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# **Behavioral Experiments of Atlantic Salmon, *Salmo Salar*, for Individualized Feeding Stations.**

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Aquaculture

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## *Preface*

Someone once said to me that aquaculture engineering is the compromise between fish behavior, biology, and the technical solutions to meet your production goals. These production goals not only include maximizing production, but doing so in a sustainable way that emphasizes fish welfare. All of these can be furthered through the use of individual monitoring based on feeding behavior. Having been an aquarium hobbyist, an avid fisherman, and later an aquaculturist I thought I understood fish behavior relatively well, but it was not until starting this project that I realized just how intelligent they truly are. At times throughout this experiment I wondered which of us, the fish or the experimenter, was more intelligent. I ran my life around this experiment for months, being at the fish lab during every meal until I figured out how to set up remote accessing, scrolling through over 500,000 photos, and the countless hours writing and editing. None of this would have been possible without the support of my advisors, the guys down at the fish lab, my friends, and of course my family. I wish I could thank each and every one of them personally, but this will have to suffice. Thank you.

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# **Behavioral Experiments of Atlantic Salmon, *Salmo Salar*, for Individualized Feeding Stations.**

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## *Abstract*

*These experiments set out to test whether Atlantic salmon, *Salmo Salar*, would pass through a narrow hatch, thus imitating the action of entering a feeding station, and whether this behavior could be influenced. In the first two experiments a tank was divided into two sides by a barrier with a small opening in the middle. To induce crossing through the opening, feedings were alternated between the sides. The tank and fish were observed via camera and the time of each crossing and the total number of crossings were recorded. In the second experiment, pavlovian conditioning was used in an attempt to influence the crossing behavior. In the last experiment two hatches were cut into the divider to determine if fish had a preference for crossing with or against the current. In the first experiment a general upward trend in crossings through the hatch occurred as the time in the experiment increased. In the second experiment the effect of pavlovian conditioning was undetermined due to aggressive territorial behavior developing within the test subjects. In the third experiment fish were shown to prefer crossing against the current. Crossing behavior was divided into four categories, a direct cross, a searching cross, a delayed cross, and a joint crossing. These behaviors can help in the design of the systems in future experiments. Lastly, the effect of the hatch on growth statistics was observed and deemed to have significant negative impact, however, the impact lessened with time.*

## **1. Introduction**

Individualized monitoring is a key component of a precision livestock farming system (PLF). The main purpose of implementing PLF is to improve animal welfare and productivity through the use of advanced monitoring systems for data collection (Benhazi, 2012). This data is a vital indicator for important husbandry decisions producers make throughout the production process. The ability to collect this data has increased greatly thanks to technological advancements in livestock monitoring systems through the use of pit tagging, RFID chips, sonar, optical detection, and imaging software (Eradus, 1999; Zion, 2012). Using this technology individualized monitoring systems for cattle, poultry, and swine have arisen (Frost, 1997; Benhazi, 2009). Typically, systems for terrestrial animals utilize a feeding station with a sensor at the entrance that monitors for an individual's unique electronic tag. Then individuals can be weighed and the amount of feed or water consumed at said station measured (Frost, 1997). As with terrestrial animals, the monitoring of the feed intake of fish allows for predictions into the

health and welfare of the stock (Conte, 2004). Developing such a system for aquacultured species, like Atlantic salmon, has proven to be more difficult as a result of their behavior (Huntingford, 2012), the three-dimensional aspects of their habitat, and the harsh treatment of monitoring equipment in the aquatic environment (Lekang, 2013).

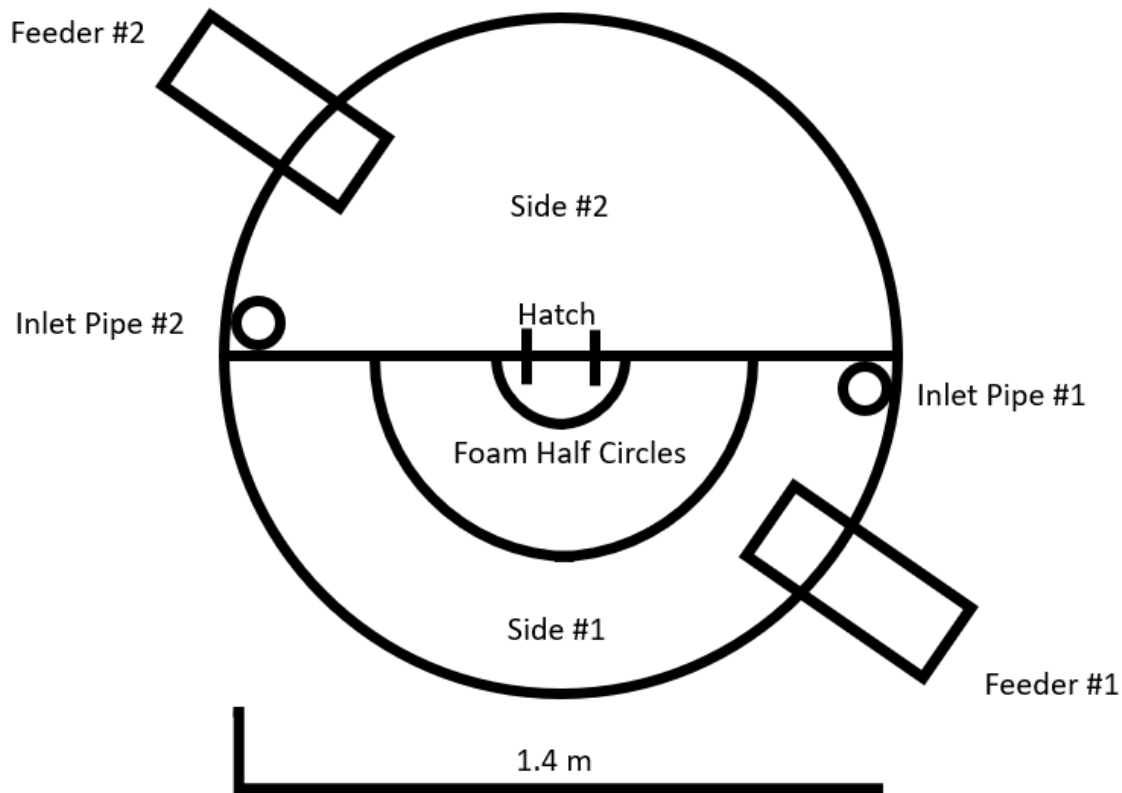
The behavior of Atlantic salmon in such a system is of particular interest. Fish behavior is a result of intrinsic drives, such as hunger and stress, combined with learned associations to extrinsic stimuli (Fernö, 2011). These stimuli are gained through the exploration of an individual's surroundings and provide crucial information about food resources and their spatial environment (Inglis, 2001). The information primacy hypothesis suggests an individual's fitness is dependent on the gathering of this information to increase the odds of satisfying its primal drives (Inglis, 2001). Behavior is thus a response to placate intrinsic drives through the utilizing this information.

The experiments described below set out to determine if the underlying behavior required for such a feeding station system exists in Atlantic Salmon, whether that behavior could be influenced, and what the effect on growth may be. The aim of the first experiment was to determine whether Atlantic Salmon would cross through a restricted opening in order to access feed, alluding to the act of entering a feeding station. The aim of the second experiment was to establish if pavlovian conditioning, or stimulus, training with light could be used to elicit the behavior. Lastly, the third experiment set out to ascertain if crossing behavior could be influenced by the flow of water through the entrance to such a system.

## **2. Materials and Methods**

### **2.1 Experimental Materials**

The first experiment was carried out between the 26th of June until the 1<sup>st</sup> of August 2018 at the NMBU, Norwegian University of Life Sciences, fish lab. Ten Atlantic Salmon, *Salmo Salar*, were selected at random from the stock. The average weight of the fish was  $139.2 \pm 19.3$  grams. They were placed in a 1,300-liter tank that was 1.4 meters in diameter and one meter tall with a slightly concave bottom sloping to a center drain (figure 1). The tank was divided in half, on the centerline, by a 7mm plexiglass sheet, that was sealed to the sides and the bottom of the tank, except where it crossed over the drain, and reached 10 cm above the water level. Two floating foam half circles were mounted to the divider on side one with the idea they would help judge the crossing behavior. The plexiglass sheet had a 20 cm by 20 cm hole cut into it roughly 45 cm above the bottom and equidistant from the sides. A "door" was installed to open and close this hatch. Two inlet pipes, one for each side, were placed in the corner of each half where the plexiglass divider met the side of the tank. These pipes had three 7mm holes drilled horizontally into the lower end to direct the water flow along the walls of the tank. The flow rates were equal on each side, 15 l/min.



**Figure #1: Drawing of the top down view of the experimental tank.**

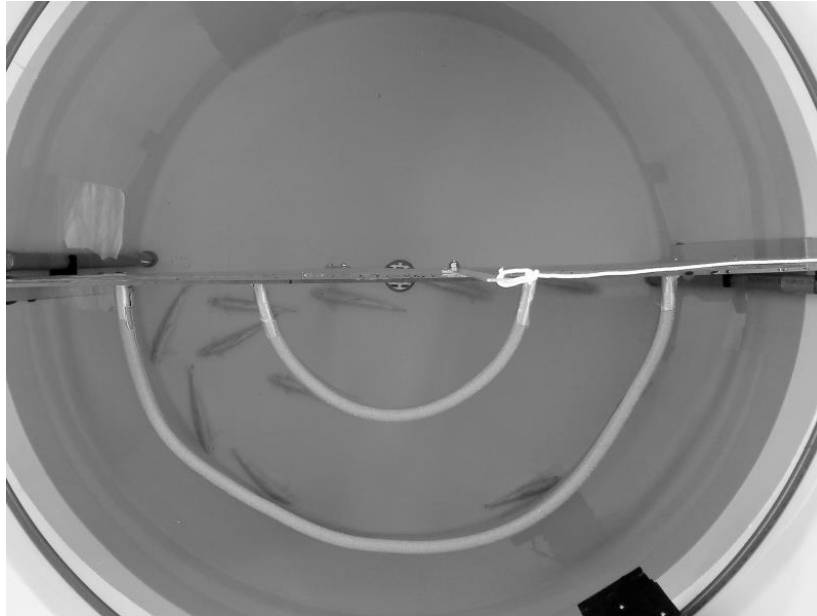
Attached to the rim of the tank were two conveyor belt feeders, one per side, in the same location in relation to the inlet pipe, about a quarter of a meter downstream. Each feeder was controlled via a timer at the socket that was programmed to their respective meal times. Skretting Nutra Olympic 2mm pellets were used throughout the experiment. Due to the feeders being mounted on the sides and the height of the divider no lid was used and the tank was left open. Instead a half meter jump guard was attached to the rim around the tank to prevent the fish from escape.

## **2.2 Experimental Design**

Fish would be weighed, measured, and then introduced to the experimental tank. A 36-hour acclimation period consisting of 24 hours of starvation and then one meal followed by an 11.5-hour break would be observed before the experiment would begin. Before the following meal the hatch door would be opened and meals would alternate between the two sides, thus beginning the experiment. Morning meals would occur at 900 on side two and at 2100 on side one. Each meal would last 30 minutes and be separated by 11.5 hours. This schedule would continue for two weeks at which point the fish would be weighed, measured, and a new feeding regimen could be calculated. The experiment would continue for another two weeks with the fish being weighed and measured upon cessation.

## 2.3 Experimental Observations

In order to observe the behavior of the fish a high definition Logitech webcam was installed above the tank that was able to capture the entire tank in one frame (image 1). This was connected to a laptop by a firewire cable that was run through the wall to a work station outside of the experiment's room. The laptop utilized a program called Scorpion Capture that allowed for programming and scheduling of the camera.



*Image 1: Top down photo from the Logitech webcam used (hatch closed)*

During the meals the camera was activated to take a time-lapse at the rate of one photo per second. This started with the meal and ended 10 minutes after each meal concluded. This series resulted in 2,400 photos per meal. Between meals the camera was set to take photos once a minute, resulting in a total of 1,360 photos per day. These photos were compiled into three periods, the morning meal, the evening meal, and non-meal. These were then analyzed to count the number of crosses between the sides. This was done by counting the fish on each side and comparing that number to the prior photo. These crosses were then recorded with their time, direction (side 1 to 2, or side 2 to 1), and the total number of fish on each side. This data was then used to calculate the total number of crosses, the crosses per period (morning/evening meals, or non-meal period), side preference, and time until the first cross of the meal. These were then analyzed using linear regression, in which the slopes and r-squared values were compared to determine their significance (Halcoussis, 2005). It was further partitioned into weekly summaries to compare the trends between the different weeks the fish were in the experiment. Standards of deviation and variance were chosen for analysis of the variability in crossing behavior over the standard error of the mean as the dispersion of daily crosses compared to the mean weekly crosses was of more interest than variability in the mean values (Barde, 2012).



In addition to the crossing data, growth statistics for the fish were calculated. The weights and lengths were recorded at the intake into the experiment, at the halfway point, and at the end of the experiment. These were used to estimate the Specific Growth Rate (SGR) (formula 1), the Feed Conversion Ratio (FCR) (formula 2), and the condition factor (Formula 3) (*Fulton, 1902*).

