THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING

A PRELIMINARY TRIAL TO DETERMINE THE FEEDING PREFERENCE OF *OREOCHROMIS NILOTICUS* (NILE TILAPIA) FINGERLINGS FOR DIFFERENT PREPARATIONS OF *LEMNA MINOR* (DUCKWEED)

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SPRING 2016

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Civil Engineering
with honors in Civil Engineering

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ABSTRACT

Hunger and malnutrition present serious complications for several areas all over the world. In 2012, about 25% of the world's children were classified as chronically malnourished; currently, almost one billion people go hungry every day (World Hunger Education Service, 2015). With a population forecast of 10 billion people in 2050, world hunger and malnutrition issues will become more severe and problematic (Worldometers.info, 2016). The finite amount of resources and space on Earth, in combination with the demands of a growing population, present an inevitable challenge that society must confront. That challenge resides in the production of basic needs (food, water, energy, shelter) for more people using fewer resources on Earth. Aquaponics, farming aquatic animals and growing crops in a single system, has been proposed by the United Nations as a potential solution to help feed the world (Somerville et al., 2014). Unfortunately, the relatively high cost and unsustainable practice of producing commercial fish feed from ocean-caught fish is currently inhibiting aquaponics from reaching its true potential (Naylor et al., 2000). A possible solution to this problem resides in the utilization of high-protein, plant-based feedstock to replace traditional fishmeal. Many omnivorous fish are known to eat aquatic plants. For example, *Oreochromis niloticus* (Nile tilapia), a fish commonly used in aquaculture (fish farming) systems, has been shown to feed on *Lemna minor* (duckweed) growing in slow-moving or stagnant water (Heuze & Tran, 2015). To provide a steady supply of duckweed for aquaponics systems year-round, it is necessary to harvest and dry the plant for extended storage. The goal of this research was to test the preference of Nile tilapia fingerlings for duckweed dried under different conditions to determine the best preparation for use in future pilot-scale aquaponics studies. The different duckweed drying methods tested in this study included: drying in an oven at 40 or 60° C, and drying in the sun. Utilizing Manly's α preference index, the results of six randomized feeding trials revealed that Nile tilapia fingerlings exhibit preference ($\alpha > 1/m$; $m = 3$) for duckweed dried at 40° C and 60° C, and exhibit an avoidance ($\alpha < 1/m$; $m = 3$) for sun-dried duckweed.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to extend many thanks to the Department of Civil & Environmental Engineering at Penn State University for their support. Specifically, I would like to extend a thousand thanks to Dr. Rachel Brennan and Ben Roman for all their assistance with this research and for their guidance through this process. Thank you also to Brett Abele for video recording the feeding trials. Thank you Dr. Robert Berghage for allowing the trials to be carried out on your Nile tilapia fingerlings. Thank you Dr. Stephanie B. Velegol for kindly agreeing to be the Faculty Reader for my thesis. Most of all, thank you to those of you who supported me and encouraged me along the way. My parents, James and Kerry, my sister, Shannon, the NB crew, you know who you are, my roommates, god bless your tolerance, and my peers in the Civil and Environmental Engineering department, thank you.

Chapter 1 Introduction and Background

Introduction

Currently, overfishing the seas occurs at an alarming rate. Overfishing exposes about four-fifths of the globe's fisheries to overexploitation, depletion, or decimation (Koster, 2012; overfishing.org). Out of necessity, aquaculture systems, farming aquatic animals, show an upward trend in popularity, especially in eastern Asia. Most Asian aquaculture systems subject their aquatic animals to high stocking densities and force the animals to swim in waste medium (Figure 1). As a result, the farmers inject high doses of antibiotics to reduce the risk of people falling ill from the consumption of these aquatic animals (Laporte, 2015).

Figure 1: An Asian tilapia farm receives dry mass chicken and pig feces (Laporte, 2015).

Since feed is one of the most expensive inputs for an aquaculture system, aquaculture's profitability directly relates to feedstock cost (El-Sayed et al., 2013). Fortunately, for the aquaculture industry, studies show that duckweed, an abundant aquatic plant, can help solve this dilemma: duckweed can yield between 20 – 44 tons of protein-rich biomass per hectare per year (Cross, 2011). Duckweed can soak up nitrogen, phosphorus, and proteins from wastewater and then be conveniently harvested and reused as a nutritious feedstock for fish such as carp, tilapia, and catfish (Landesman et al., 2011). The higher the nutrient concentration in the wastewater, the higher the protein content of the duckweed. If duckweed replaces fishmeal in aquaculture feed, the protein content of the fish may increase and potentially add to the product quality (El-Shafai et al., 2004). Incorporating duckweed into the aquaculture industry may help offset and replace the need for the often scarcer and costlier fishmeal (El-Shafai et al., 2004). The logistics of replacing conventional fish feed with duckweed relies on: 1) the ability to harvest and dry duckweed without prohibitive energy costs; and 2) the ability of fish to consume it and maintain their typical growth rate. Other studies have used a mass balance approach over set time intervals to ascertain the preference of aquatic animals for different feeds. For instance, one study allowed the fish to feed for 22 minutes, and then collected the uneaten feed to conduct a mass balance (Steele et al., 2014). A similar mass balance approach will be used in this study.

Aquaponics

Aquaponics systems are growing in popularity throughout the world in response to an increase in food demands (aquaponicssystems.org; 2013). Simply, aquaponics is the combination of hydroponics (growing plants in water, Figure 2) and aquaculture (farming aquatic animals, Figure 3). Individually, both hydroponics and aquaculture possess many advantages and some disadvantages. The downside of hydroponics is that expensive fertilizers must often be used for the plants (Malcolm & Arcaro, 2011). The downside of aquaculture is that fecal waste from fish must be removed daily (Malcolm & Arcaro, 2011). If combined together, the negative of hydroponics becomes a positive for aquaculture, and the negative of aquaculture becomes a positive for hydroponics. Thus forms the symbiotic relationship between the two systems: plants extract the nutrients from the feces of the aquatic animals, and the aquatic animals receive clean water from the filtration process of the plants. An aquaponics system's versatility lies in the diversity of applicable plants or aquatic animals it can include (Malcolm & Arcaro, 2011). If society makes an effort to optimize aquaponics, it could create a sustainable food production system to help support for the future population of Earth.

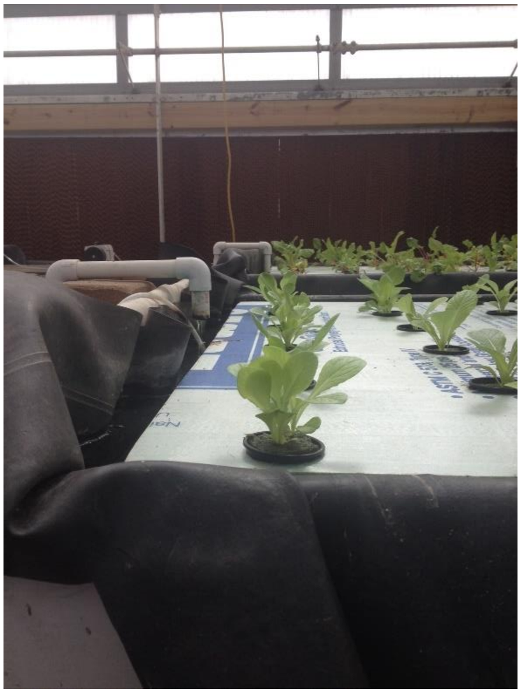


Figure 2: An example of the hydroponics subpart of a functional aquaponics system at Penn State.

Figure 3: An example of the aquaculture subpart of an operating aquaponics system at Penn State.

Lemna minor

Lemna minor, commonly known as “duckweed”, exhibits suitable properties as a fish feed for several reasons. Duckweed exemplifies a viable phytoremediation technology, and operates anywhere from complex hazardous wastes to simple municipal wastewater (Heuze & Tran, 2015). Inclusion of *Lemna minor* in a recirculating aquaculture system can result in a sharp decrease in total dissolved solids, ammonia, nitrite, and total phosphate while increasing the dissolved oxygen content of the water; furthermore, the inclusion of *Lemna minor* has been shown to result in better water quality and better growth of fish (Velichkova & Sirakov, 2013).

