# YELLOW PERCH, PERCA FLAVESCENS, GROWTH AND SURVIVAL ON DIFFERENT FEEDS AND IN LOW SALINITY ENVIRONMENTS

by

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A Thesis Submitted in

Partial Fulfillment of the

Requirements for the Degree of

Master of Science

in Freshwater Sciences and Technology

at

The University of Wisconsin-Milwaukee

May 2020

ABSTRACT

YELLOW PERCH, PERCA FLAVESCENS, GROWTH AND SURVIVAL ON DIFFERENT FEEDS AND IN LOW SALINITY ENVIRONMENTS

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The University of Wisconsin-Milwaukee, 2020

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The development of effective culture techniques in the rearing of yellow perch *(Perca flavescens)* (Mitchill 1814) has multi-lateral benefits including enriching recreational resources, helping conservation efforts, as well as providing quality sea-food production. A myriad of challenges is yet to be remediated if yellow perch aquaculture is going to prosper. One of the challenges that hinders yellow perch aquaculture is the lacking supply of high-quality fingerlings. With current techniques employed by yellow perch hatcheries, the survival of yellow perch up to 30-day post hatch (DPH) age is typically less than 10%. Furthermore, larval culture has depended on imported dry feed developed for other species of fish, as well as live feed (artemia nauplii, rotifers, and even copepods), which may not be specific to yellow perch. Thus, it is critical to investigate new hatchery techniques and diets to increase larval survival during their early life stages. The overall goal of this research is to increase the yellow perch production efficiency by developing new culture techniques for yellow perch hatchery and fingerling production. Three experiments were completed in this study.

The objective of the first experiment was to determine the best feeding practices for the yellow perch. For this, yellow perch larvae were fed artemia which had undergone a

decapsulation process, artemia which had not, as well as two formulated dry diets. These consisted of a popular commercial larval fish feed and a lab formulated larval feed. The live feed was fed in combination with dry feed as well. The diets were fed from 16 DPH to 30 DPH. The results showed that live feed performed better than the dry feeds in larval fish survival ($p<0.05$), but the decapsulated artemia had the best overall individual weight gain among live feeds. This suggests that, while non-decapsulated artemia can maintain larval perch survival, the larval perch may not be able to fully digest the unsoftened artemia, leading to less growth.

The objective of this next study was to assess whether 5-ppt saltwater had any effects on yellow perch embryos, as well as larval growth and survival, if used as an alternative to formalin for pathogen control. The conditions during embryo incubation were: Freshwater with formalin, 5-ppt saltwater with formalin, and 5-ppt saltwater without formalin. The embryos were photographed daily during development and measured for endogenous nutrition depletion from 0 days post spawn (DPS) to 7 DPS. The results showed a significantly higher endogenous nutrition diminishment among treatments with formalin than the 5-ppt saltwater without formalin ($p<0.05$). This implies that yellow perch incubated using formalin will have a lower endogenous nutrition reserve when they hatch as opposed to those that were not incubated using formalin. The second experiment also investigated the growth and survival of larval perch that were hatched after either being incubated in 5-ppt saltwater or freshwater and being reared in either a 5-ppt saltwater or freshwater environment. The feeding trial lasted for four weeks. The results of this study showed a significant increase ($p<0.05$) in growth and survival of fish in the 5-ppt saltwater environment, based on the measurement of body length and size

of fish. This result implies it may be a potential approach to use 5-ppt salinity to increase larval production of yellow perch.

The third experiment determined if 5-ppt saltwater could enhance fingerlings previously grown solely in freshwater. Yellow perch fingerlings (192 DPH) were fed a high carbohydrate diet (25% wheat flour) containing 41% protein compared to a fishmeal-based diet containing 54% protein with no added carbohydrate. The feeding trial lasted for 8 weeks in a recirculating aquaculture system run with either freshwater or 5-ppt salinity water. The study showed no significant difference in growth between treatments reared at a 5-ppt low salinity and freshwater environment. The study did however find a significantly higher feed conversion ratio (FCR) in perch raised in saltwater as well as a higher hepatosomatic index (HSI). The protein efficiency ratio (PER) was higher in perch fed the wheat flour diet compared to those fed the fishmeal diet. These results conclude that more research into the optimal amount of carbohydrate inclusion in the yellow perch diet is needed, as well as the implementation of a low salinity environment.

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| | |
|---|---|
| A | NON-DECAPSULATED ARTEMIA |
| B2 | OTIHIME B2 COMMERCIAL DIET |
| CF | FULTON'S CONDITION FACTOR |
| CI | CARCASS INDEX |
| DA | DECAPSULATED ARTEMIA |
| DPH | DAYS POST HATCH |
| DPS | DAYS POST SPAWN |
| FCR | FEED CONVERSION RATIO |
| HSI | HEPATOSOMATIC INDEX |
| L | LAB FORMULATED DIET |
| MS-222 | TRICAINE METHANESULFONATE |
| PER | PROTEIN EFFICIENCY RATIO |
| PPT | PARTS PER THOUSAND |
| VFI | VISCERAL FAT INDEX |

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# ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor Dr. Dong-Fang Deng for taking me on as her first graduate student here at the School of Freshwater Sciences and mentoring me. I have looked back at my progress from when I commenced the graduate program and your guidance has brought be a long way academically and professionally, I can't put into words how grateful I am. I also thank all of the visiting scientists that have come through the lab from China during my tenure here. Each of them has instilled a little bit of their knowledge in me from their own backgrounds and for that I am eternally thankful.

I would also like to thank Fred Binkowski for being a great source of knowledge and insight at the school. From him I have learned practical knowledge about aquaculture and fisheries that cannot be learned from any book. He helped guide me through my time here in many ways and for that I am very thankful.

Finally, I would like to thank my family and friends for their unwavering support as I pursue this endeavor, without them this would have never been possible.

A special thanks also to Dr. Jerry Kaster, Dr. Ryan Newton, Chris Ney, Mark Lausten, Linda Horbinski, Randy Metzger, Jerry Becker, Ron Bruch, Jhonatan Sepulveda, Haley Lucas, Sonya Ponzi, my colleagues at SFS and my students at Carroll University.

I also thank the Research Growth Initiative (#101X354) and UWM (Project 150-25-3150-343 PRJ93WQ) for funding my work. They are an essential resource for the spread of science education and their support provided the opportunity for me to work on this important topic.

## Introduction and Background

*Perca flavescens,* commonly known as the Yellow Perch, is a perciform fish that helps form a forage base to many freshwater ecosystems and are native to much of the north central and eastern part of the United States. Harvesting of yellow perch was once part a thriving fishery in the North American Great Lakes. According to the National Oceanic and Atmospheric Administration (NOAA), from the early-1950's to the late-1970's annual commercial landings of yellow perch from the Great Lakes could average more than 23 million pounds (NOAA, 2019). Because of this, the midwestern region of the United States has developed a large consumer and recreational interest for the species. Unfortunately, due to a rapid influx of invasive species and an altering of the food web, that number has been drastically reduced today in a status some might consider as a "crashed" fishery. (Shroyer & McComish 2000, Fulford 2006) For example, from the years 2000 – 2005, less than 9 million pounds collectively have been landed by commercial fisherman from all of the great lakes combined (NOAA 2019, FAO FishJ). The lack of a high yield commercial fishery and the great demand of this species merits the development and implementation of a viable yellow perch aquaculture sector.

Aquaculture is the fastest growing sector of food production in the world (Leal et al. 2018). Unfortunately, more research is required in order to develop techniques and practices for the yellow perch to thrive as a viable aquaculture species. While the popularity of the species cannot be understated, high quality yellow perch fingerlings suitable for aquaculture are difficult to attain due to high egg mortalities at the early life stage (El-Gawad et al. 2015). This is partly due to the species being marred with certain

difficulties pertaining to high early age mortalities with larval yellow perch raised in an intensive aquaculture system. The first of these difficulties occur after fertilization when the egg ribbon can fall victim to pathogenic oomycetes (water molds) such as *Saprolegniales* (Abd El-Gawad et al. 2016, Tiffney 1939). Without some type of pathogenic control such as salt (Sodium chloride), formalin (CH2O + Methanol), hydrogen peroxide (H2O2) or other aquatic pesticides, much of the embryos will either die or become too energy depleted to hatch (Abd El-Gawad et al. 2016, Rach et al. 1997). The second challenge is that during development and after hatching, the larval embryo contains an endogenous nutrient supply that will eventually become depleted. If the yellow perch larvae cannot adapt to an exogenous supply of energy, the larvae will die, leading to a high number of mortalities from a 7 to 10-day post hatch age (Withers et al. 2015, El Gawad et al. 2015). The third and last challenge is that prior to 28 days post hatch, the larvae usually undergo another not so understood mortality spike. Many theories about this late temporal increase in mortality are prevalent, with examples being; antagonistic intraspecific relationships, particularly cannibalism, forming from high size variability (Malison & Held 1992, Kestemont et al. 2003), or high mortality due to a small percentage of swim bladder inflation leading to a disadvantage in energetic efficiency (Craig 2008, Czesny et al. 2005, Kestemont et al. 2015). Regardless of the cause of these mortality bursts, if aquaculture practices can emerge that generally enhance the overall performance of larval perch and reduce mortalities in this early life phase, then potentially, more procedures can be researched, refined, and gradually implemented to slowly eliminate much of this early onset mortality.

The first step in reducing larval mortality is by investigating alternative methods for controlling pathogenic microbes during incubation. Formalin, a common disinfectant, made by diluting formaldehyde and mixing it with methanol and used by aquaculturists, has been shown to be effective at inhibiting pathogenic fungi associated with colonizing and reducing the viability of eggs in early stage production of fish (Schreier 1996, Rach 1997, Francis-Floyd 1996). Unfortunately, the formalin required in egg treatments is considered highly toxic, and alternative treatments are being evaluated to eventually replace this pesticide. The World Health Organization definitively classifies formalin as a carcinogen, and the Environmental Protection Agency has issued “formaldehyde regulations” citing 26 references about the carcinogenicity of formaldehyde (Buesa 2008). Looking at an alternative approach, sodium chloride has also been shown to be effective at reducing the colonizing potential of opportunistic fungi during egg incubation and is also safe and non-toxic (El-Gawad et al. 2015). Sodium chloride may also be a more natural means of incubating and rearing larval yellow perch as there is evidence that larval yellow perch may perform better in a low salinity environment than that of a freshwater one. One study demonstrated that treatments of 2-9 ppt saltwater fared far better than its freshwater counterpart in keeping larval yellow perch alive (Victoria et al. 1992). Nearly all of the larvae raised in the 2-9 ppt saltwater survived while 21.3% of larvae raised in the freshwater died. However, when used as an environment to incubate the embryos, the embryos in 7-9 ppt saltwater performed significantly worse than lower concentrations. It also states that Maryland larval perch were less sensitive to salinity than Pennsylvania perch larvae, meaning the genetic strain of yellow perch may also have an impact on larval mortality (Victoria et al. 1992). In this study, 5-ppt low

salinity saltwater was chosen as the appropriate concentration to be investigated for embryo incubation.

Prior studies involving a relative of *Perca flavescens, Perca fluviatilis* or the European perch have shown that larvae reared in low salinity solutions have demonstrated a significant increase in survival of these fish when compared to those reared in freshwater (Bein & Ribi 1994, Overton et al. 2008, Ložys 2004). These studies attribute the ability to thrive in a low salinity condition as being accompanied to the fact that many *Perca fluviatilis* early life stage fish spend most of this time period in brackish water, with a salt concentration from 0 – 5 ppt. Studies have also observed *Perca fluviatilis* migrating between brackish waters and freshwater streams to spawn (Leili 2000, Müller & Berg 1982). This behavior has been observed in the yellow perch as well (Wagner & Austin 1999). Near the Chesapeake Bay, yellow perch are often surveyed inhabiting reaches of the estuaries where there is a salinity gradient. Particularly of interest, spawning has been observed near estuarine habitat (Tsai & Gibson 1971). This implies that there could be a benefit between freshwater percids in the context of early life stage affinity to brackish water. A previous study has observed that when given a choice, juvenile yellow perch prefer a low salinity environment, suggesting that the low salinity might alleviate osmoregulatory pressures (Christensen & Grosell 2018). The European perch has been observed having the same preference (Bein & Ribi 1994, Overton et al. 2008, Ložys 2004). It is possible that yellow perch and European perch have a similar preference to low salinity environments.

In wild populations, yellow perch form a forage base in many freshwater lake food webs which naturally extend from South Carolina up to the Northwest Territories in

Canada (Page and Burr 1991). The European perch on the other hand is predatory and has a native range from Western Europe to Siberia. While the two distinct ranges suggest that they are distantly related, a common theory which scientists have supported is that both the yellow perch and European perch are two subspecies of a single circumpolar species (Thorpe 1977). The study also makes the claim that other than having a difference in the location of a pre dorsal bone, both species are biologically non-distinct. This means that they both have similar tolerances to things like salinity, temperature, and dissolved oxygen thresholds (Thorpe 1977). This would insinuate that the European perch may be physiologically similar to the yellow perch, inferring that studies pertaining to European perch and a low salinity preference may be somewhat similar to yellow perch. In the age of modern-day scientific techniques, DNA sequence comparisons show some similarity between *Perca flavescens and Perca fluviatilis* and a relatively close phylogenetic relationship (Zhan et al. 2009, Refseth et al. 1998, Leclerc et al. 2000).

The fact that both species have shown an affinity for a low saline environment during spawning could mean that the implementation of a low saline incubation and rearing period could improve performance regarding *Perca flavescens* and early life stage survival in aquaculture systems. Raising the species in a low saline environment could turn out to be standard procedure in the incubation and rearing of larval yellow perch in the future. A study assessing the potential of a low saline environment on the performance of yellow perch in an aquaculture system is warranted.

This study assessed the ontogeny, survival, and growth of *Perca flavescens* from initial fertilization, embryo incubation, and then from 5-DPH to 35-DPH during the

larval life stage in a low salinity flow-through intensive aquaculture environment. A preliminary study was also performed that assessed the optimal live feed preparation and dry feed choice for use in the low salinity larval study. The preliminary study lasted 15 days and studied the effects of 8 different diet combinations on 16 DPH larval perch to 30 DPH age.

It was then investigated how a low salinity environment affects fingerling 192 DPH-age yellow perch in a typical recirculating aquaculture rearing system provided two different diets. One was a reference diet with ingredients similar to those found in a typical yellow perch commercial diet, and the other was an experimental diet observing the effects that a 25 % substitution of wheat flour replacing fish meal has on developing fingerling yellow perch.

Sustainable and cost-efficient diet ingredient implementation is another important research topic pertaining to the future of world aquaculture. In 2000, a paper was released that essentially gave rise to investigating aquaculture nutrition practices as a major component of aquaculture research (Naylor et al. 2000). It was discovered that although the aquaculture industry benefitted from a narrative that it was helping to alleviate pressures placed on the global fisheries by providing another source of seafood, in reality the opposite was true. According to the paper, a gap in the knowledge of feeding many species of fish resulted in some feed conversion ratios of as high as ~5 kilograms of oceanic fish taken out of the oceans to produce 1 kg of desired aquaculture species (Naylor et al. 2000). This occurred because in order to provide many species of fish the proper nutrition, fish meal consisting of ground, dried, oily oceanic species of fish was seen as the best ingredient to raise farmed fish. Due to this,

fish meal was used in excess. According to this study, aquaculture was actually exacerbating the problem of overfishing by harvesting “unwanted” or “trash” fish and essentially recycling them into a desired commodity. Unfortunately, removal of this lower trophic order of fish in the forage base can have dire ecological consequences with a sudden disruption of the food web (Koen-Alonso & Yodzis 2005, Hollowed et al. 2000, Whipple et al. 2000).