***Formula 1: Specific Growth Rate.***

$$SGR = \frac{\ln(B1) - \ln(B0)}{t}$$

B<sub>1</sub> = Final Biomass

B<sub>0</sub> = Starting Biomass

t = Number of days

SGR = Daily specific growth rate

***Formula 2: Feed Conversion Ratio***

$$FCR = \frac{\text{Total Feed Given}}{B1 - B0}$$

B<sub>1</sub> = Final Biomass

B<sub>0</sub> = Starting Biomass

t = Number of days

FCR = Feed Conversion Rate

***Formula 3: Condition Factor***

$$K = \frac{W}{L^3} \times 100$$

W = Weight in grams

L = Fork Length in Centimetres

K = Condition Factor

## **2.4 Experimental Methods**

The fish were sedated using Tricaine Methanesulfonate, MS-222, then weighed and measured. From these measurements the feeding regimen was calculated. The fish were then transferred to the experimental tank and introduced to side one. The 36-hour acclimation period was observed before the experiment began. The “door” to the hatch was opened just before the 900-morning meal. From this point on meals would alternate between the two sides as described in the experimental design and observations would be made according to the regiment described

above. At the middle point of the experiment the fish were caught, sedated with Tricaine Methanesulfonate, weighed, measured, and released back into side one. This data was then used to update the feeding regimen for the final two weeks of the experiment. At the end of the experiment the fish were sedated and measured again using the same methodology.

The data collected during the first experiment omit certain days due to differences in the data recording. The decision was made to omit days 15 and 20. Day 15 was omitted because the weight sampling for the growth statistics occurred and affected the crossing behavior of the fish. Day 20 was removed from the data analysis as a camera error interrupted the recordings for three hours. Lastly, a malfunction with the foam half circles used to judge crossing behavior, (image 1, figure 1) call into question the accuracy of the measurements made from day 23 until the end of the experiment and thus were omitted.

## **2.5 Experiment 2: Materials, Methods, and Design**

The second experiment took place between the 1<sup>st</sup> and 31<sup>st</sup> of August 2018. The fish used were Atlantic Salmon, *Salmo Salar*. Ten Fish were selected at random from the stock at the NMBU, Norwegian University of Life Sciences, fish lab. The average weight of the fish was  $167.8 \pm 15$  grams. The goal of this second experiment was to see if more crosses during meal times could be induced through pavlovian conditioning or stimulus training. The stimulus selected was light. In addition to the normal room lighting, two L.E.D. lights were placed underneath each of the feeders shining downward into the water column. The lights had 12 L.E.D.s in a ring formation. These were controlled by a Raspberry-Pi computer and scheduled to be on only during the 30-minute meals. All other design parameters and methodology used for data collection and analysis were kept the same as experiment one.

During the experiment certain errors occurred that tainted various periods of the data collected. These errors were a result of multiple software malfunction and two power outages. If any part of the daily observation was affected it was not included in the analysis for the total crosses per day. Days omitted from the total crosses per day for this reason include days 2, 4, 5, 6, 15, 20, 21, 22. However, if the error occurred during only one period, such as the non-meal period, then the data for the other periods, like the meal periods, could still be used. Please note that the morning meal data for days 6 and 21 were not included, evening meal data were not included for Days 20 and 21, and non-meal period data were not included for days 2, 4, 5, 15, 20, 21, and 22. Lastly, if there was no crosses during a meal it was excluded from the analysis of the time until first cross of the meal, as such was the case during the morning meals of days 1 and 2 and the evening meals on days 7 and 9.

## **2.6 Experiment 3: Materials, Methods, and Design**

The third experiment occurred between the 3<sup>rd</sup> of October until the 1<sup>st</sup> of November 2018 at the NMBU, Norwegian University of Life Sciences, fish lab. Ten Atlantic Salmon, *Salmo Salar*, were selected at random from the stock. The average weight of the fish was  $168.6 \pm 13.4$  grams. The goal of the third experiment was to see the effect of having two hatches in the center divider and if the current flowing through each affected crossing behavior. These hatches were the same size, 20x20 cm, as those in experiment one and two. They were placed 20cm in from

the edges of the tank and 45cm above the bottom. The rest of the tank design was kept the same as the first two experiments, but the methodology for collecting pictures changed.

As there were now two hatches to cross through the direction the fish crossed through each hatch was of interest. This meant new motion detection image capture software was needed to detect which direction a fish was traveling through each hatch. The new software, I-Spy, was an open source home security software that allowed for motion detection within certain zones of the total observable area of the camera. The zones for motion detection were designated around the hatches to capture a short video whenever a fish entered the designated zone. This meant instead of calculating the crossing statistics based on a time-lapse series, as in experiments one and two, any time a fish entered the detection area a video was taken and these were used to calculate the crossing statistics. Two more crossing statistics were generated through this method of observation. As there were two hatches, hatch preference was determined by comparing the number of crosses through each hatch. Secondly, the direction of the cross, with or against the current through the hatch was determined.

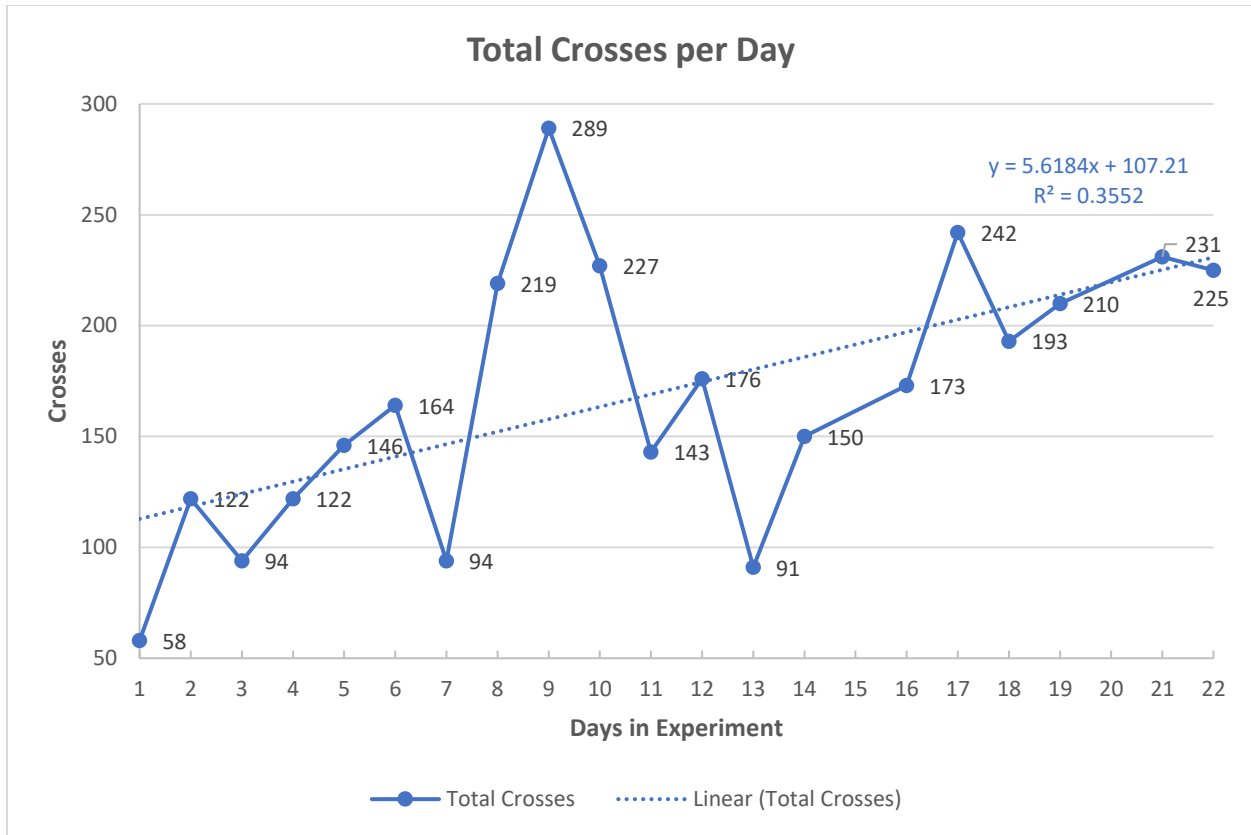
The motion detection software worked as specified above, but the number of observations greatly increased compared to the first two experiments. Unlike experiments one and two, which would generate a set number of photos because of the time lapse observation method used, the motion detection resulted in days with almost double or triple the number of observations. Steps were taken to reduce this number by adjusting the sensitivity and decreasing the motion detection area, but did little to reduce the number videos recorded. For this reason, only the first two weeks were reported on as the amount of time required to analyze the full third experiment would have been too great.

## **3. Results**

### **3.1 Experiment 1**

#### **3.1.1 Crosses Per Day**

The total number of crosses per day (figure 2) varied greatly throughout the first experiment from a high of 289 crosses on day nine to a low of 58 crosses on day one with the average crosses per day for the entire experiment being 165 (figure 2). A strong upward trend was observed as the amount of time in the experiment increased. The linear regression analysis confirms this with a trendline that has a slope of 5.62, which means that each additional day the fish were in the experiment resulted in roughly 5.62 times more crosses. The R-squared value of the trendline is .36, suggesting 36% of the variation can be explained by the model, however when outliers like day nine and 13 were removed this coefficient increases to .6 or 60%.



**Figure 2: Recorded Total Crosses Per Day**

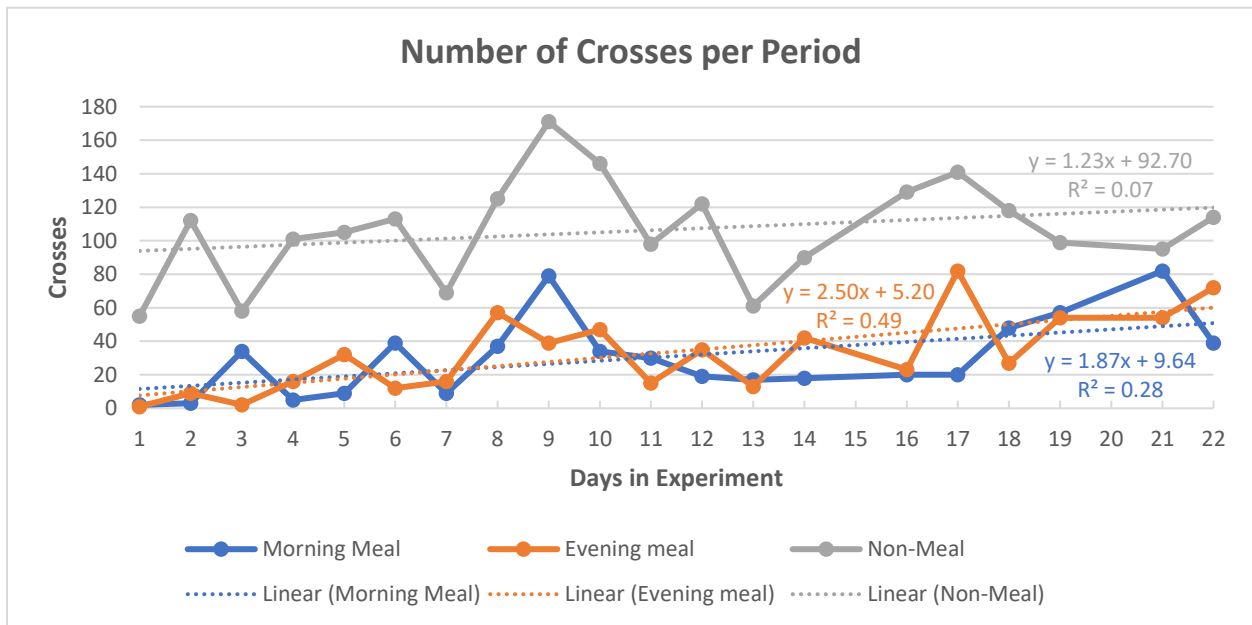
The upward trend, is also visible in the increase of the average number of crosses per day in the weekly summary (Table 1). The standard of deviation and variance are also important to note as they demonstrate when during the experiment the variation was generated. The largest variation is seen for week two which contains the day with the greatest number of crosses, day nine, and the day with the second least number of crosses, day 13. The lowest variation in the total daily crosses occurred during week three suggesting that crossing behavior became more consistent as time progressed.

**Table 1: Weekly Summary. Week 3 had 6 days as day 20 was omitted due data recording error.**

Weekly Summary: Total Crosses per Day					
Period	Average Crosses Per Day	Total Crosses	# of days	Standard Deviation	Variance
Week 1 Days 1-7	114.29	800	7	32.923	1083.92
Week 2 Days 8-14	185.00	1295	7	60.524	3663.14
Week 3 Days 16-22	212.33	1460	6	23.507	552.56

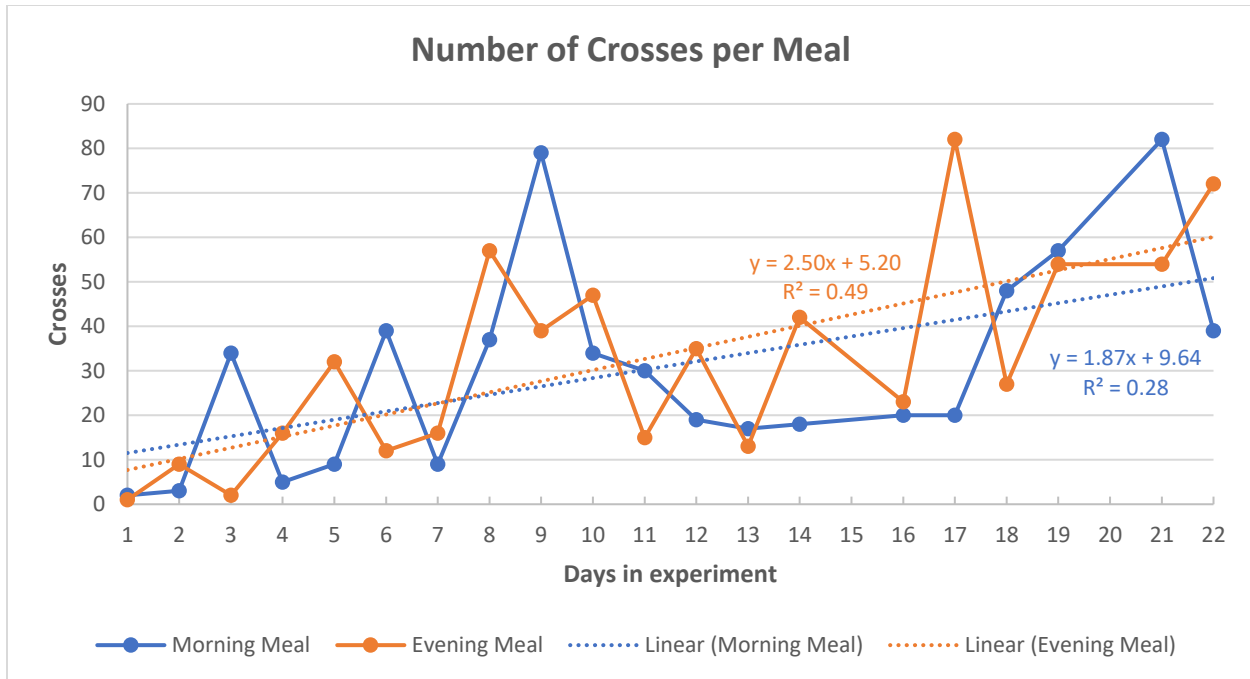
### 3.1.2 Crosses Per Period

The total number of crosses were further partitioned into the number of crosses per period (figure 3). The trendlines for each period have positive slopes alluding to the increase in the number of crosses with time in the experiment (figure 2). The slopes of the linear regression trendlines for the respective periods demonstrate how each period contributed to the overall increase in crosses. The trendline of the non-meal period has a slope of 1.23, demonstrating it contributed the least to the overall increase in crosses and had a r-squared value of .07, meaning only 7% of variation in the daily non-meal period crosses could be explained by this model (figure 3).