Duckweed can only grow on either stagnant bodies of water (Figure 4) or very slow-moving bodies of water with a velocity of less than or equal to 0.3 meters per second or 0.67 mph (Heuze & Tran, 2015).

Figure 4: ***Lemna minor*** **floating on the surface of a stagnant pond (Antieau; ecy.wa.gov).**

Duckweed grows in a variety of climates and mediums all over the world; it survives in a range of pH from about 5 to 9 (Heuze & Tran, 2015). Interestingly, because of *Lemna minor's* life cycle (it sinks to the bottom of water bodies and lays dormant until favorable conditions return), it thrives everywhere in the world except permanently frozen regions or waterless deserts (Leng et al., 1995). Although duckweed possesses a very high moisture content of up to 95%, once dry it transforms into a protein packed (15% to 40%) substance (Landesman et al., 2002). Overall, duckweed offers a sustainable feed option to nourish aquatic animals cheaply.

There are two main harvesting methods for duckweed. Duckweed "hand-skimming" refers to the process of extracting duckweed off the surface of the water with a fine meshed net by hand (Figure 5); consequently, collection takes a long time, and the initial startup cost is cheap, but the operating cost of labor is moderately high (Cross, 2013).

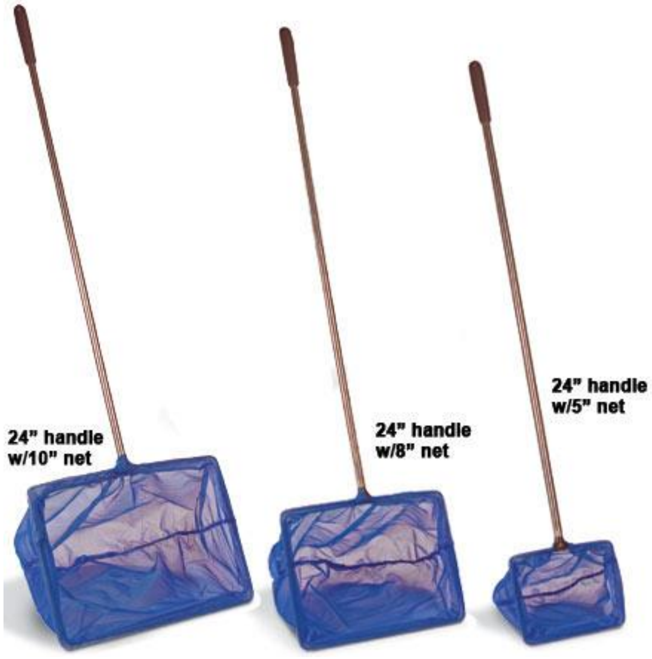


Figure 5: Three different hand skimmer sizes (www.drsfostersmith.com).

Duckweed "mechanical-skimming", the time-efficient process of extracting duckweed off of the surface of the water with machines (Figure 6), is the other common method to harvest duckweed. In mechanical skimming, the initial cost of the machinery is high while the operating cost of labor is moderately low (Cross, 2013).

Figure 6: An operating mechanical skimmer (www.aquarius-systems.com).

Two preferred methods of processing duckweed into feedstock are sun-drying and oven-drying; sun-drying has a low energy cost but takes a long time, whereas oven-drying has a moderate energy cost but finishes relatively quickly (Heuze & Tran, 2015). The dried duckweed (Figure 7) can be simply fed to animals without further processing. Grinding dried duckweed into a meal and pressing dried duckweed into pellets or flakes requires more energy to accomplish than sun or oven drying, but aquatic animals may prefer processed and dried duckweed more than unprocessed dried duckweed (Landesman et al., 2002).

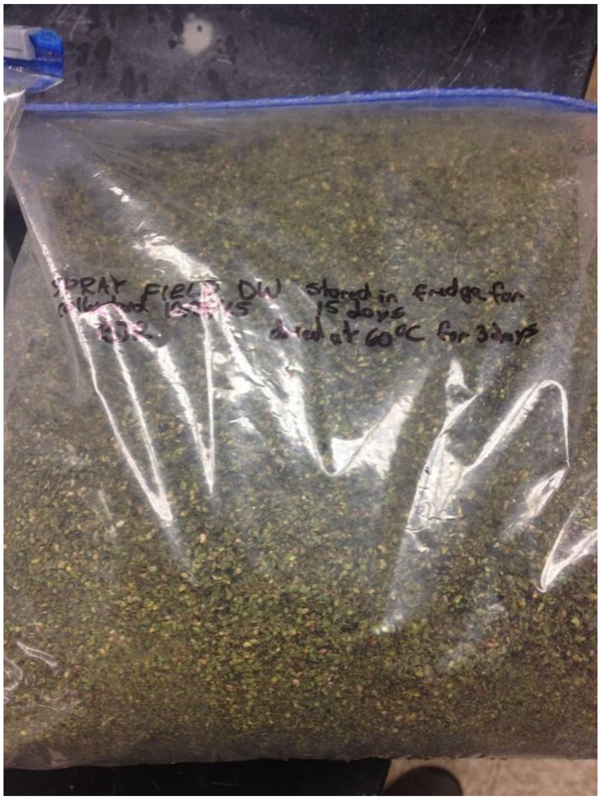


Figure 7: Dried *Lemna minor* used as a dietary replacement for conventional fish feed during the trials conducted in this study. Note its lush green color.

One of the reasons duckweed is a cheap alternative to conventional fish feed is its short doubling time. Duckweed's doubling rate resembles that of a microbe more than a typical plant: it can double its mass between 16 and 48 hours (Heuze & Tran, 2015).

Oreochromis niloticus

Oreochromis niloticus (Nile tilapia) belongs to the Cichlidae family, of the Perciformes order, in the Actinopterygii class. *Oreochromis niloticus*, shown in Figure 8, possesses the evolutionary ability to tolerate several physical parameters that other fish cannot tolerate.

Contrary to popular belief, *Oreochromis niloticus* displays feed rates of approximately 93% more activity at night than in the daytime under laboratory conditions (Fortes-Silva et al., 2010).

Figure 8: Right before a feeding trial conducted in this study, *Oreochromis niloticus* fingerlings anxiously await the addition of *Lemna minor* into their tank.

Oreochromis niloticus was selected for the aquaponics fish preference study in this work for a multitude of reasons. Nile tilapia possess an extremely efficient protein digestion system; moreover, Nile tilapia naturally feed on aquatic plants and can devour floating aquatic plants like *Lemna minor* (Khan, 2011). An economic reason to study Nile tilapia for aquaculture production is that they are able to grow to marketable size in a short period in comparison to other fish. Common sense indicates that it would be advantageous to cultivate Nile tilapia in an aquaponics system if *Lemna minor* proves successful as a fish feed because Nile tilapia fed exclusively duckweed showed higher growth rates than Nile tilapia fed conventional feed (Chowhury et al., 2008). *Oreochromis niloticus* makes a great aquatic animal for this study because of its fast growth rate and its willingness to consume *Lemna minor*.

It is important to note that prior to these trials, the Nile tilapia fingerlings were fed sinking pellets twice a day, as opposed to floating feed. It is possible that it will take them additional time to become acquainted with the floating feed. To help the fish become accustomed to floating feed quickly in this study, they were fed conventional flakes during their normal morning feeding sessions and also after each afternoon duckweed trial (Figure 9).

Figure 9: The first *Oreochromis niloticus* fingerling rushes to the surface to ingest floating conventional feed flakes.

Manly Alpha Index of Preference

By definition, preference, when occurring under random sampling, reflects any deviation from equality. Predator-prey preference depends on prey density, prey escape mechanism, predator hunger, predator searching behavior, predator density, predator effort, and water temperature (Chesson, 1978). The Manly Alpha Index of Preference (Manly's Alpha), a statistical approach useful in the estimation of predator-prey preference, can be used to predict future preference as well. A vast comparison of prey preference statistics and models concludes that Manly's Alpha is an excellent model for determining prey preference (Chipps & Garvey, 2006), and therefore was used in the determination of statistical trends in this study. For this study, Nile tilapia fingerlings were considered the predator and duckweed feed the prey.