Not only is the utilization of fish meal unsustainable from an ecological perspective, but it is also very expensive and getting more costly. Looking at historical commodity prices over the last 30 years, the price of Peruvian fish meal for example, has increased by more than 250% (Source: The World Bank). The chart can be viewed as appendix 1 on page 62. Because of the rapid rising cost associated with fishmeal, many commercial aquaculture diets rely heavily on the implementation of cheaper carbohydrates to make up part of the dry feed. A problem with this practice arises as most fish do not feed on carbohydrates in their natural environment, leading to complications that act as a detriment to aquaculturists. Providing a feed that is not specific compositionally to a given species of fish can result in a less efficient growth rate, especially in desired components of fish, like fillet size. The implementation of feed ingredients that are not suited to a specific species can also result in stunted growth, excess visceral fat, fatty enlarged livers, and other problems which can negatively contribute to the viability of raising certain species of fish.

Only by researching more cost efficient, sustainable, yet high performing ingredients for aquaculture can there be gains in the practicality of raising any aquaculture species. It was because of this reason that for the final experiment, an

investigation into the effects of replacing fish meal with wheat flour was investigated in conjunction with the performance of fingerling yellow perch in a typically used RAS system at low salinity.

# Chapter 1

## Experiment 1

## Assessment of optimal live feeding practices for larval yellow perch (*Perca flavescens)*

### *Objective and Hypothesis*

The objective of this study was to determine the optimal feeding regimen for larval perch after their endogenous nutrition is exhausted. The hypothesis of this study is: Artemia that undergoes a decapsulation process is more digestible and thus should provide better growth for larval yellow perch after their endogenous nutrition supply runs out. Furthermore, if accompanied by a proper dry feed, the growth and survival should increase even further.

### *Methods and Materials*

#### *Fish source*

All animal studies were performed in compliance with animal care protocols (15-16-#46) approved by the University of Wisconsin-Milwaukee, Institutional Animal Care and Use Committee. Yellow Perch (*P. flavescens*) were obtained from broodstocks housed at the School of Freshwater Sciences, University of Wisconsin (Milwaukee). The original broodstock was derived from the Sassafras River (Chesapeake Bay, MD) and the utilized broodstock were the UWM-SFS January breeding stock.

Spawning of the fish was performed in accordance with US Patent 7,836,852 B2 (Methods for manipulating yellow perch spawning cycles) at 13˚C. Females were individually chosen having their eggs stripped, providing one egg ribbon in a 3-liter aluminum bowl. Three males were then randomly selected having their milt extracted and mixed with the eggs to induce fertilization. This was then combined and mixed with a ~100 mL mixture of bentonite clay and water 6.4 g/L along with the egg ribbon. The fertilized eggs were then washed in the same 3-liter aluminum bowl with the freshwater tank input flow and placed into a two-gallon Ziploc brand bag filled with freshwater from the broodstock tank and kept in a tank filled with water held at a temperature of 13˚C. The same process was used to generate 8 fertilized egg ribbons. The egg ribbons were then stretched over a porous plastic grating and left to incubate for eleven days. A picture of this incubation can be seen in Figure 1. on page 37.

At the 11$^{th}$ day of incubation, the egg ribbons were manipulated for a visual assessment of structure. Hatching was facilitated by agitating the water above the egg ribbons with an acrylic rod.

Any debris from the spent eggs were removed from the tank. The water surface was skimmed to remove oil, which can impact water quality as well as larval feeding. Larvae were fed with Otihime B1 diet (larval fish diet) at 4-DPH and artemia nauplii cultured utilizing a 48-hour non-decapsulation incubation method at 1-DPH. Yellow perch were raised in a flow through water system until use for this study at 16-DPH.

***Larval fish placement***

Yellow perch larvae 16-DPH were transported to stock tank and subsequently distributed into a flow through aquaculture system containing 23 tanks distributing dechlorinated water. The tanks have a diameter of 44 cm and were filled to a depth of 23 cm for a volume of 35.0 L. The outflow of each tank was a perforated 1 ½ inch diameter x 12 cm long PVC central column wrapped in 425 sized mesh to slow outflow and protect the fish from escape. Each tank was stocked with 200 fish by counting 50 per tank until fully stocked. All surplus fish were placed in a 4-foot stock tank for initial sampling. After 24 hours, any mortalities from the research tanks were removed and subtracted from the initial stocking of 200. These mortalities were attributed to having died due to the stress of stocking rather than the study. The initial number of fishes per tank was determined as any dead fish after 24 hours subtracted from 200.

One hundred and fifty larval fish (16 DPH) were retrieved from the stock tank and weighed using an analytical balance for initial measurements. The average individual weight of these fish was measured as 8.4 (± 0.9) mg per fish. This weight would be used as the initial weight for the experiment. Fifty random perch were also measured for initial length. The individual length average was 7.7 (± 0.5) mm.

Each tank had a constant flow-through of dechlorinated water at a flow rate of 0.3 L/minute. Water temperature was maintained between 20-22 ˚C. A Google random number generator was used to randomly allot the treatments to each tank. The feeding trial lasted for 15 days from 16-DPH to 30-DPH. Measurements of yellow perch were taken to evaluate survival and individual growth.

***Formulated feeds:***

The commercial diet used in this experiment was Otohime B2 diet from Marubeni Nishin feed company in Japan. This feed was a common feed used by the UWM – SFS broodstock facility and commercial hatcheries to feed larval yellow perch. The particle size was 360-650 µm. A formulated diet (L) was also prepared and tested in this study. The feed crumbled to a size of 400-600 µm. The nutritional composition of all feeds is presented as Table 2. on page 20.

***Live feed treatment preparation method:***

The live feed investigated in this experiment were artemia nauplii cysts sourced from the Great Salt Lake in Utah, U.S.A. Two artemia nauplii incubation methods were examined for optimal larval survival and growth in this experiment; decapsulated artemia (DA) which incubates for 21 hours, and a non-decapsulation artemia (A) incubation method which incubates for 21 hours.

Decapsulation of artemia: Five grams of artemia cysts was hydrated in 500 ml of 27-29 ˚C water for one hour. The hydrated cysts were collected by filtering the solution through a fine mesh bag. The hydrated cysts were then placed in a 0.27 molar solution of sodium hypochlorite (Chlorox bleach) in water. The hydrated cysts were vigorously hydrated for 8-12 minutes until the cysts turn into a bright orange color. The cysts were then rinsed thoroughly with water. After this, the cysts were placed into 500 mL of a 5 micromolar solution of sodium thiosulfate in water for 30 seconds to neutralize the sodium hypochlorite then rinsed thoroughly again. The cysts were then placed into a glass container with 2.5 L of 25-ppt aerating saltwater held at a temperature between 27-29 degrees C. The artemia were then ready to be fed from 21 – 28.5 hours of incubation, between the first and last feeding.

1. Non-decapsulation of artemia: Five grams of artemia cysts were placed into 2.5 L of 25-ppt aerating saltwater held at a temperature between 27-29 degrees C. After 21 hours of incubation, a 60-watt incandescent light bulb was shone on the bottom of the jar for one minute to attract hatched artemia to congregate at the bottom of the jar. A siphoning tube was then used to extract all hatched artemia from the jar and any discarded eggs floating on the surface of the water is discarded. The collected hatched artemia were then fed to the fish.

***Dietary Treatments***

Yellow perch were fed with 8 diets:

1. Decapsulated artemia 2. non-decapsulated artemia 3. A combination of a commercial feed (B2 Otihime) and DA (B2+DA), 4. A combination of B2 and A (B2+A). 5. A combination of L and DA (L+DA). 6. A combination of L and A (L+A). 7. (B2) 8. (L). All dietary treatments were randomly assigned with three tanks except the test diet L was fed to two tanks only.

***Larval Fish Maintenance***

For the combination feed treatments, 40 mL of homogenized artemia and 65 mg of dry feed was fed to each tank per feeding. For the fish fed DA or A, only 80 mL of A or DA was fed to each tank per feeding. For the treatments fed with dry feed, 130 mg of feed was fed per feeding.

Fish fed the dry feed or artemia were fed six times daily at (9:00, 10:30, 12:00, 13:30, 15:00, and 16:30) by hand. For the combination feeding treatments, the fish were fed

the dry feed at 9:00, 12:00 and 15:00, with the artemia being dispensed at 9:15, 12:15, and 15:15.

Leftover feed and waste solids were siphoned one hour after the last feeding into a bucket and the mortality of fish was recorded. Dissolved oxygen (DO) > 8.0, pH (8.3-8.6), and total ammonia nitrogen (TAN) < 0.02 mg/L, were measured initially and in 6-day intervals until final sampling of fish. Water temperature was monitored daily and recorded.

***Data collection and statistics***

The length of the first 50 larval fish caught per tank was measured using a metric ruler and the weight of all surviving fish was obtained using an analytical scale accurate to 0.0001 g.

Fulton's Condition Factor was generated using the following equation:

$$\text{Fulton Condition factor (CF)} = 100^* \text{ body weight (g)/body length (cm)}^3$$

Survival per tank was determined using equation:

$$\% \text{ Survival} = (\#\text{ initial fish}/\ \#\text{ final fish}) *100$$

Statistical analysis of experiment 1 analyzed the total growth rate and survival using a one-way ANOVA. Data are presented as mean ± SE, n=3. All assumption violations were checked before statistical analysis. Significant differences ($p<0.05$) that occurred among ANOVA groups were compared using a Duncan multiple range comparison test for pairwise comparisons. Pair-wise comparisons were considered significant when $p<0.05$. Statistical analysis was performed using SPSS 26.0 for windows (IBM).

## *Results*

The growth and survival performance of yellow perch was presented in Table 1. There was no significant difference among the survival of larvae fed the two live artemia diets, non-decapsulated (A), and decapsulated artemia (DA), ($p > 0.05$), both of which had the highest survival among all treatments. The survival from these two treatments was significantly higher ($p<0.05$) than those fed the lab diet (L), A+L, and DA+B2 (commercial feed). There was however a significant difference among the final body weight of fish fed the two live feeds. DA had a significantly bigger size than A. The survival of larvae fed dry feeds combined with the live feed varied but was ultimately all statistically similar. Pertaining to formulated feeds combined with artemia, the lab formulated diet showed the highest survival when combined with DA. When DA was combined with the B2 commercial diet, the perch had the highest individual weight, but a relatively low survival.

The final body length of the larval perch was the highest among the treatments fed DA, DA+B2, and DA + L. The DA + L, and the DA + B2 had the longest length among dry diets fed in combination with artemia. The length was also significantly different among the live feed, DA and A treatments, with DA treatments having a significantly longer length. The condition factor was similar for the perch fed all test diets.

| Treatment | Survival (%) | Final body weight (mg) | Final body length (mm) | CF |
|---|---|---|---|---|
| **A** | 74.5 ± 1.2 [a] | 22.1 ± 0.4 [c] | 14.7 ± 0.0 [b] | 0.696 ± 0.01 [a] |
| **DA** | 73.6 ± 2.3 [a] | 43.2 ± 7.6 [b] | 17.3 ± 0.5 [a] | 0.834 ± 0.14 [a] |
| **DA + L** | 61.9 ± 5.9 [ab] | 31.0 ± 0.9 [bc] | 16.7 ± 1.2 [ab] | 0.666 ± 0.15 [a] |
| **A + B2** | 61.0 ± 6.4 [ab] | 26.4 ± 3.8 [bc] | 13.4 ± 1.8 [bc] | 1.097 ± 0.97 [a] |
| **B2** | 56.5 ± 6.9 [ab] | 23.4 ± 2.8 [c] | 12.0 ± 1.1 [c] | 1.354 ± 0.50 [a] |
| **A + L** | 51.8 ± 2.2 [b] | 22.1 ± 0.4 [c] | 12.2 ± 1.8 [c] | 1.630 ± 0.64 [a] |
| **DA + B2** | 45.5 ± 11.6 [b] | 62.1 ± 6.8 [a] | 16.3 ± 1.9 [ab] | 1.434 ± 0.43 [a] |
| **L** | 43.1 ± 2.1 [b] | 22.0 ± 2.6 [c] | 14.4 ± 0.37 [b] | 0.737 ± 0.01 [a] |

*Table 1: Growth performance of yellow perch fed different diets from 16 -30 day post hatch. Treatments sharing a letter have no statistically significant difference p > 0.05. (n=3, ± SE). The lab-based diet fed solely by itself was trialed in duplicate. Treatments: A: Non-Decapsulated artemia, DA: Decapsulated artemia, B2: Otohime commercial B2 Diet, L: Formulated lab diet. Initial individual weight: 8.402 mg.CF: Conditioning factor*

## *Discussion*

### *Live feeds*

The results from the experiment demonstrate that live feeds, when fed independently of a dry feed provide the best survival of any treatment, but the chosen method of preparation for the live feed may come with a cost to growth. According to the data, decapsulated artemia fed without any dry feed had a similar survival to non-decapsulated artemia, but there was a significant difference in the individual weight of each treatment, with the DA method being associated with a higher individual weight than the A. This could be due to that during decapsulation, the cyst capsule and the shell of the organism becomes softened so that the whole cyst and shell can be consumed and fully digested (Lim et al. 2002, Sorgeloos et al. 1977). The DA also had a higher condition factor than the A, which described a higher weight to length relationship, suggesting that the fish had more girth than the A. The non-decapsulated

live diet does not soften the shell, meaning that the cyst shell was not consumable, and a majority of the artemia may pass undigested through the larvae (Sorgeloos et al. 1977). The resultant data support this notion. If using the same amount of artemia cysts for early larval rearing, providing a diet in which a portion of the feed is nutritionally unavailable comes at a developmental cost. Due to the relatively low nutrition of live artemia cysts and nauplii compared to a formulated diet, a high amount of artemia must be consumed in order to meet larval nutritional requirements, thus it is imperative that the larvae can extract as much nutrition from the artemia as possible (Vanhaecke et al. 1983). Hence, practices that optimize the digestion of artemia are necessary for better larval perch culture. It is also important to try to implement a formulated feed at this life stage to provide a more nutrient rich food source for early development. This experiment helped to inform the decision of using the DA method for all larval trials pertaining to the yellow perch for the optimal survival as well as growth in any larval trial moving forward.

***Formulated feed***

All formulated diets fed solely by themselves had a relatively poor performance pertaining to both survival and individual growth compared to the live feeds. These results fit expectations as at 16-DPH, the larval perch may not yet be fully weaned off live feed and may not be able to fully depend on a formulated diet. Making a switch to a formulated diet provides larvae a much more nutrient dense diet than feeding solely on artemia. This can be seen in the condition factor values, which typically have a higher CF than those larvae which fed solely on artemia.

As for the lab formulated diet, it yielded higher than expected results. Nutritionally, it had a lower protein and lipid content than the B2 diet, but the lab-formulated diet had a higher moisture content. The B2 commercial diet also most likely has underwent a high-pressure extrusion method which makes the feed more hardened, and the water stability significantly higher than the lab formulated diet extrusion method. While the commercial feed may hold its form better, the softer lab-formulated larval feed could potentially be more palatable and may have better performance pertaining to digestion. The results from this study demonstrated the importance of developing a larval feed specific to the yellow perch.

The B2 commercial feed when fed in conjunction with decapsulated artemia was associated with the highest individual growth of all treatments. This result comes with some questions that would make for good follow-up experiments. The most important question is “If the treatment had mediocre survivability but a rapid individual growth, and the biggest fish, then could a high variability in size have led to a higher tendency of cannibalism within the tanks?” It is known that yellow perch larvae can attain cannibalistic tendencies if they develop more rapidly than other fish in their environment (Post & Evans 1989, Tarby 1974). This could happen if a portion of the larvae start feeding on the nutrient rich B2 diet, as those fish theoretically would grow faster than larvae which only feed on the less nutrient dense artemia. This might be the best interpretation of the results, as the B2 commercial feed fed in conjunction with the DA had the highest individual growth.

