do not capture the entire pen population, but they provide access to data on individual fish (e.g., swimming speed) that is not available when using population-level echo-sounding. Continuous monitoring of fish positioning can provide a more comprehensive view of the effects of environmental conditions on swimming behavior and consequently, on welfare. In the present study, implanted acoustic tags were used to monitor the three-dimensional positioning of salmon for one month during the fall at a commercial farm in Nova Scotia, Canada. The main research questions of this study were:

i. Can high resolution 3D fish location be used to infer swimming behavior in the cage environment?
ii. Can 3D location be used to develop metrics related to fish behavior?
iii. What is the relationship of behavioral indicators to environmental conditions in the net pen?

2.3. Methods

2.3.1. Study Site

Shelburne Bay, located in southwest Nova Scotia (Canada), is 7.4km wide at the open mouth, narrowing landward and then branching to Shelburne Harbor over a distance of 15.5km (Fig. 2.1). The outer bay is somewhat protected by McNutt's Island. Seawater temperature has a typical north temperate range of 0-3°C in February to a maximum of 14-18°C in August (seatemperature.org). Temperature changes can be rapid due to wind-induced upwelling of cold shelf water. The small Shelburne River enters the bay at the head of the harbor, but the outer bay has salinity values (30 PSU) typical of inshore Nova Scotia.

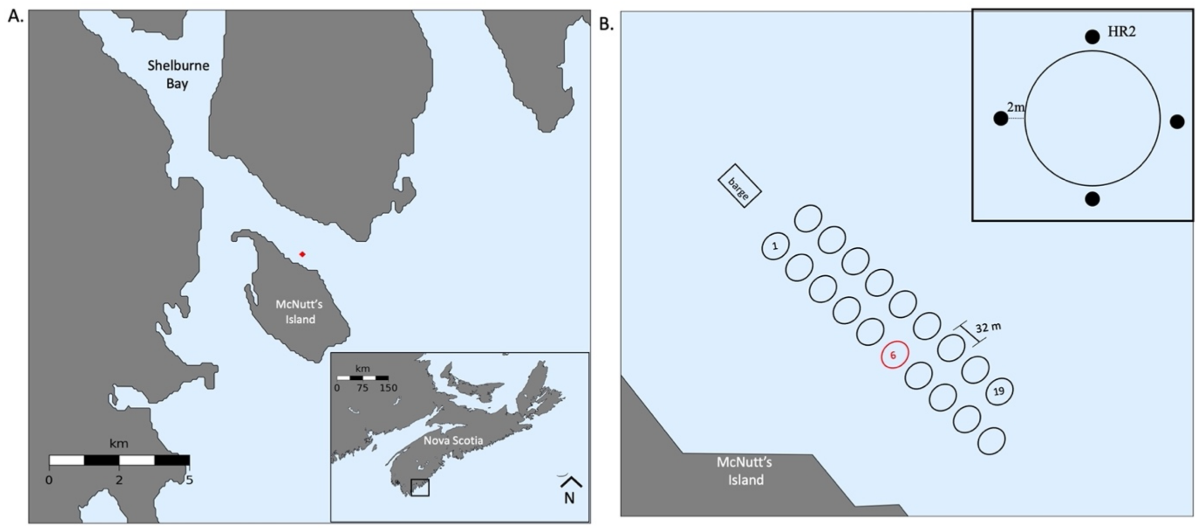

Figure 2.1: Study site map. A) Regional map of Shelburne Bay with site location marked in red, and inset showing location in Nova Scotia, Canada. B) Zoomed site map with farm orientation and net pen schematic; study net pen highlighted in red, inset showing schematic.

Atlantic salmon are farmed on the northeast side of McNutts Island (Kelly Cove Salmon), a farm area of about 50,000 m^2 (Fig. 2.1A). During the study period (day of year 274 - 304), the site contained 19 active net pens, each of them with a diameter of ~32 m, a depth of 11 m, and an average stocking density of 12.09 kgm^{-3} (~20,000 individuals). The site has a feed barge on the north end, and net pens in two equal columns of 10, with pen 20 being empty (Fig. 2.1B).

2.3.2. Acoustic Tags

Implanted acoustic tags (Vemco V9P-180, InnovaSea Systems Inc, Bedford, Nova Scotia, Canada) were 9 mm in diameter, 26 mm in length, and 4g in air, transmitting at a frequency of 180 kHz. Tags were surgically implanted in 15 Atlantic salmon on day of year 229, and receivers ran out of battery on day of the year 361. Although 15 fish are a small percentage of the net pen population, studies show individual salmon response to environmental factors reflects group behavior in aquaculture conditions (Oppedal *et al*., 2011; Stehfest *et* *al*., 2017). In addition, a higher density of tags causes interference in acoustic transmission and potential loss of data (Al-Dharrab *et al*., 2013).

Adult salmon (73-78 cm length and 3kg weight) were retrieved from net pen 6, located in the center of the farm, and implanted with acoustic tags by a Cooke Aquaculture veterinarian in compliance with the Dalhousie University Animal Care Council. Before each surgery, all surgical instruments were disinfected using a Vikron solution, and activated V9P acoustic tags were immersed in a chemical disinfectant bath of Beta iodine for a minimum of 20 minutes. Each fish was collected from the cage using a dip net and placed in a 40 mgL^{-1} bath of TMS (MS-222) 3-aminobenzoic acid ethyl. After 5-10 minutes, when visual signs of sedation (loss of equilibrium and no reaction to touch; Moore *et al*., 1990) fish were transferred to a cradle with fresh seawater bathed over the gills. A 1-1.5 cm incision was made slightly offset from the linea alba and anterior to the pelvic girdle, parallel to the midline. The tag was inserted into the coelomic cavity and closed with an absorbable 3-0 monofilament sterilized suture. An interrupted surgical knot was applied to the incision every 5 mm. Once the incision was closed, the fish was transferred to a 350-gallon recovery tank with fresh seawater constantly supplied. Recovery averaged about 40 minutes and subsequent release were determined when opercular movement, equilibrium, and locomotory movements were observed (Moore *et al*., 1990).

Each tag was set to transmit a 2D position [x, y] every 3 seconds for the duration of the experiment. A pressure sensor within the tags was used to measure depth (z dimension). Eight HR2 receivers (InnovaSea Systems Inc.) were deployed on the NSEW axes outside of net pen in a two-layer array and used to triangulate horizontal position (Fig. 1B). The top 4 HR2s were deployed at 2 meters depth in the net pen and the bottom 4 HR2s were deployed at 9 meters depth. Among the 15 tagged fish, data were unusable from 5 tags due to tag malfunction or fish mortality. Three tags malfunctioned, noted by the lack of response of the pressure sensor (constant depth over time), and two tags were ascribed to dead individuals. The data from these 5 tags were not used in the analysis. Over the tagging period (132 days) mortality was 13%, which is lower than average long-term (100+ days) studies (Macaulay *et al*., 2021). Data from the HR2 receivers were downloaded upon retrieval but fish tags were discarded in processing following harvest.

2.3.3. Additional data

Additional fish data, such as feeding time and mortality rate, were collected from farm logs. Feeding was completed twice a day, morning and evening, by an automated feed system with submerged cameras to inform when the population was satiated. Weather data, including wind speed and direction, were retrieved from the nearby Sandy Point weather station, about 7 km from the study site (Environment and Climate Change Canada). Temperature and DO were recorded every 5 minutes by real-time, wireless sensors (AquaMeasure, InnovaSea Systems Inc.). The data were stored onto cloud-based storage in real-time. Sensors were deployed within the study cage with 4 sensors at 2 m depth in NSEW axes, one sensor in cage center at 4 m depth, and 4 sensors at 8 m depth in NSEW axes.

2.3.4. Data Processing

Raw data collected from the receivers were downloaded and sent to InnovaSea for processing. Data were returned in an [x, y, z] format with a time stamp out to 10^{-6} seconds. The positioning was then normalized by representing [0, 0, 0] as the approximate cage center at the surface. Absolute error values for horizonal positioning is about 1 fish length, approximately 1m, and absolute error for depth is about 7.5 cm. An error value was provided with each location via the horizontal error position (HPE), which is the theoretical horizontal error calculated using 3 receivers that create an array. The HPE value can range from 0-2, where 2 is higher error sensitivity, and 0 is less error sensitivity. In this dataset, HPE ranged from 0.06 – 0.6 with a mean of 0.31, after filtering the top 10% out.

The three-dimensional distance travelled by the fish between two consecutive time records (d_{12}) was calculated based on [x, y, z] positioning following:

$$d_{12} = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2} \qquad (1)$$

where $[x_1, y_1, z_1]$ to $[x_2, y_2, z_2]$ are the positions for the two records. Velocity between those two consecutive acoustic records (v_{12}) was calculated as:

$$v_{12} = \frac{d_{12}}{t_2 - t_1} \qquad (2)$$

where d_{12} is the distance travelled by the fish between times t_1 and t_2. Distance from the center of the net pen (d_{center}) was calculated for each acoustic record following:

$$d_{center,1} = \sqrt{{x_1}^2 + {y_1}^2} \qquad (3)$$

where x_1 and y_1 are the x and y positions of the first acoustic record. Turning angle from the fish's point-of-view ($turn_{13}$) was calculated considering three consecutive acoustic records as:

$$turn_{13} = 180 - \cos^{-1}\left(\frac{{d_{12}}^2 + {d_{23}}^2 - {d_{13}}^2}{2 d_{12} d_{23}}\right) \qquad (4)$$

where d_{12} is the distance from $[x_1, y_1, z_1]$ to $[x_2, y_2, z_2]$, d_{23} the distance from $[x_2, y_2, z_2]$ to $[x_3, y_3, z_3]$, and d_{13} the distance from $[x_1, y_1, z_1]$ to $[x_3, y_3, z_3]$. Due to the way acoustic tags transmit data, a time filter was applied to use only acoustic records with time stamps within 5 seconds of each other to ensure that only consecutive records were used in the calculations. This filtering reduced the number of detections but ensured the high quality of the data. Given the high temporal resolution of the tags, ~3 seconds, discarding detections is acceptable when the outcome is reducing the uncertainty of the dataset.

2.3.5. Statistical Analyses

Although the tagging period lasted 132 days (day of the year 229-361), the study period was reduced to one-month (day of year 274-304). This was done both to ensure that the fish had enough time to recover after the surgery and for statistical consistency (in comparing periods of similar lengths around key environmental changes). This one-month period captured two environmental changes, 1) fall overturn and disruption of water column stratification, and 2) a storm. The abrupt change in temperature and DO caused by the fall overturn allowed the study period to be split into two periods (see Results). A storm event was identified on 298-299 when wind speeds averaged 30 ± 8 kmh^{-1}. The fish positioning variables during the storm period were compared to the observations two days before the storm (296-297) and two days after the storm (300-301). The dataset was also divided into two categories regarding feeding activity: feeding and non-feeding times.

The 4 fish positioning variables, distance from center, swimming depth, swimming speed, and turning angle, were compared (a) before and after the fall overturn event and (b) between feeding and non-feeding periods using a Wilcoxon signed-ranks test. The storm event required the use of a Friedman test to detect differences across multiple groups (before, during, after). Spectral analysis was performed to examine periodicity in depth and velocity. A Wilcoxon signed-ranks test was used to compare periodicity of day against night to test the significance of those cycles. All data processing was completed using Python Spyder 3.7 (Python Software Foundation, https://www.python.org/).

2.4. Results

2.4.1. Fish Survival

Other than survivorship, the effects of surgery and the tags on fish behavior are difficult to assess. Although there is inter-individual variation in the different measures of fish position/swimming, the spread is not large, and no fish stand out as being obviously incapacitated. In addition, it is expected that poorly recovered fish would not persist in the population. Therefore, it could be concluded that the 10 remaining fish recovered and displayed normal swimming responses.

2.4.2. Background swimming movement

Four variables were examined in individual fish including (a) distance from center of the net pen [m], (b) swimming depth [m], (c) swimming velocity [ms^{-1}], and (d) turning angle [º] (Eqs. 1-4; Fig. 2.2). The tagged population averaged a distance of 9.1 ± 3.5 m from the cage center during the study period, with a maximum of 20.6 m and a minimum of <1m (Fig. 2.2A). Based on cage diameter, the maximum distance from the center should be ~16m, but the net is flexible, and some distention due to flow is expected. Fish 5 and 9 are highlighted in Figure 2.2 to focus on individual responses. Fish 5 is the most extreme case for three of the four fish variables. Fish 9 is a representation of the majority of the population and therefore was chosen to show a more detailed view of an 'average' fish.