Manly's Alpha calculation hinges on two equations that depend on if the prey replaces itself or not. For example, if shrimp are the prey, the test falls under the category of a Type One Selection Experiment (T.O.S.E.), because they quickly reproduce and replace themselves (Manly, 1974). In the current study, duckweed does not replace itself; therefore, a Type Two Selection Experiment (T.T.S.E.), also known as variable prey preference, is appropriate. Equation 1 shows the final form of a T.T.S.E.:

$$\alpha_i = (\log P_i) / \sum_{i=1}^{m} log\, P_i \qquad (1)$$

where:

i = prey type i

j = prey type j

α_i = Manly's alpha

$P_i = e_i/n_i$ prey type i

$P_j = e_j/n_j$ prey type j

e_i = prey type i at the end

n_i = prey type i at the start

e_j = prey type j at the end

n_j = prey type j at the start

m = total number of prey types

If Manly’s alpha for a particular prey type equals more than (1/m), then that prey type exhibits preference. If Manly’s alpha for a particular prey type is less than or equal to (1/m), then that prey type lacks preference and represents avoidance (Viljoen et al., 2013). Since Manly’s alpha is derived from a realistic biological model, it holds more use for prediction than other models; consequently, Manly’s alpha was used as a prediction tool in this study (Chesson, 1978).

Prior Work

Full details on the collection, drying, and storage of each type of duckweed used in this study are provided in Appendix A. In brief, duckweed was manually collected from small ponds in the Penn State spray field, which is located approximately one mile north of Penn State University (University Park, PA). The spray field receives treated effluent from the Penn State wastewater treatment plant (WWTP), which is irrigated over the land before percolating through the soil and recharging the local water supply. Some low-lying areas in the spray field fill with this wastewater effluent, and duckweed naturally grows on these ponds. Because the spray field is irrigated with treated wastewater effluent (low concentration of nutrients) instead of primary municipal wastewater (high concentration of nutrients), the duckweed in this study is expected to contain a relatively low protein content (approximately 14.5%), based on previous tests conducted in the lab. Consequently, throughout the feeding trials, the Nile tilapia fingerlings received normal fish feed once in the morning and once in the afternoon after completing the trial for the day to ensure they accrued proper daily nutrition.

The Conceptual System at a Glance

Combining aquaculture, hydroponics, and humans together harmoniously may lead toward a conceptual system that is sustainable. Duckweed readily absorbs vital nutrients from municipal wastewater. Nile tilapia feed on aquatic plants like duckweed. Nile tilapia can provide food for humans as well as provide fertilization for vegetables via their feces. Vegetables filter water for Nile tilapia and provide food for humans. Using duckweed as a feed for the fish in an aquaponics system completes a sustainable cycle; therefore, if Nile tilapia willingly consume duckweed, then the conceptual system may come to reality (Figure 10).

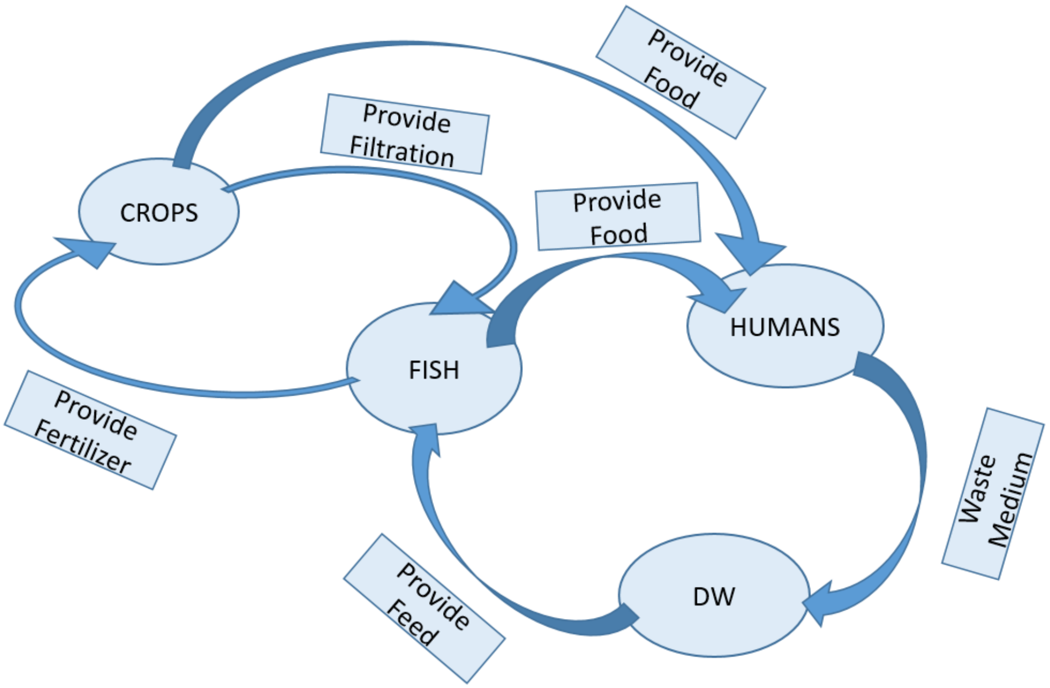


Figure 10: A networking web illustrates the conceptual system of this study. Note that humans receive a net gain of one input as a result of this complex cycle. DW = duckweed.

Chapter 2 Purpose and Hypothesis

Purpose

Aquaponics utilization reduces the consumption of fresh water in agriculture; as a result, aquaponics may help reduce stresses on global fresh water resources. Aquaponics systems use 90% less water for plants than traditional ground irrigation (Malcolm & Arcaro, 2011). In addition to global concerns for fresh water, as populations and food demands increase, space and resources decrease. Because of food scarcity, equatorial and coastal regions throughout the world suffer from undernourishment (*World Hunger Education Service*, 2015). Developing regions rely heavily on a fish-based diet and suffer tremendously from the over-harvesting of the oceans. Aquaponics may help relieve scarcity issues with fresh water and food. Correct optimization and operation of an aquaponics system may result in reduced freshwater use, as well as increased food production at more sustainable energy levels.

The main purpose of this research is to determine the feeding preference of Nile tilapia fingerlings for duckweed dried under different conditions. Studying Nile tilapia fingerlings is important because the profitability of an aquaculture system may be directly linked to the starting age of the fish in the system. A Nile tilapia fingerling costs about $1.25, and an adult Nile tilapia costs about $3.00 (tilapiafingerlings.com, 2016). The fingerlings' nutrition is an important factor in the aquaculture industry. If dried, the optimum vitamin retention of duckweed is obtained between 43° C and 46° C, and for every pound of duckweed dried at this temperature, 1000 BTUs are needed (Fakhoorian, 2012). Therefore, it is expected that the 40° C duckweed will have a higher nutritional value than the 60° C duckweed, but it may cost more to produce if it takes longer to dry. In order for the conceptual system (Figure 10) to work, the feed component (duckweed) relies on the preference of the Nile tilapia. Utilizing the data from the feeding trials, pilot-scale production and preparation of the preferred duckweed type(s) should generate the highest possible success rate for the conceptual system in future work.

Hypotheses

Hypothesis One - Sundried duckweed will have the lowest consumption rate by Nile tilapia fingerlings due to poor physical appearance, resulting in avoidance. The sundried duckweed appears ashy, so it may seem undesirable to the Nile tilapia fingerlings. In addition, the sundried duckweed appears to clump together and form clusters that may not fit in the fingerlings' mouths.

Hypothesis Two - The 40° C and 60° C duckweed consumption rates will be approximately the same, resulting in preference of Nile tilapia fingerlings for both. Both products possess very similar smells and appearances.

Hypothesis Three - Given enough time and repeated exposure, the Nile tilapia fingerlings will adapt and readily consume any preparation type of duckweed.