As for the lab-formulated diet, it could provide a baseline to start adjusting the composition for a yellow perch specified larval diet.

## *Conclusion*

This study demonstrated that decapsulated artemia promoted growth better than larvae fed non-decapsulated artemia. A potential mechanism for these results is that the DA is better digested by yellow perch larvae. Pertaining to larval rearing and survival, this study suggested that the best feed option would be decapsulated artemia fed through 30 DPH, but this may have impacts on the weaning of larval yellow perch from live feed to a formulated diet. This experiment also suggested that a more improved feed specific to the larval yellow perch could be developed in order to replace commonly used commercial diets. This study demonstrated that the lab formulated diet showed potential in the possible development of a larval yellow perch feed and should be explored further.

| Diet | Particle Size (µm) | Moisture % | Protein % | Lipid % | Ash % |
|---|---|---|---|---|---|
| B2 (commercial) | 360-650 | 5.6 | 55.5 | 18.1 | 12.9 |
| L1 (lab) | 400-600 | 11.0 | 49.0 | 14.0 | 7.6 |
| Decapsulated A | ~ | 84.7 | 8.9 | 2.43 | 0.8 |

Lab test diet contain (g/100 g): menhaden fishmeal (25), casein (24), gelatin (6), squid meal (5), wheat gluten (4), wheat starch (15), oil mixture (soy oil: fish oil: lard = 1:1:1) (6), lecithin (6), cholesterol (0.12), sodium alginate (2), carboxyl methylcellulose (1.83), ascorbyl palmitate (0.05), choline chloride (0.05), glucosamine (0.15), calcium phosphate dibasic (1), haematococcus algae powder (2), vitamin premix (1.5), mineral premix (0.15), and betaine hydrochloride (0.15).

*Table 2: Nutritional composition of B2 commercial diet, lab made diet, and decapsulated artemia live feed diet. Methods for retrieval (AOAC 1990).*

Table 2: Nutritional composition of diets used in chapter 1.

# Chapter 2

## Experiment 2

## Effect of a low salinity 5-ppt environment on the survival and growth performance of yellow perch (*Perca flavescens*), at early life stage

### *Objectives and Hypothesis*

The first objective of this study was to determine the effect of a 5-ppt salinity on the development of yellow perch embryos. This would be tested as a form of pathogen control when compared to the commonly used aquaculture pesticide Parasite-S brand (37%) formalin. The second objective was to determine if the low salinity environment during incubation and larval rearing after hatching has any effects on the growth and survival of yellow perch up to 30 days post hatch. The hypothesis of this study is: A 5-ppt low salinity environment during incubation will conserve the endogenous nutrition of embryonic yellow perch when compared to the commonly used pesticide formalin. Consequently, larval perch raised in a low salinity 5-ppt environment will have higher growth and better survival than those raised primarily in freshwater.

### *Methods and Materials*

#### *Fish source*

All animal studies were performed in compliance with animal care protocols (15-16-#46) approved by the University of Wisconsin-Milwaukee, Institutional Animal Care and Use Committee. Yellow Perch (*P. flavescens*) were obtained from broodstocks housed at the

University of Wisconsin – School of Freshwater Sciences facility (Milwaukee, WI). The original broodstock was derived from the Sassafras River (Chesapeake Bay, MD) and the utilized broodstock were the UWM-SFS January breeding stock.

Spawning of the fish was performed in accordance with US Patent 7,836,852 B2 (Methods for manipulating yellow perch spawning cycles) at 13 degrees C. Six males were chosen having their milt combined and mixed with a 330 micromolar sucrose milt extender (Cayman Chemical company). Next, A female perch was chosen and stripped of eggs into a tared aluminum bowl. The ribbons were weighed and then mixed with 50 mL of the milt extender solution for fertilization. This was then combined and mixed with a ~100 mL mixture of bentonite clay and water 6.4g/L along with the egg ribbon. The fertilized eggs were then washed with freshwater from the broodstock tank and placed into a two-gallon Ziploc brand bag filled with freshwater from the same broodstock tank. The bag containing the fertilized eggs was then kept in an adjacent tank filled with water held at a temperature of 13 degrees C. This process was repeated until nine females were stripped of eggs and the eggs fertilized. All male and female fish chosen were weighed and euthanized with an overdose (1g/L) of tricane methane-sulfonate (MS-222 Sigma-Aldrich Corp.) and kept at -80C for further analysis.

***Preparation of 5 ppt salinity water***

Water preparation: Aquarium salt (Instant Ocean, Blacksburg, VA) was weighed and administered daily to two reservoirs (680 L water each) yielding a 10 part per thousand (ppt) concentration saline water. Each reservoir supplied 10 ppt saltwater to each of three tanks with a flow rate of 0.15 L/minute (LPM) for a sustained delivery period of twenty-four hours. The saltwater reservoirs were purged and refilled once daily.

Dechlorinated freshwater was also introduced to each of these tanks at a rate of 0.15 LPM to yield 5 ppt saltwater. Freshwater treatments were operated with the freshwater flow at 0.3 LPM. Light aeration ensured even mixing of the freshwater and saltwater, and a Hach brand salinity refractometer was used to record daily salinity levels. Each of the 12 tanks was filled with water to a volume of 25 L and the turnover rate was ~80 minutes for each tank. The design of the experimental system can be seen as figure 2. on page 38.

Incubation treatment: Three treatments were conducted to evaluate embryo development under different water conditions: 1) freshwater treated with formalin; 2) 5 ppt water with no formalin; 3) 5 ppt water treated with formalin.

***Embryo incubation trial***

Eight fertilized egg ribbons were used in this study. Each of the fertilized egg ribbons were divided into four equal portions of 20 cm. Each portion from the same egg ribbon was assigned to one of the incubation treatments. The egg ribbons were pinned to a plastic porous substrate and sampled daily to be photographed for recording the embryonic development.

The temperature regime during the embryo incubation follows the method described by US Patent 7,836,852 B2 (Methods for manipulating fish spawning cycles). The actual temperatures are graphed and described in the appendix as Appendix 2 on page 63. In order to adjust the temperature to a required level, inputs from the saltwater and freshwater sources had to combine and meet a required temperature during the experiment. The saltwater reservoir was kept at 20˚C and the freshwater temperature

was adjusted to maintain a required temperature of each tank. All tanks were recorded for water temperature daily.

Sampling collection: Egg samples for all treatments were collected at 0 days post spawn (DPS), 1 DPS, 3 DPS, 4 DPS, 7 DPS, and prior to hatching for investigation of whole egg, chorion, yolk sac, and oil drop size. These samples were frozen at -80˚C.

Hatching: Hatching was performed with a rod agitating the water above the egg ribbon which allowed the majority of embryos to escape. A small sample of larvae from each egg ribbon for all treatments was collected and photographed for developmental analysis.

The saltwater eggs with no pathogen control were hatched at 8 days post spawn (DPS). The freshwater + formalin and saltwater + formalin treatments were hatched at 10 DPS.

***Larval Feeding Trial***

A two-factorial design (two incubation systems * two culture systems) was applied to test the effect of incubation and larval culture systems as well as the interactive effects of the two system conditions on the performance of larval fish. There were four treatments including: freshwater + formalin incubated larval fish in either a freshwater or a 5-ppt saltwater rearing condition, and 5-ppt saltwater incubated larval fish in either a freshwater or 5-ppt saltwater rearing condition.

Larval perch from the freshwater + formalin treatment and larval perch from the Saltwater treatment were stocked at 7 DPH and 5 DPH respectively. Each treatment of embryonic treated larvae was placed in 3 replicates of either 5 ppt saltwater or freshwater. Each of the 12 tanks were randomized with six-freshwater treatments and

six 5-ppt saltwater treatments. The larvae were stocked 50 at a time by five people rotating tank by tank until each tank had 500 larvae. This ensured randomization of fish selection. All counting was performed with a tally counter.

Any dead fish were replaced during the 48 hours after stocking.

***Fish Maintenance***

Marubeni Nissin Otohime™ (Tokyo, Japan) brand B1 (200-360 um) larval feed was utilized as the dry feed in this trial, and INVE™ brand decapsulated artemia sourced from the Great Salt Lake, Utah U.S. was used as the live feed. In order to ensure the same feeding regimen for all treatments, timed revolving feeders were implemented to control the feed amount, feeding time and duration of the dry feed. Twelve 85 gallon per hour (GPH) submersible water pumps with uniform hose length were also used to transport the Live brine shrimp to each of the twelve tanks and were controlled by timers as well. The dry feed was programmed to drop 5 minutes before the artemia in order to facilitate behavioral feeding conditioning toward dry feed. The feeding regimen is described as Appendix 1B on page 64.

Live Feed Preparation: A preliminary study was conducted in order to establish that a combination of decapsulated artemia and the Japanese commercial diet Otohime B1 has a correlation with better growth in larval yellow perch when compared to non-decapsulated artemia. Decapsulation and an eighteen-hour initial incubation was determined to be the preferred method of preparation for the artemia nauplii live perch feed. Refer to the previous study for decapsulated artemia preparation method. The salinity was decided to be lowered from the concentration in the preliminary study (25

ppt) to 18 ppt to keep the salinity in the experimental tanks from raising during the multiple automatic feedings.

Live Feed Implementation: All six jars of artemia were collected after 18 hours of incubation and they were pooled together into a 5-gallon plastic bucket and transported into the room where the trial was being conducted. Any artemia left from the previous day was discarded. The artemia was then aerated and distributed among six jars. Each jar had two identical 85 GPH submersible water pumps each plugged into the same programmable timer set for a determined amount of live artemia distribution at a given time. At 14:00, new artemia would start incubating for the next day's feed. Two meters of 3/8" clear vinyl PVC tubing dispersed live artemia nauplii from the pump to the tanks with the given feed schedule. The feed distribution chart for this study can be viewed as Appendix 1B on page 64.

Water quality was monitored once a week from three random tanks in the system using an API® water quality testing kit and a YSI multi-meter. Water quality testing and highest measured levels (mg/L) include: TAN < 0.02, nitrite ($NO_2$) < 0.25, DO > 8.4, and pH = (8.3 – 8.7). No water quality tests fell outside of acceptable ranges.

The lighting in the research room was kept at 14-hours on and 10-hours off with lights closest to the research tanks blocked with black plastic sheeting. From 9:00 to 10:00, the preset light condition was set to simulate dawn, then full lighting was implemented from 10:00 to 22:00, then lights that simulated dusk lasted from 22:00 – 23:00, with the lights being turned off at 23:00 and darkness lasting until 9:00 the next day. The timing for the lighting ensured that all duties required by this experiment could be met given the researcher's schedule. All tanks were cleaned with a squeegee once daily at 11:00

and siphoned, followed by replacement and cleaning of the central pipe outflow screens.

### *Sample Collection and Analysis*

Live Feed: The artemia was dispersed between an 18-hour post incubation age and a 42-hour post incubation age from initial feeding after artemia replacement at 16:00 to the final feeding after replacement at 14:00 the following day. In order to analyze the difference between live feed dispersal times and age-related size and composition differences, three replicates of live decapsulated artemia were photographed at 18, 24, 36, and 48 hours post decapsulation. The photographs were used to establish a hatching rate for the artemia at different stages of incubation. Live artemia were then measured at their widest point of frame and also for total area for each age group with IMAGEJ™ photography analysis software. These measurements could then be compared against the gape of the larval perch and used to determine the percentage of available artemia for consumption at 5 and 7 DPH for freshwater and saltwater raised larval perch respectively. Representative samples of each age group of artemia were then frozen in liquid nitrogen and preserved for compositional analysis.

Broodstock: The eight females and six males chosen as the broodstock parents in this study were initially weighed and measured before being placed in -80˚C. The viscera were then removed to obtain a carcass weight and the viscera weighed to obtain a visceral weight.

Embryo: Embryo samples were collected on the 0 DPS, immediately after fertilization of embryos, 1 DPS, 3 DPS, 4 DPS, 6 DPS, and prior to hatching, cutting a small segment

of the developing egg ribbon daily with a scissors and taking photographs on an Olympus® SZX-7 Stereoscopic photographic microscope. All samples were stored at -80˚C for analysis.

The photos were later analyzed using the photograph analysis software ImageJ© for egg development by measuring the egg size, chorion size, yolk sac, and the oil drop daily. Sample photos of this process can be viewed as figure 3 & 4. on pages 39 & 40. The egg component sizes were then transferred to a spreadsheet for further statistical analysis.

Larvae: At the end of the trial all fish were collected by net and transferred into a 2-liter pitcher containing 500 mL of water. They were then poured through a tared mesh basket, which was dried with a paper towel. Larval fish were euthanized by overdose of MS-222, then collected, to be weighed for a total weight per tank. The fish were then placed on a light box and photographed with a scale. The pictures were used to measure the body size and length of each fish individually using IMAGEJ™. An image of this process can be viewed as figure 5. on page 41. The fish were stored at -80˚C until use.

Proximate Composition: The nutrient composition of the broodstock, embryo, and larvae were determined following the methods by (AOAC, 1990). Moisture was assessed using freeze drying to less than 10%, followed by thermal drying to a constant weight in an oven at 105˚C for 24 h. The ash content was determined by combustion in a high heat oven at 600˚C for 2 h. The crude protein was determined by the combustion method (ECS 4010 Nitrogen/protein analyzer, Costech Analytical Technologies, Inc) with the nitrogen content multiplied by 6.25 for a total protein content. The crude lipid content

was determined utilizing a Soxhlet extraction method (Soxtec 8000 extraction unit, Foss) in which dried samples were boiled petroleum ether heated to 55˚C.

***Data Calculation and Statistics***

Embryo: Final embryo component size was measured and recorded as well as a depletion or growth amount calculated for each treatment between 0 DPS and 7 DPS. The depletion was calculated for the oil drop and yolk sac by the following formula: where $_c$ = specific embryo component area $mm^2$ (oil drop, yolk sac, etc…)

$$(Initial_c – Final_c) / Initial_c)*100$$

The growth of the chorion and total egg was calculated using the following formula:

$$(Final_c - Initial_c) / Initial_c)*100$$

Statistical analysis was performed on eight ribbons which were sectioned to equal lengths and placed into each of the three treatments. Because each ribbon section was partially dependent on the original ribbon for performance in the trial, a matched pairwise Student's T-test analysis of means was performed three times between the treatments. Data are presented as mean ± SE, n=8.

Larval: A Fulton condition factor and survival % were calculated using the following equations:

$$\text{Fulton condition factor (CF} = 100* \text{ body weight (g)/body length (cm)}^3$$

Survival per tank was determined using equation:

$$\text{Survival} = (\#\text{ initial fish}/\ \#\text{ final fish}) *100$$

Statistical analysis of experiment 2 analyzed larval growth and survival using a two-way ANOVA. Data are presented as mean ± SE, (n=3). All assumption violations were checked before statistical analysis. Comparisons using a Fisher LSD test were considered significant when $p<0.05$. Statistical analysis was performed using SPSS 26.0 for windows (IBM).

## *Results*

This experiment had two facets to its design, embryo development as well as larval development. Therefore, two separate sets of data were constructed to represent the results.

### *Embryo components*

Embryo components associated with endogenous nutrition (oil drop and yolk sac) were smaller in treatments exposed to formalin ($p > 0.05$). They were smaller than those in the 5-ppt treatment without formalin ($p<0.05$). The embryo chorion and total egg size on the other hand were significantly larger in freshwater, Table 3.