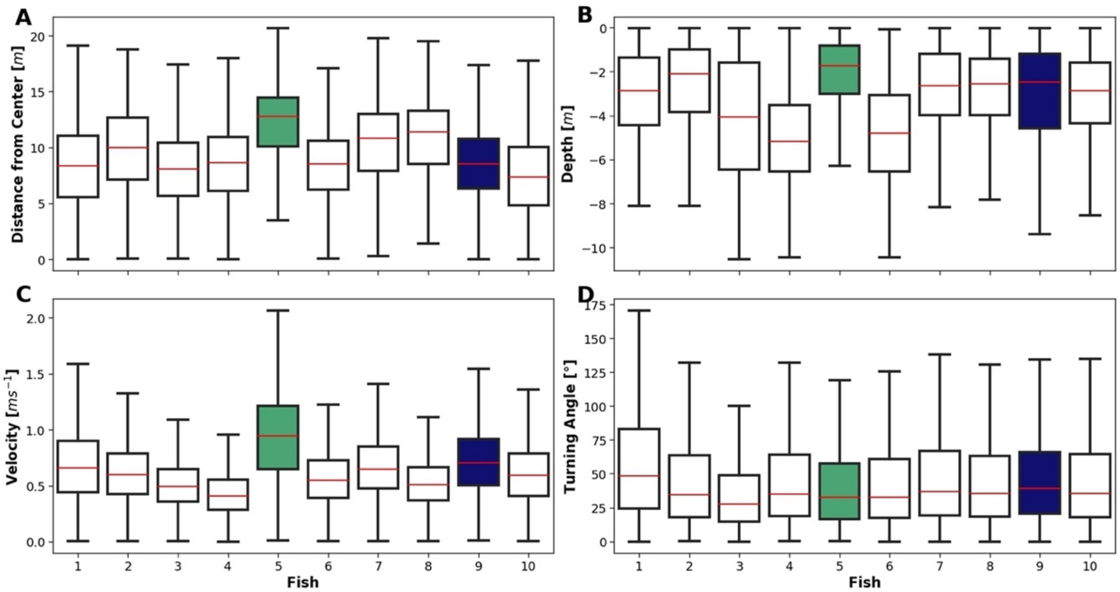

Figure 2.2: Boxplots of four behavioral variables for individual fish during the study period where the red line is median, box incorporates 25-75th percentile, and error bars mark maxima and minima. Green shaded box represents fish 5 and blue shaded represents fish 9. A) Distance from center [m]. B) Depth [m]. C) Velocity [ms^{-1}]. D) Turning Angle [º].

Heatmaps of horizontal fish position based on % of observations in a given location (Fig. 2.3) show examples of the two emphasized individuals. In both cases, the maximum density of observations is less than 0.5%, indicating that the fish evenly occupied most of the net pen. Fish 5 showed the highest density of observations at distances of about 10-15 m from the net pen center (Fig. 2.3A). Fish 9 occupied more centered positions than Fish 5, with regions of higher relative density at distances of about 5-10 m from the center (Fig.

2.3B). The directionality of the swimming vectors demonstrates that both fish 5 and 9 fish swam in a counter-clockwise direction (Fig. 2.3C, D).

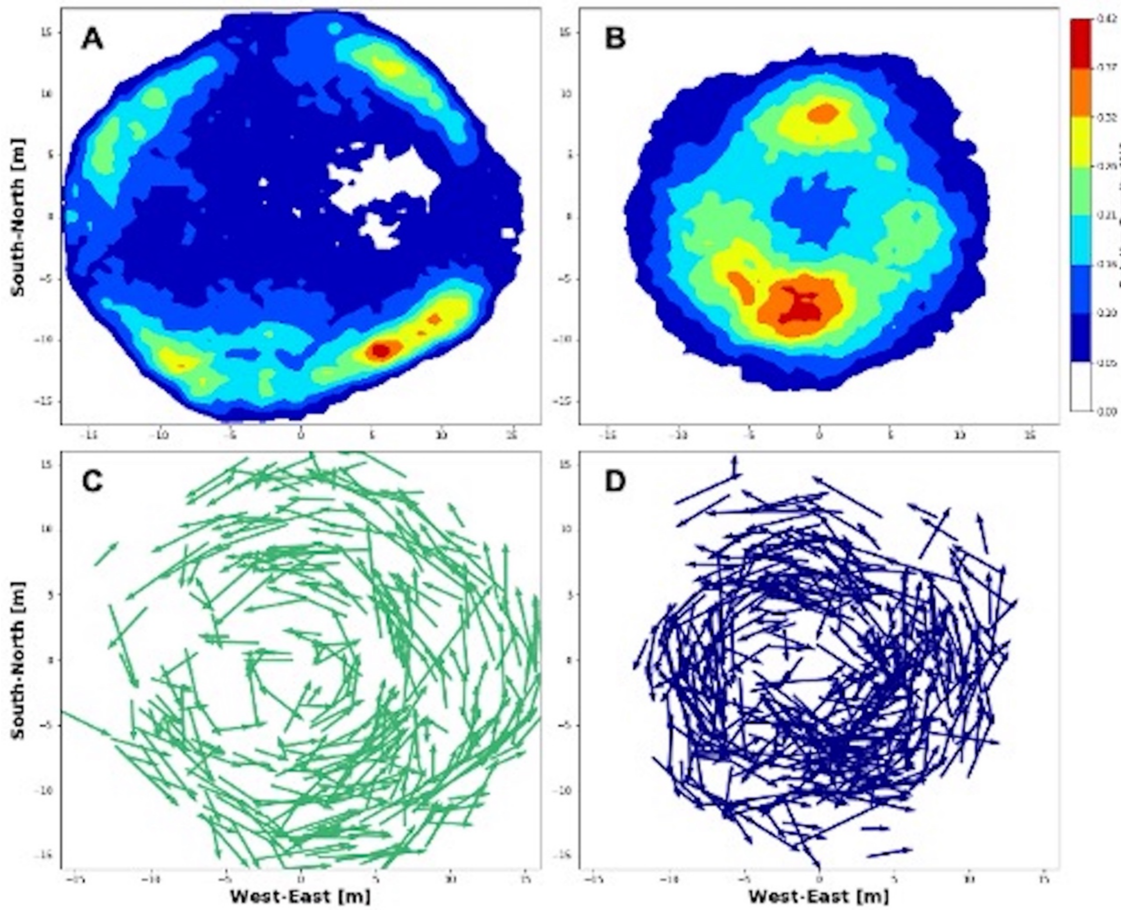

Figure 2.3: Horizontal plots of two tagged fish. A) Heatmap of fish 5 during October where blue shows low relative density [% occupancy] and warmer colors represent higher relative density. B) Heatmap of fish 9 during October. C) Vector plot of fish 5 during a 12-hour period in October. D) Vector plot of fish 9 during a 6-hour period in October.

The tagged population averaged 3.6 ± 2.2 m swimming depth below the water surface with a maximum of 10 m and minimum at the water surface (Fig. 2.2B). Jumping out of the water was not recorded since the tags only operate in water. A time series of Fish 5 and 9 showed a clear periodicity related to time of day with greater depths during the day, and shallower depths at night (Fig. 2.4A). Despite the temporal similarity between the vertical position of these two fish, Fish 9 migrated distinctly deeper in the net pen than Fish 5. Spectral density analysis (one-hour rolling mean) of each individual identified a strong peak at 24 hours for 70% of the tagged fish, with the other 30% having two peaks on either side of the 24-hour mark, ranging between 22.5-25.5 hours (Fig. 2.4B). Fish 9 also showed a slight peak at 8 hours. In general, the tagged population depth was deeper during daylight, 2.95 ± 0.03 m, than at night, 2.05 ± 0.07 m (Wilcoxon signed-ranks, $p < 0.001$).

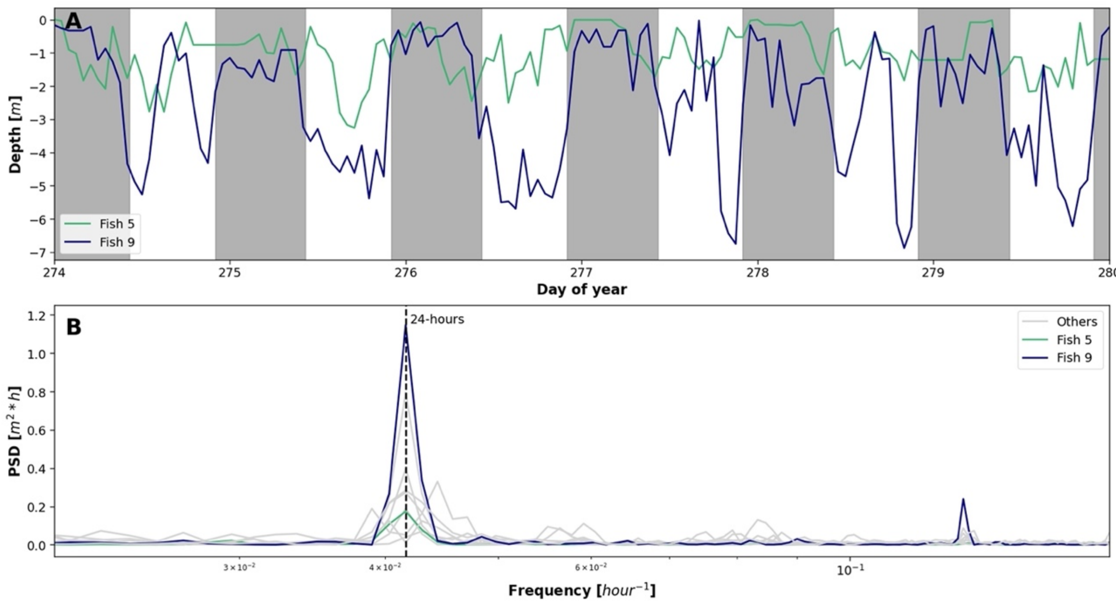

Figure 2.4: Spectral analysis of tagged fish depth [m]. A) One-week one hour rolling mean time series of two individuals: Fish 5 (green) and Fish 9 (blue) with night highlighted in gray. B) Power spectral density plot using one hour rolling mean of all individuals highlighting fish 5 & 9 where frequency is in hours.

Swimming velocity [ms^{-1}] averaged 0.61 ± 0.38 ms^{-1} with a maximum of 2.0 ms^{-1} and a minimum remaining almost still (Fig. 2.2C). A time series of velocity for Fish 9 showed the highest velocity occurred during evening, while for Fish 5 no obvious pattern was observed (Fig. 2.5A). Similar to the results obtained for depth, spectral density analysis (one-hour rolling mean) of velocity identified a strong peak at 24 hours for 50% of the individuals (Fig. 2.5B). Swimming speed among the tagged population was significantly higher during daylight than at night (Wilcoxon signed-ranks, $p = 0.00$). The other half of individuals show peaks on either side of 24 hours ranging between 23-25.75 hours. Turning angle [°] was used to measure the change in direction of the fish trajectory (Fig. 2.2D). The population averaged 46.82 ± 38.2° deviation from their trajectory with a maximum of 179.9°, indicating a U-turn, and a minimum of 0°, indicating no change in swimming direction. Mean turning angle was highly consistent among the tagged fish.

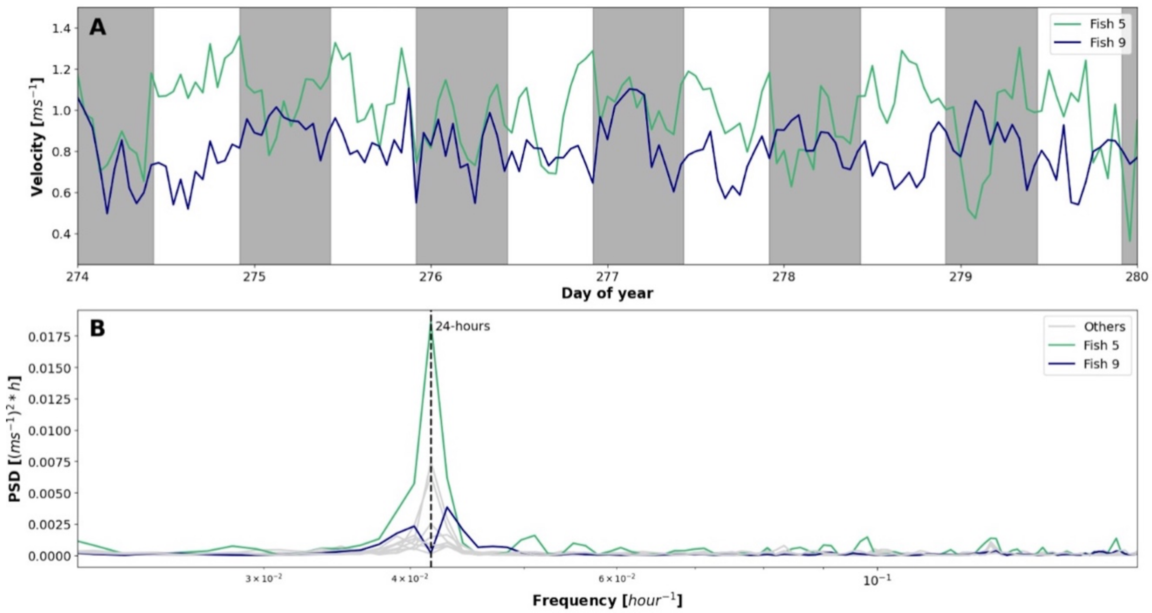

Figure 2.5: Spectral analysis of tagged fish velocity [ms^{-1}]. A) One-week one hour rolling mean time series of two individuals: Fish 5 (green) and Fish 9 (blue) with night highlighted in gray. B) Power spectral density plot using one hour rolling mean of all individuals highlighting fish 5 & 9 where frequency is in hours.