Chapter 3 Materials and Methods

Duckweed Preparation

All duckweed used in this study was collected by hand-skimming the ponds in the spray field, and then rinsing with cold tap water over an old window screen to remove impurities. From there, the drying procedures were as follows. The sundried duckweed was dried by the sun (9.4 – 17.4° C) on the window screen outside (unprotected from the wind, over grass) for 10 days. The 40° C duckweed was dried in a laboratory oven at 40° C for 3 days. The 60° C duckweed was stored in the refrigerator for 15 days (while waiting for available ovens), and then dried in a laboratory oven at 60° C for 3 days. After drying, all duckweed was stored in sealed plastic bags in the lab at room temperature for 5 months.

Oreochromis niloticus

The Nile tilapia fingerlings used in this study were raised at a conventional hatchery (White Brook Tilapia Farm in Smithfield, Missouri), and were about four weeks old (approximately 1 g each) when they were shipped to Penn State University. The fingerlings were allowed to acclimate for two weeks in 10-gallon aquariums on campus before the trials began. A week prior to the trials, ammonia levels were high (causing about 30% of the original fish to die), but they returned to normal before the onset of the trials. Prior to the trials, each tank was fed 1.5 grams of TetraMin Plus (shrimp flavor), once in the morning and once in the afternoon. The weight of the average fingerling at the onset of the feeding trials is unknown. The average fingerling weight per tank after the study can be found in Table 6.

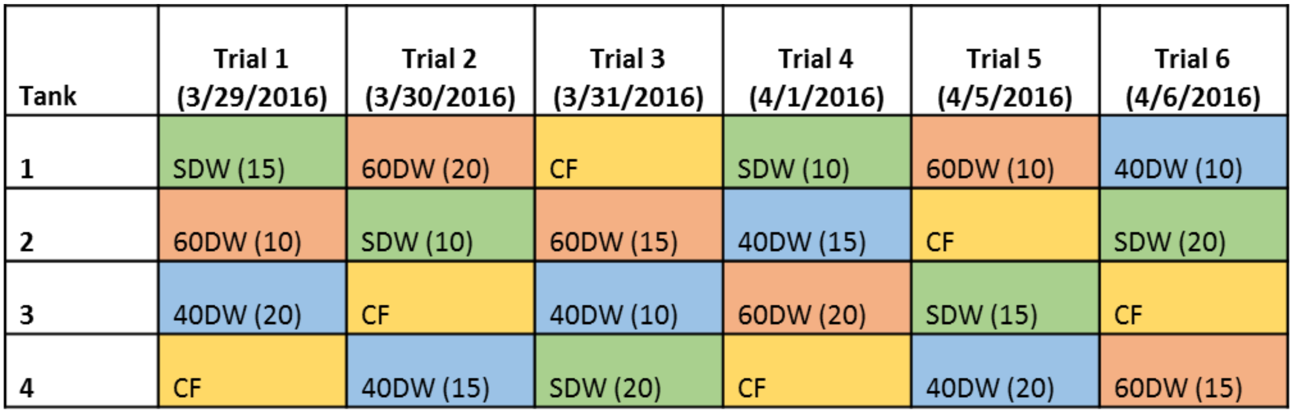
Experimental Setup

The experiment lasted for a total of 10 days. The experimental setup for the feeding trials consisted of four, 10-gallon aquarium tanks (T1-T4) with 10-14 Nile tilapia fingerlings in each tank, as follows: T1 = 14 fingerlings; T2 = 10 fingerlings; T3 = 10 fingerlings; and T4 = 11 fingerlings (Appendix D, Figure 16). Aeration lines, filtration systems, artificial plants, red igneous rocks, and temperature monitoring devices were used to help mimic a natural aquatic environment for the fish. Approximately 1.5 g of each type of duckweed (sun, 40° C, and 60° C) was weighed for each tank in the lab prior to each trial, to ensure equal weights of each duckweed were placed in each tank (Legner & Murray, 1981). Immediately before each trial, the aquarium filter power cords were unplugged to prevent the duckweed from clogging the filtration system. In addition, artificial floating foliage and tank lids were removed to allow easy access to the surface of the water.

Duckweed feedstock was added to each tank and the fingerlings allowed to feed for a randomized time interval according to Table 1. A random schedule was created to ensure unbiased feeding trials and statistical validity (Table 1). After each specific time interval had elapsed, the surface of the water was hand-skimmed with a small, green aquarium net to recollect the uneaten duckweed (Appendix D, Figure 15). Conventional feed (1 g) was added to the tanks at the end of each trial ensure the fingerlings received their daily dietary needs.

The duckweed collected from each aquarium was allowed to air-dry for one day and then oven-dried at 40° C for two days. The oven-dried duckweed was transferred to a desiccator for 30 minutes to allow for moisture-free cooling and then weighed.

Table 1: Randomized schedule used for the Nile tilapia feeding trials in this study. SDW = Sundried duckweed; 40DW = duckweed dried at 40° C; 60DW = duckweed dried at 60° C; CF = Conventional/Control feed. (#) = the amount of time (min) that the feed was allowed to remain in the tank before removal.



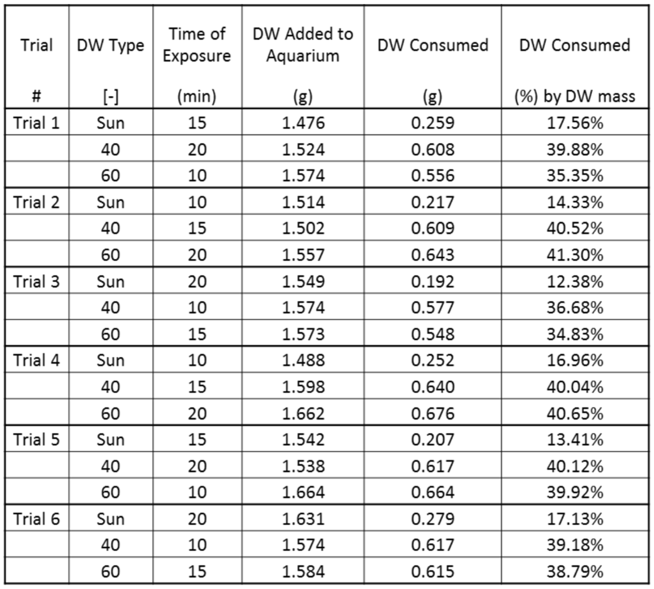
Tank	Trial 1 (3/29/2016)	Trial 2 (3/30/2016)	Trial 3 (3/31/2016)	Trial 4 (4/1/2016)	Trial 5 (4/5/2016)	Trial 6 (4/6/2016)
1	SDW (15)	60DW (20)	CF	SDW (10)	60DW (10)	40DW (10)
2	60DW (10)	SDW (10)	60DW (15)	40DW (15)	CF	SDW (20)
3	40DW (20)	CF	40DW (10)	60DW (20)	SDW (15)	CF
4	CF	40DW (15)	SDW (20)	CF	40DW (20)	60DW (15)

Chapter 4 Results and Discussion

Results

The results of the experiment show a statistical preference trend for the 40° C duckweed and the 60° C duckweed, but not for the sundried duckweed. The consumption (percent by mass) of the sundried duckweed by Nile tilapia fingerlings only ranged between 12% and 18% (Figure 11, Table 2). Whereas, the consumption (percent by mass) of the 40° C duckweed ranged between 36% and 41%, and the 60° C duckweed ranged between 34% and 42% (Table 2, Figure 11). Clearly, the 40° C and 60° C preparation types of duckweed perform between two to three times better than the sundried duckweed for the Nile tilapia fingerlings.

Table 2: Mass of duckweed (DW) consumed by Nile tilapia fingerlings during the trials, organized by drying method.