The reduction in oil drop or yolk size from initial to 7-DPS was significantly higher in embryos exposed to formalin compared to embryos from non-formalin exposure treatment, Table 4. For both endogenous nutrition components, the yolk sac had a higher reduction in size as opposed to the oil drop.

| Treatment | Oil Drop Size ($mm^2$) | Yolk Sac Size ($mm^2$) | Chorion Size ($mm^2$) | Total Egg Size ($mm^2$) | Moisture (%) |
|---|---|---|---|---|---|
| **F + Form** | 0.208 ± 0.01 [b] | 0.582 ± 0.03 [b] | 4.89 ± 0.3 [a] | 9.02 ± 0.8 [a] | 98.5 ± 0.1[a] |
| **5-ppt** | 0.228 ± 0.01 [a] | 0.644 ± 0.02 [a] | 2.86 ± 0.2 [b] | 5.91 ± 0.3 [b] | 97.4 ± 0.2[b] |
| **5-ppt + Form** | 0.213 ± 0.01 [b] | 0.608 ± 0.02 [b] | 2.87 ± 0.1 [b] | 6.27 ± 0.2 [b] | 97.3 ± 0.4[b] |
| **Initial** | 0.380 ± 0.01 | 1.24 ± 0.03 | 2.07 ± 0.0 | 3.92 ± 0.1 | |

*Table 3: Components of embryos at 7-day post spawn. Similar letters indicate no significant difference in the statistical analysis, pairwise comparison Student's t-test, p > 0.05, n=8, mean embryo component sizes at 7 DPS are represented. Treatments: F + Form: Freshwater + Formalin, 5-ppt: Saltwater, 5-ppt + Form: Saltwater + Formalin, Initial: Initial average size.*

| Treatment | Oil Drop (%) | Yolk Sac (%) | Chorion (%) | Total Egg (%) |
|---|---|---|---|---|
| **Freshwater + Formalin** | - 45.2 ± 1.4 [b] | - 52.8 ± 2.0 [b] | 136.5 ± 9.7 [a] | 131.0 ± 9.7 [a] |
| **5-ppt Saltwater** | - 39.8 ± 1.6 [a] | - 47.8 ± 1.9 [a] | 38.6 ± 6.4 [b] | 51.3 ± 6.0 [b] |
| **5-ppt Saltwater + Formalin** | - 43.9 ± 1.2 [b] | - 50.8 ± 1.5 [b] | 39.0 ± 4.3 [b] | 60.6 ± 5.4 [b] |

*Table 4: Size percentage change from initial size to 7 days post spawn. Similar letters indicate no significant difference in the statistical analysis p > 0.05, pairwise comparison Student's t-test, n=8, mean % changes from initial size are represented. Treatments: F + Form: Freshwater + Formalin, 5-ppt: Saltwater, 5-ppt + Form: Saltwater + Formalin, Initial: Initial average size.*

### *Larval Results*

Salinity during embryonic development and the rearing environment significantly influenced the growth of larval fish ($p < 0.05$). However, there was also no interaction among embryos that were raised in alternating environments. The yellow perch incubated and reared in 5-ppt showed the highest amount of growth while those larvae incubated and reared in a freshwater environment displayed the lowest. Survival was significantly influenced by the rearing environment with the highest survival being seen in a 5-ppt environment, Table 5.

| Environment | | Length | Body weight | Survival |
|---|---|---|---|---|
| Incubation | Rearing | (cm) | (g) | (%) |
| | | **Individual treatment means** | | |
| 5-ppt | Freshwater | 1.32 | 33.4 | 5.13 |
| 5-ppt | 5-ppt | 1.46 | 39.9 | 18.00 |
| Freshwater | Freshwater | 1.13 | 27.3 | 4.00 |
| Freshwater | 5-ppt | 1.36 | 32.2 | 8.67 |
| Pooled SE | | 0.03 | 2.4 | 2.67 |
| | | **Means of main effect** | | |
| 5-ppt | | 1.39[a] | 36.7[a] | 11.57 |
| Freshwater | | 1.25[b] | 29.8[b] | 6.33 |
| | 5-ppt | 1.41[a] | 36.0[a] | 13.33[a] |
| | Freshwater | 1.23[b] | 30.4[b] | 4.57[b] |
| | | **ANOVA: P-values** | | |
| Incubation | | 0.01 | 0.02 | 0.14 |
| Rearing | | 0.002 | 0.045 | 0.025 |
| Environment*Rearing | | 0.28 | 0.73 | 0.23 |

*Table 5: The final average length, weight, and survival of larval perch from 5-DPH – 33-DPH (formalin treatments), and from 7-DPH – 35-DPH (5-ppt treatment). Similar letters show no significant difference in the statistical analysis, p > 0.05, two-way ANOVA, n=3, represented as treatment means ± SE.*

### *Final Area*

These results show that yellow perch embryos incubated in 5-ppt water had the highest measured body area when the resultant larvae were reared in a 5-ppt environment. Refer to table 6. There was also no interaction among embryos that were raised in alternating environments, but this shows there was a larger body area attributed to

when either incubated or reared in a 5-ppt environment. The yellow perch incubated and reared in a freshwater environment displayed the smallest body area.

| Environment | | Avg. Area ($cm^2$) |
|---|---|---|
| Incubation | Rearing | |
| | | **Individual treatment means** |
| 5-ppt | Freshwater | 0.245 |
| 5-ppt | 5-ppt | 0.311 |
| Freshwater | Freshwater | 0.166 |
| Freshwater | 5-ppt | 0.265 |
| Pooled SE | | 0.02 |
| | | **Means of main effect** |
| 5-ppt | | $0.278^{a}$ |
| Freshwater | | $0.216^{b}$ |
| | 5-ppt | $0.288^{a}$ |
| | Freshwater | $0.205^{b}$ |
| | | **ANOVA: P-values** |
| Incubation | | 0.02 |
| Rearing | | 0.005 |
| Environment*Rearing | | 0.58 |

*Table 6: The final average per fish area of larval perch from 5-DPH – 33-DPH (formalin treatments), and from 7-DPH – 35-DPH (5-ppt treatment). Similar letters show no significant difference in the statistical analysis, p > 0.05, two-way ANOVA, n=3, represented as treatment means ± SE. No letters indicate no statistical difference among groups, two-way ANOVA*

## *Discussion*

### *Embryo*

The results from the embryo measurements suggest that the oil drop and yolk sac diminishment in size is linked to whether formalin was used as a pathogen control

treatment. According to the data, treatments that were exposed to a formalin dosing regimen showed a higher size depletion than those eggs that were not exposed. This could indicate that eggs incubated with 5-ppt saltwater might maintain an endogenous nutrition supply better than those incubated under control of formalin, and thus have more longevity to be able to switch to an exogenous nutritional supply post hatch.

The measurements of the endogenous nutrition components also showed that the yolk sac size diminished to a greater extent than that of the oil drop among all treatments. This implies the embryos consumed the protein rich yolk sac more than the lipid rich oil drop at this stage. This information helps to provide baseline information for aquaculture nutritionists to develop feed for the broodstock or larval fish culture of yellow perch. Furthermore, if embryos could be analyzed for fatty acid and amino acid content, these proportions of lipid and protein consumption at the larval early life stage could prove to be valuable. The growth of the chorion and total egg showed a link between significant increases in the size in those eggs incubated in freshwater opposed to those that were incubated in saltwater. This could potentially be due to osmoregulatory properties of each treatment. Embryonic fish eggs contain suspended colloidal nutrients which provide for a higher solute concentration within the egg (Cotelli et al. 1988, Iwamatsu 2004). This may cause lysis of the egg and subsequently a larger total egg and chorion size. Providing a 5-ppt environment may act as an osmotic buffer creating a more stable equilibrium and less lysis. This becomes more evident when looking at the moisture content of each treatment. The eggs which were incubated in freshwater had a significantly higher moisture content than those that were incubated in saltwater. This implies that freshwater incubation leads to a higher water retention, and possibly a

dilution of the nutrients within the egg or greater osmotic pressure on the developing embryo.

***Larval***

The two-factorial design of this experiment took into account the method of incubation to see how this affects the larval growth through the first 33-35 DPH. The results indicate that the method of incubation has a significant effect on the yellow perch performance during rearing. Larvae that were incubated in 5-ppt had a significant increase in size. When reared in 5-ppt, the larval yellow perch had a significant increase in both growth and survival. The best growth and survival of all treatments were larval yellow perch which were incubated in 5-ppt and reared in 5-ppt. Unfortunately, during the course of the experiment, operator error caused a high amount of mortalities in one replication for the larvae incubated and reared in 5-ppt. If it had not been for this, it is an opinion that incubation would have been shown to be associated with higher survival as well.

One factor which may have contributed to the higher growth and survival in a 5-ppt saltwater condition is that the live decapsulated artemia nauplii survived much better in this environment as opposed to a freshwater one. It was observed that artemia left in 5-ppt saltwater samples could be seen surviving up to 24-hours after sampling. Because of this, larval perch in a 5-ppt environment exhibited a grazing behavior while those in a freshwater environment tended to display an erratic darting behavior. This could be due to artemia nauplii accumulating to high density in a 5-ppt environment, whereas the artemia were seen to die off within the freshwater environment over the course of one hour.

## *Conclusion*

This study has demonstrated that during incubation, yellow perch embryos that are not exposed to a formalin pathogenic dosing have a lower diminishment of endogenous nutrition components than those that had been exposed to formalin. This would suggest that a non-formalin pathogen control for yellow perch embryo incubation should be utilized, and one potential substitution is 5-ppt low saline water. It also demonstrated that embryos which develop in freshwater tend to have a higher water retention and are linked to a bigger chorion and total egg size. This should be investigated further to determine the effects.

Pertaining to larval rearing conditions, this study demonstrated that from hatching through the first 33 DPH & 35 DPH, 5-ppt low salinity water provides better survival and growth when compared to freshwater. Considering that yellow perch larvae are associated with a high mortality in the first 30-DPH, this process for rearing yellow perch could be beneficial. Though it is still necessary to understand how rearing yellow perch in a low salinity environment has impacts at later life stages. Typically, fingerling yellow perch are attained from larvae raised in a freshwater system, so a 5-ppt low salinity environment should be further investigated for its use in early life stage yellow perch aquaculture. This should also be investigated in a RAS system and aquaponics to determine its feasibility in practical aquaculture applications.

Chapter 2 Figures

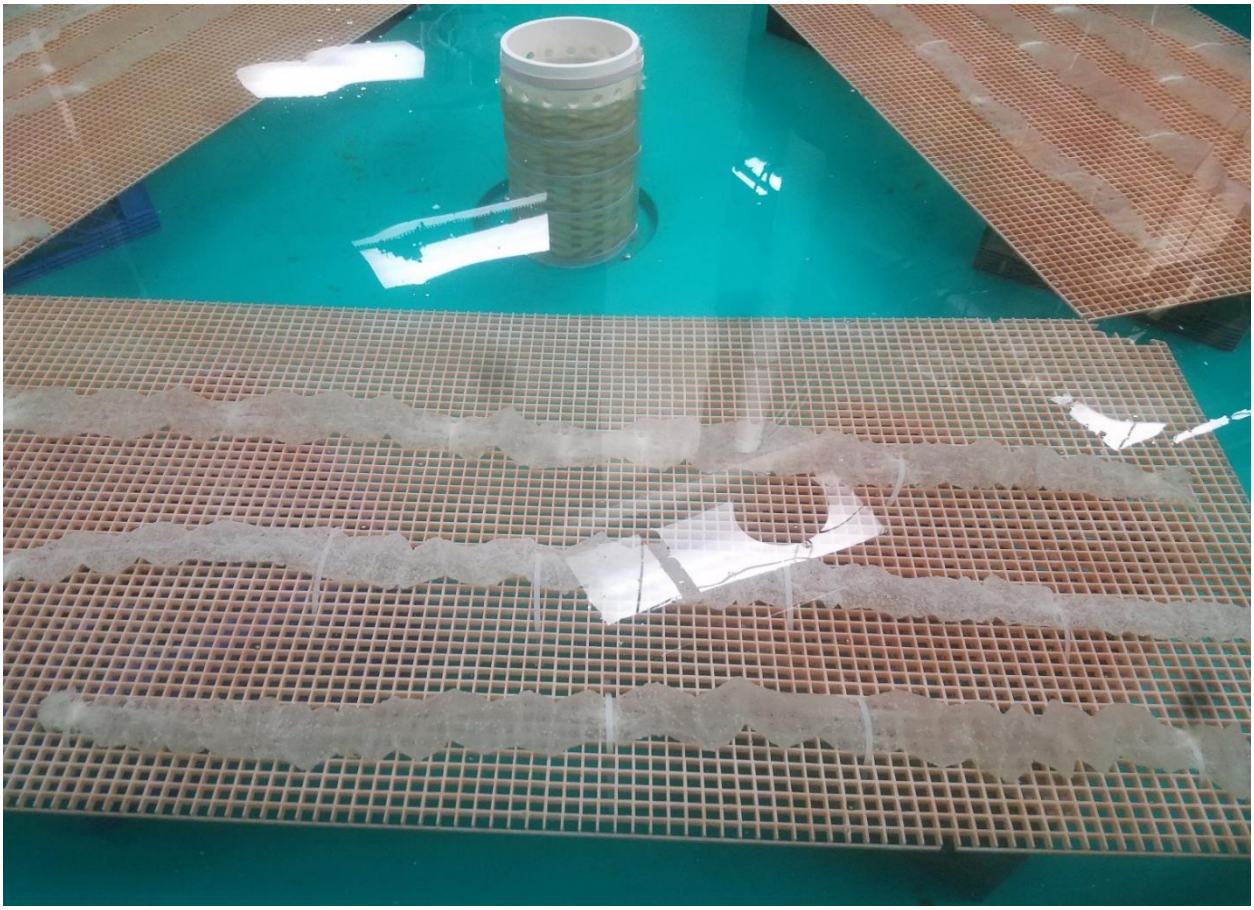


Figure 1: Example of incubating yellow perch egg ribbons.