**Figure 3: Number of Crosses per Period**

The evening meal trendline has a slope of 2.5 which means that each additional day resulted in 2.5 times more crosses during the evening meal (figure 3 & 4). The increase in crosses of this period contributed the most to the overall upward trend in total crosses (figure 2). The trendline for the evening meal crosses had a r-squared value of .49, meaning 49% of the variation can be explained by this model. The evening meal had a maximum of 82 crosses on day 17, a minimum of one cross on day one, and an average of 32.4 crosses per meal. The morning meal crosses contributed the second most to the overall increase as seen with a trendline slope of 1.87 and a r-squared value of .28 (figure 3 & 4). The morning meal period had a maximum of 82 crosses on day 21, a minimum of two crosses on the day one, with an average of 30 crosses per meal throughout the experiment.



**Figure 4: Number of Crosses per Meal**

The weekly summary for the number of crosses in the Morning Meal period show an increase in the average number of crosses per meal reaching a maximum during week three (table 2). However, the largest increase in crosses occurred between weeks one and two, on average there were 18 more crosses per morning meal in week two than in week one. At the same time though the amount of variation in the number of crosses between the morning meals grew as well, thus decreasing the r-squared value. The variation grew the most between weeks one and two, but peaked in week three.

**Table 2: Weekly Summary for the Number of Morning-Meal period crosses per week. Week 3 had 6 days as day 20 was omitted due data recording error.**

Weekly Summary: Morning Meals					
Period	Average Crosses per meal	Total Crosses	# of days	Standard Deviation	Variance
Week 1 Days 1-7	14.43	101	7	14.24	202.82
Week 2 Days 8-14	33.43	234	7	20.06	402.53
Week 3 Days 16-22	44.33	266	6	21.62	467.56

The increase in the number of crosses per evening meal for each week, (table 3), reflects the upward trendline in the period crosses (figures 3 & 4) and the increase in total crosses (figure 2). The largest difference in the average evening meal crosses was seen between weeks one and

two with an increase of 22.86 crosses on average, whereas the increase from week two to week three was 16.57 crosses. The variance also rose as the experiment progressed, just as with the morning meals (table 2). However, the variation increased more gradually than that of the morning meal (Tables 3).

**Table 3: Weekly Summary of Evening-Meal period crosses per week. Week 3 had 6 days as day 20 was omitted due data recording error.**

<b>Weekly Summary: Evening Meals</b>					
<b>Period</b>	<b>Average Crosses per meal</b>	<b>Total Crosses</b>	<b># of days</b>	<b>Standard Deviation</b>	<b>Variance</b>
<b>Week 1 Days 1-7</b>	12.57	88	7	9.71	94.25
<b>Week 2 Days 8-14</b>	35.43	248	7	15.00	225.10
<b>Week 3 Days 16-22</b>	52.00	312	6	21.50	462.33

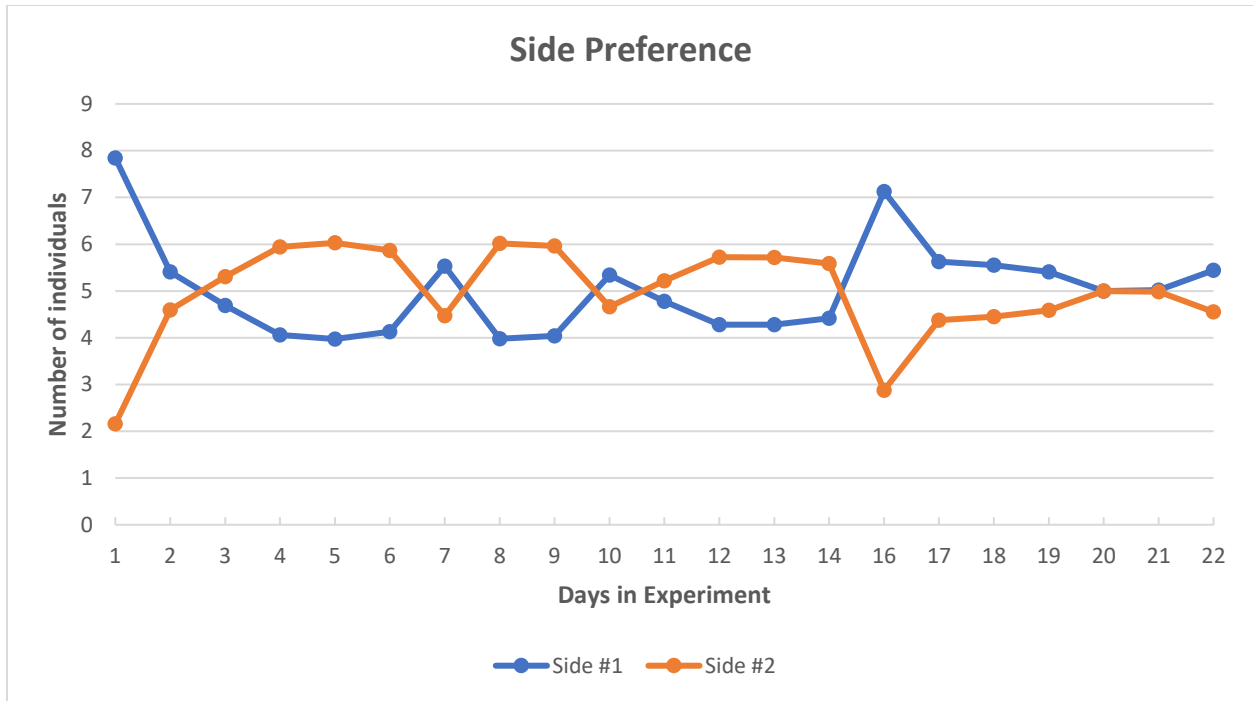
The weekly summary for Non-meal period crosses (table 4) demonstrates the same increase in the average number of crosses from week one to week two, but no increase is observed between week two and three. As there is still an upward trend in the total number of crossing (figure 2) through week three (table 1) the growth must be a result of the increase in meal time crossings (figure 4). In addition, the large variances seen in the non-meal period crossings for week one and week two compared to those from the meal time crosses shows that the majority of variation within the experiment is a result of the non-meal period.

**Table 4: Weekly Summary for the Number of NON-Meal period crosses per week. Week 3 had 6 days as day 20 was omitted due data recording error.**

<b>Weekly Summary: Non-Meals</b>					
<b>Period</b>	<b>Average Crosses between meals</b>	<b>Total Crosses</b>	<b># of days</b>	<b>Standard Deviation</b>	<b>Variance</b>
<b>Week 1 Days 1-7</b>	87.57	613	7	23.93	572.53
<b>Week 2 Days 8-14</b>	116.14	813	7	33.95	1152.41
<b>Week 3 Days 16-22</b>	116.00	696	6	15.98	255.33

### 3.1.3 Side Preference

As the number of fish on each side at the time of a cross was recorded, the average number of fish on each side for each day could be calculated (figure 5). This statistic allows for an analysis of any environmental stimuli or occurrences of dominant behavior that may impact crossing behavior and the distribution of individuals. For example, if one side had worse water quality the fish would be expected to stay away from that side.



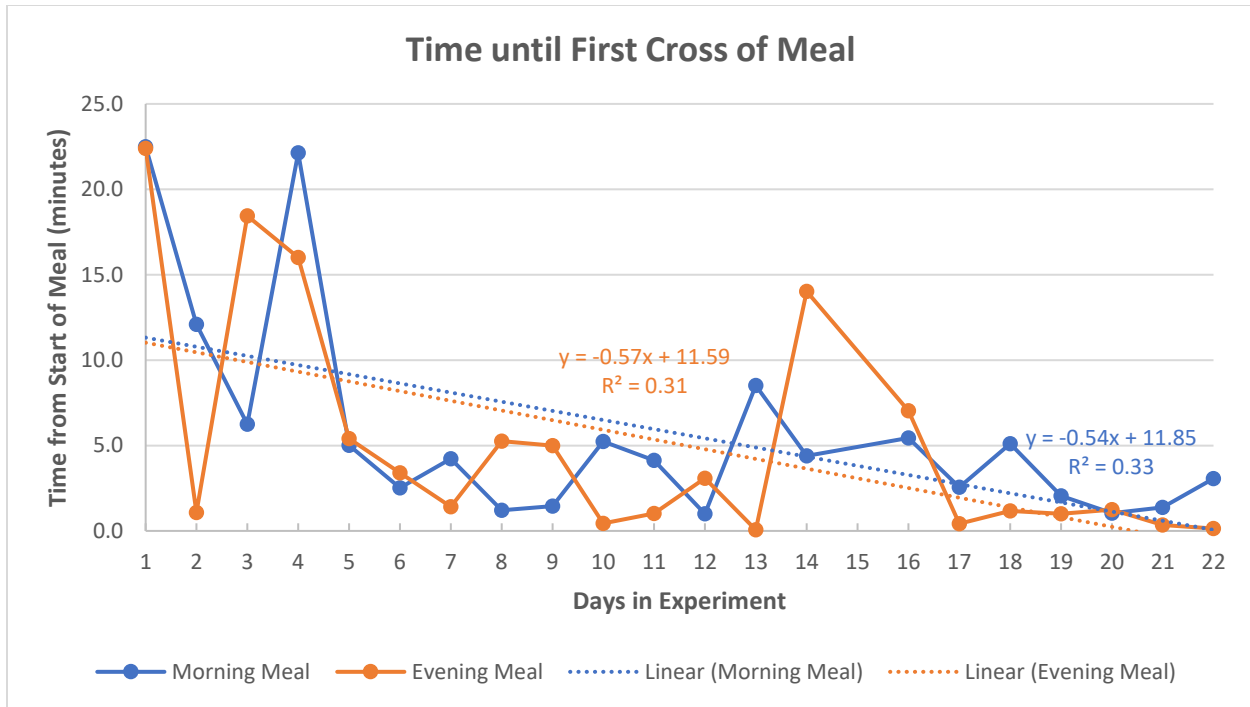
**Figure 5: Side preference for each Day**

The average number of fish per side varied throughout the experiment, but it is evident that the fish did not prefer one side over another, actively alternating between the two (figure 5). However, one note is that after removing the fish to weigh in on day 15 the fish were all reintroduced to the experiment onto side one and took longer to return to an equilibrium between the sides, as seen in the spike in number of individuals in side one on day 16.

### 3.1.4 Time Until First Cross of the meal

The time until the first cross of the meal was observed in order to determine whether the fish would learn how to use the hatch when it was most important. The total crosses and subsequently the crosses for each period are a function of the number of fish that understood how the hatch works and willingness to use it, but the speed of the first cross demonstrates the fish can sense the feeding cues and use the hatch appropriately and timely. A downward trend is visible throughout the experiment, for both meal periods, which alludes to fish understanding and utilizing the hatch better as time goes on (figure 6). The slopes of the linear regression trendlines for both meals averages to  $-0.555$ , illustrating that with each day in the experiment the fish cross twice as fast as the prior day. The r-squared values are also roughly the same with an average of  $.32$ , showing that the models account for roughly 32% of the total variation.





**Figure 6: The time until the first cross of the meal**

### 3.1.5 Growth Statistics

The weight, length, and calculated condition factor for the 10 fish used in the experiment increased with time (table 5). The standards of deviation from the intake until the weigh in on the 16<sup>th</sup> increased for the length and weight of the fish. This alludes to more variation being generated as the fish entered the experiment and learned how to cross between the sides. Compared to the period between the 16<sup>th</sup> and the end of the experiment on the 1<sup>st</sup>, these standards of deviations are relatively constant thus showing no new variation is generated during this period.