Trial #	DW Type [-]	Time of Exposure (min)	DW Added to Aquarium (g)	DW Consumed (g)	DW Consumed (%) by DW mass
Trial 1	Sun	15	1.476	0.259	17.56%
	40	20	1.524	0.608	39.88%
	60	10	1.574	0.556	35.35%
Trial 2	Sun	10	1.514	0.217	14.33%
	40	15	1.502	0.609	40.52%
	60	20	1.557	0.643	41.30%
Trial 3	Sun	20	1.549	0.192	12.38%
	40	10	1.574	0.577	36.68%
	60	15	1.573	0.548	34.83%
Trial 4	Sun	10	1.488	0.252	16.96%
	40	15	1.598	0.640	40.04%
	60	20	1.662	0.676	40.65%
Trial 5	Sun	15	1.542	0.207	13.41%
	40	20	1.538	0.617	40.12%
	60	10	1.664	0.664	39.92%
Trial 6	Sun	20	1.631	0.279	17.13%
	40	10	1.574	0.617	39.18%
	60	15	1.584	0.615	38.79%

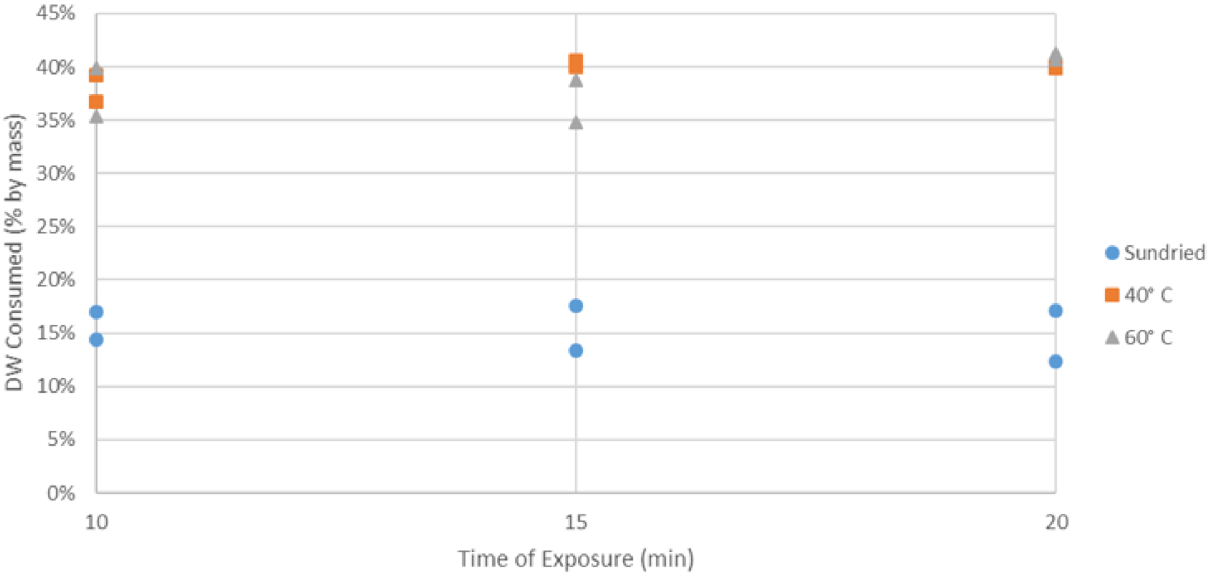


Figure 11: Consumption of duckweed (DW, % by mass) by Nile tilapia fingerlings over varying exposure times from 10 to 20 minutes. Sundried duckweed exhibited a lower consumption rate than the virtually identical consumption rates of the 40° C and 60° C duckweed.

Since the mass of the fish is directly correlated to feedstock consumption, the data must be analyzed by normalizing the amount of duckweed consumed to the fish (mass) for each trial. Figure 12 illustrates that the duckweed consumption rate of the fingerlings was fairly constant over the course of the study.

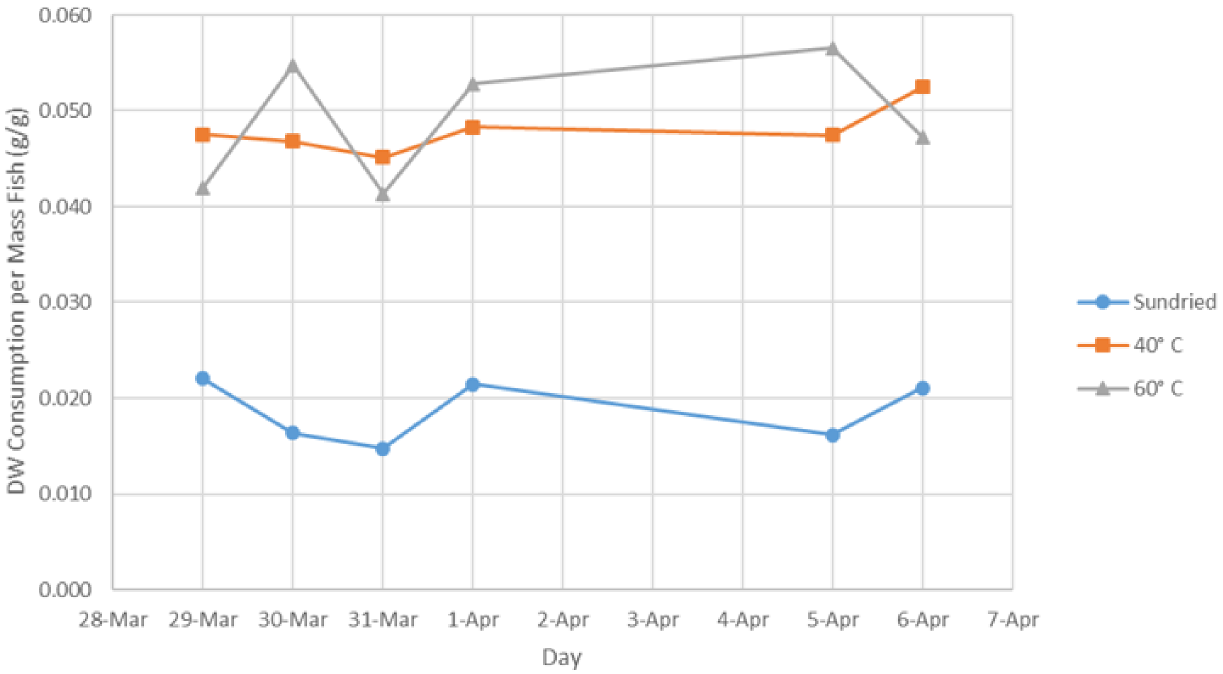


Figure 12: Duckweed consumption normalized per fingerling (mass) over time.

The Manly's alpha statistical model was used to determine preference versus avoidance for the three preparation types of duckweed in this experiment. Manly's alpha for the sundried duckweed was determined to be 0.14 ± 0.02 for the whole trial period, indicating avoidance. In contrast, Manly's alpha for the 40° C and 60° C duckweed were 0.43 ± 0.01 and 0.42 ± 0.02, respectively, indicating preference of Nile tilapia fingerlings for these feedstocks (Table 3). Over the course of the study, Manly's alpha remained fairly constant for the different feed types: the fingerlings showed a constant preference for the 40° C and 60° C duckweed, and a constant avoidance for the sundried duckweed (Figure 13).

Table 3: Summary of Manly's alpha over the course of the study for n = 6 feeding trials. Preference is determined if Manly's alpha is greater than (1/m), otherwise preference is not indicated.