2.4.3. Fish movement in relation to external variables

The rapid change in temperature and DO associated with the fall overturn and breakdown of the seasonal thermocline allowed division of the dataset into two well-defined periods with distinct and relatively stable conditions in terms of temperature and DO. Days 274-289 are defined as 'Before' when temperatures ranged from 12 °C to 17 °C and DO from

5.0 mgL^{-1} to 7.2 mgL^{-1}, and days 290-304 are defined as 'After', with temperature ranging from 6.5 °C to 10 °C and DO from 6.8 mgL^{-1} to 8.3 mgL^{-1} (Fig. 2.6).

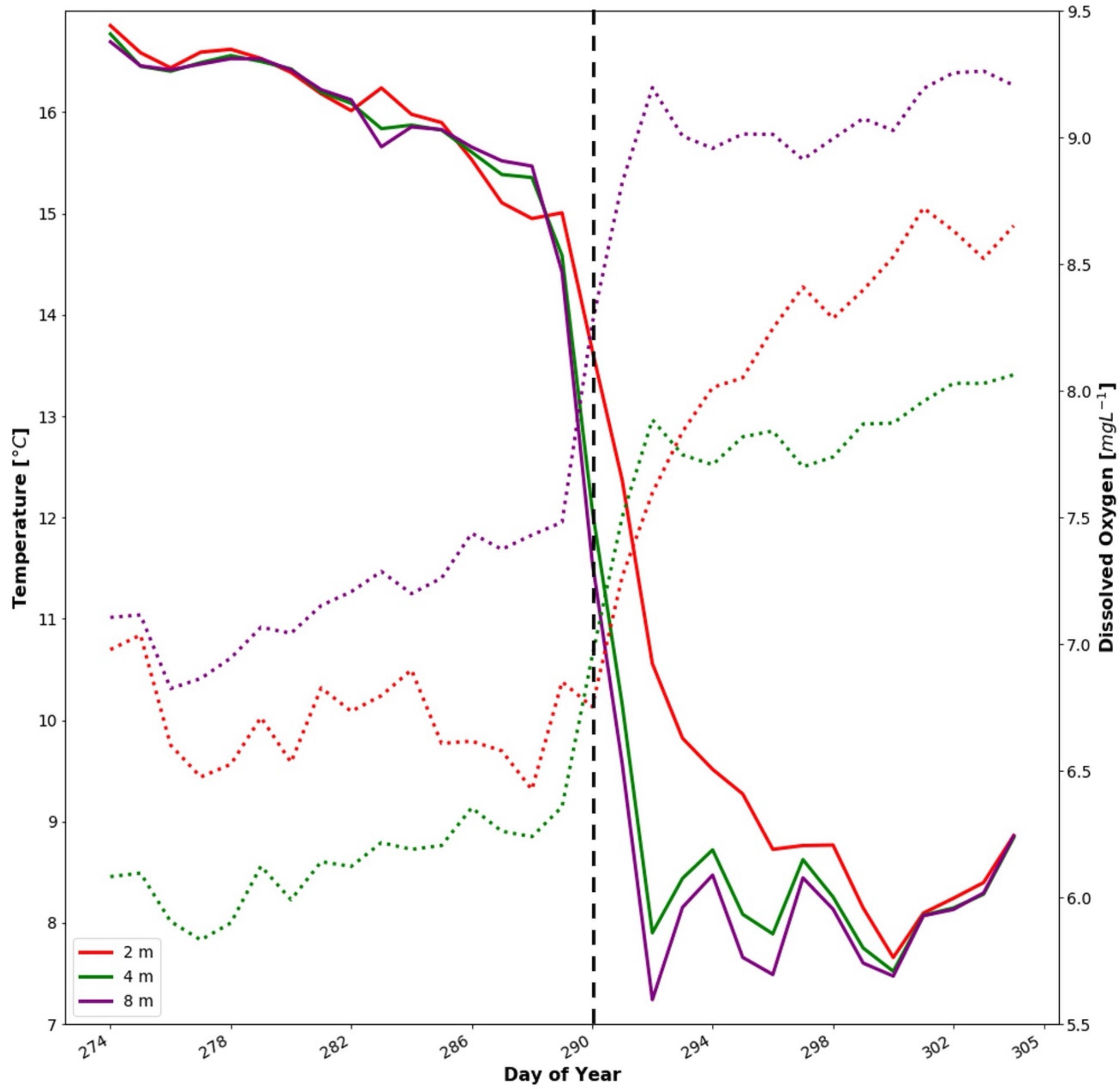

Figure 2.6 Daily average of temperature [°C] (solid lines) and dissolved oxygen [mgL^{-1}] (dotted lines) measured in study cage at 2 m (red), 4 m (green), and 8 m (purple) depth (adapted from Burke et al., 2021). Vertical black line denotes split between before and after the fall overturn.

Comparison of the four fish positional variables between these two periods showed that for distance from center [m], 80% of tagged individuals moved slightly towards the inner part of the net pen after the fall overturn, with a relative change between 0.8% and 15% of their previous position (Fig. 2.7A). In contrast, Fish 1 and 10 moved towards the edge of the net pen by a relative change of 0.33% and 4.55%, respectively. As a population, the fish moved towards the center with a significant decrease from a mean of 9.4 ± 1.4 m (Before) to 8.7 ± 1.3 m (After; $p<0.05$, Table 2.1). Examination of depth distribution before and after the overturn revealed greater inter-individual variability than distance from center (Fig. 2.7B), which resulted in non-significant differences in depth ($p=0.284$, Table 2.1). Swimming velocity showed a pattern similar to horizontal distance, with 90% of individuals decreasing velocity by 8.3-33.8% after the fall overturn (Fig. 2.7C). Before the change in DO and temperature, the mean velocity of the tagged population was 0.69 ± 0.15 ms^{-1}, which decreased significantly to 0.57 ± 0.11 ms^{-1} after the fall overturn ($p<0.01$, Table 2.1). Fish 8 was the only individual in which a 7% increase in velocity occurred after the overturn. High inter-individual variability in turning angle (Fig. 2.7D) resulted in a non-significant difference at the tagged population level between periods ($p=0.092$, Table 2.1).

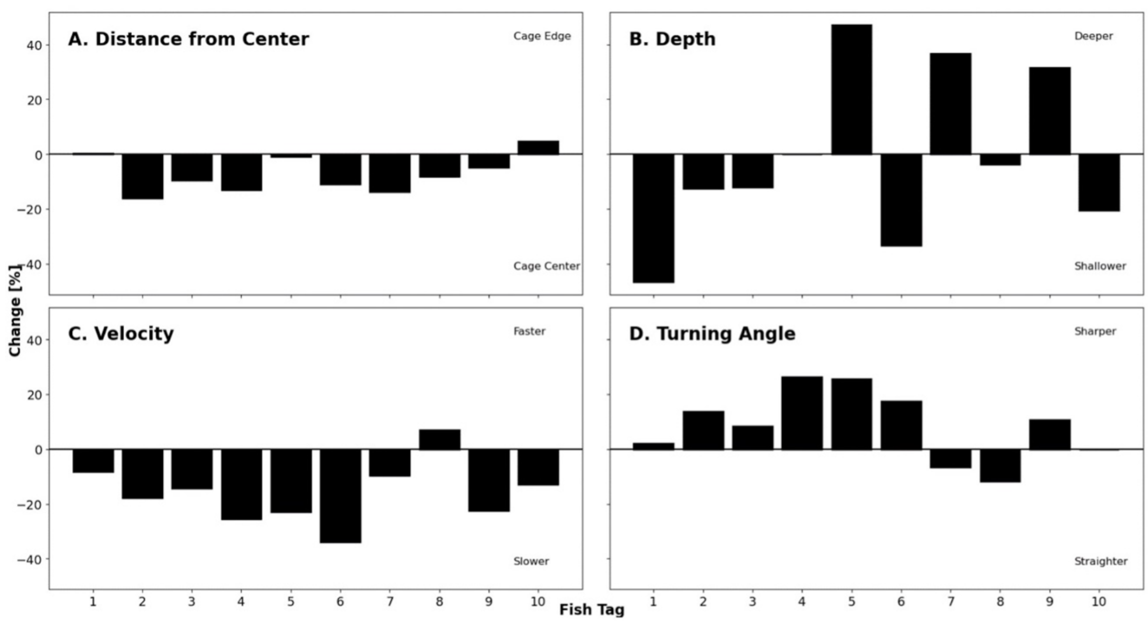

Figure 2.7: Percent change of individual fish comparing before the fall overturn (1-16 October) temperature and DO change to after (19-31 October). A) Change in distance from center where a positive change represents a shift towards cage edge. B) Change in depth where a positive change signifies movement towards the bottom. C) Change in velocity where an increase in velocity is represented by a positive change. D) Change in turning angle with a positive change representing a sharper turning angle.

The same four fish variables were used to compare the effect of feeding (Fig. 2.8). Feeding was defined as any time period that feed was distributed within the net pen, while before feeding is any period in which fish were not being fed. No statistical differences were observed in terms of distance from the center (Fig. 2.8A) before and during feeding periods ($p=0.240$, Table 2.1). Depth increased for 80% of the individuals by 1.7-40.4% during feeding (Fig. 2.8B). In contrast, Fish 4 and 10 moved towards the surface by 1.8% and 8.7%, respectively. Depth for the tagged population averaged 2.9 ± 1.2 m from the surface for non-feeding and 3.4 ± 1.1 m for feeding periods, leading to a significant difference ($p<0.05$, Table 2.1). All individuals increased in velocity between 1.2-19.7% during feeding (Fig. 2.8C). As a population, the mean velocity before feeding periods was 0.64 ± 0.13 ms^{-1}, which increased significantly up to 0.72 ± 0.18 ms^{-1} during feeding ($p<0.01$, Table 2.1). All individuals showed smaller turning angles, between 2.7 and 36.1% less, while being fed (Fig. 2.8D). Population turning angle while feeding averaged 41.0 ± 5.6 °, significantly different from nonfeeding, which averaged 46.8 ± 5.0 ° ($p<0.01$, Table 2.1).

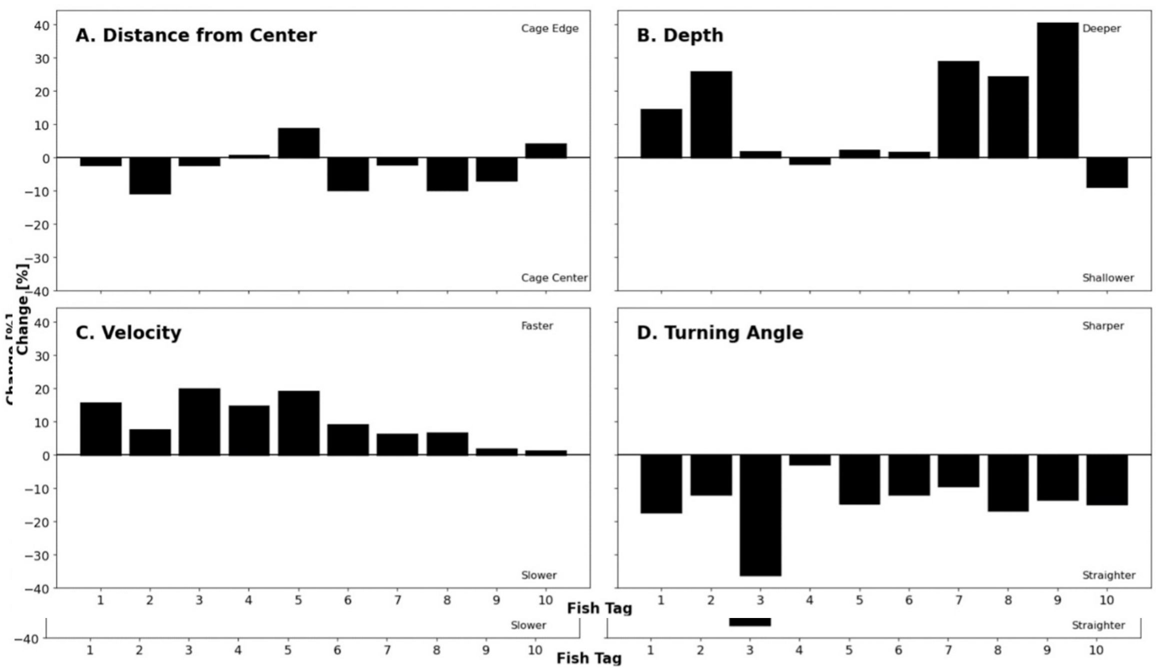

Figure 2.8: Percent change of individual fish comparing feeding times to non-feeding times. A) Change in distance from center where a positive change represents a shift towards cage edge. B) Change in depth where a positive change signifies movement towards the bottom of the net pen. C) Change in velocity where an increase in velocity is represented by a positive change. D) Change in turning angle with a negative change characterizing a straighter turning angle.

A storm event characterized by 30 ± 8 kmh^{-1} winds was observed after the fall overturn during days 298 and 299, which allowed for a comparison before, during, and after the storm. Before the storm event, temperature ranged from 7.5 °C to 8.7 °C and DO from 7.7

mgL^{-1} to 9.0 mgL^{-1}; while after the storm, temperature ranged from 7.4 °C to 8.0 °C and DO from 7.8 mgL^{-1} – 9.2 mgL^{-1}. No significant differences were observed for any fish variable when comparing before, during, and after the storm (Table 2.1). Averaging across these three periods, distance from center was 8.9 ± 1.3 m, depth 2.9 ± 1.2 m, velocity 0.55 ± 0.12 ms^{-1}, and turning angle 53.1 ± 9.2 °.