**Table 5: Growth Sampling Data**

26.6.18 Intake				16.7.18 Weigh In				1.8.18 End			
Fish	Weight (g)	Length (cm)	Condition Factor (K)	Fish	Weight (g)	Length (cm)	Condition Factor (K)	Fish	Weight (g)	Length (cm)	Condition Factor (K)
1	182	25.5	1.10	1	159	23.5	1.23	1	254	27	1.29
2	123	21.5	1.24	2	183	24.5	1.24	2	173	24	1.25
3	124	21.5	1.25	3	160	23.5	1.23	3	207	25	1.32
4	116	21.5	1.17	4	168	24	1.22	4	176	23.5	1.36
5	129	22.25	1.17	5	149	22	1.40	5	179	24	1.29
6	144	23.5	1.11	6	143	22.5	1.26	6	203	25	1.30
7	148	24	1.07	7	143	22.5	1.26	7	188	24.5	1.28
8	130	22.5	1.14	8	238	26	1.35	8	199	25	1.27
9	144	24	1.04	9	118	20.5	1.37	9	142	21.5	1.43
10	152	24.5	1.03	10	158	23	1.30	10	233	26.5	1.25
<b>Average</b>	139.2	23.075	1.13	<b>Average</b>	161.9	23.2	1.29	<b>Average</b>	195.4	24.6	1.30
<b>Sum</b>	1392	230.75	11.32	<b>Sum</b>	1619	232	12.85	<b>Sum</b>	1954	246	13.05
<b>Standard Deviation</b>	19.26	1.42	0.08	<b>Standard Deviation</b>	31.81	1.49	0.07	<b>Standard Deviation</b>	31.81	1.54	0.05

Comparing these samples, the Specific Growth rate can be calculated (table 6). During the first period 26/6-16/7 the specific growth rate was less than half of the expected value. This was anticipated as the fish have to learn how to get through the hatch to receive both meals, but less than half is a statistically significant decrease in growth. The second period showed an improvement in SGR, but still well below the expected value. Another important production statistic calculated was the Feed Conversion Ratio (Table 6). The FCR follows the same trend as the SGR. During the first period it was much higher than expected, as the fish had to learn how to use the hatch to access the feed, but the FCR decreased during the second period, illustrating that the fish were getting more feed as seen in the increase in crossing behavior during meal times (figure 4).

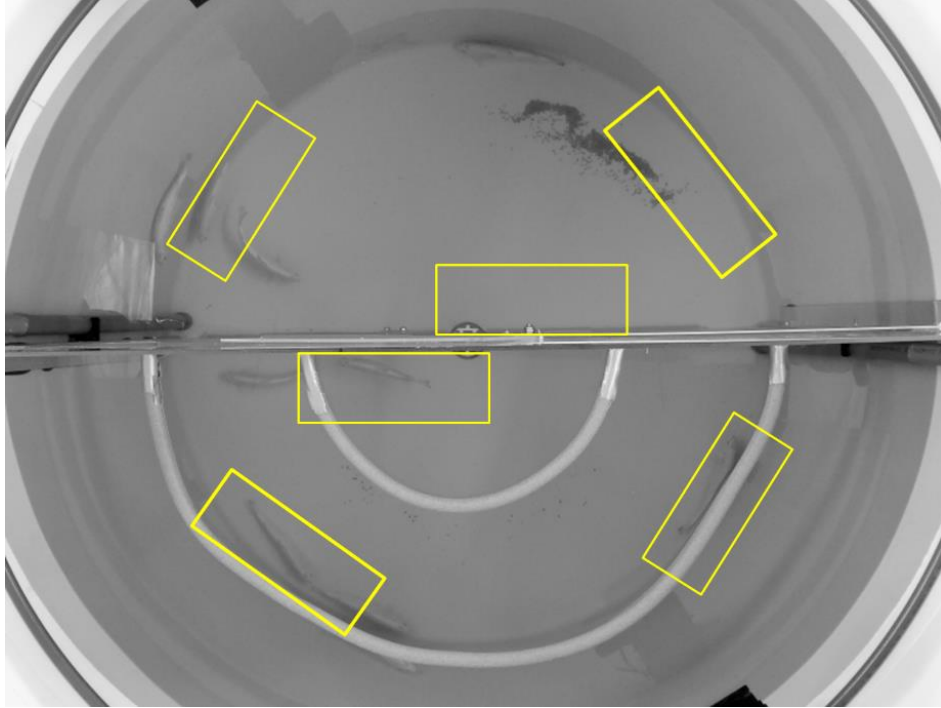
**Table 6: Expected SGR and FCR compared to the Calculated values for the two periods.**

<b>Dates</b>	<b>Expected SGR</b>	<b>Calculated SGR</b>	<b>Expected FCR</b>	<b>Calculated FCR</b>
<b>26/6-16/7</b>	<b>1.92%</b>	<b>0.76%</b>	<b>1</b>	<b>1.75</b>
<b>16/7-1/8</b>	<b>1.90%</b>	<b>1.18%</b>	<b>1</b>	<b>1.51</b>

### **3.1.6 Behavioral Observations**

As the experiment focused on the ability of fish to cross through a hatch it was observed that there were multiple types of crosses that occurred. The first type of cross was a searching cross, in which a fish would swim with its nose against the plexiglass until it eventually found the opening and proceeded through. The area of the search typically occurred from the inside edge of the outer foam float to the edge of the inner foam float on the far side of the hatch (image #1). The search pattern varied more in depth than in length. The second type of cross was a delayed cross. A fish would be stationary, swimming in the water column within the inner foam float, usually below or to the side of the hatch, but never above it. After some time, the fish would then move towards the hatch and swim through. In contrast to the delayed cross there was also a direct cross. This is when the fish would swim from outside the outer foam float and pass directly through the hatch. The last type of cross was a joint cross. This occurred when one fish using one of the first three types of crosses would pass through the hatch and subsequently another fish would cross through typically using a modified direct or modified searching cross.

When fish were not crossing through the hatch they typically were located along the sides of the tank and plexiglass divider, mostly along the bottom. In image two the favorite positions of fish were marked by yellow squares. They would orient themselves into the current and hold steady until they were chased out of position by another fish, cross to the other side, or they would switch to another position. The most common reason to move was a fish being chased out of its resting position. This dominance was seen during the feedings although its influence on feeding habits and side preference were not measured here as no individual monitoring system was used. As shown in the side preferences (figure 5), fish did not prefer a specific side and would swim back and forth multiple times until they found a position in which to rest.

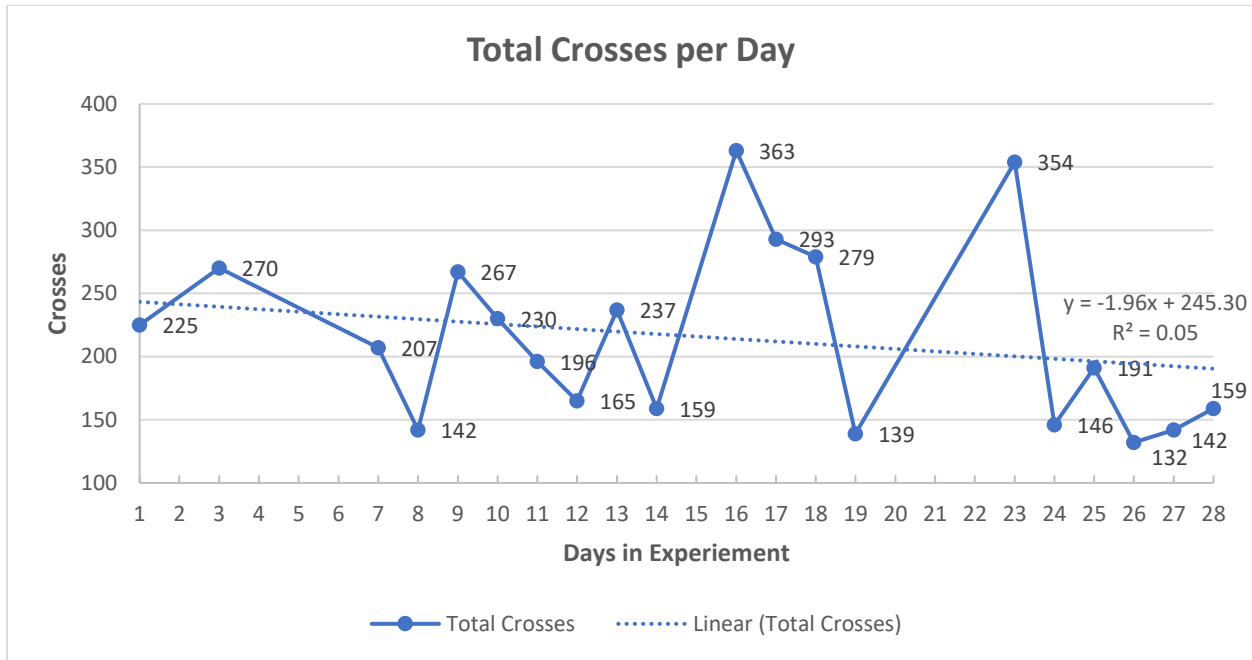


*Image 2 Locations of most common resting positions*

## **3.2 Experiment 2**

### **3.2.1 Crosses Per Day**

The total number of crosses per day for experiment two varied the greatest of the three experiments from a high of 363 crosses on day 16 to a low of 132 crosses on day 26 with the average crosses per day for the entire experiment being 215 (figure 7). In contrast to the first experiment a general downward trend was observed as the amount of time in the experiment increased. The linear regression model produced a trendline with a slope of -1.96, which confirms that each additional day the fish were in the experiment resulted in almost 2 times less crosses. The R-squared value of the trendline however is .05, suggesting that only 5% of the variation can be explained by the model and illustrating that it is not a good fit.



**Figure 7: Recorded Total Crosses Per Day**

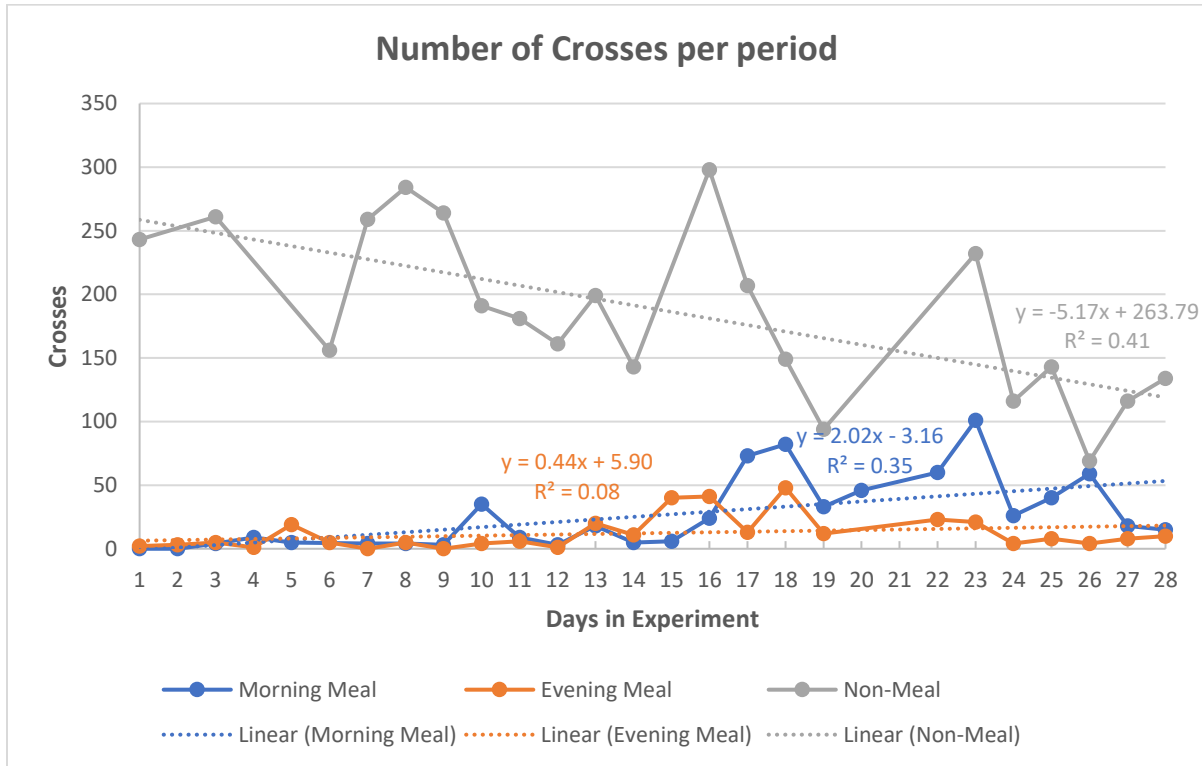
The downward trend in total crosses, is seen in the decrease in the average number of crosses between weeks one and two and weeks three to four in the weekly summary (Table 10). From week one to week three the standard of deviation and variance both increased (table 10). However, the accuracy of this observation can be called into question as the number of days in each of these periods varies due to the sampling errors. The largest variation is seen in week three, as it contains the day with the greatest number of crosses, day 16, and the day with the second least number of crosses, day 19 (Table 7). The large amounts of variation directly affect the ability of the regression analysis model to explain the total variation and thus decreases the r-squared value.