Type [-]	Avg Manly Alpha [-]	Standard Deviation [-]	1/m; m = 3 [-]	Preference? Y/N
Sun	0.14	0.02	0.33	N
40	0.43	0.01	0.33	Y
60	0.42	0.02	0.33	Y

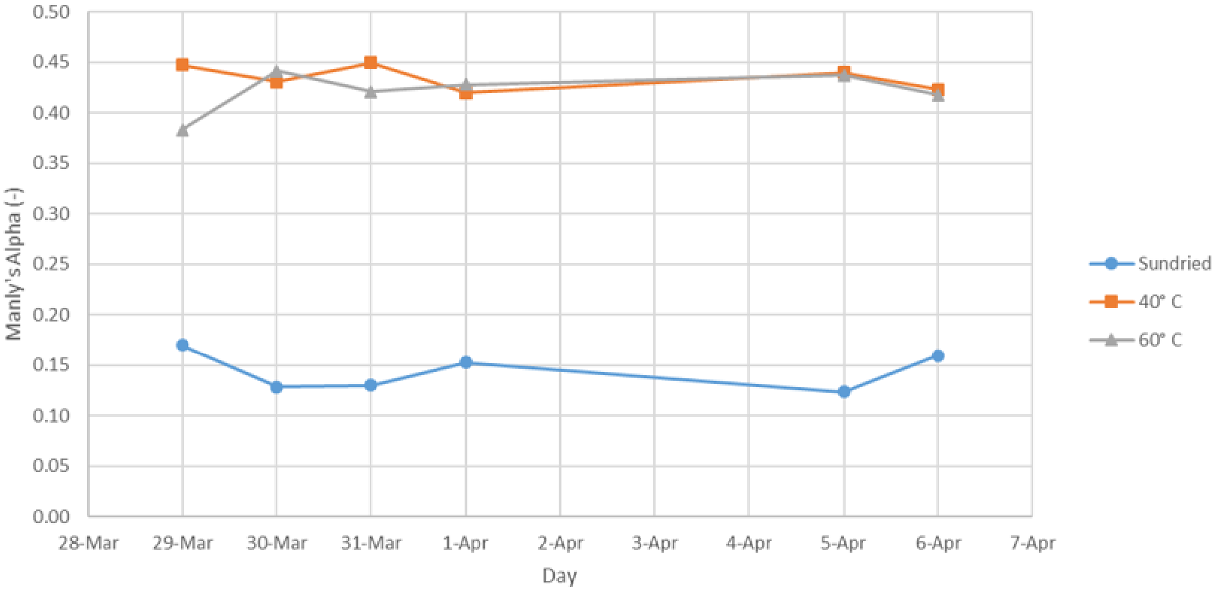


Figure 13: Manly's alpha over time for each preparation type of duckweed.

Discussion

The Nile tilapia fingerlings consistently consumed the sundried duckweed less than the two other preparation types. The ashy color of the sundried duckweed in comparison to the greener color of the 40° C and 60° C duckweed preparation temperatures may be an explanation. Indeed, the color of the feed has been shown to make a difference in fish feeding preference (Steele et al., 2014). Contrary to these results, certain literature claims that Nile tilapia fingerlings do not exhibit a preference to feed colors (El-Sayed et al., 2013). Additional color preference studies for Nile tilapia fingerlings may determine if the feed color affects preference. Another potential difference in preparation types may be feed odor. In the fishing industry, many anglers rely on odorous sprays to attract fish (BPS Direct, L.L.C., 2016). It is possible that the difference in odor between the preparation types of duckweed can influence the preference of the Nile tilapia fingerlings.

The sundried duckweed possessed hair-like fibers that forced it to clump together and form a concentration of duckweed over a single area (Figure 14). Looking up at the surface of the tank at the sundried duckweed, the Nile tilapia fingerlings may have seen that the mass of feed casts a shadow (larger than any individual fingerling), potentially resembling a large predator at the surface. The fingerlings may fear the massive shadow above them, and decide to keep their distance from the sundried duckweed. Adult Nile tilapia (24 inches) may be less fearful and more likely to consume the large clumps of sundried duckweed (tilapiafingerlings.com, 2016). Hence, the density of the feed may correlate to preference.

Figure 14: A photograph of the sundried duckweed amassed in heaps on the surface of the tank during a feeding trial. Note the absence of Nile tilapia fingerlings.

It was seen that competition for food was fierce during the feeding trials. Spatial and resource partition techniques aid the Nile tilapia fingerlings in decreasing competition. Spatial partitions decrease competition when one animal takes a piece of territory over another in hopes of establishing a safe refuge. Resource partitions decrease competition when an animal develops different feeding habits; for instance, a day versus night feeding habit or an aggressive versus a patient feeding style (Haynes et al., 2011). Fingerlings that claim the bottom of the tank may not respond well to the floating duckweed, and this presents a potential concern for future work.

The time separation between feeding trials presents another possible effect on the results. This study's feeding trials lasted four days straight, then followed a three-day break, and concluded with two more days of trials. The foraging rate of fish has been shown to develop an asymptote after an average of seven continuous days of feeding trials (Steele et al., 2014). Unfortunately, a true forage rate to determine the optimal preference of Nile tilapia fingerlings for duckweed could not be verified in this study because duckweed was not fed continuously for a long enough duration to develop an asymptote (Figure 12).

Lastly, outer environmental color may skew the feeding preference results. Although the Nile tilapia fingerlings used in these feeding trials satisfied the theoretical isolation time to acclimatize to their environment (3 weeks), the clear aquarium tanks may have disturbed the fingerlings' feeding habits, since background color (light disturbs more than dark) has been shown to affect the behavior of Nile tilapia (El-Sayed et al., 2013).

Chapter 5 Conclusions and Recommendations

The data from the feeding trials enabled some conclusions regarding the preference and avoidance of the Nile tilapia fingerlings for different preparations of duckweed. A longer continuous trial sequence (at least seven days) would help validate the results and conclusions of this experiment. To confirm the validity of this feeding preference study, I strongly recommend repeating the feeding trials several times to either confirm or deny the strength of the current data trends.

Conclusions

Hypothesis One - Sundried duckweed will have the lowest consumption rate by Nile tilapia fingerlings due to poor physical appearance, resulting in avoidance. Statistical analysis confirms this hypothesis as Manly's alpha for sundried duckweed indicates avoidance.

Hypothesis Two - The 40° C and 60° C duckweed consumption rates will be approximately the same, resulting in preference of Nile tilapia fingerlings for both. From this study, it is strongly evident that the 40° C and 60° C duckweed preparation types confirm hypothesis two. The causation of the statistical equality may be a result of the similar smells and appearances of the two types. However, odor and appearance factors require future studies to accurately confirm the causation of hypothesis two's confirmation.

Hypothesis Three - Given enough time and repeated exposure, the Nile tilapia fingerlings will adapt and readily consume any preparation type of duckweed. Unfortunately, hypothesis three cannot be confirmed nor denied because of the insufficient time of exposure of this short study. The minimum time for foraging adaptability averages about seven continuous days of feeding trials. Even though this study lasted only 4 straight days, the statistics show a clear trend among the data consumption rates versus time for all three preparation types of duckweed.

Recommendations

It is recommended that the trials be repeated to ensure that the possible errors did not sway the data. Due to the consistent avoidance of the sundried duckweed, I recommend to either modify it to add more appeal for the Nile tilapia fingerlings, or throw it out of the duckweed preparation arsenal altogether. Even though the preference for duckweed dried at 40° C and at 60° did not significantly differ statistically, I recommend that future optimization efforts focus on the 40° C preparation type since higher nutrition is retained at 40° C. Further work is needed to determine the energy requirements for drying duckweed at this temperature. Overall, I recommend the use of these results, and to continue with the implementation of the conceptual system for future work.

Future Work

From the results and conclusions of this feeding trial, future work by other students should include optimization of the 40° C duckweed and its incorporation into the "Future Self-sustaining Eco-Machine Aquaponics System" (Future SEAS) at Penn State. Future SEAS intends to optimize the management of food, energy, and water in an aquaponics system. The system remediates municipal wastewater, produces fish, and provides vegetables with a reduction in energy and water inputs relative to conventional systems. Overall, this study ensures a step in the right direction towards successful dietary inclusion of *Lemna minor* for *Oreochromis niloticus* in the Future SEAS system.

Appendix A: Duckweed collection, drying, and storage

Table 4: A compilation of the duckweed collected prior to the feeding trials. ID #1 was the 60° C duckweed, #4 was the 40° C duckweed, and #7 was the sundried duckweed chosen for the feeding trials.