*Table 2.1: Mean values and standard deviation for each fish variable during environmental events. Test statistic for temperature/DO and feeding is for Wilcoxon signed-ranks test in pairwise comparison, while test statistic for storms is for Friedman's test. P-values with * denotes significant values, p < 0.05. N = 10 for all scenarios. For environmental events, temperature/DO is before and after the fall overturn, feeding is before and during feeding events, and storm is before, during, and after a wind event. See text for details.*

Environmental Event	**Fish Variable**	**Mean Before**	**Mean During**	**Mean After**	**Test Statistic**	**p-value**
Temperature/DO	Distance from Center [m]	9.4±1.4	--	8.7±1.3	4.0	0.016*
	Depth [m]	3.1±1.4	--	2.7±1.0	17.0	0.284
	Velocity [ms^{-1}]	0.69±0.15	--	0.57±0.11	1.0	0.006*
	Turning Angle [°]	45.4±5.7	--	48.8±5.3	11.0	0.092
Feeding	Distance from Center [m]	9.2±1.4	8.9±1.6	--	16.0	0.240
	Depth [m]	2.9±1.2	3.4±1.1	--	8.0	0.046*
	Velocity [ms^{-1}]	0.64±0.13	0.72±0.18	--	<0.001	0.005*
	Turning Angle [°]	46.8±5.0	41.0±5.6	--	<0.001	0.005*
Storm	Distance from Center [m]	8.6±1.5	9.1±1.4	9.0±1.1	2.6	0.272
	Depth [m]	2.8±1.1	2.9±1.5	3.1±1.2	0.20	0.904
	Velocity [ms^{-1}]	0.54±0.13	0.57±0.14	0.55±0.11	2.66	0.263
	Turning Angle [°]	50.0±10.7	52.0±8.5	57.1±7.5	5.0	0.082

2.5. Discussion

The combination of receiver array and acoustic tags has offered a 3-dimensional view of Atlantic salmon swimming behavior and positioning in a commercial net pen. This detailed information has provided further understanding of fish behavioral response to different environmental stimuli. Previous studies have described vertical movement (Fernö *et al.,* 1995; Juell & Fosseidengen, 2004; Johansson *et al*., 2006 & 2009; Føre *et al*., 2017) and behavior (Martins *et al*., 2011; Oppedal *et al*., 2011; Castanheira *et al*., 2017) of cultured salmon, however measuring movement in 3-dimensions is less common (Cubitt *et al*., 2003).

2.5.1. Swimming movement

There was a distinguishable circular, counterclockwise swimming pattern within the group of Atlantic salmon. The circular swimming pattern is affected by water currents, but directionality may change among net pens or farms (Juell, 1995; Johansson *et al*., 2014). A shoal, or a group of fish remaining together, adopts a polarized swimming pattern to minimize the possibility of collisions, and deviations from this pattern by individuals can affect the group reaction (Martins *et al*., 2011). Within a shoal, there are 'traffic rules' used by all individuals, and consequently, the analysis of a few individuals can provide insight into group behavior. The counterclockwise swimming pattern observed in this study is an example of schooling behavior as in the above definition, suggesting that group behavior can be inferred from individual tags.

There was a distinct diel rhythm in depth with swimming nearer the surface at night, compared to the day. Diel rhythms in depth in salmon farming have been linked to light intensity, feed anticipation, and the apparent threat of predation (Oppedal *et al*., 2001 & 2007; Juell & Fosseidengen, 2004; Johansson *et al*., 2006 & 2009). The diel rhythm observed in this study ranged approximately 0-6 m, corresponding to the upper half of the net pen. This observation matches previous studies where populations remain in the upper half of net pens, regardless of site depth (Fernö *et al*., 1995; Juell & Fosseidengen, 2004; Johansson *et al*., 2006 & 2009; Føre *et al*., 2017). Net pens in Norway are up to 50 m deep and located in fjords, where in Atlantic Canada net pens are on average about 6-15 m deep.

The avoidance of net pen bottom observed could characterize anti-predator evasion since large piscivorous fish have been detected under net pens (Dempster *et al*., 2009), or a natural biological response (Tanaka *et al*., 2005).

A diel rhythm in swimming speed, with higher velocities during the day was also observed in this study. Previous studies have found swimming speeds to range between 0-2 ms^{-1}, similar to the values observed in this study, with the highest speeds during the day (Juell & Westerberg, 1993; Andrew *et al*., 2002; Føre *et al*., 2011 & 2018b). Swimming speeds are customarily faster during the day than at night due to light availability (Oppedal *et al*., 2001) because Atlantic salmon rely heavily on vision while swimming (Oppedal *et al*., 2011). The tagged salmon in this study thus followed the expected pattern previously described in the literature with greater depths and higher velocity during daylight hours and lower velocity and lesser depths during nighttime (Fernö *et al*., 1995; Andrew *et al*., 2002; Johansson *et al*., 2006; Oppedal *et al*., 2011; Føre *et al*., 2011 & 2018b).

2.5.2. Fish movement in relation to external variables

Following the rapid temperature and DO change halfway through the study period due to the fall turnover, individuals showed a shift towards the cage center with a decrease in velocity but no significant change in depth distribution. Changes in proximity toward the center, although significant, were small and on the order of one fish length. It is difficult to assign causality to this minor shift, but associated results for swimming speed are consistent with an expected decrease caused by a drop in temperature (Hvas *et al*., 2017).

During feeding, there was an increase in depth while individuals increased speed and decreased turning angle. During daylight hours, fish tend to swim deeper in net pens but ascend towards the surface when motivated by feed (Fernö *et al*., 1995; Oppedal *et al*., 2001), contrary to this study where the opposite occurred. It is likely more crowded at the surface during feeding, an observation apparent even to a casual observer. An increase of speed during feeding is indicative of foraging behavior to capture food pellets nearby (Martins *et al*., 2011). A decrease in turning angle is suggestive of straighter paths toward food pellets. Although turning angle was calculated from adjacent swimming vectors, turning is a continuous process in part dictated by cage diameter and thus the tightness of circular swimming.

During a storm with wind speeds between 35 and 45 kmh^{-1}, there were no significant movements horizontally or vertically in the tagged group, suggesting that fish behavior was not affected by the storm. This does not rule out effects on fish at higher wind speeds. The movement of net pens during storms has become a concern for farmers due to the possibility of increased net movement causing damage to fish as well as generally leading to increased stress. Although no responses on these four variables were significant from this storm, it does not neglect other stressors, e.g., fin and scale damage, that could cause physical damage not seen with movement data.

2.5.3. Conclusions

This study used detailed positioning data to catalog behavioral responses using fish position to calculate swimming speed and vertical/horizontal location. There are a variety of external variables available to evaluate as determinants of behavior including oxygen, temperature, and wind. In addition, husbandry variables including feeding provide further basis for comparison. Nonetheless, the net pen environment is confined and the scope for behavioral responses is limited. Some of the changes in defined behaviors were subtle and difficult to assess with respect to causality. Previous studies have confirmed the use of acoustic tags in field experiments at commercial fish farms and have found common swimming patterns (e.g., circular swimming pattern and diel vertical movement) (e.g., Føre *et al*., 2017; Muñoz *et al*., 2020; Macaulay *et al*., 2021). This study is one of the few employing tags to resolve 3D positioning, but also able to incorporate high frequency data on both horizontal and vertical movement. As fish farming continues to embrace precision aquaculture (Føre *et al*., 2018a; O'Donncha & Grant, 2019), further delineation of both fish behavior and its environmental forcing will be possible, as well as input into husbandry management.

High frequency acoustic tags provide a detailed temporal view of fish positioning within aquaculture net pens; however, some data loss is possible due to the signal interference caused by the high density of fish in the cages (Al-Dharrab et al., 2013). Although only 3 receivers are needed to triangulate fish positioning, a total of 8 receivers were placed in this study to minimize data loss. One of the assumptions to triangulate positioning, and minimize error, is to have stationary receivers. In this study, the receivers were attached to the gear that keeps the cage in place, which was assumed to be the most stationary option. Although this gear is not completely stationary, the short-term drift of the receivers should be negligible compared to fish movement during the 2 or 3 consecutive datapoints (~6 seconds) used for the mathematical calculation of positioning, velocity, and turning angle. The use of a reference tag on the ocean bottom could be used to quantify this potential error in future studies.

The study intended to use position-based behaviors to examine the response to apparent stress by conditions such as storms. However, stress is manifest by morphological and physiological responses assessed by operational welfare indicators (OWI), including fin and scale health as well as other metrics of condition (Martins *et al*., 2011; Oppedal *et al*., 2011; Noble *et al*., 2018). OWIs would provide complementary information that if compiled appropriately could be used to understand the welfare of farmed Atlantic salmon (Stien *et al*., 2013). Positioning data provides the function-based information needed to understand how changes in conditions affect changes in behavior and ultimately welfare. Although this study examined changes in behavior with respect to documented events such as storms and the fall overturn, these events are not necessarily judged as extreme. In comparison, OWIs which depend on mostly negative morphological changes are reflective of rather severe impacts on fish condition. This study has provided a highly resolved data set on farmed salmon behavior that creates a baseline for responses to the ambient environment. The study posited that these data would provide a sensitive response to external conditions, however more extreme events such as severe storms might yield a more obvious behavioral response. This approach results in a quantitative set of metrics with which to approach fish behavior. As oceanic conditions such as storminess and hypoxia become more common in a changing climate, it is anticipated a wider range of response in fish swimming and positioning behavior.

Climate change is leading to increased sea surface temperatures, decrease oxygen, and increased frequency and intensity of storms (Handisyde *et al*., 2006; Keeling *et al*., 2009; Johannesen *et al*., 2020). An understanding of fish behavioral response to these variables provides further management options including cage and mooring design, feeding regimens, oxygen supplementation, and site selection. Research progress in an increasing sensor and data-rich farm environment will inevitably lead to better fish welfare and more sustainable aquaculture.

CHAPTER 3 : THE EFFECTS OF OXYGEN SUPPLEMENTATION ON FARMED ATLANTIC SALMON (*SALMO SALAR*) BEHAVIOR USING ACOUSTIC TELEMETRY

3.1. Abstract

Climate change is leading to worldwide ocean temperature rise and increased occurrence of low oxygen events. Dissolved oxygen and water temperature play a crucial role on the growth and health of fish becoming determining factors of welfare. In order to counteract the effects of low oxygen events, farms worldwide have begun to experiment with oxygen supplementation systems. In this study, high resolution, high frequency acoustic tags were used to monitor movement of Atlantic salmon (*Salmo salar)* at a commercial farm where an oxygen supplementation system was installed. A two-month study period was selected which allowed characterization of fish movement during and after the oxygen supplementation project. The positioning of 15 fish was recorded using high temporal resolution (3 s). Fish movement was characterized by calculating four fish variables: velocity [ms^{-1}], distance from the center of the cage [m], turning angle [°], and relative measurements of depth [m]. During the oxygen trial, all tagged individuals recorded slower swimming velocities than after the trial despite that during the trial water temperatures were between 11.2°C to 13.8°C. Seventy-seven % of the tagged population swam nearer to the cage edge during the trial than after, and 85% displayed straighter swimming patterns during the trial than after. Lastly, during the trial, 85% of the tagged population swam shallower than after the trial. Although causality cannot be related to the oxygen supplementation experiment, this study highlights that increased farm technology can provide more insight into the effects of oxygenation on fish behavior.

3.2. Introduction

The solubility of oxygen in seawater is dependent on pressure, salinity, and temperature where warmer temperatures decrease the solubility of oxygen. Furthermore, temperature is an essential environmental variable controlling metabolic rate in fish (Stehfast *et al*., 2017). Consequently, temperature affects dissolved oxygen in two ways, directly though solubility and indirectly via metabolic rate, as increased temperatures increment oxygen demand (Brett, 1972; Stehfast *et al*., 2017). Dissolved oxygen (DO) affects critically the growth and health of farmed fish (Elliott & Elliott, 2010). For example, at 15°C, when DO levels fall below 6.0 mgL^{-1}, Atlantic salmon (*Salmo salar*) appetite decreases, while below 4.0 mgL^{-1}, anerobic metabolism is activated to decrease oxygen demand (Burt *et al*., 2012; Remen *et al.*, 2012; Dempster *et al*, 2016; Oldham *et al*., 2018). The response to hypoxic levels of DO is also a function of the duration and the frequency of the exposure. In short-term exposure, individuals show physiological stress that manifests as decreased swimming speed and appetite (Oldham *et al.*, 2018). Extended exposure to hypoxic conditions results in slower swimming speeds indicating a management of energy-demanding processes such as feed uptake and immune function (Martins *et al*., 2011; Oppedal *et al*., 2011). Ultimately, prolonged exposure to low DO can result in elevated mortalities (Johansson *et al.*, 2006; Remen *et al*., 2012, 2016; Gamperl *et al*., 2020). Salmon can avoid low DO conditions by vertically migrating within net pens to avoid unfavorable conditions (Johansson *et al*., 2006); however, other factors, such as fish density and water temperature, have been reported to outweigh the effect of DO (Johansson *et al*., 2006; Stehfest *et al*., 2017).