**Table 7: Weekly Summary. Week 1 had 3 days as days 2, 4, 5, and 6 were omitted due data recording errors. Week 3 had 4 days as days 15, 20, and 21 were omitted due sampling and data recording errors cause by a power outage. Week 4 had 6 days as day 22 was omitted due to feeder malfunction cause by the power outage.**

Weekly Summary: Total Crosses per Day					
Period	Average Crosses Per Day	Total Crosses	# of days	Standard Deviation	Variance
Week 1 Days 1-7	234.00	702	3	26.50	702.00
Week 2 Days 8-14	199.43	1396	7	43.16	1863.10
Week 3 Days 15-21	268.50	1074	4	81.26	6602.75
Week 4 Days 22-28	187.33	1124	6	76.83	5903.22

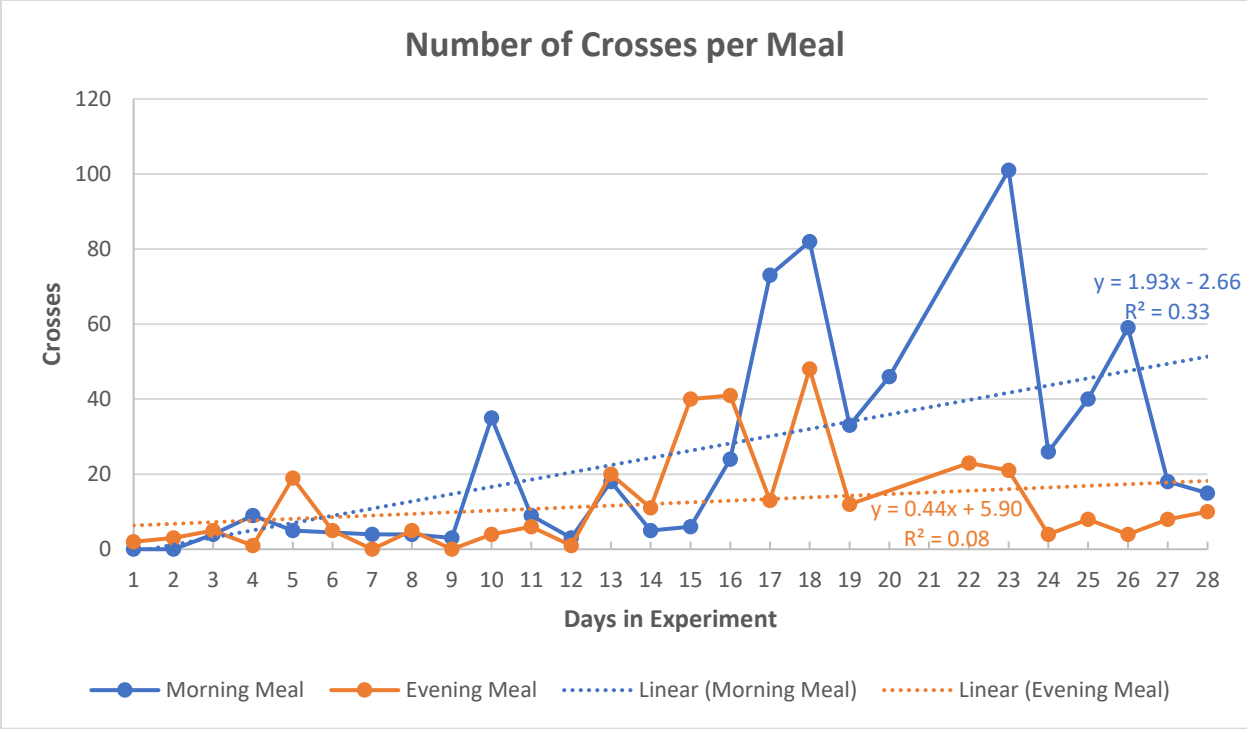
### 3.2.2 Crosses Per Period

The total number of crosses were further partitioned into the number of crosses per period (figure 8). The slopes of the linear regression trendlines for the respective periods demonstrate how each period contributed to the downward trend in total crosses. The trendline for the non-meal period has the largest impact as it has a negative slope of -5.17 and an r-squared value of .41 (figure 8). This confirms the decrease in the total number of crosses with relatively good accuracy as 41% of the variation can be explained by the model (figure 7).



**Figure 8: Number of Crosses per Period**

The morning meal trendline has a slope of 2.02 which means that each additional day in the experiment resulted in roughly two times more crosses during the morning meal (figure 8 & 9). The increase in crosses of this period decreased the impact of the downward trend in non-meal crosses on the overall trend in the total crosses (figure 7). The morning meal trendline had a r-squared value of .35, meaning 35% of the variation can be explained by this model (figure 8 & 9). The morning meal period had a maximum of 101 crosses on day 23, no crosses on days one and two, and an average of 26 crosses per meal throughout the experiment. The evening meal had a maximum of 48 crosses on day 18, no cross on days seven and nine, and an average of 12 crosses per meal. The evening meal period crosses contributed the least to the total crosses. Although an upward trend is observed the slope of the trendline was small at .44. This shows time in the experiment had little effect on increasing the number of crosses for this period, but the r-squared value of .08 demonstrates the model does little to explain the variation seen during this period (figure 9).



**Figure 9: Number of Crosses per Meal**

The period crosses were broken down per week to analyze where the most variation occurred and when major changes in crossing behavior took place. Throughout the experiment the average number of crosses during the morning meal per week increased reaching a maximum during week four (table 8). The largest increase in the average crosses per morning meal took place between weeks two and three, 33 more crosses on average (figure 8). However, at the same time the amount of variation in the number of crosses grew the largest over this same period. The increase in variances between the morning meals from week one to week four contributed to the lower r-squared value, but the upward trend in average crosses per meal permits the model to fit relatively well with a r-squared of .35 (figure 9).

**Table 8: Weekly Summary for the Number of Morning-Meal period crosses per week. Week 1 had 6 as day 6 was omitted due a data recording error. Week 3 had 6 days as day 21 was omitted due to a power outage at the farm.**

Weekly Summary: Morning Meal					
Period	Average Crosses Per Meal	Total Crosses	# of days	Standard Deviation	Variance
Week 1 Days 1-7	3.67	22	6	3.09	9.56
Week 2 Days 8-14	11.00	77	7	10.97	120.29
Week 3 Days 15-21	44.00	264	6	26.63	709.00
Week 4 Days 22-28	45.57	319	7	28.21	795.67

An increase in the average number of crosses per evening meal, (table 9), can be seen from weeks one until three. Weeks one and two have relatively close averages for the crosses per meal, but the largest difference was seen between week two and week three with an increase of 24.09 crosses on average. This increase however disappears as the average crosses decreases from week three to week four by 19.66, thus contributing to the small slope of the trendline (figure 9). The variance also peaks in week three at 230.96 (table #). It is this variance and the decrease in average crosses from week three to four that brings the models r-squared value down, as it has difficulties to explain the variability in average crosses.

**Table 9: Weekly Summary of Evening-Meal period crosses per week. Week 3 had 5 days as days 20 and 21 were omitted due to a power outage at the farm.**

<b>Weekly Summary: Evening Meal</b>					
<b>Period</b>	<b>Average Crosses Per Meal</b>	<b>Total Crosses</b>	<b># of days</b>	<b>Standard Deviation</b>	<b>Variance</b>
<b>Week 1 Days 1-7</b>	5.00	35	7	5.98	35.71
<b>Week 2 Days 8-14</b>	6.71	47	7	6.36	40.49
<b>Week 3 Days 15-21</b>	30.80	154	5	15.2	230.96
<b>Week 4 Days 22-28</b>	11.14	78	7	7.18	51.55

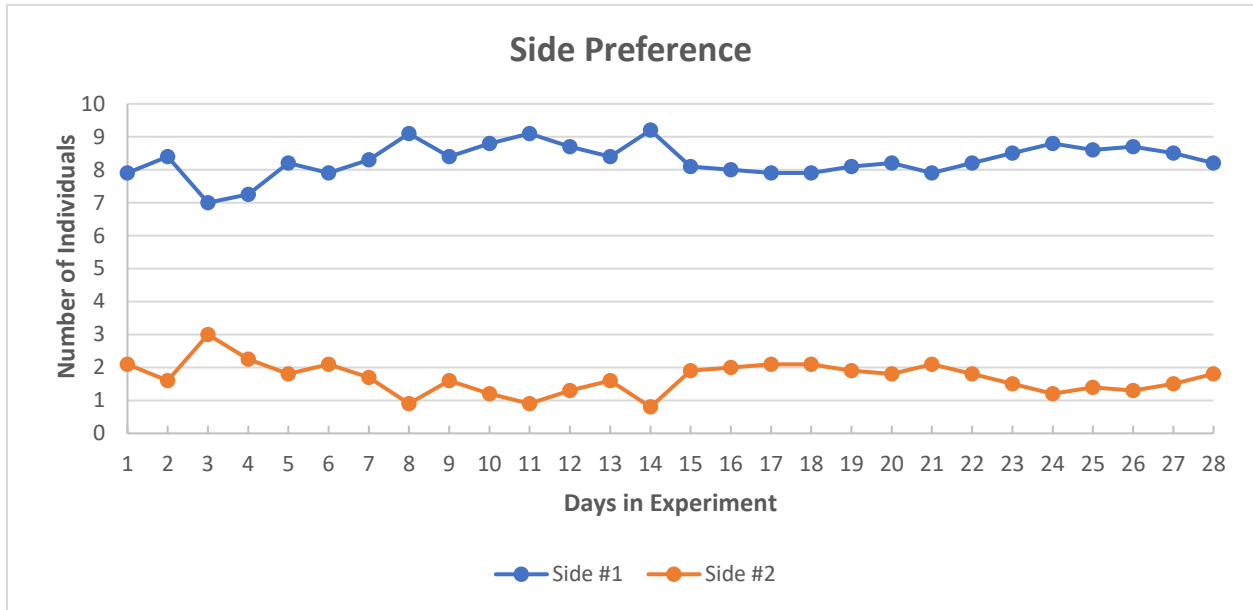
The Non-meal period crosses contributed the most to the downward trend of the experiment (figure 7). A decrease in the average number of crosses from week one to week four is observed, decreasing the most between week three and Week four by 52 crosses (table 10). The strength of this downward trend is echoed by the slope of the non-meal trendline, (figure 8), demonstrating that as time in the experiment increased the less crosses occurred. The large variances, especially week three, contributed to the decrease in the r-squared value to .41, but despite large variances the model still explains 41% of the total variation.

**Table 10: Weekly Summary for the Number of Non-Meal period crosses per week. Week 1 had 4 days as days 2, 4, and 5 were excluded due to data recording errors. Week 3 had 4 days as days 15, 20, and 21 were omitted due sampling and data recording errors cause by a power outage. Week 4 had 6 days as day 22 was omitted due to feeder malfunction cause by the power outage.**

<b>Weekly Summary: Non-Meal</b>					
<b>Period</b>	<b>Average Crosses between Meals</b>	<b>Total Crosses</b>	<b># of days</b>	<b>Standard Deviation</b>	<b>Variance</b>
<b>Week 1 Days 1-7</b>	229.75	919	4	43.15	1861.69
<b>Week 2 Days 8-14</b>	203.29	1423	7	48.24	2327.06
<b>Week 3 Days 15-21</b>	187.00	748	4	75.52	5703.50
<b>Week 4 Days 22-28</b>	135.00	810	6	49.25	2425.33

### 3.2.3 Side Preference

The side preference of the fish in experiment two was clear (figure 10). The majority of the fish could be found on side one throughout the duration of the experiment. This statistic demonstrates that there was some negative stimulus that impacted the distribution between the two sides. Through the observational data this was determined to be a result of the dominant behavior by one fish.

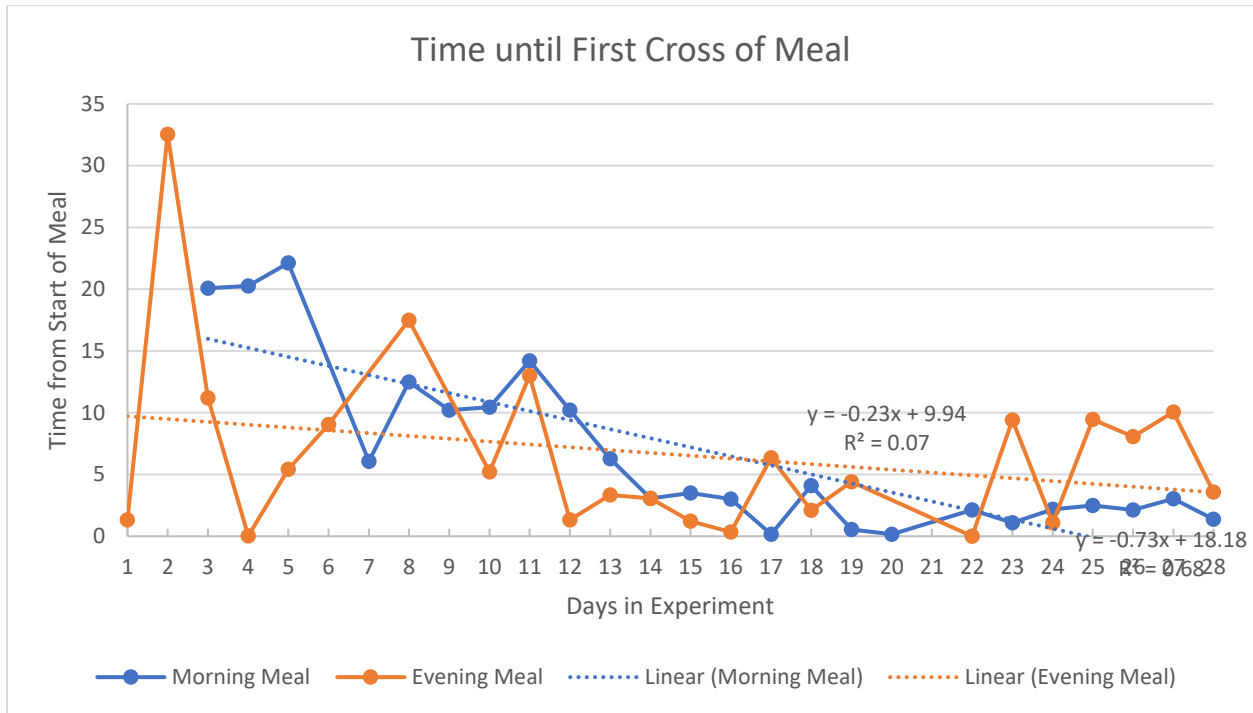


*Figure 10: Side preference for each Day*

### 3.2.4 Time Until First Cross of the Meal

A downward trend in the time until the first cross of the meal is visible throughout the experiment, for both meal periods, which alludes to fish understanding and utilizing the hatch better as time in the experiment increased (figure 11). The slope of the linear regression model's trendline for the morning meal was the largest, at  $-0.73$ , with an  $r$ -squared value of  $.68$ . The fit of the trendline for this period however is exaggerated as days one and two were omitted due to no crossing occurring during these meals. The evening meal trendline had a slope of  $-0.23$  and a low  $r$ -squared value of  $.07$ , suggesting that the model did not explain the total amount of variation (figure 11).