ID	Location	Pre-dry Storage	Pre-dry Storage Time	Post-dry Storage	Post-dry Storage Time	Date Collected	Drying Method	Drying Temp	Drying Time	Duckweed Mass	Additional Drying
#	[-]	[-]	days	[-]	[-]	[-]	[-]	° C	days	g	[-]
1	Spray field	Fridge	15	Plastic bag in lab	5 months at room temp	10/8/2015	Oven	60	3	764.582	
2	Spray field			Plastic bag in lab	5 months at room temp	10/8/2015	Sundried	11.83	9	250.208	36 hours in a 40° C Oven
3	Spray field	Fridge	1.5	Plastic bag in lab	5 months at room temp	10/8/2015	Sundried	11.83	9	134.316	48 hours in a 40° C Oven
4	Spray field			Plastic bag in lab	5 months at room temp	10/8/2015	Oven	40	3	132.274	
5	Spray field			Plastic bag in lab	5 months at room temp	9/21/2015	Oven	60	3	1295.739	
6	Spray field			Plastic bag in lab	5 months at room temp	9/21/2015	Oven	40	3	101.884	
7	Spray field			Plastic bag in lab	5 months at room temp	9/21/2015	Sundried	11.41	9	34.298	

Appendix B: Governing Equations and Sample Calculations

Manly's alpha (trial 1, sundried):

$$\alpha_i = (\log P_i)/\sum_{i=1}^{m} log\, P_i$$

$$P_1 = \frac{e_1}{n_1} = \frac{1.217}{1.476} = 0.824$$

$$P_2 = \frac{e_2}{n_2} = \frac{0.917}{1.524} = 0.601$$

$$P_3 = \frac{e_3}{n_3} = \frac{1.018}{1.574} = 0.647$$

$$\alpha_{trial\ 1,\ sundried} = (\log 0.824)/(\log 0.824 + log 0.601 + log 0.647)$$

$$\alpha_{trial\ 1,\ sundried} = 0.17$$

Duckweed used (trial 1, sundried, Table 4):

(Falcon tube with duckweed mass) – (Falcon tube mass after feeding trial) = (Duckweed used in the feeding trial)

14.907 g – 13.431 g = 1.476 g

Duckweed collected (trial 1, sundried, Table 5):

(Weigh boat with dried duckweed) – (Empty weigh boat) = (Duckweed collected after feeding trial)

3.111 g – 1.894 g = 1.217 g

Fish mass (for Tank 1, Table 6):

(Fish 1 mass + Fish 2 mass + Fish 3 mass + Fish 4 mass) / (# of Fish weighed) = Average fingerling mass per tank

(8 g + 23 g + 5 g + 11 g) / 4 = 11.75 g

Duckweed (g) consumed (trial 1, sundried):

(Duckweed used) – (Duckweed collected) = (Duckweed consumed)

1.476 g – 1.217 g = 0.259 g

Duckweed (% by mass) (trial 1, sundried):

(Duckweed consumed) / (Duckweed used) = Duckweed consumed (% by mass)

(0.259 g / 1.476 g) * 100 = 17.56 %

Duckweed consumption rate (trial 1, sundried, Table 7):

Duckweed consumed (g) / Time of exposure (min) = Duckweed consumption rate (g/min)

(0.259 g / 15 min) = 0.017 (g/min)

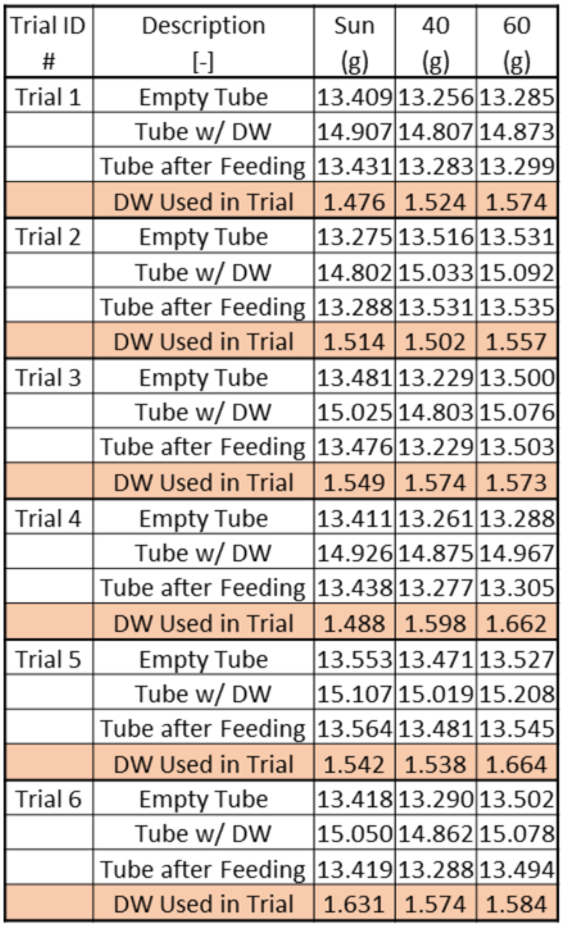
Duckweed consumed per mass (trial 1, sundried, Table 7):

Duckweed consumed (g) / Average fish weight (per tank) (g) = Duckweed consumed per fish mass (g/g)

(0.259 g / 11.75 g) = 0.022 (g/g)

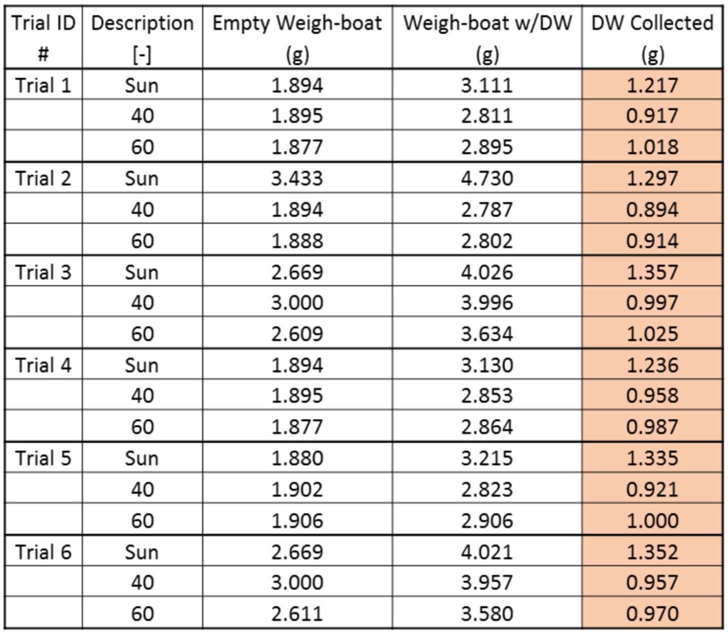
Appendix C: Raw Data

Table 5: Spreadsheet used to calculate the amount of duckweed (DW) used per feeding trial for each DW type.