In the past few years, mass mortality events have occurred at fish farms with causes attributed to poor water quality, disease, and farm sites infested with sea lice. In 2018, over a million farmed salmon died within six months in Australia, intensified by stress from high water temperatures and low oxygen causing diseases to be spread more easily throughout the population (Galea *et al*., 2018). In 2019, a mass mortality event occurred off the south coast of Newfoundland in which low DO and high temperatures along with sea lice treatments most likely led to high mortality rates (Fisheries and Marine Institute of Memorial University of Newfoundland, 2020). In 2019, sites in western Scotland experienced high mortality rates due to low DO combined with sea lice treatments and the presence of cardiomyopathy syndrome and pancreas disease (Edwards, 2019). In 2020, more than 200 000 farm salmon died from an algal bloom in Clayoquot Sound, British Columbia, Canada (Oceanographic Magazine, 2020). In southern Chile in 2020, high mortality rates were reported several times in a month, believed to be due to poor water quality from a harmful algal bloom (Evans, 2020). Since ocean temperature will continue to rise due to climate change, low oxygen events and harmful algal blooms, which can perpetuate low oxygen, are predicted to occur more often and for a longer duration (Gobler *et al*., 2017).

Demand for Atlantic salmon is steadily increasing worldwide (FAO, 2018) and aquaculture sites are increasing cage size and stocking density (Oldham *et al*., 2018). As the cage size increases, the surface area to volume ratio decreases reducing water exchange within net pens (Klebert *et al*., 2013). A simple model by Aure *et al*. (2009) estimates DO levels within aquaculture net pens as a function of stocking density, cage size, and current speed. The model predicts a drop from 90% of ambient levels in a 30 m diameter cage to 50% in a 60 m cage with stocking density at 15 kgm^{-3} and 6 cms^{-1} current speed. In order to counteract the negative effects of low DO on fish welfare, farms worldwide have recently employed oxygen supplementation to maintain recommended levels of DO. One method involves pumping nanobubbles of oxygen gas by hose-based diffusers, which can have different configurations, placed at depth inside net pens. Another method involves pumping water into a barged-based system, oxygenating the water within the barge system and then reintroducing the oxygenated water into the net pen. Due to the innovative nature of this technology, there are unknown behavioral effects introduced by pumping oxygen into sea cages. Studies have been performed to understand the effect of oxygen supplementation on physiology and behavior of juvenile salmon in smolt rearing systems. Some physiological effects have been recorded in hatcheries ranging from gas bubble disease to changes in swimming behavior (Kramer, 1987; Clarke *et al*., 2009; Espmark & Baeverfjord, 2009; Espmark *et al.*, 2010). Some examples of changes in swimming behavior include changes in schooling (Shelton and Johnstone, 1995), and vertical and horizontal distribution

(Espmark & Baeverfjord, 2009). However, to the best of our knowledge, no studies have been published in active Atlantic salmon sea cages.

In Chapter 2, I used acoustic tags to collect high frequency data on salmon positioning in three dimensions within marine cages (Stockwell *et al.*, accepted). This research allowed determination of variables such as swimming speed as measures of salmon behavior in relation to feeding storms, and destratification of the water column, providing a baseline for quantifying salmon behavior with this approach. Using identical methods, although at a different site, the aim of the present study was to investigate if oxygen supplementation affected Atlantic salmon (*Salmo salar*) behavior at a commercial aquaculture site in Nova Scotia (Canada). For this purpose, fish behavior was inferred from acoustic tags implanted in 15 individuals, which provide continuous three-dimensional positioning data. To understand the effects of supplemental oxygen conditions on farmed fish, it was assumed that behavioral responses can be related to changes in biological performance as a consequence of suboptimal environmental conditions (Martins *et al.*, 2011). Accordingly, environmental conditions that lead to behavioral modifications may be important in context of welfare.

3.3. Methods

3.3.1. Study Site

The study site is located in a semi-exposed bay off the Aspotogan Peninsula which separates the larger St. Margarets Bay and Mahone Bay, on the South Shore of Nova Scotia (Fig. 3.1A). Saddle Island provides protection to the farm from the mouth of the St. Margarets Bay. Seawater temperature at the site ranges from 0 – 3°C in the winter and early spring, with warmer waters ranging from 17 – 22°C in August and September.

Dissolved oxygen at site is ~ 4.5 mgL^{-1} in September, and ~ 12 mgL^{-1} in April (*pers comm* Cooke Aquaculture). The study period was from 2 October until 25 November 2019.

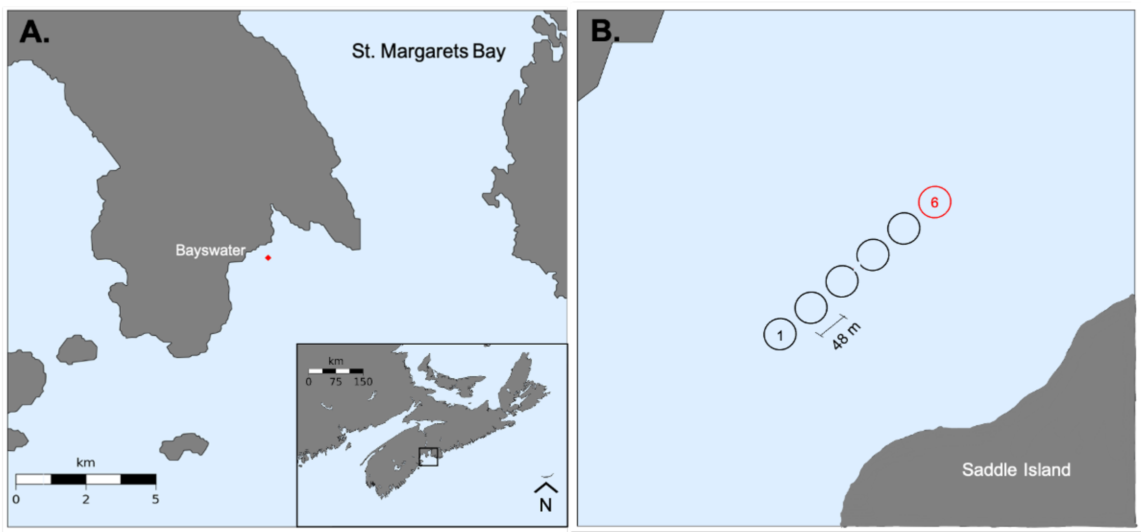

Figure 3.1: Site map of Saddle Island, Nova Scotia. A) Regional map where red marker shows site location and inset of Nova Scotia shows location of the site in St. Margarets Bay. B) Site schematic with study cage outlined in red.

The salmon farm is one column of 6 net pens, each measuring 48 m diameter, running from southwest to northeast (Fig. 3.1B). Maximum depth is approximately 11 m to cage bottom. The site had a stocking density of approximately 10 kg m^{-3} during the study period. Feeding was by vessel-based blowers twice a day for approximately 30 minutes at each net pen.

3.3.2. VEMCO Acoustic Tags

VEMCO acoustic tags (model V9P-180, InnovaSea Systems, Inc, Bedford, Nova Scotia, Canada) are 9 mm in diameter, 26 mm length, and 4g in air. 15 Atlantic salmon were surgically implanted with acoustic tags. Previous studies show that individual responses of salmon to environmental factors reflect group behavior in aquaculture settings (Oppedal *et al.*, 2010; Stehfest *et al.*, 2017). Furthermore, a higher density of acoustic tags could interfere with successful transmission and possible loss of data (Al-Dharrab *et al.*, 2013).

Adult salmon (60-72 cm and 4.5 kg) were retrieved from net pen #6, located on the northeastern edge of the farm. The surgical procedure followed Stockwell *et al.* (submitted); in brief, before each surgery, all instruments were disinfected using Vikron solution. Each individual was collected using a dip net and immersed, for about 2-10 minutes, in a 40 mgL^{-1} bath of TMS (MS-222) 3-aminobenzoic acidethy until visual signs of sedation were reached (no reaction to touch and loss of equilibrium; Moore *et al*., 1990). A 1-1.5 cm incision was made slightly offset from the linea alba, parallel to the midline, where the tag was inserted into the coelomic cavity. The opening was closed with an absorbable 3-0 monofilament sterilized suture using an interrupted surgical knot applied every 5 mm. Surgery averaged 6 minutes and fish were then placed in a 350-gallon recovery tank until opercular movement, equilibrium and locomotory movements were observed (averaging about 45 min; Moore *et al.*, 1990). All fish handling and surgery were performed by a certified veterinarian and in compliance with Dalhousie University Animal Care Council.

The acoustic array consisted of eight Vemco HR2 receivers deployed on a NSEW axis in two layers at 2 and 7m depth outside of net pen. All fish tags were equipped with a pressure sensor to measure depth, and the HR2 array was used to triangulate horizontal positions of each fish. The tags were programmed to transmit the resulting data of 3D position [x, y, z] every 3 seconds during the duration of the study. Data were then stored on HR2 receivers until the end of experiment when it was downloaded. Only relative changes in depth were used in the analyses due to uncertainty with the accuracy of absolute depth data based on a mismatch of recorded depth (mean = 17.4±0.60) and depth of the cage (mean = 8.31±3.02). In order to assess the uncertainty of the third dimension in velocity calculations, comparison between 2-dimensional and 3-dimensional velocity was performed, indicating complete agreement (3-dimensional velocity = 0.99 x 2-dimensional velocity + 0.006, n = 323433, r^2 =0.99, p = 0.001).

3.3.3. Oxygen Supplementation

An oxygen supplementation trial ran from 2 October 2019 until 7 November 2019, during which compressed oxygen was continuously supplied to all cages on site. The study cage was supplied with 93% oxygen and 7% argon gas mixture at an average flow rate of 136 $Lmin^{-1}$, with T = 15°C and P = 101.325 kPa. The system is a hose-based diffuser (NetOx, Bio Marine AS, Norway) shaped into 4 rings and placed at 7 m depth within the study cage

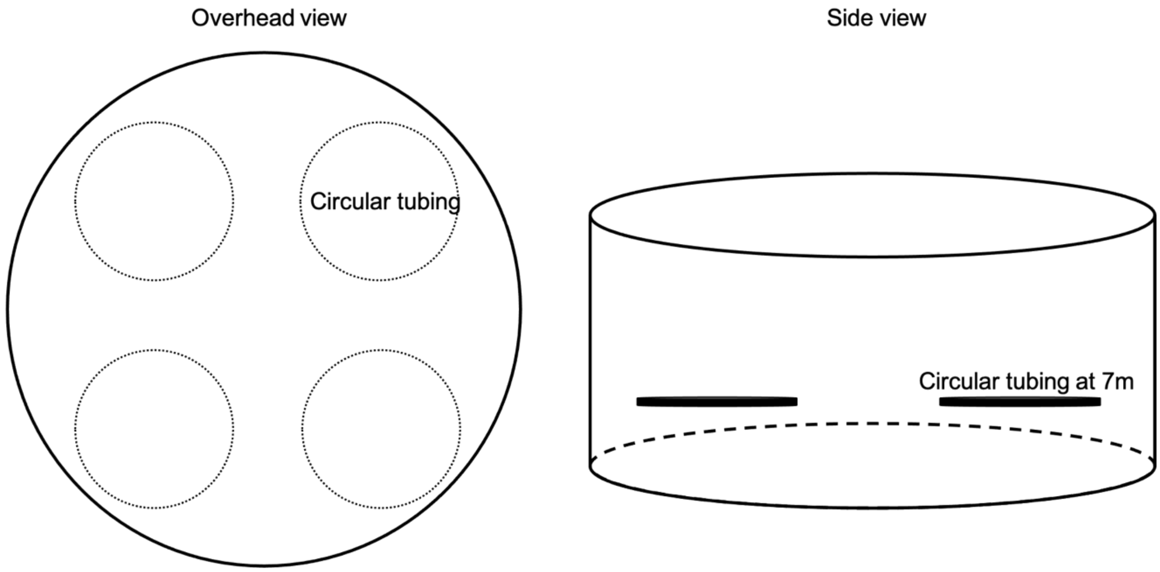

Figure 3.2: Schematic of oxygen supplementation system. An overhead view showing four circular tubing releasing oxygen gas mixture, and a side view showing the location within the net pen of the tubing.

(Fig. 3.2). Diesel generators and compressed gas were housed in an onsite barge southeast of the cages with one barge supplying 3 cages. No control cage without supplementation was available due to the necessity of reducing fish mortalities during low DO months.

3.3.4. Additional data

Additional husbandry data, including feeding days and mortality rate were collected by Cooke Aquaculture (Kelly Cove Salmon Ltd.). Temperature and dissolved oxygen data were recorded using AquaMeasure wireless sensors (InnovaSea Systems, Inc., Bedford, Nova Scotia, Canada). Two sensors were placed at 2 and 7m depth in the center of the net pen. Data were collected every 2-3 minutes, acoustically transmitted to on-site data hub, and stored on a cloud-based server.