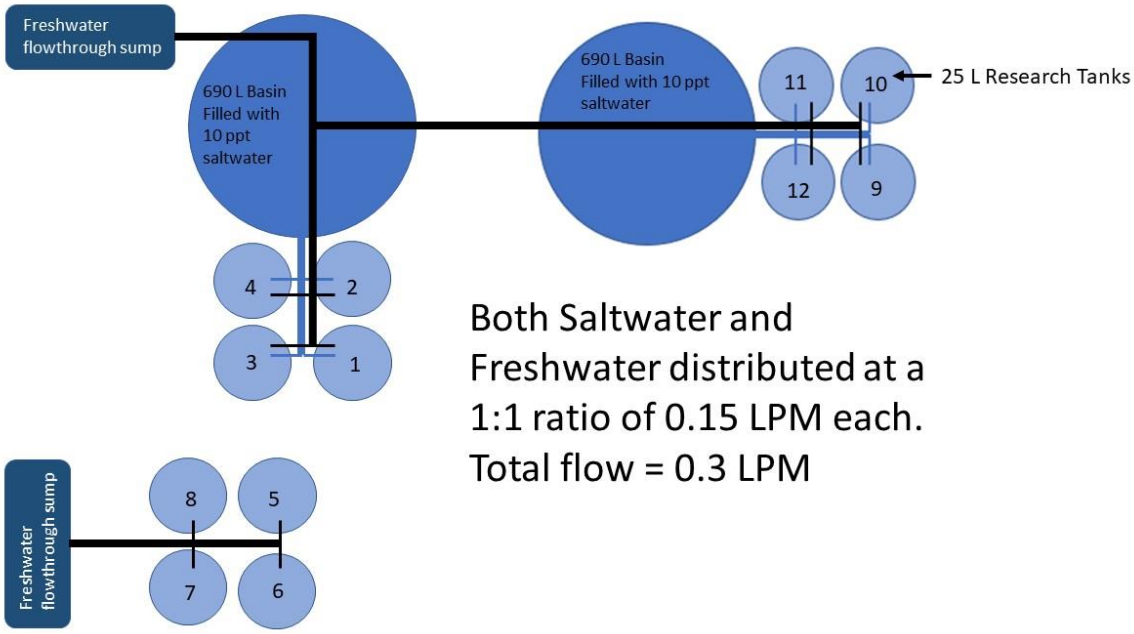
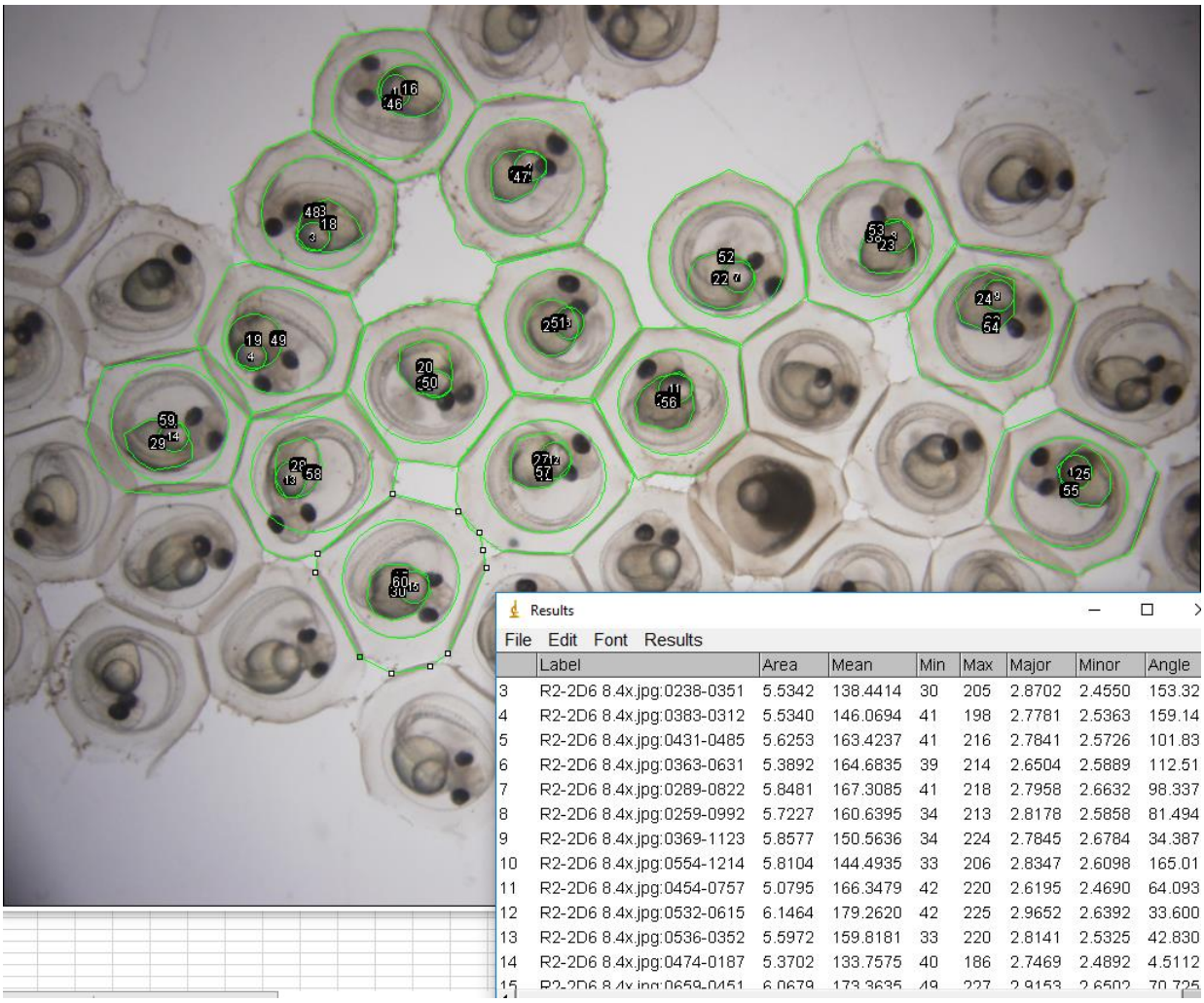



Figure 2: Diagram of the flow through saltwater system used for experiment 2



Results

File Edit Font Results

| | Label | Area | Mean | Min | Max | Major | Minor | Angle |
|---|---|---|---|---|---|---|---|---|
| 3 | R2-2D6 8.4x.jpg:0238-0351 | 5.5342 | 138.4414 | 30 | 205 | 2.8702 | 2.4550 | 153.32 |
| 4 | R2-2D6 8.4x.jpg:0383-0312 | 5.5340 | 146.0694 | 41 | 198 | 2.7781 | 2.5363 | 159.14 |
| 5 | R2-2D6 8.4x.jpg:0431-0485 | 5.6253 | 163.4237 | 41 | 216 | 2.7841 | 2.5726 | 101.83 |
| 6 | R2-2D6 8.4x.jpg:0363-0631 | 5.3892 | 164.6835 | 39 | 214 | 2.6504 | 2.5889 | 112.51 |
| 7 | R2-2D6 8.4x.jpg:0289-0822 | 5.8481 | 167.3085 | 41 | 218 | 2.7958 | 2.6632 | 98.337 |
| 8 | R2-2D6 8.4x.jpg:0259-0992 | 5.7227 | 160.6395 | 34 | 213 | 2.8178 | 2.5858 | 81.494 |
| 9 | R2-2D6 8.4x.jpg:0369-1123 | 5.8577 | 150.5636 | 34 | 224 | 2.7845 | 2.6784 | 34.387 |
| 10 | R2-2D6 8.4x.jpg:0554-1214 | 5.8104 | 144.4935 | 33 | 206 | 2.8347 | 2.6098 | 165.01 |
| 11 | R2-2D6 8.4x.jpg:0454-0757 | 5.0795 | 166.3479 | 42 | 220 | 2.6195 | 2.4690 | 64.093 |
| 12 | R2-2D6 8.4x.jpg:0532-0615 | 6.1464 | 179.2620 | 42 | 225 | 2.9652 | 2.6392 | 33.600 |
| 13 | R2-2D6 8.4x.jpg:0536-0352 | 5.5972 | 159.8181 | 33 | 220 | 2.8141 | 2.5325 | 42.830 |
| 14 | R2-2D6 8.4x.jpg:0474-0187 | 5.3702 | 133.7575 | 40 | 186 | 2.7469 | 2.4892 | 4.5112 |
| 15 | R2-2D6 8.4x.jpg:0659-0451 | 6.0679 | 173.3635 | 49 | 227 | 2.9153 | 2.6502 | 70.725 |

Figure 3: Example of 6 DPS (Saltwater treatment) ribbon with all components (oil drop, chorion, and total egg) measured using Image J software. Measurements are included in mm² for easy transfer to a spreadsheet. Figures 3. and 4. are of equal scale.

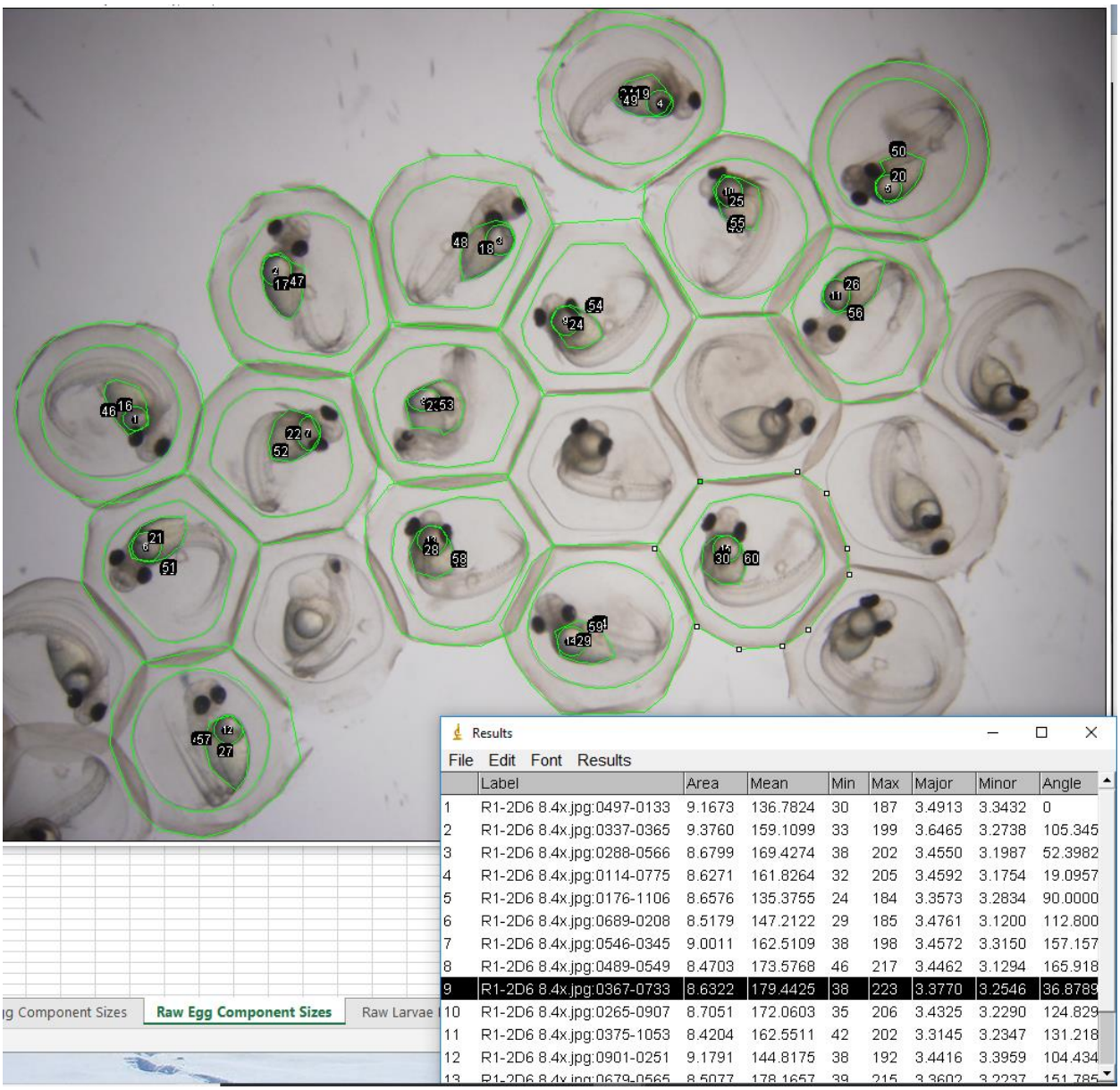



Figure 4: Example of 6 DPS (freshwater + formalin treatment) ribbon with all components (oil drop, chorion, and total egg) measured using Image J software. Measurements are included in mm² for easy transfer to a spreadsheet. The freshwater and formalin treatments make for a larger egg. Figures 3. and 4. are of equal scale.

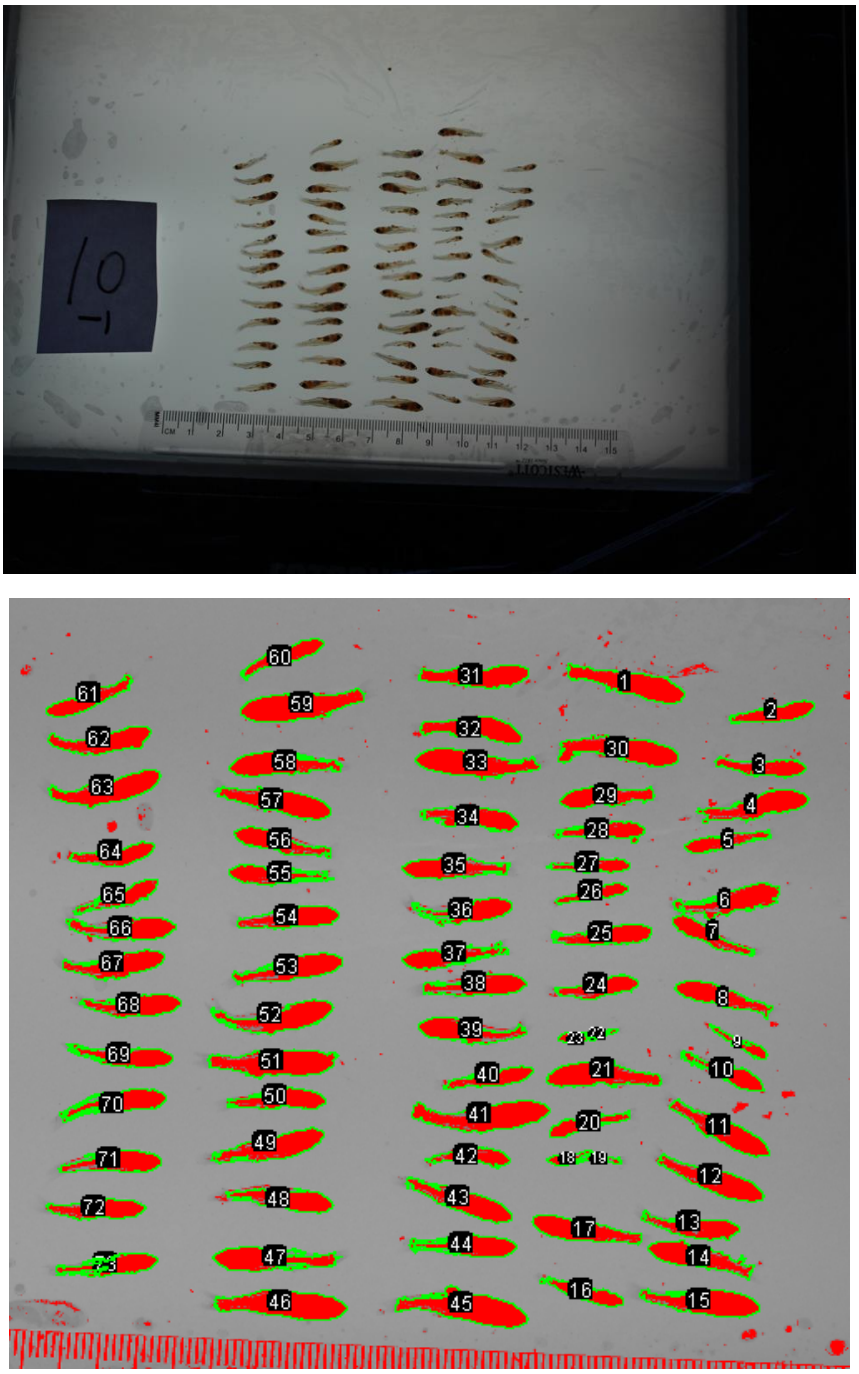


Figure 5: Example of final larval measurement technique using ImageJ photo analysis software

# Chapter 3

## Experiment 3

## Effect of water salinity and dietary protein level on yellow perch cultured in a recirculating aquaculture system

### *Objective and Hypothesis*

The objective of this study was to determine if a low salinity 5-ppt environment, when introduced at a later stage of life for yellow perch, could still increase growth and feed efficiency. This study assessed the yellow perch at the fingerling life stage and was conducted in a recirculating aquaculture system design commonly used by aquaculturists. The study also assessed the growth and efficiency of fingerling perch fed a high fish meal diet and a high carbohydrate diet.