**Figure 11: The time until the first cross of the meal**

### 3.2.5 Growth Statistics

The weight, length, and condition factor for the ten fish used in the experiment increased with time (table 11). The average weight increased the most during the last two weeks of the experiment, 48.8 grams, compared to 37.5 grams between the intake and the middle point. The standards of deviation for the length and weight of the fish increased throughout the experiment, which alludes to more variation being generated by the tank design. The largest increase in the standard of deviation of the weight occurred during the last two weeks of the experiment, 14.61 g. This increase demonstrates that some fish were able to use the hatch to gain access to both meals, while some were not and thus their growth suffered. The condition factor of the fish increased slightly throughout the experiment, suggesting that all individuals could still be considered in good health.

**Table 11: Growth Sampling Data**

1.8.18 Intake				17.8.18 Weigh In				31.8.18 End			
Fish	Weight (g)	Length (cm)	Condition Factor (K)	Fish	Weight (g)	Length (cm)	Condition Factor (K)	Fish	Weight (g)	Length (cm)	Condition Factor (K)
1	166	24.5	1.13	1	234	27.5	1.13	1	303	28.5	1.31
2	168	24.5	1.14	2	233	27	1.18	2	254	28.5	1.10
3	173	23.5	1.33	3	212	27	1.08	3	319	29	1.31
4	188	25	1.20	4	229	26.5	1.23	4	218	26	1.24
5	185	25.5	1.12	5	177	25	1.13	5	222	27	1.13
6	148	24.5	1.01	6	196	24.5	1.33	6	225	25.5	1.36
7	149	23.5	1.15	7	178	24.5	1.21	7	229	26	1.30
8	174	23.5	1.34	8	234	27	1.19	8	291	29	1.19
9	179	25.5	1.08	9	187	25.5	1.13	9	276	28.5	1.19
10	148	23	1.22	10	173	24.5	1.18	10	204	25.5	1.23
<b>Average</b>	167.8	24.3	1.17	<b>Average</b>	205.3	25.9	1.18	<b>Average</b>	254.1	27.35	1.24
<b>Sum</b>	1678	243	11.71	<b>Sum</b>	2053	259	11.79	<b>Sum</b>	2541	273.5	12.36
<b>Deviation</b>	15.04	0.89	0.11	<b>Deviation</b>	25.89	1.22	0.07	<b>Standard Deviation</b>	40.50	1.49	0.08

The growth data, (table 11) was then used to calculate the specific growth rate (table 12). During the first period 1/8-17/8 the specific growth rate was well below the expected value. This was anticipated as the fish had to learn how to get through the hatch to receive both meals. The second period showed an improvement in SGR, but still did not reach the expected value. Another important production statistic calculated was the Feed Conversion Ratio (Table 12). The FCR follows the same trend as the SGR. During the first period it was much higher than expected, but the FCR comes down during the second period, illustrating that the more feed was converted to weight during this time.

**Table 12: Expected SGR and FCR compared to the Calculated values for the two periods.**

Dates	Expected SGR	Calculated SGR	Expected FCR	Calculated FCR
<b>1/8-17/8</b>	<b>1.90%</b>	<b>1.34%</b>	<b>1</b>	<b>1.45</b>
<b>17/8-31/8</b>	<b>1.90%</b>	<b>1.52%</b>	<b>1</b>	<b>1.27</b>

### 3.2.6 Behavioral Observations

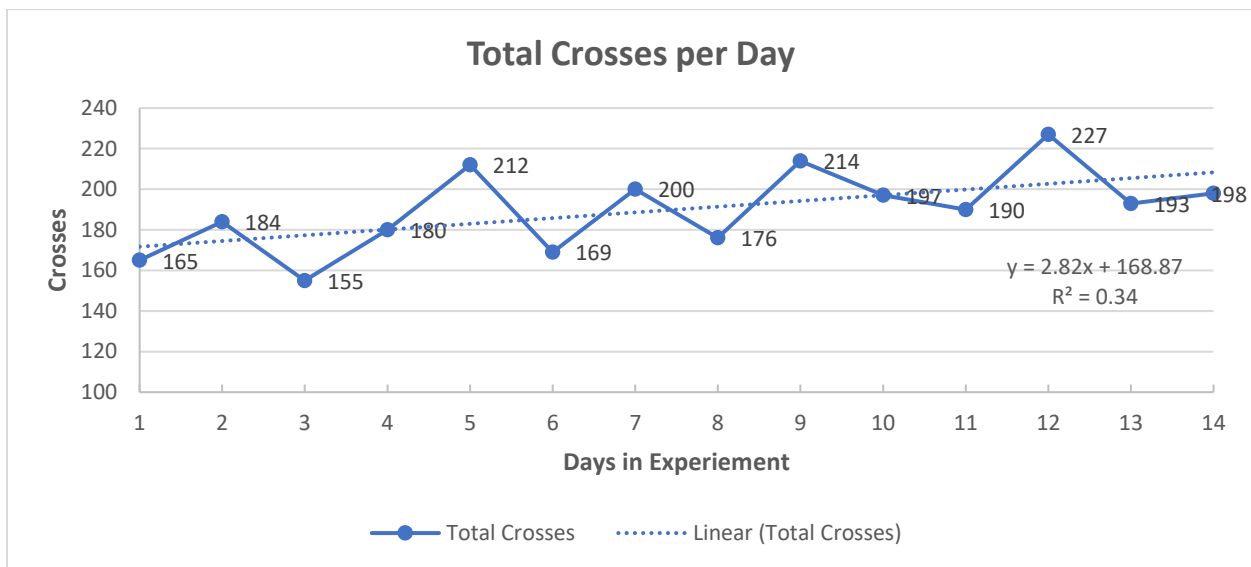
The crossing behavior of the fish in experiment two echoed that observed in experiment one. The four types of crosses, searching, delayed, direct, and joint, were seen again. The resting positions (image 2) appeared to be the same as well. The major differences between these experiments were the observed occurrences of dominant behavior. There was visibly more dominant behavior on side two, as seen in the disparity in side preferences (figure 10). The ability to confirm the same individual was responsible for these occurrences was not possible as individual identification was not used. However, from the experimenter’s observations the identity of the individual was relatively confident. The behavior witnessed was that this fish would reside mostly of side two with very few crossings to side one. It would chase any other individual that would cross into side two until it would cross back to side one or the dominant

fish would go back to its resting position before continuing the chase again after some time. This dominant behavior would cease if the number of fish on side two increased above three fish. This chasing behavior resulted in a modified direct cross or a frantic searching cross by the chased individual. This behavior did not seem to be influenced by the period, meal or non-meal, and the dominant individual would give chase regardless of the time of day.

### 3.3 Experiment 3

#### 3.3.1 Crosses Per Day

The total number of crosses per day in the third experiment (figure 12) did not vary as much as the first two experiments. The total crosses had a high of 227 crosses on day 12, a low of 155 crosses on day three, and an average of 190 crosses (figure 12). An upward trend was observed as the amount of time in the experiment increased. The linear regression analysis confirms this with a trendline slope of 2.82. The R-squared value of the trendline is .34, suggesting 34% of the variation could be explained by the model.



**Figure 12: Recorded Total Crosses Per Day**

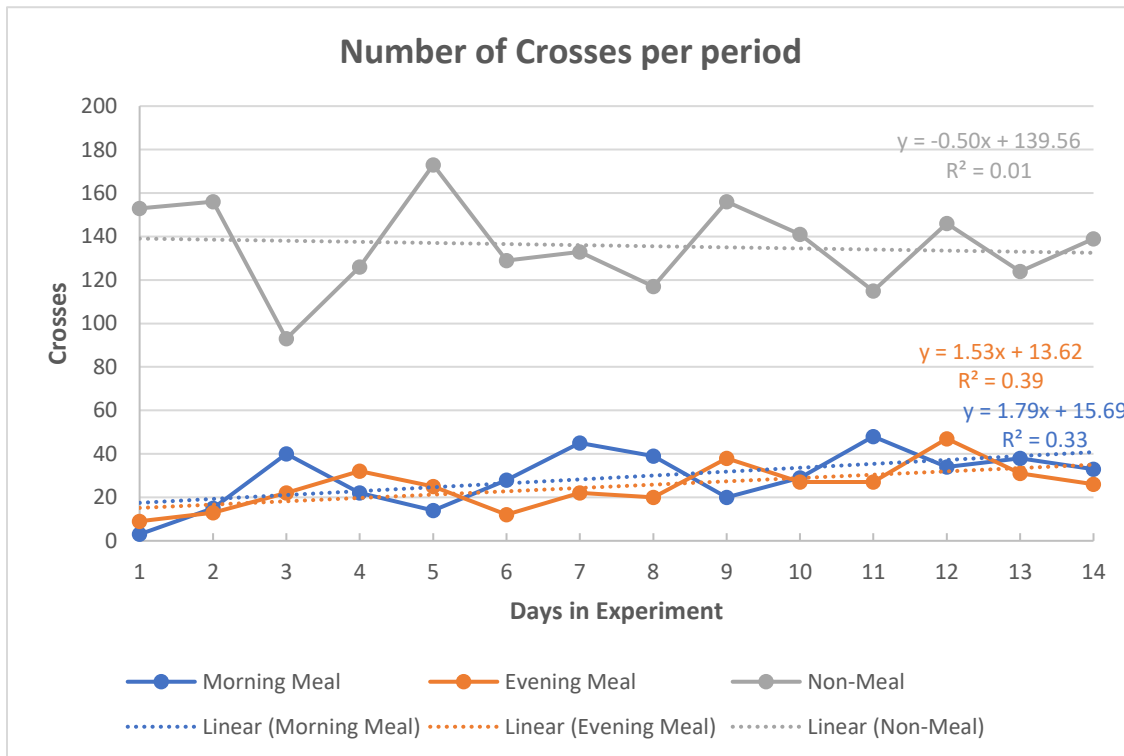
The upward trend, is also visible in the increase of the average number of crosses per day in the weekly summary (Table 13). The standard of deviation and variance are also important to note as they demonstrate the exact extent of the variation within and between the weekly averages. The largest variation is seen for week one which contains the day with the least number of crosses, day three, and day five which had the third most crosses. The variance and standard of deviation both decreased between weeks one and two showing that the number of crosses per day were becoming more consistently dispersed.

**Table 13: Weekly Summary.** Week 3 had 6 days as day 20 was omitted due data recording error.

Weekly Summary: Total Crosses per Day					
Period	Average Crosses Per Day	Total Crosses	# of days	Standard Deviation	Variance
Week 1 Days 1-7	180.71	1265	7	18.55	343.92
Week 2 Days 8-14	199.29	1395	7	15.4	237.06

### 3.3.2 Crosses Per Period

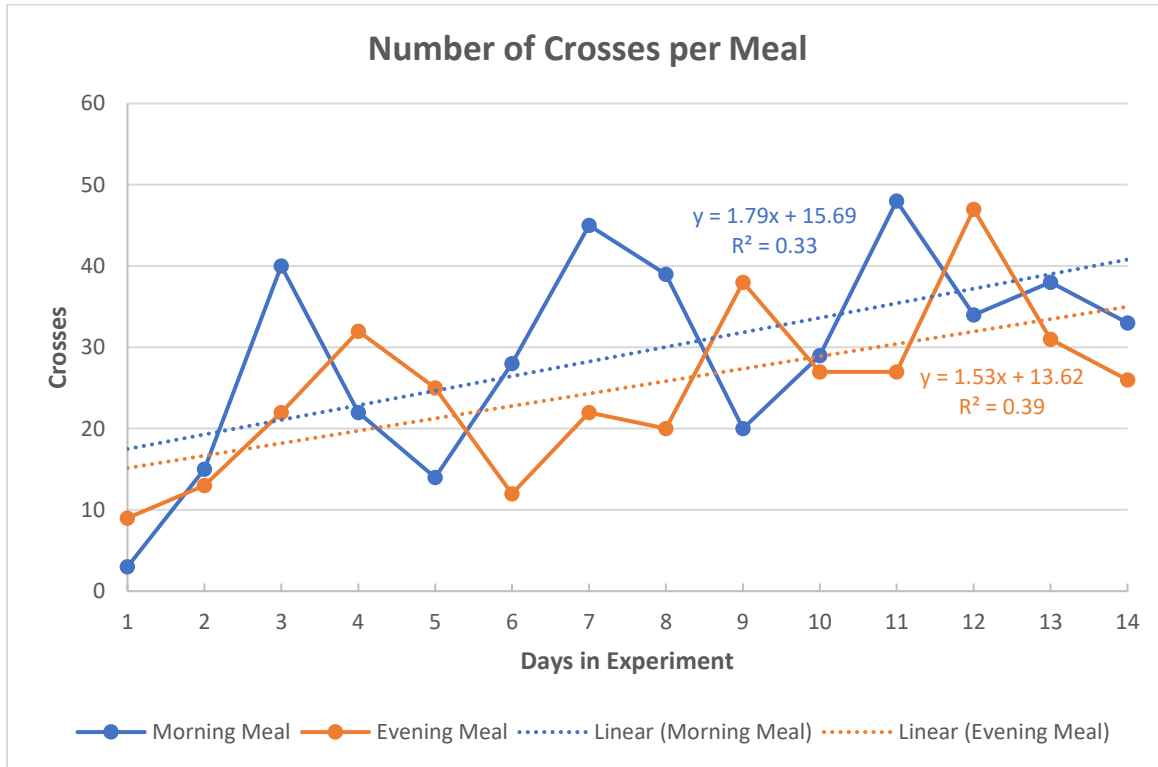
The total number of crosses were further partitioned into the number of crosses per period (figure 13). The slopes of the linear regression trendlines for the respective periods demonstrate how each period contributed to the overall increase in crosses. The trendline of the non-meal period has a slope of -.5, demonstrating crosses during this period decreased over time, contrasting the upward trend in total crosses. This period had a r-squared value of .01, meaning only 1% of variation could be explained by this model (figure 13).