3.3.5. Data Processing

Initial processing of raw data was completed by VEMCO and data were returned in an [x, y, z] format with a datetime stamp out to 10^{-6} seconds. The system was then normalized using HR2 locations with [0, 0, 0] representing the estimated cage center at the surface. Two error values were provided with each fish position, 1) root mean square error (RMSE), and 2) horizontal error position (HPE). RMSE is a time-based error component where a higher value signifies a longer than estimated return time indicating a multipath signal. RMSE ranges from 0-5, with 0 representing a straight path with no reflection or refraction of the signal, and 5 representing a high reflection or refraction of the signal. RMSE was used to filter data as to disregard outliers by removing the highest 10% of the data, a value of 0.32 or greater. HPE is a relative horizontal error and is calculated using 3 receivers that create an array. The HPE value can range from 0-2, where a higher error sensitivity is represented by a higher number. This error value was used to filter data as to disregard outliers by removing the top 10%, a value of 0.6679 or higher. Mortality during the production cycle within the study cage was about 11%. Among the 15 tagged fish, data were unusable from 2 individuals due to poor quality and quantity. Thirteen data sets were usable out of the original 15 tagged individuals, with no mortalities in the tagged individuals.

The two-dimensional fish swimming distance between two consecutive time acoustic records, d_{12}, was calculated using [x, y] positioning following:

$$d_{12} = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2} \qquad (1)$$

where $[x_1, y_1]$ to $[x_2, y_2]$ are the positions for two consecutive acoustic records. Further, velocity between those two consecutive records, v_{12} over sequential times t_1 and t_2 was calculated as:

$$vel_{12} = \frac{d_{12}}{t_2 - t_1} \qquad (2)$$

Distance from center of the net pen, d_{center}, was calculated for each acoustic position following:

$$d_{center,1} = \sqrt{{x_1}^2 + {y_1}^2} \qquad (3)$$

Turning angle from the fish point-of-view, $turn_{13}$, was calculated using three consecutive acoustic records as follows:

$$turn_{13} = 180 - \cos^{-1}\left(\frac{{d_{12}}^2 + {d_{23}}^2 - {d_{13}}^2}{2 d_{12} d_{23}}\right) \qquad (4)$$

where, d_{23} is the distance from $[x_2, y_2]$ to $[x_3, y_3]$, and d_{13} the distance from $[x_1, y_1]$ to $[x_3, y_3]$. A time filter was applied to ensure that only acoustic records within 5 seconds were used in all calculations. This ensured a high quality of data by reducing the number of detections used, which is acceptable given the high temporal resolution of the data (~ 3 seconds).

3.3.6. Statistical Analyses

The study period was divided into two periods, during the oxygen trial (2 October – 7 November) and after the oxygen trial (8 – 25 November). A pairwise comparison of position variable between these two periods involving each fish was conducted using a Wilcoxon signed-ranks test. In order to resolve the periodicity of depth and velocity, spectral analysis was conducted using signal power content versus frequency. A Wilcoxon signed-ranks test was used to compare periodic fish variables (speed and depth) between day- and nighttime using daylight hours to indicate day and night. The relationship between fish variables and environmental variables were examined with linear regression fit to daily averages. Saturation oxygen concentration was calculated based on measured temperatures. All data processing was completed in Python Spyder 3.7 (Python Software Foundation, https://www.python.org/).

3.4. Results

3.4.1. Environmental conditions

During the study period, temperature ranged from 7.7 to 13.8°C and dissolved oxygen (DO) from 6 to 9.3 mgL^{-1} (Fig. 3.3). An extended record from sensors at 2 and 7m (Burke *et al*., in prep) indicated strong temperature stratification until the end of August, when the water column became mixed. The water column did not generally cool at that time but continued to warm into early September after which it began the decline shown herein. Temperatures were similar between 2 m and 7 m depth during the study. DO fluctuated during the oxygen trial, usually below saturation values, ranging from 6 to 8.4 mgL^{-1} at 7m and 7.9 to 8.7 mgL^{-1} at 2 m. There was a general decline in DO during late October, reaching a minimum of 6.0 mgL^{-1} on 31 Oct (Fig. 3.3B). Beyond this minimum, there was an increasing trend which persisted through the end of oxygenation, beginning even before the sharper decline of temperature at the overturn. After the oxygen supplementation trial, DO increased, as temperature decreased, from 7.5 until 8.9 mgL^{-1} at 7m and 8.3 – 9.3 mgL^{-1} at 2m by the end of the study period. There was a similar DO pattern between 2m and 7m with 2m having slightly higher concentrations throughout the study. Saturated DO was calculated based on temperature and assuming 100% saturation increased across the study period ranging from 8.3 – 9.5 mgL^{-1}. It is difficult to determine the extent to which the

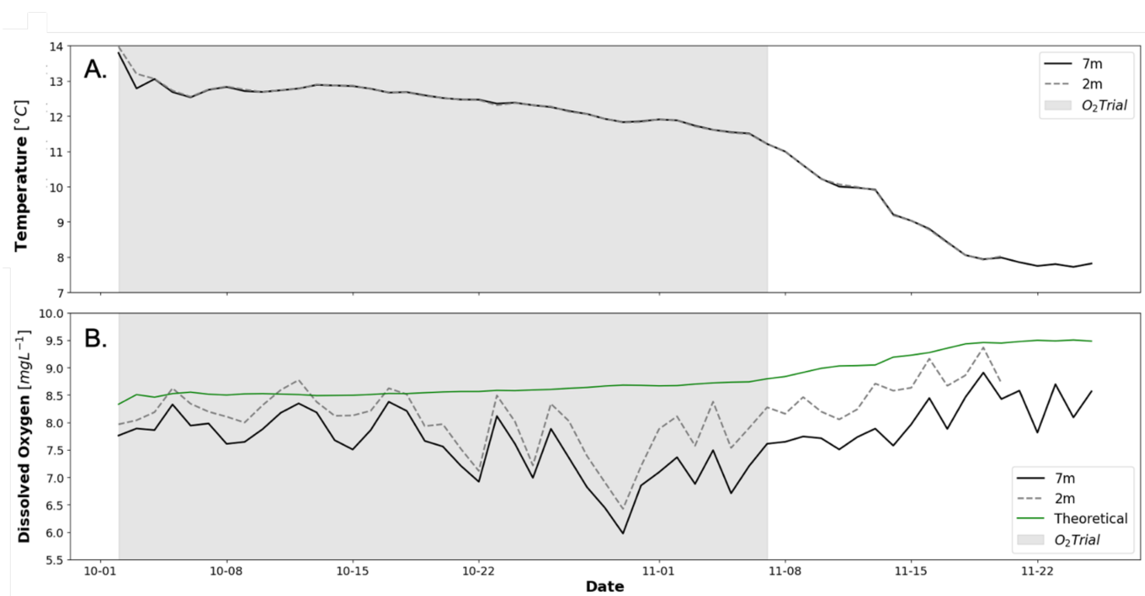

Figure 3.3: Temperature and dissolved oxygen daily averaged time series during study period. Gray shaded area shows when the oxygen supplementation trial was running. A) Temperature [°] with solid black line showing 7m depth and dashed gray line showing 2m depth, B) Dissolved oxygen [mgL^{-1}] with solid black line showing 7m depth and gray dashed line showing 2m depth with the solid green line showing saturation concentration.

system increased oxygen above ambient since there are no reference data without supplemental oxygen, but the DO was at least maintained above the critical level of 6.0 mgL^{-1} allowing fish to continue to feed during the expectedly low DO months.

3.4.2. Background swimming movement

Four fish variables were calculated to describe individual fish behavior, including (a) velocity [ms^{-1}], (b) distance from center of cage [m], (c) turning angle [°], and (d) depth [m], although only relative values of depth were considered in the analyses. All movement and activity variables were largely consistent within the tagged population, despite averaging across the oxygenation period and subsequently. Swimming velocity averaged 0.72 ± 0.39 ms^{-1} for the tagged population during the study period (Fig. 3.4A). Spectral density analysis of velocity (one-hour rolling) showed a strong peak at 24 hours for 77% of the tagged individuals (Fig. 3.5A). The remaining three fish showed peaks ranging from 22 to 25 hours. In general, swimming speed was significantly higher during the day (0.77 ms^{-1}) than at night (0.69 ms^{-1}; $p = 0.003$). Spectral density analysis of relative depth (one-

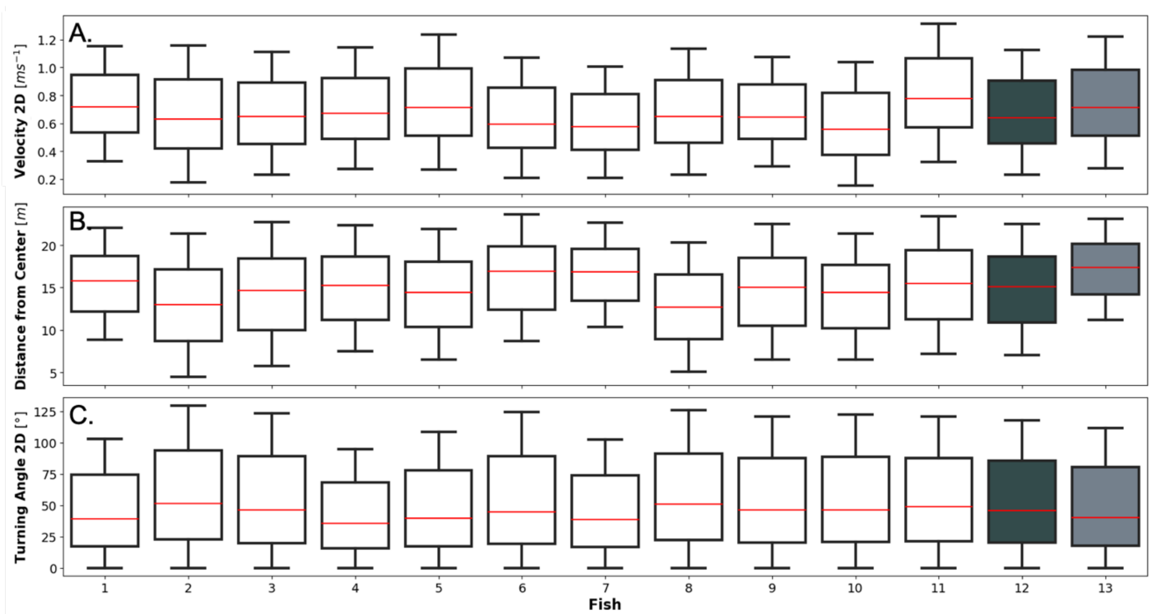

Figure 3.4: Boxplots of three fish variables for each individual during the study period where center line represents the median, box incorporates 25-75th percentiles, and error bars represent standard deviation. A) Velocity [ms^{-1}]. B) Distance from center [m]. C) Turning Angle [°]. Fish 12 and 13 are colored with shades of gray to highlight two individuals who represent the high and low ends of the population's spectrum.

hour rolling) exhibited a strong peak at 24 hours for 69% of individuals with the remaining

4 individuals displaying peaks ranging from 23 to 25.5 hours (Fig. 3.5B). Depth among the tagged population was deeper during daylight than at night ($p < 0.0001$).

The tagged population averaged 14.52 ± 5.1 m from the cage center during the study (Fig. 3.4B). Heatmaps of horizontal fish positioning are based on percent of observations in a given location. In both cases, relative density was less than 0.5% at a single location since the fish uniformly occupied the net pen. Two individuals are highlighted because they are representative of the range of swimming behaviors found in the population. Fish 12 and 13 held a higher relative density on the southeast and southwest edges of the net pen (Fig. 3.6A, B) and Fish 13 swam in a more circular pattern (Fig. 3.6D). Fish 12 had a less clear circular swimming pattern (Fig. 3.6C) and spent some time near the center of the cage. Overall, however, preference was away from cage center which is representative of the range of the population. An absence of data from the northeastern section may be an artifact of the array not detecting there. Turning angle was used to measure the change in bearing of fish trajectory (Fig. 3.4C). The population averaged 53.25 ± 44.12°, where 0° denotes a straight line in the same direction, and 180° indicates a U-turn. The directionality of swimming vectors for the same two individuals, Fish 12 and 13, indicated

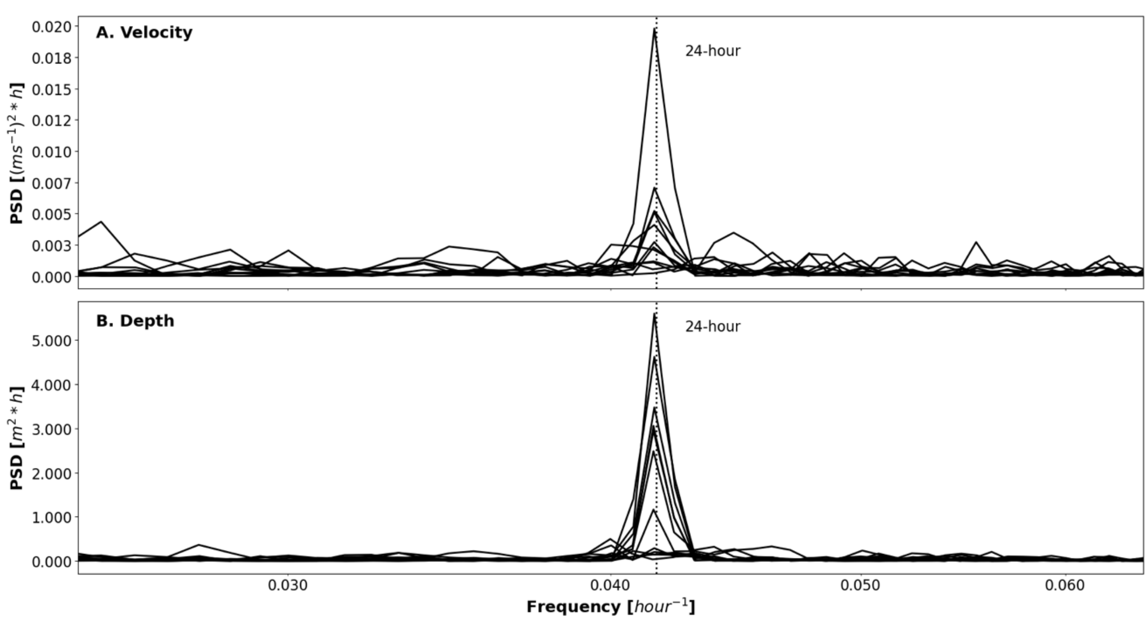

Figure 3.5: Power spectral analysis of A) velocity [ms^{-1}] and B) depth [m] using one hour rolling mean of all individuals.

counterclockwise movement for both (Fig. 3.6C, D), which was also observed for all tagged individuals.