The hypothesis for this study is that the low salinity 5-ppt saltwater environment would enhance growth, as it was observed in the larval feeding study. An implementation of a high carbohydrate diet (25%) would impact the growth of yellow perch because a carnivorous fish may have a lower capacity of utilizing carbohydrate.

### *Methods and Materials*

#### *Fish source and breeding*

All animal studies were performed in compliance with animal care protocols (18-19-#34) approved by the University of Wisconsin-Milwaukee, Institutional Animal Care and Use Committee. Yellow Perch (*P. flavescens*) were obtained from broodstocks housed at the University of Wisconsin – School of Freshwater Sciences facility (Milwaukee, WI). The

original broodstock was derived from the Choptank River (Chesapeake Bay, MD) and the utilized broodstock were the UWM-SFS March breeding stock. The procedure for spawning followed the same protocol as experiment 1: Effect of different feeds on the growth and survival of yellow perch from 16 to 30 days post hatching.

The UWM - SFS broodstock facility reared these yellow perch hatchlings to fingerling size and an age of 192-DPH or roughly 6 months before the feeding trial.

***Experimental treatments***

A two-factorial design (two rearing systems * two feeds) was applied to investigate the effect of the two rearing conditions, the two test diets, and the interaction effect between the rearing conditions and the two different types of feed. There were four treatments including: 1) Fingerlings raised in freshwater with a high protein diet 2) Fingerlings raised in freshwater with a high carbohydrate diet 3) Fingerlings raised in 5-ppt water with a high protein diet 4) Fingerlings raised in a 5-ppt water with a high carbohydrate diet.

***RAS salinity, source, implementation and recording***

Instant Ocean brand aquarium salt (Blacksburg, VA) was used to prepare the salinity for one RAS system. To maintain the salinity of 5-ppt for the 1400 L capacity of the RAS, 7 kg of salt was first diluted down with 15 L of freshwater in a 5-gallon bucket, mixed, and poured into the RAS sump at a rate of 2 L/hour throughout a period of 8-hours. After the salt was added into the pump, a HACH brand salinity refractometer calibrated to 0-ppt for de-ionized water was used to monitor the salinity. Throughout the conditioning of the biofilter, both freshwater and 5-ppt saltwater RAS systems were monitored for efficiency

of nitrification with no apparent difference in performance. The description of the design of recirculating aquaculture is given as Appendix 2C on page 66. A description of the biofilter maturation process is given as Appendix 3C on page 67.

***Fish Maintenance***

Roughly 400 fingerling yellow perch 192-DPH were transported from the UWM – SFS broodstock facility to the aquaculture research facility housing the dual RAS systems. Three hundred uniformly sized fish were selected and then placed into a 4-foot diameter tank attached to the RAS circulation. Fifteen fish were stocked into each of the culture tanks to be conditioned for 8 day before the feeding trial. During conditioning, the fish were fed 7.5 g/day of a 1:1 ratio of Ziegler commercial perch feed with a particle size of 2 mm, and the high protein reference diet used in the study with a particle size of 1.5 mm. The tanks were held at a water flow of 3 L/minute and kept at a water temperature of 23 - 25˚C. At the end of the conditioning period, the fish were withdrawn from feeding. All fish were removed from the experimental tanks and pooled in the 4-foot diameter tank. Twelve yellow perch were weighed and stocked into each tank. Each diet was randomly assigned to three tanks within a system. The initial body weight for each of the 12 fish per treatment was 41.9 g ± 0.6 g, n=3. Initial weights of the stocked fingerlings can be viewed as table 9. on page 55.

Diet preparation and distribution: Two diets were prepared at UWM – SFS in this experiment. Both diets were produced using a cold extrusion method based on the formulation presented in table 10. on page 56. The non-carbohydrate diet was the control diet and contained 545 g/kg of protein, the diet containing carbohydrate, the test diet, had 383 g/kg of protein. The control diet had a lipid content of 50 g/kg while the

test diet had 70 g/kg. All feed ingredients were pulverized to <400 µm, then weighed and mixed using a Hobart stand mixer (K5-SS Hobart Corporation, Troy Ohio) yielding a homogeneous mixture. The dry mixture was first combined with 250 g/kg hot water (80˚C), then with the oils to form a fully homogenized dough. The dough was then extruded through a Kitchen Aid stand mixer meat grinder attachment. The extruded ingredients were then gently broken up by hand and placed in aluminum foil trays. The trays were then placed in an oven set to 85˚C for 20 min to incite gelatinization and binding of the carbohydrate. The diets were then dried at 21˚C for 24-hours, crumbled and sieved to the desired size (1.5-3 mm in length). This would serve as the feed used in the feeding trial. All diets were stored at 4˚C and sealed in zipper bags until use.

Fish were weighed initially and every three weeks until final sampling on week 8 in order to provide the proper feeding rate and food size. From weeks 1-3, the feed diameter size was 1.5 mm and from weeks 3-8, the feed size was changed to 3mm.

Initially, each tank was fed 1.5% body weight daily, with the feed increased by 0.5% per week to compensate for fish growth. After the third week, once all fish in each system were weighed for growth rate, the feed was pre-weighed based on a percentage of each tank’s biomass.

In order to distribute an accurate amount of feed, a daily feed amount was pre-weighed using a fisher precision balance accurate to 0.01 g and placed into a 50 mL plastic centrifuge tube labelled for a designated tank. Timed-rotating feeders (TRF’s) were designed for the RAS to drop a designated amount of feed into each tank and were set to distribute feed for 5 seconds each feeding at 9:00, 11:30, 14:00, and 16:30 respectively with a cleanup rotation distributing any leftover feeds at 17:00. A 5 second

feeding meant the feeder ring would perform one full rotation on the feeder. Each of the 12 tanks had a TRF attached above the water and was cleaned and loaded every day at 8:30. The feeders were loaded by distributing each tank's feed allotment evenly around the feed ring once at 8:30 every day.

Periodic weighing of fish and cleaning of system: Fish were weighed initially, at 21 days, at 42 days, and for final sampling at 56 days. Weighing of the fish was completed one 6-tank RAS system at a time. All fish from each tank were caught with a net and put into a specific tank designated 32-Gallon Rubbermaid™ bucket. The RAS water was then diverted at a flow rate of 1-LPM to each of the 6 32-Gallon Rubbermaid™ buckets containing 12 fish each. The weighing was completed using a perforated bucket fit inside a solid bucket holding 10-liters of water. Fish were captured out of the 32-gallon buckets and put into the perforated bucket. The perforated bucket was then lifted out of the solid bucket holding water, with excess water being wiped off with paper towel, and the fish dumped into a tared bucket on a Fisher™ brand precision high capacity balance accurate to 0.1g. The weight of each tank's fish was then recorded and the fish returned to the 32-gallon bucket.

During the weighing, the RAS system was fully drained with the radial desolidifier solids purged. The biofilter was drained to half of its capacity, but still left to aerate the K1 MBBR (moving bed bioreactor) media. At this point all of the tanks were cleaned using a hose and any debris washed out of the system. The PVC outflow screens were also sprayed down and replaced. After cleaning the system, the water was monitored to match the prior temperature and the RAS system was refilled until full recirculation was achieved. Both RAS systems had their fish weighed and systems cleaned in this

fashion. After both RAS systems were refilled, the fish were removed from the 32-gallon buckets and returned to their respective research tanks.

To establish a 5-ppt salinity gradient for the treatment tanks after weighing, 6 kg of salt was pre-weighed in a 5-gallon bucket with water from the RAS dissolved in it. The salt water was slowly redistributed within the RAS over 8 hours, and supplemental salt ~ 1 kg was slowly added after to re-establish a 5-ppt gradient.

Temperature and Water quality monitoring: Temperature was maintained between 23 – 25˚C by utilizing 4 submersible 200-watt aquarium water heaters in the RAS sump (API brand). Water quality was monitored daily using an API® Master Water Quality Test Kit and a YSI multi-meter. Tests and highest recorded measurements (mg/L) include TAN < 0.02, NO2 < 0.5, nitrate (NO3) < 140, total dissolved solids (TDS) < 360, DO > 8.2, and pH = 8.3 - 8.6. No water quality parameters recorded fell outside of acceptable ranges.

***Sample collection***

At the end of the 56-day feeding trial, the fish were not fed. All fish from each tank were collected, weighed, and recorded for fish number. They were then returned to their respective tanks. Initially 3 fish were randomly selected, measured for length and weight, then euthanized with an overdose of MS-222 and stored at -80˚C for nutritional analysis. Next, four fish from each tank were used for mucous collection. Four fish were collected and briefly dried on a paper towel, and placed into a tared zipper bag for 2 minutes. The fish were then scraped both sides slightly for mucous collection. Mucous samples were weighed and stored at -80˚C. The fish were weighed and killed by

severing the spinal column cord. Fish were stored in zipper bags and placed into a -20˚C freezer.

After 42 hours from the last feeding, 3 fish were anesthetized with a 200-ppm dose of MS-222. They had their blood drawn by a 22-gauge 1mL syringe. Whole blood glucose levels were recorded using a CVS® Advanced 1 Glucose meter. After blood collection the fish were euthanized with an overdose of MS-222 and dissected for liver, viscera fat and carcass weight. The whole viscera were removed and weighed as well as the liver and visceral lipid. The whole gastrointestinal tract and gill also collected. All samples collected were then frozen in liquid nitrogen and subsequently stored in a -80˚C freezer until use for analysis.

***Final Calculations***

A final percentage weight gain, protein efficiency ratio, hepatosomatic index, visceral fat index, carcass to body ratio, Fulton's condition factor, and feed conversion ratio had been calculated for this experiment.

A percent weight gain was calculated as:

$$(w_t - w_i)/ w_i * 100$$

A protein efficiency ratio (PER) was calculated as:

(Fish weight gain, g)/(protein fed, g)

The hepatosomatic index (HSI) was calculated as:

100 * (liver weight, g)/(body weight, g)

The visceral fat index (VFI) was calculated as:

$$100 * (\text{visceral fat weight, g})/(\text{body weight, g})$$

The ratio of carcass to body is given as:

$$100 * (\text{carcass weight})/(\text{total body weight, g})$$

A Fulton condition factor (CF) is given as:

$$\text{Condition factor} = 100* \text{ body weight (g)}/\text{body length (cm)}^3$$

A feed conversion ratio (FCR) was also calculated for all tanks using the equation:

$$FCR = [(TF_g/(\text{total weight gain, g})]$$

In this equation $TF_g$ is the total feed given in grams over the course of the period.

No mortalities were observed during the feeding trial period

Statistical analysis of experiment 3 analyzed fingerling growth and survival using a two-way ANOVA. Data are presented as mean ± SE, n=3. All assumption violations were checked before statistical analysis. Comparisons were considered significant when $p<0.05$. Statistical analysis was performed using SPSS 26.0 for windows (IBM).

## ***Results***

The results for percentage weight gain showed no significant difference between the two RAS environments $p > 0.05$ Table 7. Yellow perch cultured in freshwater had a significantly lower feed conversion ratio (FCR) than those cultured in 5 ppt $p < 0.05$. Both diets in this study had no effect on the FCR, and there was no significant interaction between the diets or the environment pertaining to the FCR. The PER showed a significant difference among the two diets, with a lower PER in fish fed the

fishmeal diet than the fish fed the wheat flour-based diet. The CF for all treatments were all similar.

### *Relative growth, FCR, PER, and CF*

| Environment | Diet | Weight Gain (%) | FCR (Ratio) | PER (Ratio) | CF ($g/cm^3$) |
|---|---|---|---|---|---|
| | | **Individual treatment means** | | | |
| 5-ppt | Fishmeal | 113.2 | 1.30 | 1.569 | 1.29 |
| 5-ppt | Wheat Flour | 116.7 | 1.30 | 1.961 | 1.33 |
| Freshwater | Fishmeal | 126.4 | 1.17 | 1.410 | 1.26 |
| Freshwater | Wheat Flour | 118.7 | 1.25 | 2.093 | 1.35 |
| Pooled SE | | 4.0 | 0.04 | 0.05 | 0.03 |
| | | **Means of main effect** | | | |
| 5-ppt | | 122.5 | $1.30^{a}$ | $1.71^{b}$ | 1.31 |
| Freshwater | | 115 | $1.21^{b}$ | $1.83^{a}$ | 1.31 |
| | Fishmeal | 119.8 | 1.24 | $1.49^{b}$ | 1.28 |
| | Wheat Flour | 117.7 | 1.27 | $2.03^{a}$ | 1.34 |
| | | **ANOVA: P-values** | | | |
| Environment | | 0.09 | 0.048 | <0.025 | 0.94 |
| Diet | | 0.61 | 0.39 | <0.001 | 0.22 |
| Environment*Diet | | 0.19 | 0.28 | 0.082 | 0.62 |

*Table 7: Final weight gain percentage, feed conversion ratio, protein efficiency ratio, and condition factor of yellow perch fed 56 days under different conditions. Similar letters show no significant difference in the statistical analysis, $p > 0.05$,two-way ANOVA, no letters indicate no statistical difference among groups n=3, represented as treatment means ± SE. Initial body weight: 41.74g ± 0.13*

The HSI was significnatly higher in fingerlings fed the wheat flour diet as opposed to the fish meal diet Table 8. The visceral fat index and carcass to body weight percentage on the other hand showed no significant difference among all treatments.

### *Morphology parameters*

| Environment | Diet | HSI (%) | VFI (%) | Carcass Index (%) |
|---|---|---|---|---|
| | | **Individual treatment means** | | |
| 5-ppt | Fishmeal | 1.24 | 7.53 | 85.33 |
| 5-ppt | Wheat Flour | 1.67 | 6.88 | 85.42 |
| Freshwater | Fishmeal | 1.21 | 7.51 | 84.44 |
| Freshwater | Wheat Flour | 1.53 | 7.82 | 81.98 |
| Pooled SE | | 0.06 | 0.40 | 0.05 |
| | | **Means of main effect** | | |
| 5-ppt | | 1.45 | 7.20 | 85.38 |
| Freshwater | | 1.37 | 7.67 | 83.21 |
| | Fishmeal | $1.23^{b}$ | 7.52 | 84.89 |
| | Wheat Flour | $1.60^{a}$ | 7.35 | 83.70 |
| | | **ANOVA: P-values** | | |
| Environment | | 0.2 | 0.29 | 0.12 |
| Diet | | <0.001 | 0.69 | 0.37 |
| Environment*Diet | | 0.36 | 0.27 | 0.34 |

*Table 8: Final hepatosomatic index, viscerosomatic index, and carcass to body percentage for the 56-day feeding trial. Similar letters show no significant difference in the statistical analysis, p > 0.05, no letters indicate no statistical difference among groups, two-way ANOVA, n=3, represented as treatment means ± SE.*

## *Discussion*

The results of this study show that yellow perch which had been incubated and reared in freshwater to 192 DPH age have no significant advantage when reared in a 5-ppt low salinity saltwater RAS. The study shows that the freshwater RAS had better

performance with the FCR. Through the first 3 weeks of the trial, the yellow perch raised in the 5-ppt had better growth, but after a 3-week weighing, fish in the 5-ppt system showed a marked decrease in appetite and appeared somewhat lethargic compared to the freshwater counterpart. This could be due to the 5-ppt acting as a mild stressor compared to the freshwater system. This was further demonstrated in the time required to anesthetize the fish during the final sampling. It took more than 3 times the amount of time to anesthetize the freshwater fish as opposed to the saltwater fish. This could also be due to other factors as well.