**Figure 13: Number of Crosses per Period**

The evening meal trendline has a slope of 1.53 which means that each additional day resulted in 1.53 times more crosses during the evening meal (figure 13 & 14). The increase in crosses of this period contributed to the second most to the overall upward trend in total crosses (figure 12). The trendline for the evening meal crosses had a r-squared value of .39, meaning

39% of the variation can be explained by this model. The evening meal had a maximum of 47 crosses on day 12, a minimum of nine cross on day one, and an average of 25 crosses per meal. The morning meal crosses contributed the most to the overall increase in total crossing with a trendline slope of 1.79 and a r-squared value of .33 (figure 13 & 14). The morning meal period had a maximum of 48 crosses on day 11, a minimum of 3 crosses on the day one, and an average of 29 crosses per meal throughout the experiment.



**Figure 14: Number of Crosses per Meal**

The period crosses were broken down per week to analyze when crossing behavior increased and where the most amount variation was generated (Table 14). For the first two weeks of experiment three the average number of crosses per meal increased. In addition, the amount of variation in the number of crosses between the morning meals decreased, contributing to a higher r-squared value.

**Table 14: Weekly Summary for the Number of Evening-Meal period crosses per week.**

<b>Weekly Summary: Evening Meal</b>					
<b>Period</b>	<b>Average Crosses Per Day</b>	<b>Total Crosses</b>	<b># of days</b>	<b>Standard Deviation</b>	<b>Variance</b>
<b>Week 1 Days 1-7</b>	19.29	135	7	7.629	58.20
<b>Week 2 Days 8-14</b>	30.86	216	7	8.305	68.98

The increase in the number of crosses per evening meal for each week, (table 14), reflects the upward trendline in the period crosses (figures 13 & 14) and the increase in total crosses for experiment three (figure 12). Between the two weeks the average number of evening meal crosses increased by 11.57 crosses per meal, which is only a bit more than the increase in the average crosses per morning meal, 10.57 (table 15). The variance of evening meal crosses also rose as the experiment progressed (table 14), in contrast to the morning meals which decreased (table 15).

**Table 15: Weekly Summary of Morning-Meal period crosses per week.**

<b>Weekly Summary: Morning Meal</b>					
<b>Period</b>	<b>Average Crosses Per Day</b>	<b>Total Crosses</b>	<b># of days</b>	<b>Standard Deviation</b>	<b>Variance</b>
<b>Week 1 Days 1-7</b>	23.86	167	7	13.83	191.27
<b>Week 2 Days 8-14</b>	34.43	241	7	8.09	65.39

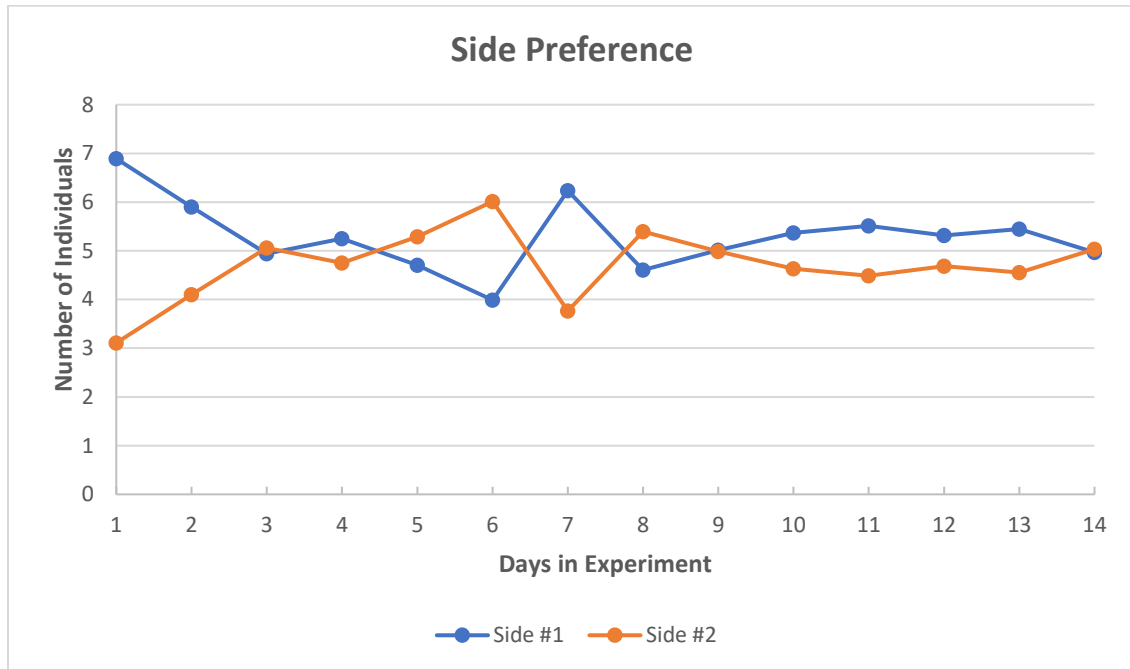
The weekly summary for Non-meal period crosses (table 16) shows a slight downward trend is observed between week one and week two. As there is still an upward trend in the total number of crossing (figure 12) this must be a result of the increase in meal time crossings (figure 14). In addition, the large variances seen in the non-meal period crossings compared to those for the meal time crosses show that the majority of variation within the experiment is a result of non-meal period.

**Table 16: Weekly Summary for the Number of NON-Meal period crosses per week.**

<b>Weekly Summary: Non-Meal</b>					
<b>Period</b>	<b>Average Crosses Per Day</b>	<b>Total Crosses</b>	<b># of days</b>	<b>Standard Deviation</b>	<b>Variance</b>
<b>Week 1 Days 1-7</b>	137.57	963	7	24.05	578.25
<b>Week 2 Days 8-14</b>	134.00	938	7	14.40	207.43

### 3.3.3 Side Preference

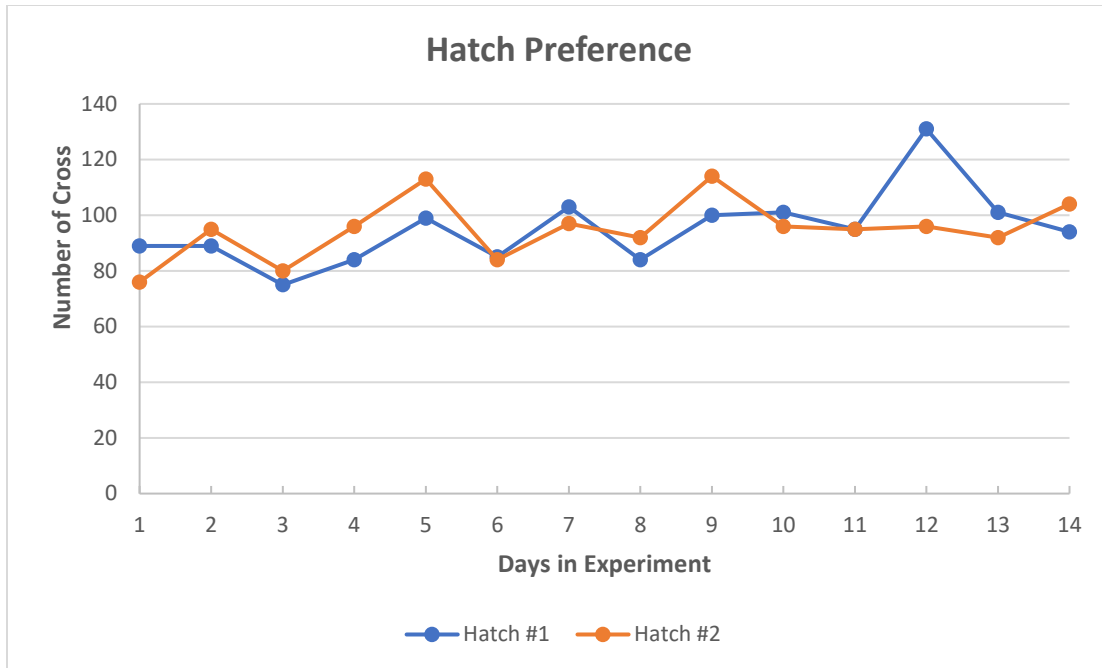
The fish's daily side preferences for the experiment were calculated based on the observations made at each crossing (figure 15). All fish were introduced to side 1, but already on the first day an average of three fish were on side two during each of the observations. The average number of fish per side varied throughout the experiment, but it is evident that the fish did not prefer one side over another, as the fish actively alternated between the two (figure 15). This statistic allowed for an analysis of any environmental stimuli or occurrences of dominant behavior that could have impacted crossing behavior, for example if one side had worse water quality the fish would be expected to stay away from that side. As there was no clear preference it was deemed that there was little to no environmental or dominance effect.



*Figure 15: Side preference for each Day*

### 3.3.4 Hatch Preference

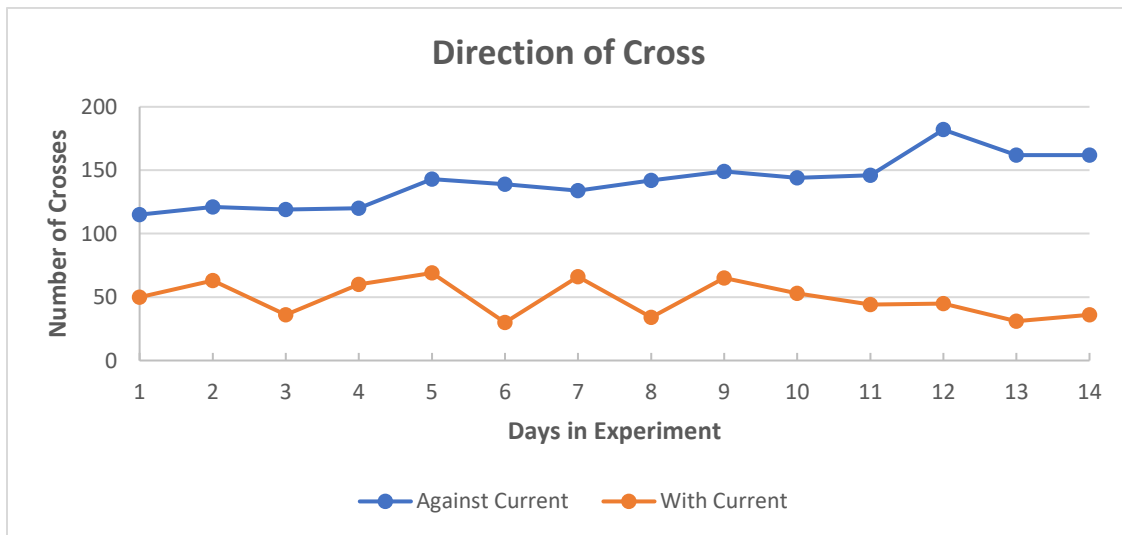
As with the Side preference the hatch preference was calculated for the third experiment (figure 16). This was to determine if there were any environmental stimuli or unforeseen design flaws that would affect the crossing behavior. As there is no clearly preferred hatch, except maybe hatch one on day 12, it can be deemed that the fish used the hatches non-disparately and thus crossing behavior was more impacted by the current through the hatch and general behavior.



**Figure 16: Hatch preference for each Day**

### 3.3.5 Direction of Crossing

As there appeared to be no hatch preference based on environmental stimuli or engineering errors, (figure 16), it can be determined that the flow of current through the hatch was the major factor for fish in choosing which hatch to use. The number of crosses through the hatch against the current greatly outnumbered the number of crossing with the current (figure 17), by almost 2:1.

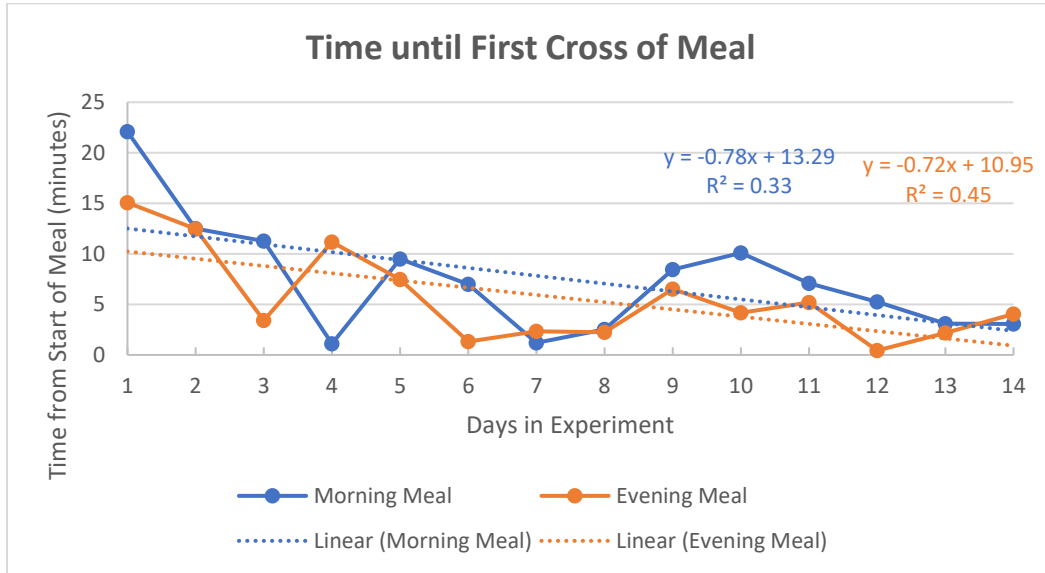


**Figure 17: Direction of Crossings**



### 3.3.6 Time Until First Cross of the Meal

A downward trend is visible throughout experiment three, for both meal periods, with the amount of time until the first cross of the meal decreasing with the number of days in the experiment, which alludes to fish understanding and utilizing the hatch better as time goes on (figure 18). The slopes of the linear regression trendlines average to  $-0.75$ , illustrating this downward trend for both meals. The  $r$ -squared values, however, differed with the evening meal having a value of  $.45$  and the morning meal with  $.33$ . This shows that the model for the evening meal explains 12% more of the total variation than the morning meal.