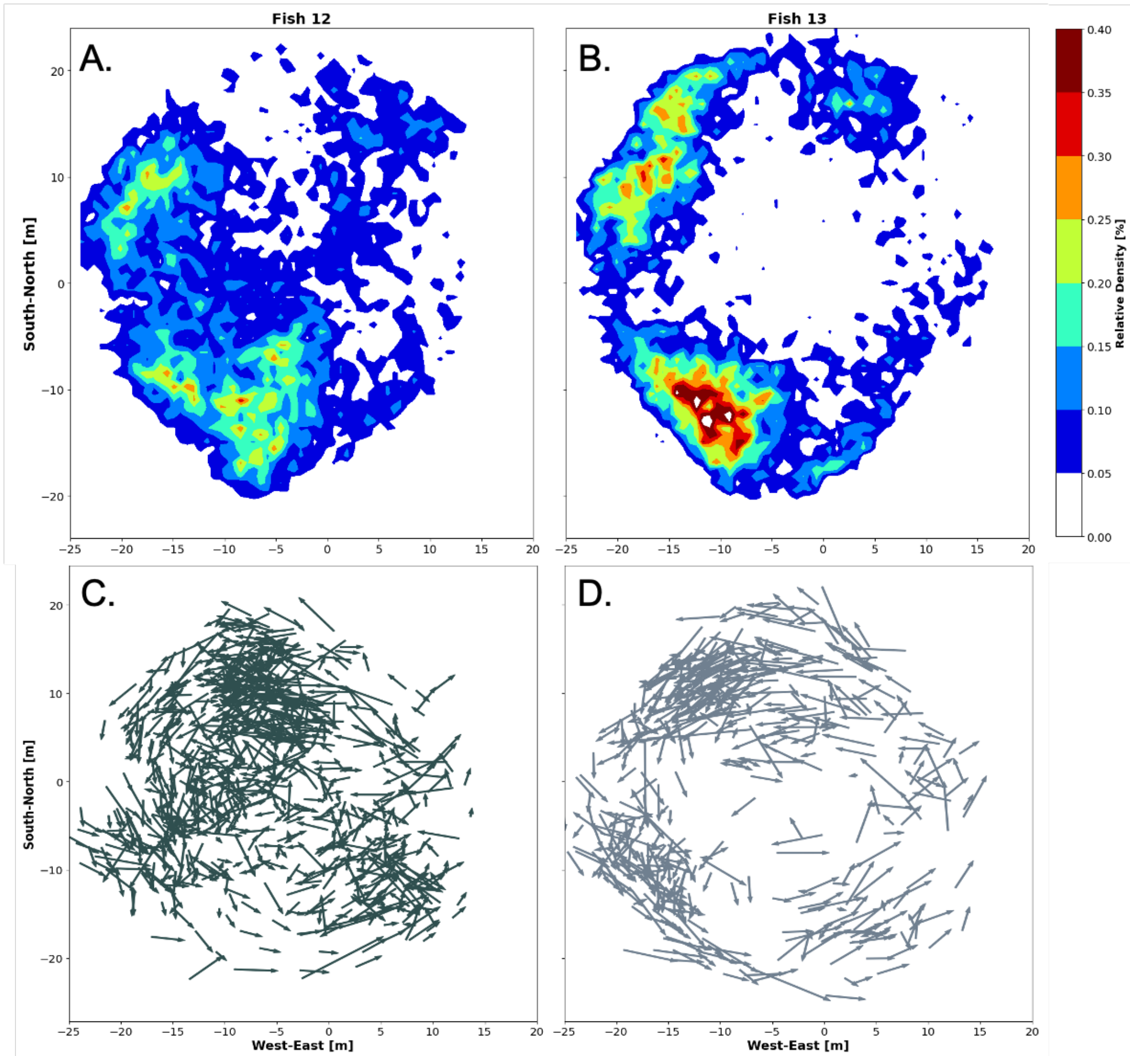

Figure 3.6: Horizontal plots of two tagged individuals which are representative of the range of behaviors from the population. A) Heatmap of fish 12 during the study period. B) Heatmap of fish 13 during study period. C) Vector plot of fish 12 from 31 Oct 2019 to 18 Nov 2019. D) Vector plot of fish 13 from 31 Oct 2019 to 18 Nov 2019.

3.4.3. Swimming during Oxygen Trial

Behavioral responses before and after oxygenation were largely consistent, with never more than 2 fish of 13 displaying contrary results. During the oxygen trial, all tagged individuals displayed slower swimming velocities with a relative reduction of 5 – 23% compared to after the trial (Fig. 3.7A). As a population, the swimming velocity

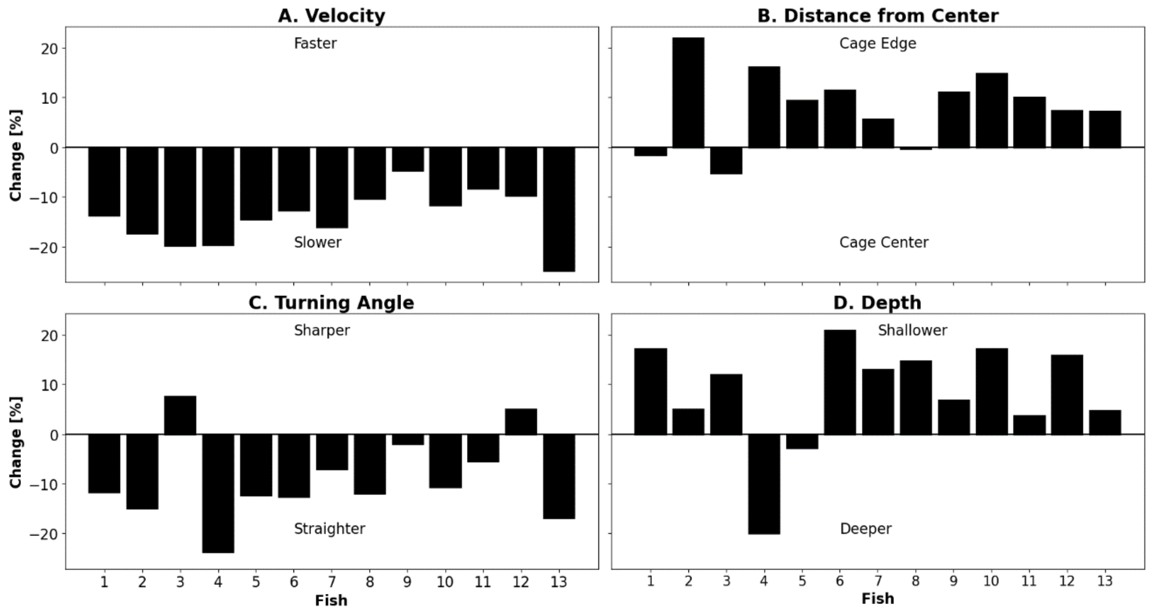

Figure 3.7: Percent change of individuals comparing during oxygen trial (2 Oct – 7 Nov) to after the trial (8 – 25 Nov). A) Change in velocity where a positive change indicated faster swimming speed. B) Change in distance from center where a positive change signifies a movement towards cage edge. C) Change in turning angle where a sharper turning angle is represented by a positive change. D) Change in depth where a positive change represents shallower swimming depths.

significantly changed from 0.66 ± 0.34 ms^{-1} during the trial to 0.79 ± 0.44 ms^{-1} after the trial, a significant change in pairwise comparisons ($p<0.001$, Table 3.1). During oxygen supplementation, 77% of tagged individuals showed a change towards the edge of the cage with a relative change of 6 – 22% compared to the values after the trial (Fig. 3.7B). The remaining 27% of the individuals moved towards the cage center with only a slight relative change of 0.3-5.2%. At the population level, the distance from center changed significantly from 15.3 ± 5.0 m during the trial to 13.74 ± 5.17 m after the trial (pairwise $p<0.01$, Table 3.1). Turning angle in 85% of tagged individuals displayed straighter swimming angles during the trial with a relative change of 2 – 24% compared to after the trial (Fig. 3.7C).
The remaining 15% of the population showed opposite trends with sharper turning angles during the trial, with relative changes of 5.1 and 7.6% (Fig. 3.7C). When considering the whole population, the turning angle changed from 49.84° ± 42.54° during the trial compared to 56.65° ± 45.7° after the trial (pairwise $p<0.01$, Table 3.1). During the trial, 85% of the tagged population swam significantly shallower than after the trial with a

relative change of 3 – 21% (Fig. 3.7D; $p<0.05$, Table 3.1). Fish 4 and 5 responded in opposite by swimming deeper by a relative change of 19.9% and 2.7%, respectively.

*Table 3.1: Mean values and standard deviation for each fish variable during and after the oxygen trial. Although means are expressed as a summary statistic, a Wilcoxon signed-rank test was used in pairwise comparisons for each fish. N = 13 for all scenarios. *only relative changes in depth were analyzed.*

Fish variable	**Mean During Oxygen Trial**	**Mean After Oxygen Trial**	**Test statistic**	**P-value**
Velocity [ms^{-1}]	0.66 ± 0.34	0.79 ± 0.44	<0.001	<0.001
Distance from Center [m]	15.3 ± 5.0	13.74 ± 5.17	6.0	<0.01
Turning Angle [°]	49.84 ± 42.54	56.65 ± 45.7	7.0	<0.01
Depth	*	*	14.0	<0.05

Linear regression indicated a negative relationship between swimming velocity and temperature for all individuals in the tagged population ($p<0.001$). with slope ranging from -0.016 to -0.065. Fish 12 and 13 were chosen to represent the highest and lowest slope values in the tagged population (Fig. 3.8). Fish 12 had a flatter slope ($y = -0.016x + 0.89$, $R^2 = 0.1$, $p < 0.05$) whereas fish 13 was on the opposite end of the spectrum ($y = -0.065x + 1.5$, $R^2 = 0.56$, $p < 0.001$). A linear regression was also explored between swimming velocity and oxygen with no significant relationships identified.

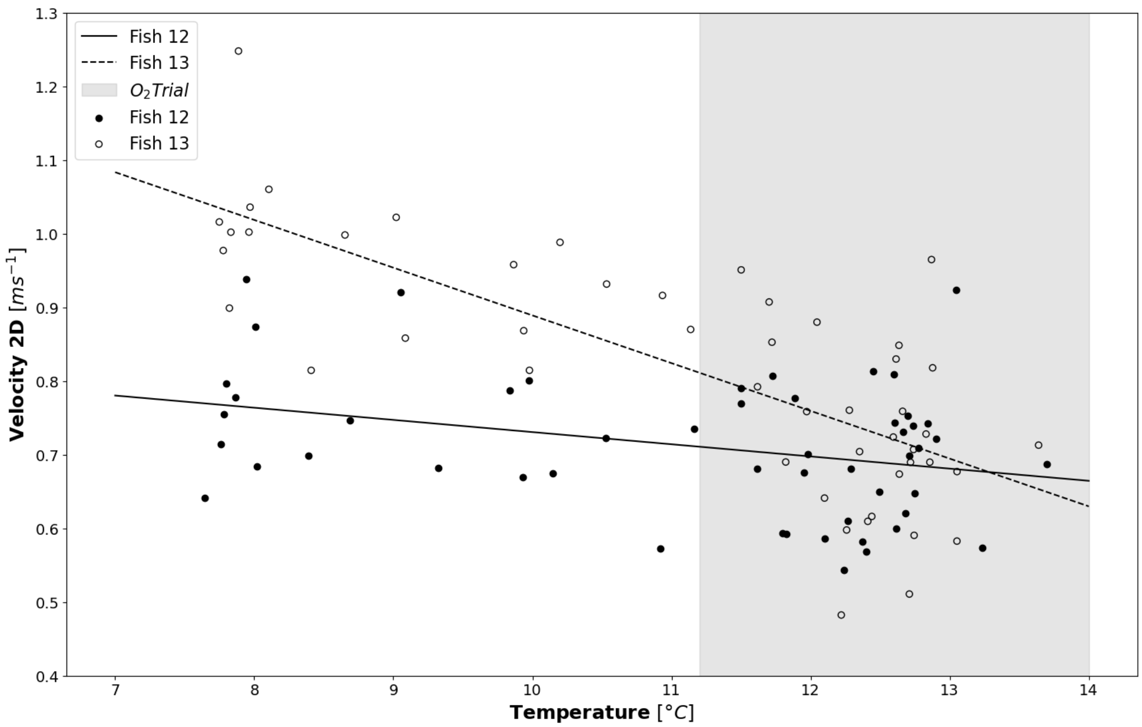

Figure 3.8: Linear regression for daily averages comparing temperature and velocity of two individuals, fish 12 (closed circles, solid line) and 13 (open circles, dotted line). The shaded gray region indicates temperatures associated with the oxygen supplementation trial.