Pertaining to the wheat flour diet and the higher protein fishmeal diet, no significant difference pertaining to FCR was observed in the high protein diet and freshwater treatment. The hepatosomatic index was also lowest in the high protein diet, showing that livers became enlarged on a high wheat flour carbohydrate diet. This could be due to the fact that in nature yellow perch consume virtually no carbohydrates, meaning that their digestive system is not specialized to digest these compounds leading to larger livers (Wilson 1994). The high carbohydrate diet had a significantly higher PER, indicating protein was used more efficiently for growth in these fish, however, yellow perch fed the high fishmeal diet used more protein from the feed as an energy source. Thus, the result of this study demonstrated the protein sparing effect by carbohydrates within this testing condition. These results could be due to the 8-week trial length, so a longer trial period would be required to confirm this finding.

Both of the RAS systems biofilters performed well at nitrification, something noteworthy is that in the 5-ppt system, the $NO_2$ never accumulated past 0.25 mg/L, where as in the freshwater system, $NO_2$ would accumulate to 0.5 mg/L and plateau. More investigation

into the cause of this should be considered. One other question is whether the higher salt concentration had any impact on the gills of the fingerling perch. As to be expected, the TDS measurements in the 5-ppt saltwater RAS remained at 5000 mg/L higher than that of the freshwater RAS due to the dissolved salt. This higher TDS may have had a negative effect on the gills. In future studies, it is an opinion that any study that investigates a 5-ppt environment on freshwater fingerling fish should perform a histology on the gills to see any impacts if any.

## *Conclusion*

This experiment demonstrated that fingerlings procured from a freshwater hatchery are not necessarily supplemented by a 5-ppt low salinity environment. In fact, the 5-ppt may negatively affect production of yellow perch cultured in a RAS. One theory on the initial accelerated growth followed by a decrease in performance after weighing could be that the method by which the fish were weighed have diminished the efficiency of the biofilter leading to a decline in water quality. There was a marked decrease in performance of the fingerlings in the 5-ppt environment before and immediately after weighing. This may indicate that fish from the 5-ppt RAS may not be as tolerant to handling as the freshwater counterpart, so this shouldn't go unignored. The study also showed that the wheat flour had no effect on the amount of visceral fat or the carcass to body ratio, but did enlarge the livers when compared to a high protein diet and was generally not impacted by the environment. If the study had lasted longer, some significant impacts may have been observed. This study does demonstrate that you can get a higher PER using carbohydrate as a substantial ingredient in the yellow perch diet, making it a good source for energy, but the right level of carbohydrate is required

to minimize liver damage. It is still unanswered how fingerlings incubated as embryos and raised in a 5-ppt saltwater system could have fared in this study. It would be necessary to run this study again using yellow perch that had been acclimated to a low salinity environment as well as those which had been not been acclimated, in order to see if the decrease in performance was due to the stress of not being acclimatized.

Chapter 3 Tables

| Treatment | Environment | Initial fish weight (12 fish) | Initial body weight |
|---|---|---|---|
| **Diet** | **Tank #** | **(g)** | **(g)** |
| 1 | Freshwater 2 | **503.6** | **42.0** |
| 1 | Freshwater 3 | **495.7** | **41.3** |
| 1 | Freshwater 6 | **501.4** | **41.8** |
| **Diet 1** | **Average** | **500.2** | **41.7** |
| 2 | Freshwater 1 | **496.2** | **41.4** |
| 2 | Freshwater 4 | **510.7** | **42.6** |
| 2 | Freshwater 5 | **501.7** | **41.8** |
| **Diet 2** | **Average** | **502.9** | **41.9** |
| **Freshwater Tanks** | **Average** | **502.6** | **41.9** |
| 1 | Saltwater 2 | **494.8** | **41.2** |
| 1 | Saltwater 4 | **498.1** | **41.5** |
| 1 | Saltwater 5 | **509.9** | **42.5** |
| **Diet 1** | **Average** | **500.9** | **41.7** |
| 2 | Saltwater 1 | **496.7** | **41.4** |
| 2 | Saltwater 3 | **505.3** | **42.1** |
| 2 | Saltwater 6 | **495.8** | **41.3** |
| **Diet 2** | **Average** | **499.3** | **41.6** |
| **Saltwater Tanks** | **Average** | **500.5** | **41.7** |

Table 10: ***Initial weights per tank for experiment 3:*** Effect of a low salinity 5-ppt environment on the survival and growth performance of fingerling yellow perch (*Perca flavescens*) in a recirculating aquaculture system

| Yellow perch diet | Diet 1 | Diet 2 |
|---|---|---|
| | (%) | (%) |
| | | |
| **Ingredients** | | |
| Menhadden meal (63%) | 58.0 | 35.0 |
| Wheat gluten (90% protein) | 6.0 | 6.0 |
| Corn protein concentrate (63%) | 20.0 | 17.2 |
| Wheat flour | | 25.0 |
| CaHPO4.2H2O | 1.0 | 1.0 |
| Mineral premix | 1.6 | 4.0 |
| Vitamin premix | 3.0 | 3.0 |
| Soy Lecithin | 1.0 | 1.0 |
| Menhaden oil | 3.0 | 5.0 |
| Corn oil | 2.0 | 2.0 |
| Choline chloride | 0.1 | 0.1 |
| Cr2O3 | 0.7 | 0.7 |
| CMC:sodium alginate=1:1 | 3.6 | |
| Total (g) | 100.0 | 100.0 |

Table 11: ***Ingredient composition for the two diets in experiment 3:*** Effect of a low salinity 5-ppt environment on the survival and growth performance of fingerling yellow perch (*Perca flavescens*) in a recirculating aquaculture system

Literature Citations


1. Abd El-Gawad, E. A., Shen, Z. G., & Wang, H. P. (2016). Efficacy of formalin, iodine and sodium chloride in improvement of egg hatching rate and fry survival prior to the onset of exogenous feeding in yellow perch. *Aquaculture research*, *47*(8), 2461-2469.

2. AOAC., (1990). Official Methods of Analysis of the Association of Chemists. Analysis of the Association of Chemists, Washington, DC., 223-225, 992-995.

3. AOAC. (2000). *Official Methods of Analysis.* 17th ed. Gaithersburg, Maryland, USA, AOAC International.

4. Bein, R., & Ribi, G. (1994). Effects of larval density and salinity on the development of perch larvae (Perca fluviatilis L.). *Aquatic Sciences*, *56*(2), 97-105.

5. Binkowski F.B (2010) Methods for manipulating yellow perch spawning cycles, US Patent 7,836,852 B2, UWM Research foundation.

6. Buesa, R. J. (2008). Histology without formalin?. *Annals of diagnostic pathology*, *12*(6), 387-396.

7. Christensen, E. A. F., & Grosell, M. (2018). Behavioural salinity preference of juvenile yellow perch Perca flavescens. *Journal of fish biology*, *92*(5), 1620-1626.

8. Cotelli, F., Andronico, F., Brivio, M., & Lamia, C. L. (1988). Structure and composition of the fish egg chorion (Carassius auratus). *Journal of ultrastructure and molecular structure research*, *99*(1), 70-78.

9. Craig, J. F. (2008). *Percid fishes: systematics, ecology and exploitation*. John Wiley & Sons.

10. Czesny, S. J., Graeb, B. D., & Dettmers, J. M. (2005). Ecological consequences of swim bladder noninflation for larval yellow perch. *Transactions of the American Fisheries Society*, *134*(4), 1011-1020.*

11. El-Gawad, E. A. A., Shen, Z. G., & Wang, H. P. (2015). Efficacy of formalin, iodine and sodium chloride in improvement of egg hatching rate and fry survival prior to the onset of exogenous feeding in yellow perch. *Aquaculture Research*, *1*, 9.

12. FAO. (2018). The State of World Fisheries and Aquaculture 2018-Meeting the sustainable development goals.

13. Francis-Floyd, R. (1996). *Use of formalin to control fish parasites*. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.

14. Fulford, R. S., Rice, J. A., Miller, T. J., & Binkowski, F. P. (2006). Elucidating patterns of size-dependent predation on larval yellow perch (Perca flavescens) in Lake Michigan: an experimental and modeling approach. *Canadian journal of fisheries and aquatic sciences*, *63*(1), 11-27.

15. Hollowed, A.B., Bax, N., Beamish, R., Collie, J., Fogarty, M., Livingston, P., et al. (2000). Are multispecies models an improvement on single-species models for measuring fishing impacts on marine ecosystems? ICES J. Mar. Sci. 57: 707–719.

16. Hopkins, K. D. (1992). Reporting fish growth: A review of the basics 1. *Journal of the world aquaculture society*, *23*(3), 173-179.

17. Iwamatsu, T. (2004). Stages of normal development in the medaka Oryzias latipes. *Mechanisms of development*, *121*(7-8), 605-618.

18. Kestemont, P., Jourdan, S., Houbart, M., Mélard, C., Paspatis, M., Fontaine, P., ... & Baras, E. (2003). Size heterogeneity, cannibalism and competition in cultured predatory fish larvae: biotic and abiotic influences. *Aquaculture*, *227*(1-4), 333-356.

19. Kestemont, P., Dabrowski, K., & Summerfelt, R. C. (Eds.). (2015). *Biology and culture of percid fishes: principles and practices*. Springer.*

20. Koen-Alonso, M., & Yodzis, P. (2005). Multispecies modelling of some components of the marine community of northern and central Patagonia, Argentina. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*(7), 1490-1512.

21. Leal, M. C., Rocha, R. J. M., Rosa, R., & Calado, R. (2018). Aquaculture of marine non-food organisms: what, why and how?. *Reviews in Aquaculture*, *10*(2), 400-423.

22. Leclerc, D., Wirth, T., & Bernatchez, L. (2000). Isolation and characterization of microsatellite loci in the yellow perch (Perca flavescens), and cross-species amplification within the family Percidae. Molecular Ecology, 9(7), 995-997.

23. Leili, J. A. R. V. (2000). Migrations of the perch (Perca fluviatilis L.) in the coastal waters of western Estonia. In *Proceedings of the Estonian Academy of Sciences, Biology and Ecology* (Vol. 49, No. 3, pp. 270-276). Estonian Academy Publishers.

24. Lim, L. C., Cho, Y. L., Dhert, P., Wong, C. C., Nelis, H., & Sorgeloos, P. (2002). Use of decapsulated Artemia cysts in ornamental fish culture. *Aquaculture Research*, *33*(8), 575-589.

25. Ložys, L. (2004). The growth of pikeperch (Sander lucioperca L.) and perch (Perca fluviatilis L.) under different water temperature and salinity conditions in the Curonian Lagoon and Lithuanian coastal waters of the Baltic Sea. *Hydrobiologia*, *514*(1-3), 105-113.*

26. Malison, J. A., & Held, J. A. (1992). Effects of fish size at harvest, initial stocking density and tank lighting conditions on the habituation of pond-reared yellow perch (Perca flavescens) to intensive culture conditions. *Aquaculture*, *104*(1-2), 67-78.

27. Müller, K., & Berg, E. (1982). Spring migration of some anadromous freshwater fish species in the northern Bothnian Sea. *Hydrobiologia*, *96*(2), 161-168.*

28. Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M. C., Clay, J., ... & Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature*, *405*(6790), 1017.

29. NOAA. (2019). Great Lakes Commercial Fishery Landings Reports, statistics generated by website retrieved from: https://www.fisheries.noaa.gov/topic/resources-fishing#commercial-fishing

30. Overton, J. L., Bayley, M., Paulsen, H., & Wang, T. (2008). Salinity tolerance of cultured Eurasian perch, Perca fluviatilis L.: effects on growth and on survival as a function of temperature. *Aquaculture*, *277*(3-4), 282-286.*

31. Page, L.M. and B.M. Burr. 1991. A Field Guide to Freshwater Fishes. Houghton MifflinCo. New York, NY. 432 pp

32. Post, J. R., & Evans, D. O. (1989). Experimental evidence of size-dependent predation mortality in juvenile yellow perch. *Canadian Journal of Zoology*, *67*(2), 521-523.

33. Rach, J. J., Howe, G. E., & Schreier, T. M. (1997). Safety of formalin treatments on warm-and coolwater fish eggs. *Aquaculture*, *149*(3-4), 183-191.

34. Refseth, U. H., Nesbø, C. L., Stacy, J. E., Vøllestad, L. A., Fjeld, E., & JAKOBSEN, A. K. (1998). Genetic evidence for different migration routes of freshwater fish into Norway revealed by analysis of current perch (Perca fluviatilis) populations in Scandinavia. *Molecular Ecology*, *7*(8), 1015-1027.

35. Schreier, T. M., Rach, J. J., & Howe, G. E. (1996). Efficacy of formalin, hydrogen peroxide, and sodium chloride on fungal-infected rainbow trout eggs. *Aquaculture*, *140*(4), 323-331.

36. Shroyer, S. M., & McComish, T. S. (2000). Relationship between alewife abundance and yellow perch recruitment in southern Lake Michigan. *North American Journal of Fisheries Management*, *20*(1), 220-225.*

37. Sorgeloos, P., Bossuyt, E., Laviña, E., Baeza-Mesa, M., & Persoone, G. (1977). Decapsulation of Artemia cysts: a simple technique for the improvement of the use of brine shrimp in aquaculture. *Aquaculture*, *12*(4), 311-315.

38. Tarby, M. J. (1974). Characteristics of yellow perch cannibalism in Oneida Lake and the relation to first year survival. *Transactions of the American Fisheries Society*, *103*(3), 462-471.

39. Tiffney, W. N. (1939). The host range of Saprolegnia parasitica. *Mycologia*, *31*(3), 310-321.

40. Thorpe, J. 1977. Morphology, physiology, behavior and ecology of Perca fluviatilis L.and P. flavescens Mitchill. J. Fish. Res. Board Can. 34: 1504-1511

41. Tsai, C. F., & Gibson, G. R. (1971). Fecundity of the yellow perch, Perca flavescens Mitchill, in the Patuxent River, Maryland. *Chesapeake Science*, *12*(4), 270-274.

42. Vanhaecke, P. A. U. L., Lavens, P. A. T. R. I. C. K., & Sorgeloos, P. A. T. R. I. C. K. (1983). International study on Artemia. XVII. Energy consumption in cysts and early larval stages of various geographical strains of Artemia. *Ann Soc R Zool Belg*, *113*(2), 155-164.

43. Victoria, C. J., Wilkerson, B. S., Klauda, R. J., & Perry, E. S. (1992). Salinity tolerance of yellow perch eggs and larvae from coastal plain stream populations in Maryland, with comparison to a Pennsylvania lake population. *Copeia*, 859-865.

44. Wagner, C. M., & Austin, H. M. (1999). Correspondence between environmental gradients and summer littoral fish assemblages in low salinity reaches of the Chesapeake Bay, USA. *Marine Ecology Progress Series*, *177*, 197-212.