*Figure 18: The time until the first cross of the meal*

### 3.3.7 Growth Statistics

The weight and length of the ten fish used increased throughout the experiment (table 17). However, the weight and length increased more between the midpoint and the end than from the intake until the midpoint,  $42.08\text{g}$  and  $1.89\text{cm}$  per individual compared to  $28.6\text{g}$  and  $1.4\text{cm}$ . This trend however was not seen in the condition factor, which decreased slightly throughout the experiment, but was still within healthy parameters.

**Table 17: Growth Sampling Data**

3.10.18 Intake				18.1018 Weigh In				1.11.18 End			
Fish	Weight (g)	Length (cm)	Condition Factor (K)	Fish	Weight (g)	Length (cm)	Condition Factor (K)	Fish	Weight (g)	Length (cm)	Condition Factor (K)
1	176	24.0	1.27	1	193	23.5	1.49	1	244.3	26.5	1.31
2	157	22.5	1.38	2	199	24.5	1.35	2	232.9	26	1.33
3	187	23.5	1.44	3	189	24.5	1.29	3	242.4	26	1.38
4	152	23.0	1.25	4	197	25.5	1.19	4	231.1	25.5	1.39
5	187	23.5	1.44	5	204	25.0	1.31	5	228.8	26	1.30
6	164	23.5	1.26	6	193	24.0	1.40	6	247.6	26.5	1.33
7	155	21.5	1.56	7	191	24.5	1.30	7	242	27.5	1.16
8	158	22.5	1.39	8	209	25.0	1.34	8	259.7	27	1.32
9	181	24.0	1.31	9	215	25.5	1.30	9	224.7	26.5	1.21
10	169	23.0	1.39	10	182	23.0	1.50	10	N/A	N/A	N/A
Average	168.60	23.10	1.37	Average	197.20	24.50	1.34	Average	239.28	26.39	1.30
Sum	1686.00	231.00	13.69	Sum	1972.00	245.00	13.44	Sum	2153.50	237.50	11.73
Standard Deviation	13.39	0.77	0.10	Standard Deviation	9.87	0.82	0.09	Standard Deviation	10.95	0.60	0.07

Comparing these samples, the Specific Growth rate can be calculated (table 18). During the first period 3/8-18/8 the specific growth rate was a little more than half of the expected value. This was anticipated as the fish have to learn how to get through the hatches to receive both meals, but this was a statistically significant decrease in growth. The second period showed an improvement in SGR, but still well below the expected value. The second important production statistic calculated was the Feed Conversion Ratio (Table 18). The FCR follows the same trend as the SGR. During the first period it was much higher than expected, as the fish had to learn how to use the hatch to access the feed, but the FCR decreased during the second period, illustrating that the fish were getting more feed as seen in the increase in crossing behavior during meal times (figure 14).

**Table 18: Expected SGR and FCR compared to the Calculated values for the two periods.**

Dates	Expected SGR	Calculated SGR	Expected FCR	Calculated FCR
3/8-18/8	1.90%	1.04%	1	1.78
18/8-1/11	1.90%	1.32%	1	1.53

### 3.3.8 Behavioral Observations

The behavior of the fish in experiment three aligned most with that of experiment one. The same four major crossing types were observed along with similar resting positions. There were also fewer occurrences of dominant behavior, which is visible in the side preference of the experiment (figure 15). One key observation was that the fish in experiment three seemed to start crossing earlier and more consistently during meal times than the first two experiments. This can be seen in the larger values for the average daily crosses per meal and the lower standards of deviations (tables 14 & 15). In addition, the speed at which the fish crossed during a meal decreased more rapidly than the other two experiments as seen in the slopes of the trendlines for the time until the first cross (figures 18).

## 4. Discussion

### 4.1 Experiment 1

The results show that Salmon were capable of crossing through the hatch and that this behavior increased with time. This is in contrast to reports that salmon were afraid of performing a similar action, moving through subsurface grading equipment (Fjæra, 1993). The origin of this crossing behavior is a result of primal motivators that can be broken into two categories; intrinsic drives such as hunger, threat, competition, and stress, or extrinsic reasons relating to prior experiences and stimuli from the environment (Fernö, 2011). The response to intrinsic drives has been well documented in Atlantic salmon. For example, the response of Atlantic salmon to a predatory threat results in avoidance, seeking shelter, or freezing in place (Einum, 1997). In sea cages salmon are intrinsically motivated to maximize their environmental conditions by spatially distributing in response to cues such as light, temperature, salinity, and dissolved oxygen levels (Oppedal, 2011). However, in recirculating aquaculture systems these intrinsic drivers are limited as a result of the highly controlled environment (Damsgård, 2004). By testing for side preferences, it was determined that the equal distribution of fish between the sides showed there were no environmental effects specific to one region.

The most common intrinsic drivers in aquaculture are hunger and competition. These can be correlated to each other and the feeding scheme of the operation (Noble, 2007). In response to hunger and competition fish can respond aggressively, avoid confrontation, or engage in exploration and foraging behavior (Huntingford, 2012). This exploration is vital for individuals to gain information about food resources and their spatial environment, thus contributing to the extrinsic drivers for certain behaviors (Inglis, 2001). This is part of the information primacy hypothesis that suggests an individual's fitness is dependent on the gathering of information on the environment to increase the odds of satisfying its primal drives (Inglis, 2001). This information is then interpreted by the fish and can result in learned behaviors through the association of stimuli with a negative or positive outcome. In salmon these learned behaviors can be as complex as using visual cues for associating the probability of the presence of feed (Braithwaite, 1996). These new behaviors can then be acquired by other fish in the experiment through the observations of its companions (Helfman, 1984). This type of learning is illustrated by the observations of joint crossings in this experiment and the increase of crossings as the time in the experiment continued.

The intrinsic drives of hunger and competition caused fish to gather spatial information of the environment, thus discovering the hatch and how to use it. The observations by cohorts gave rise to and cemented the crossing behavior in this experiment as evidenced by the increase in total crossings. This new foraging behavior could then be implemented in response to competition and hunger (Huntingford, 2012). In this experiment competition and hunger were a result of the limited feed availability fish would experience if they remained only on one side. In addition, salmon in this experiment were stocked at low densities and aggressive feeding behaviors as a result of low stocking densities has been demonstrated (Turnbull, 2005).

While competition and hunger are two reasons to adopt crossing behavior, it is also likely that feeding stimuli played an important role in crossing as well. Salmon use a large variety of extrinsic environmental stimuli associated with feed to dictate foraging behavior, as seen with visual cues (Braithwaite, 1996). Atlantic salmon can also smell the amino acids used in feed (Sutterlin, 1971) and can associate these olfactory stimuli as a cue to begin foraging behavior (Warburton, 2011). Furthermore, Salmon could potentially sense the sound and or the vibrations of the feeders starting up at the beginning of a meal and use it as a cue to begin foraging (Tlusty, 2008). The ability to measure the effect intrinsic drives have compared to extrinsic environmental stimuli on the behavior of Atlantic salmon is difficult to quantify and needs further study. However, in this experiment it was deemed that a combination of extrinsic foraging cues along with the intrinsic drive to satisfy hunger were the most important as the increase in crossings during meal times were responsible for the majority of the total crossing behavior. A capability to learn the particular stimuli associated with the start of a meal was also observed as the time until the first cross of a meal decreased as the time in the experiment increased. This demonstrates that the crossing behavior is an innate response to intrinsic and extrinsic drivers through the use of understanding the environment to increase an individual's fitness.

## **4.2 Experiment 2**

Experiment one demonstrated that crossing behavior in Atlantic Salmon is initially a response to internal drives that transitions into a learned behavior increasing with the presence of external stimuli during meal times. Experiment two attempted to use additional external stimuli to influence the crossing behavior through pavlovian conditioning. The ability for such conditioning has been shown to exist in Atlantic Salmon through the use of sound and light during feedings (Tlusty, 2008; Lines, 2008). Light was deemed as the most appropriate stimuli as it has been shown that the frequency and intensity of light can elicit behavior (Koike 1987), directly affect the spatial distribution of Atlantic Salmon in sea cages (Juell, 2004), and potentially used for voluntary fish transport (Lekang, 1995). The effects of such conditioning on crossing behavior in this experiment were indecisive.

Experiment two had fewer average meal time crossings per day than experiment one. In addition, the slopes of the trendlines illustrate that the number of crossings during meals with the conditioning stimulus increased comparably to or less than those during meals without it. However, when the growth statistics are compared, experiment two has the highest SGR and the lowest FCR of the three experiments, alluding to the impression that fish had more access to feed. However, during the observations of meals on side two the majority of fish remained on side one demonstrating that pavlovian conditioning did not occur in this experiment in contrast to the prior research evidencing that Atlantic salmon possess this capability (Lines, 2008). This could be a result of the conditioning stimulus not being associated with the reward. However, the stimulus was comparable to those used in prior research (Lines, 1997). Therefore, the reason conditioning did not occur could be an outcome of the dominant and territorial behavior of individuals observed in experiment two. This aggressive behavior has been shown to be a result of low stocking densities (Turnbull, 2005). In addition, with the limited availability of feed on each side of the tank, aggressive behavior would be expected to increase and the establishment of

feeding territory could occur (Symons, 1968). The development of territoriality is seen in the disproportionate side preference of fish in this experiment. This demonstrates one of the key issues with the implementation of a feeding station. More dominant individuals will monopolize this area as there are lots of rewards and little incentives to leave. It is interesting to note that this behavior did not develop to the same degree in either of the other two experiments. It is for these reasons that experiment two should be performed again to investigate the effect of pavlovian conditioning on crossing behavior, while minimizing the occurrence of dominance and territoriality.

### **4.3 Experiment 3**

As with experiment two, the third experiment explored the effect of the environment of the tank design on crossing behavior. The use of two hatches not only allowed for more opportunities for the salmon to cross between the sides, but also the ability of stimuli to be transmitted. The effect of the increased opportunity to cross could not be determined. Comparing experiment three to the first two weeks of experiment one and two, the average total crosses per day were larger than that of experiment one, but lower than that of experiment two. However, there were more average crosses per meal in experiment three than in two and for the majority of experiment one, excluding the second week of morning meals.

The two hatches not only allowed for more crossing opportunities, but as water flowed through the hatches olfactory cues associated with feed (Sutterlin, 1971), sensations such as pellets falling into the water and vibrations from the feeders (Tlusty, 2008), along with the current itself affected the behavior of the fish. The effects of the transmission of environmental stimuli associated with feeding can be seen in the decrease of time until the first cross of the meal during experiment three. The negative slopes of the trendlines for the time until the first cross for each meal are significantly steeper than those in experiment one. The results of this can also be seen comparing growth data for the first two weeks, with experiment three having a larger calculated SGR. This suggests that the increased transmission and subsequent detection of environmental stimuli, not the increase in the ability to cross, was responsible for more crossing behavior.

The flow of the current carrying these stimuli through the hatches was also analyzed to judge the effect on crossing behavior. Salmon have been shown to favor swimming against the current in recirculating aquaculture systems (Huntingford, 2012). Prior research has shown that the use of flow can be used to influence swimming direction (Fjæra, 1993). This was observed in crossing behavior as well. The number of crosses between the two sides was much larger through the hatch that allowed the fish to cross against the current compared to that with the current. This result is in line with research done to use current to stimulate voluntary movement (Lekang, 1996). This manipulation of swimming behavior has important implications for future designs of feeding stations as it alludes to how fish will enter and exit the system.

## 5. Conclusion

The aim of the first experiment set out to demonstrate that Atlantic salmon possessed the capability to learn the behavior of crossing through a hatch to access feed, thus alluding to the act of entering a feeding station. This was established in all three experiments as the average crosses per meal increased throughout the duration of the trials. In addition, the increase in meal time crosses were larger than the increases seen in non-meal time crossings, illustrating that salmon could use this behavior to maximize their access to feed. The aim of increasing this crossing behavior through the use of pavlovian conditioning was undetermined. When comparing crossing statistics between the three experiments there was no effect of conditioning seen, thus more research is needed. The influence of current on crossing behavior was shown in experiment three. As the majority of crosses occurred against the current, future designs of feeding stations could take advantage of this relationship. Lastly, the negative effects of such a system on growth were revealed to decrease as time in the experiment increased. Further study is needed to see if with more time in the experiment would growth reach comparable levels to current aquaculture methods. These experiments demonstrate that the behaviors required for a feeding station system, like those used for individual monitoring in precision livestock farming of terrestrial animals, exist in Atlantic salmon and how the design of such a system could be influenced through the use of environmental stimuli.

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