3.5. Discussion

By combining acoustic tags with a receiver array, a 3-dimensional view of Atlantic salmon positioning and swimming behavior was obtained in a commercial net pen setting. This detailed information has provided evidence of a notable response of fish behavior to an oxygen supplementation system. Previous studies of fish behavior using 3-dimensional acoustic telemetry have been conducted in aquaculture sites (i.e., Cubitt *et al*., 2003; Føre *et al*., 2011; Stockwell *et al*, accepted), but this study reports for the first time the effects of supplemental oxygen system on behavior in farmed salmon.

3.5.1. Swimming movement

While consideration of the oxygen trial on fish behavior is of interest, a generalized comparison of our overall results with previous research is also useful. Velocity ranged from 0 – 2.46 ms^{-1}, similar to previous studies where swimming speed ranged from 0 – 2 ms^{-1}, where velocity is standardized to the length of the fish (Juell & Westerberg, 1993; Andrew *et al*., 2002; Føre *et al*., 2011 & 2018b; Stockwell *et al*, accepted). The upper range of velocity is higher than reported in a study using identical hardware at another Nova Scotia salmon farm (Stockwell *et al*, accepted) where the upper limit was 2 ms^{-1}. The present study demonstrated a distinct diel rhythm in swimming velocity, with higher speeds during the day and slower speeds in the evening. Previous studies have found similar patterns with velocity, with the fastest velocities during the day (Juell & Westerberg, 1993; Andrew *et al*., 2002; Føre *et al*., 2011 & 2018b; Stockwell *et al*, accepted). Atlantic salmon rely heavily on vision while swimming (Oppedal *et al*., 2011), which explains the higher swimming velocities with light availability (Oppedal *et al*., 2001). Depth also showed a distinct diel rhythm with shallower depths at night, and deeper positioning during the day. This pattern in depth has been observed in previous studies and it has been linked to light intensity, feed anticipation, and apparent threat of predation (Oppedal *et al*., 2001 & 2007; Juell & Fosseidengen, 2004; Johansson *et al*., 2006 & 2009; Stockwell *et al*, accepted). Therefore, the tagged fish in this study followed the expected pattern previously described in literature, with greater depths and higher velocities during the day and lower velocities and shallower depths during the night (Fernö *et al*., 1995; Andrew *et al*., 2002; Johansson *et al*., 2006; Oppedal *et al*., 2001; Føre *et al*., 2011 & 2018b; Stockwell *et al*, accepted).

There was a consistent, circular, counterclockwise swimming pattern in all individuals of the population. Although directionality can vary between farms or even between cages (Juell, 1995; Johansson *et al*., 2014), all Atlantic salmon individuals from the same cage tend to remain together and adopt a polarized swimming pattern, which define the ‘traffic rules’ within the cage. Any deviations from these rules can cause a group reaction which can have implications for schooling (Martins *et al*., 2011). The existence of a common swimming pattern for all individuals in the population allows the extrapolation of behavioral responses from individual to the population level; although each individuals movement is unique and can be termed as a micropersonality (Bailey *et al*., 2021). The counterclockwise pattern observed in this study is an example of schooling behavior which indicates that group behavior can be inferred from individual movement.

3.5.2. Oxygen Supplementation Trial

Additional oxygen was supplied into the study net pen during late summer and into fall. DO concentrations during the trial were close to and sometimes above oxygen saturation, indicating oxygen levels were improved within the net pen while the system was working. However, when DO concentrations fell to lowest levels in late October, they remained below oxygen saturation. Oxygen supplementation systems in which nanobubbles of oxygen gas are introduced into seawater are not intended to increase DO concentrations, but instead maintain DO levels at a biologically important level. Low oxygen events are common at fish farms in coastal Nova Scotia (Burke *et al*., 2021) and, fortunately, DO did not drop below 6.0 mgL^{-1}, the minimum level required by Atlantic salmon to continue to feed at this temperature range. Furthermore, the lowest DO during the oxygenation time series caused no significant changes in the behavioral response variables when compared to the previously higher oxygen state (analysis not shown). Therefore, although there is no reference cage without a supplementation system, the fact that DO did not drop below critical levels and behavior did not changed during the lowest DO suggest that the supplemental oxygen system was successful.

Four fish variables were used to describe fish movement: velocity, distance from center, turning angle, and depth. Although we focus on the oxygen trial, the end of supplementation coincided with a temperature drop. Therefore, there are two simultaneous changes in culture conditions: end of oxygen addition and a decrease in temperature. In our previous work at another site without added oxygen (Stockwell *et al.* accepted), a comparison between swimming behavior before and after the fall overturn, an oceanographic event that is characterized by an unstratified water column and rapid temperature decrease, was conducted based on acoustic tags and identical methods in the present study. In that study, individuals also moved towards cage center slightly when temperatures dropped. This suggests the likelihood that changes in horizontal positioning observed in the present study were not due to the oxygen system. No significant difference was found in swimming behavior for counterclockwise directionality, depth or turning angle in the previous study as a result of the fall overturn. In the present work, in addition to swimming slower during oxygenation, fish swam straighter and shallower. Among behavioral variables assessed during the oxygen trial, swimming speed was the only one with complete consistency for all fish, with slower speeds during the trial when water temperatures were also higher. Contrarily, previous laboratory studies on salmonid behavior have found an increase in swimming speed with temperature (e.g., Brett, 1967; Tang & Boisclair, 1995; Oppedal *et al*., 2001; Hvas *et al*., 2017). Moreover, in a previous Nova Scotia study with similar methods, temperature and swimming velocity also showed a positive correlation (Stockwell *et al.*, accepted). In the present study, the negative correlation between swimming speed and temperature, and the upward movement toward the surface, is potentially due to the presence of supplemental oxygen/infrastructure in the net pen, suggesting potential effects on fish behavior.

3.5.3. Implications and future prospects

This study employed detailed fish positioning to catalog behavioral responses using calculated swimming velocity and horizontal location. There are many external environmental variables that can affect behavior, such as oxygen and temperature, as well as farm procedures, such as oxygen supplementation. The confined environment found in net pens restricts the range for behavioral responses. Some changes in identified behaviors are subtle and difficult to assess with known causation. The use of 3D acoustic tags in field experiments at commercial fish farms have found common swimming patterns such as diel vertical movement and circular swimming patterns (e.g., Føre *et al*., 2017; Muñoz *et al*., 2020; Macaulay *et al*., 2021). To the best of our knowledge, this study is the only study in which acoustic tags were used to incorporate high frequency data to understand possible changes in fish behavior when oxygen supplementation systems are implemented in commercial aquaculture sites.

The study used position-based behaviors to observe the response to an oxygen supplementation system. A response in swimming velocity with temperature was found to be contrary to previous studies where no oxygen system was installed. Therefore, the change in swimming speed is most likely caused by this system, which could be a result of stress induced by the oxygen supplementation (Ritola *et al*., 2002). Stress is also manifested by morphological and physiological responses which can be understood using operational welfare indicators (OWI). Fin and scale health, as well as other metrics of condition, would provide a fuller picture of apparent stress due to the oxygen supplementation system (Martins *et al*., 2011; Oppedal *et al*., 2011; Nobel *et al*., 2018). Furthermore, in order to apply OWIs to positioning, a better understanding of their combined effect on behavior and importance of each indicator is required (Stien *et al*., 2013).

Climate change is triggering increased sea surface temperatures and decreased dissolved oxygen, leading to more sites requiring supplemental oxygen (Keeling *et al*., 2009; Johannesen *et al*., 2020). Our work suggests behavioral changes associated with oxygen supplementation. However, the implications of these changes for feeding, growth, and fish health are not yet clarified. The development of farm sensing technology associated with intervention such as oxygenation provides further scope for management options leading to improved fish welfare and more sustainable aquaculture.

CHAPTER 4 : DISCUSSION

Acoustic telemetry provides information on fish positioning. These data can be used to assess behavior, and from behavior we can infer welfare. The function-based definition of welfare, as defined in the introduction as fish health not being pushed passed their biological thresholds, which was used throughout these studies (Chapters 2 & 3), helped to define behavior for each study population at each study site. Behavior was defined using four fish variables 1) depth, 2) swimming speed, 3) distance from center of cage, and 4) turning angle. These variables help to create an overall picture of fish movement behaviour, but do not directly provide insight on welfare; however, the application of this method under different environmental conditions provide insight into the effects of stressors on behavior, which have implications for welfare. In order to further the understanding of the effects of these changing environmental conditions on welfare, operational welfare indicators (OWIs) could provide visual signs of stress that movement data is not capable of detecting.

OWIs provide a framework for assessing welfare of fish by utilizing a limited number of parameters to determine the overall health of individuals (Stein *et al*., 2013). Some of these parameters include visible abnormalities such as scale loss, gill and eye lesions, and overall body shape/condition which can provide farmers with early warning indicators of health Martins *et al*., 2011). OWIs rely on the assumption that if the fish looks healthy then it is probably healthy, if the fish looks like it is not thriving then there is most likely an underlying problem. This information paired with movement behavior could lead to a better understanding of fish welfare and health (Stein *et al*., 2013).

With current methods of visual inspection, which involve manually capturing individuals or attempting to spot individuals through a submerged camera, having real-time assessment of fish welfare is nearly impossible. Despite the limitations of acoustic telemetry, tagging a small population, as used in these studies, is a feasible solution to understanding of cage behavior. With a real-time application of this information, along with frequent OWIs report, an overall index of health of aquaculture cages can inform farmer decision making.

4.1. Lessons Learned

During chapter 3, I attempted to place a stationary tag at the cage edge in order to understand absolute error and any cage drift that may have occurred. However, that was not successful due to placing the tag later than the study period and using a cinderblock in hopes it could within movement, which it did not. If I were to attempt that again, I would find a more secure method for installing a stationary tag, independent of the cage, and not interfering with its acoustic signal.

Some lessons I learned through the coding processes that lead the decisions I did were arduous. With high temporal resolution, any time step could theoretically be studied, meaning this dataset has many more opportunities to be analyzed and new results to be discovered. I decided to do every minute averages for each positioning because I believe it gives the best of both worlds, seeing the smaller movements, but also still seeing the big picture. If say a 3 second time scale was used, a smaller period would need to be analyzed as to not be overwhelmed with the shear amount of data as well as to get a better understanding of smaller movements individuals can make. But also, a larger time scale could have been chosen to show the big picture movement, such as 5 minutes or so. I think for a larger time scale, this would be useful for large oceanographic events over longer periods of time, such as warming oceans over a year or so, which would require analyzing the data from both chapters 2 & 3 to get a better understanding. Another major learning area was integrating all the data sources into one file and making sure that all the time zones match up. Often, environmental data collected from weather stations, or on-site data collected by farmers are in local time, but acoustic tags and many other oceanographic tools collect in UTC and proper alignment of the data is necessary to properly understand the correlation of the datasets.

Furthermore, the process when troubleshooting new technology with a company involves flexibility on the researcher side, understanding that the company doesn't necessarily understand where there might be problems as well as how to fix problems that are introduced. For example, in chapter 2, some tags were unusable due to technical problems, and once they were notified, they worked to make sure it didn't happen again, which it did not in chapter 3. There were also a lot of back-and-forth between myself and VEMCO when processing the first dataset. I ran through the dataset and identified anomalies or any problems with each tag and came prepared to a meeting to have a discussion or what could be explained with my error or misunderstanding, and any problems which we could not identify that way became a discussion of what could be done on the coding ending, and what needed to be fixed in the experimental set up. Without this open communication between us and them, the analysis would not have been as smooth, and problems would not have been addressed for the second study.

4.2. Conclusions

Through these studies (Chapters 2 & 3), I have established baseline behaviors of farmed Atlantic salmon in Nova Scotia. I was able to determine how feeding, fall overturn, storms, and oxygen supplementation can affect behavior. As the climate continues to change, more extreme environmental events are likely to occur more often. Chapters 2 & 3 provided an insight into how behavior is affected now by these changes and what to expect for future events. The oxygen supplementation project was a completely unique study and will start to become more common as ocean temperature continue to increase. The findings from Chapter 3 provide new information to the field and with further investigation a more complete picture of the impact of the oxygen system on fish behavior can be understood. With the integration of OWIs to the results of Chapters 2 & 3, a more comprehensive picture of fish health will become available leading to improved welfare. This thesis has filled an important knowledge gap in the field of cultured Atlantic salmon behavior.

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