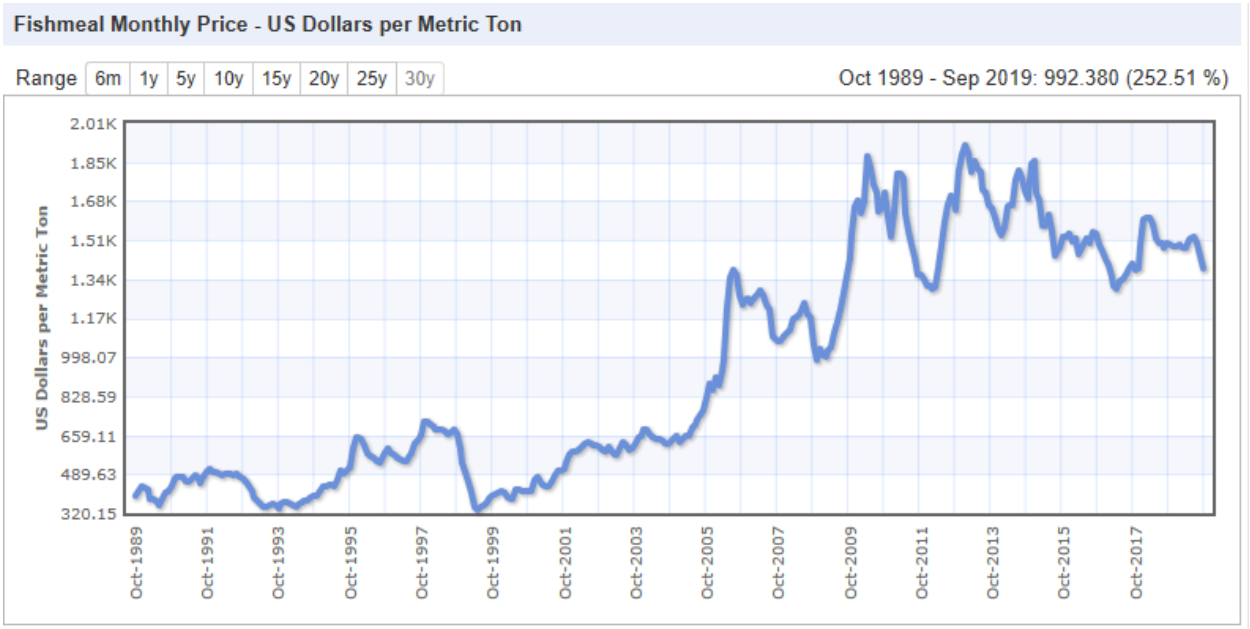
45. Whipple, S.J., Link, J.S., Garrison, L.P., and Fogarty, M.J. (2000). Models of predation and fishing mortality in aquatic ecosystems. Fish Fish. 1: 22–40.

46. Wilson, R. P. (1994). Utilization of dietary carbohydrate by fish. *Aquaculture*, *124*(1-4), 67-80.

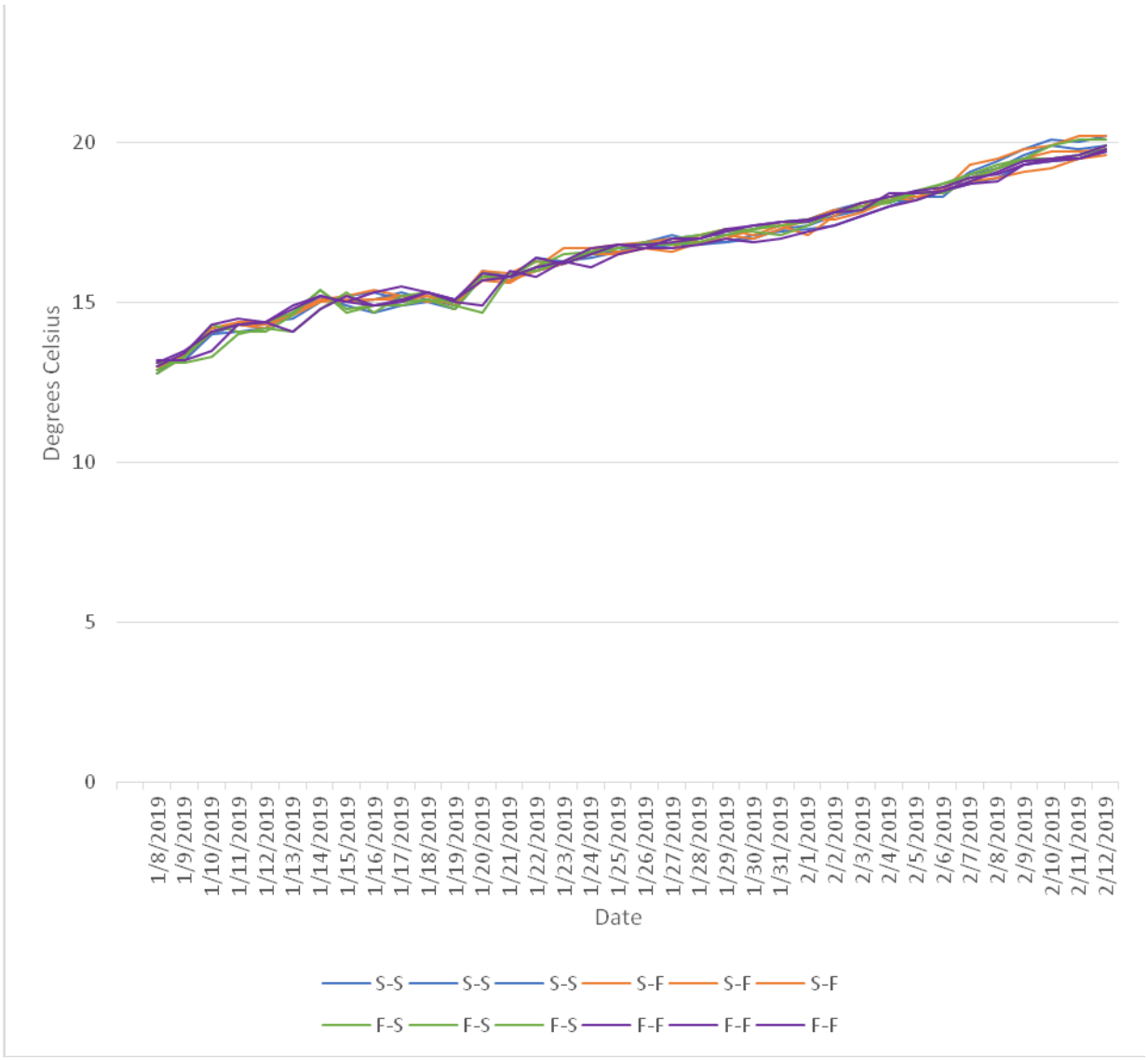
47. Withers, J.L., Sesterhenn, T.M., Foley, C.J., & Troy, C.D. (2015). Diets and growth potential of early stage larval yellow perch and alewife in a nearshore region of southeastern Lake Michigan. *Journal of Great Lakes Research, 41, 197-209.*

48. Zhan, A., Wang, Y., Brown, B., & Wang, H. P. (2009). Isolation and characterization of novel microsatellite markers for yellow perch (Perca flavescens). *International journal of molecular sciences*, *10*(1), 18-27.

Appendix A: Graphs




Appendix 1: ***The price trend in Peruvian fish meal according to the World Bank data over the last 30 years.*** Visual graph retrieved from: www.indexmundi.com/commodities, data source: www.worldbank.org.




Appendix 2: ***Temperatures per treatment for experiment 2:*** Effect of a low salinity 5-ppt environment on the survival and growth performance of yellow perch (*Perca flavescens*), at early life stage. The rising temperature followed the temperature regime instructed by US Patent 7,836,852 B2 (Methods for manipulating yellow perch spawning cycles).

S-S: Saltwater incubation to saltwater larval rearing treatment

S-F: Saltwater incubation to freshwater larval rearing treatment

F-S: Freshwater incubation to saltwater larval rearing treatment

F-F: Freshwater incubation to freshwater larval rearing treatment

# Appendix B: Schedules

| | Feeding from 1/25/19 to 2/31/19 | | | |
|---|---|---|---|---|
| | Each tank | | | |
| Time | Otohime | Time | Artemia | Notes |
| 9:45am | 0.1g **B1** | 9:50am | 150 ml | **Lights on at 10:00 am** |
| 11:55pm | 0.1g **B1** | 12:00pm | 150 ml | |
| 1:55pm | 0.1g **B1** | 2:00pm | 150 ml | **Cleaning cycle and diet replacement after 2pm, dry diet all fed by 2pm** |
| 3:55pm | 0.1g **B1** | 4:00pm | 150 ml | |
| 5:55pm | 0.1g **B1** | 6:00pm | 150 ml | **Artemia is made for next day** |
| 7:55pm | 0.1g **B1** | 8:00pm | 150 ml | |
| 9:55pm | 0.1g **B1** | 10:00pm | 150 ml | **Lights off at 12:00 am** |
| 2:55am | 0.1g **B1** | 3:00am | 150 ml | |
| 6:55am | 0.1g **B1** | 7:00am | 150 ml | |
| Total/day: | 0.9g **B1** | Total/day: | 1,350 ml | |
| | | | | |
| | Feeding from 2/1/19 to 2/7/19 | | | |
| | Each tank | | | |
| Time | Otohime | Time | Artemia | Notes |
| 9:45am | 0.2g **B1** | 9:50am | 150 ml | **Lights on at 10:00 am** |
| 11:55pm | 0.2g **B1** | 12:00pm | 150 ml | |
| 1:55pm | 0.2g **B1** | 2:00pm | 150 ml | **Cleaning cycle and diet replacement after 2pm, dry diet all fed by 2pm** |
| 3:55pm | 0.2g **B1** | 4:00pm | 150 ml | |
| 5:55pm | 0.2g **B1** | 6:00pm | 150 ml | **Artemia is made for next day** |
| 7:55pm | 0.2g **B1** | 8:00pm | 150 ml | |
| 9:55pm | 0.2g **B1** | 10:00pm | 150 ml | **Lights off at 12:00 am** |
| 2:55am | 0.2g **B1** | 3:00am | 150 ml | |
| 6:55am | 0.2g **B1** | 7:00am | 150 ml | |
| Total/day: | 1.8g **B1** | Total/day: | 1,350 ml | |
| | | | | |
| | Feeding from 2/8/19 to 2/14/19 | | | |
| | Each tank | | | |
| Time | Otohime | Time | Artemia | Notes |
| 9:45am | 0.15g **B1**/0.15g **B2** | 9:50am | 150 ml | **Lights on at 10:00 am** |
| 11:55pm | 0.15g **B1**/0.15g **B2** | 12:00pm | 150 ml | |
| 1:55pm | 0.15g **B1**/0.15g **B2** | 2:00pm | 150 ml | **Cleaning cycle and diet replacement after 2pm, dry diet all fed by 2pm** |
| 3:55pm | 0.15g **B1**/0.15g **B2** | 4:00pm | 150 ml | |
| 5:55pm | 0.15g **B1**/0.15g **B2** | 6:00pm | 150 ml | **Artemia is made for next day** |
| 7:55pm | 0.15g **B1**/0.15g **B2** | 8:00pm | 150 ml | |
| 9:55pm | 0.15g **B1**/0.15g **B2** | 10:00pm | 150 ml | **Lights off at 12:00 am** |
| 2:55am | 0.15g **B1**/0.15g **B2** | 3:00am | 150 ml | |
| 6:55am | 0.15g **B1**/0.15g **B2** | 7:00am | 150 ml | |
| Total/day: | 1.35g **B1**/1.35g **B2** | Total/day: | 1,350 ml | |
| | | | | |
| | Feeding from 2/15/19 to 2/20/19 | | | |
| | Each tank | | | |
| Time | Otohime | Time | Artemia | Notes |
| 9:45am | 0.4g **B2** | 9:50am | 150 ml | **Lights on at 10:00 am** |
| 11:55pm | 0.4g **B2** | 12:00pm | 150 ml | |
| 1:55pm | 0.4g **B2** | 2:00pm | 150 ml | **Cleaning cycle and diet replacement after 2pm, dry diet all fed by 2pm** |
| 3:55pm | 0.4g **B2** | 4:00pm | 150 ml | |
| 5:55pm | 0.4g **B2** | 6:00pm | 150 ml | **Artemia is made for next day** |
| 7:55pm | 0.4g **B2** | 8:00pm | 150 ml | |
| 9:55pm | 0.4g **B2** | 10:00pm | 150 ml | **Lights off at 12:00 am** |
| 2:55am | 0.4g **B2** | 3:00am | 150 ml | |
| 6:55am | 0.4g **B2** | 7:00am | 150 ml | |
| Total/day: | 3.6g **B2** | Total/day: | 1,350 ml | |

Appendix 1B: ***Feeding regimen and schedule for entirety of experiment 2:*** Effect of a low salinity 5-ppt environment on the survival and growth performance of yellow perch (*Perca flavescens*), at early life stage.

Appendix C: Descriptions

***Dry feed implementation***

In order to ensure that the dry feed would drop at a desired time in a determined quantity, timed feeders needed to be designed and developed to handle these tanks. To create the timed-rotating feeder (TRF), a 2 rotation per minute (RPM) high torque electronic motor, designed for cork-screw vending machines, was utilized to drive a 15-cell feeder disc which when rotated would drop feed through a hole. The motor was provided power by a 600 milliamp 9-volt adapter and housed in 1 ½ inch PVC. In order to attach the feeder to the side of a given tank, a 2-inch “C” clamp attached to the feeder could clamp onto the side of a tank. All feeders were plugged into a power strip connected to a Sweeny™ programmable timed feeder console. This design allows feed to be dropped at a designated time for a desired amount of time, meaning a preset accurate feeding weight fed multiple times per day at a chosen time.

Appendix 1C: ***Description of design of timed rotating feeders.***

***Recirculating aquaculture system design***

In order to test the efficacy of a 5-ppt saltwater environment compared to a freshwater one in a recirculating aquaculture system (RAS), two identical aquaculture systems had to be made with the same exact components. Two Rubbermaid™ 65-gallon stock tanks were used as each system's water sumps each housing the 7000 LPH submersible pumps which provided water through a ¾" diameter PVC pipe, channeled through an 18-watt ultraviolet light aquarium sterilizer and outflow controlled by ¼" PVC ball valves. The water was discharged into each of six 160-Liter square research tanks with rounded corners, a width of 61 cm filled to a depth of 41 cm. PVC mesh screens with ¼" holes were used to block the outflow into a 1 ½" PVC pipe which fed into a radial cone desolidifier. The radial cone desolidifier had a 1 ½" PVC outflow equipped with a ball valve in which collected solids could be purged from the system. The height of the desolidifier also controlled the depth of the research tanks. From the radial cone desolidifier the water flowed into an 85-gallon silo-shaped biofilter filled with 2-kilograms of dry weight K1 plastic moving bed biofilter media (MBBR). From the biofilter, the filtered water was discharged into the Rubbermaid™ 65-gallon stock tank, closing the loop and causing recirculation. A 4-foot circular tank with an independent flow return to the RAS sump was attached to the end of the system for use as a conditioning tan. The tank was capable of circulating the same water as the RAS, but had the capability of running separately from the system as well.

Appendix 2C: ***Description of design of RAS system for experiment 3:*** Effect of a low salinity 5-ppt environment on the survival and growth performance of fingerling yellow perch (*Perca flavescens*) in a recirculating aquaculture system

### *Recirculating aquaculture system conditioning*

To be able to condition the RAS system biofilters, with a total water capacity of 1400 liters per 6 tank system, 20.6 g of dry weight NH4Cl was diluted into each system to a desired concentration of 5 mg/L or ppm. The weight was based on the molecular weight of NH4 in the compound. The saltwater RAS was kept at a salinity of 5 ppt for the duration of the biofilter conditioning. The system was then left to cycle with water quality measurements being taken every other day. After 25 days when the total ammonia nitrogen (NH3/NH4) and nitrite (NO2), the most detrimental aquaculture compounds, were efficiently being removed from the water, the system was ready for use pertaining to experiment 3 in this thesis. At this point fish were ready for transport into the 4-foot conditioning tank attached to the RAS system.

Appendix 3C: ***Description of the conditioning of the RAS system biofilter for experiment 3:*** Effect of a low salinity 5-ppt environment on the survival and growth performance of fingerling yellow perch (*Perca flavescens*) in a recirculating aquaculture system)