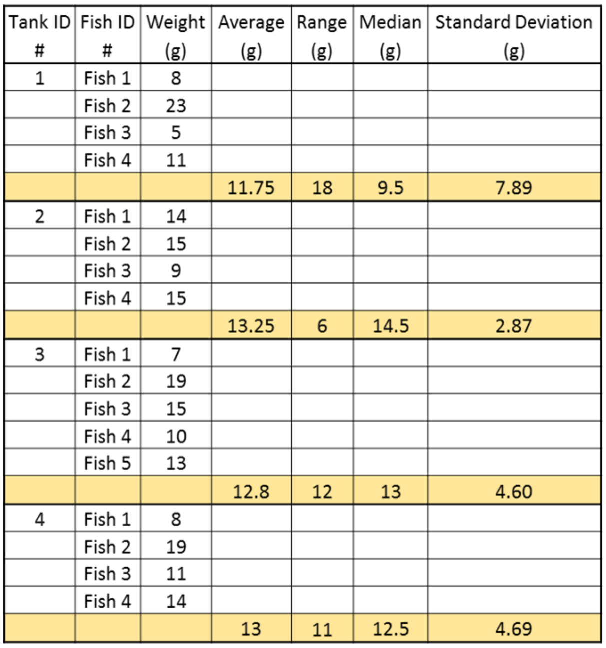
Trial ID #	Description [-]	Sun (g)	40 (g)	60 (g)
Trial 1	Empty Tube	13.409	13.256	13.285
	Tube w/ DW	14.907	14.807	14.873
	Tube after Feeding	13.431	13.283	13.299
	DW Used in Trial	1.476	1.524	1.574
Trial 2	Empty Tube	13.275	13.516	13.531
	Tube w/ DW	14.802	15.033	15.092
	Tube after Feeding	13.288	13.531	13.535
	DW Used in Trial	1.514	1.502	1.557
Trial 3	Empty Tube	13.481	13.229	13.500
	Tube w/ DW	15.025	14.803	15.076
	Tube after Feeding	13.476	13.229	13.503
	DW Used in Trial	1.549	1.574	1.573
Trial 4	Empty Tube	13.411	13.261	13.288
	Tube w/ DW	14.926	14.875	14.967
	Tube after Feeding	13.438	13.277	13.305
	DW Used in Trial	1.488	1.598	1.662
Trial 5	Empty Tube	13.553	13.471	13.527
	Tube w/ DW	15.107	15.019	15.208
	Tube after Feeding	13.564	13.481	13.545
	DW Used in Trial	1.542	1.538	1.664
Trial 6	Empty Tube	13.418	13.290	13.502
	Tube w/ DW	15.050	14.862	15.078
	Tube after Feeding	13.419	13.288	13.494
	DW Used in Trial	1.631	1.574	1.584

Table 6: Raw data for duckweed (DW) collected at the end of each trial per preparation feed type.



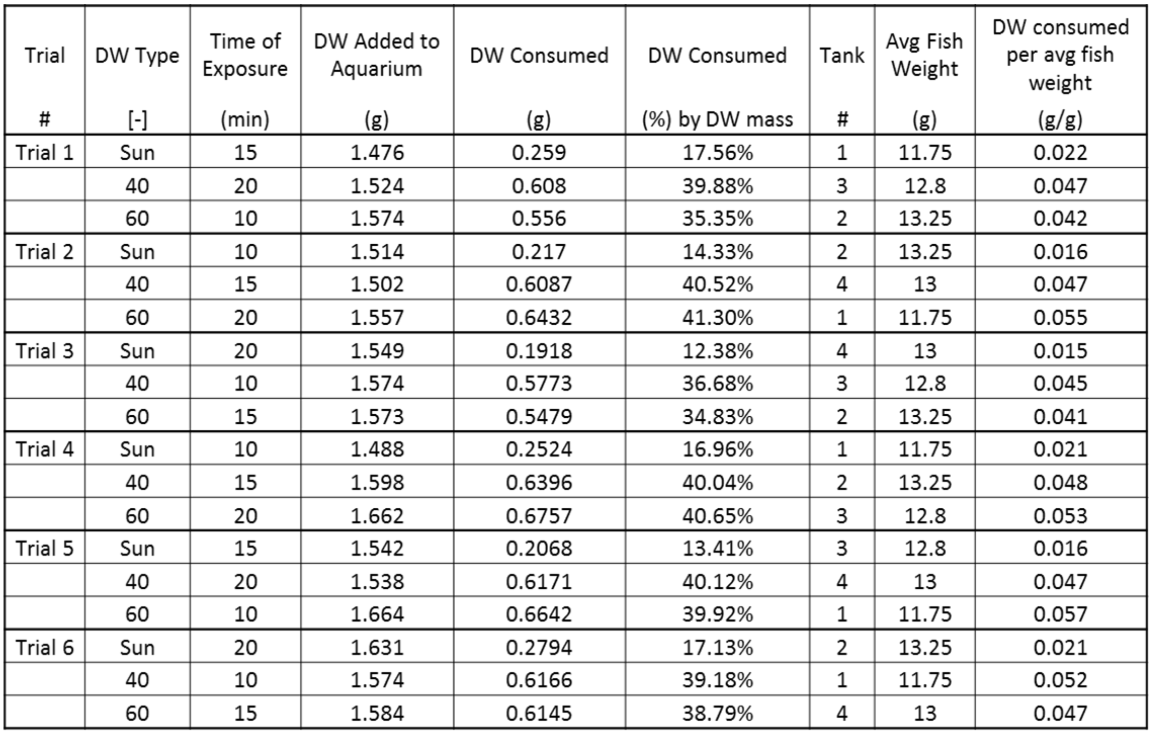
Trial ID #	Description [-]	Empty Weigh-boat (g)	Weigh-boat w/DW (g)	DW Collected (g)
Trial 1	Sun	1.894	3.111	1.217
	40	1.895	2.811	0.917
	60	1.877	2.895	1.018
Trial 2	Sun	3.433	4.730	1.297
	40	1.894	2.787	0.894
	60	1.888	2.802	0.914
Trial 3	Sun	2.669	4.026	1.357
	40	3.000	3.996	0.997
	60	2.609	3.634	1.025
Trial 4	Sun	1.894	3.130	1.236
	40	1.895	2.853	0.958
	60	1.877	2.864	0.987
Trial 5	Sun	1.880	3.215	1.335
	40	1.902	2.823	0.921
	60	1.906	2.906	1.000
Trial 6	Sun	2.669	4.021	1.352
	40	3.000	3.957	0.957
	60	2.611	3.580	0.970

Table 7: Raw data for calculating the average weight, range, median and standard deviation of the fingerlings in each tank after the trials.



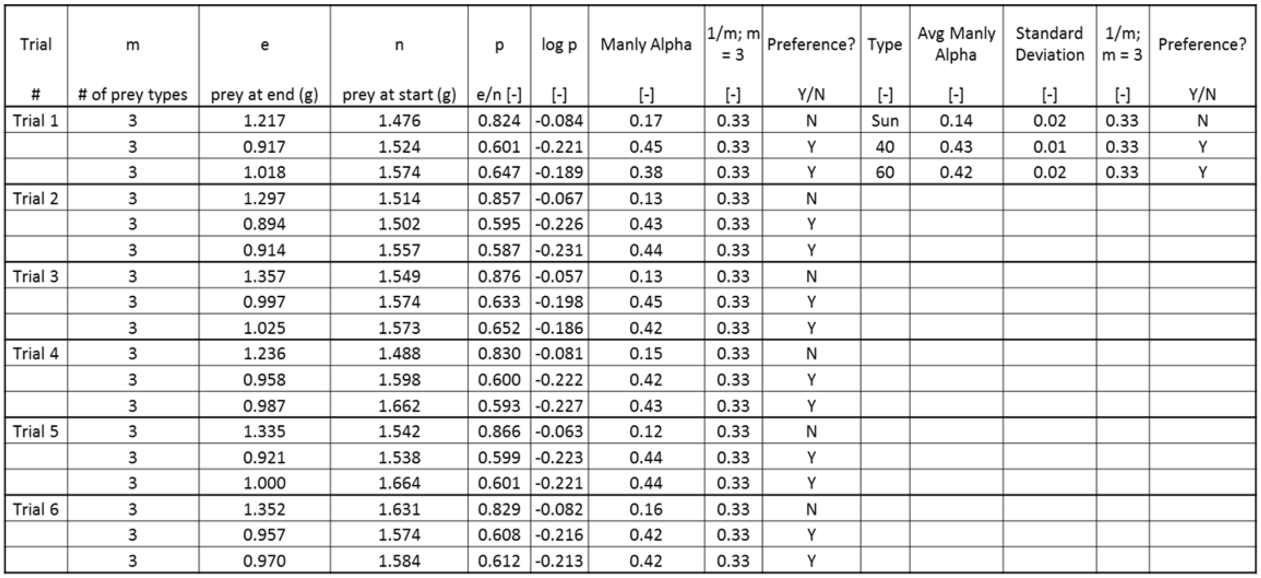
Tank ID #	Fish ID #	Weight (g)	Average (g)	Range (g)	Median (g)	Standard Deviation (g)
1	Fish 1	8				
	Fish 2	23				
	Fish 3	5				
	Fish 4	11				
			11.75	18	9.5	7.89
2	Fish 1	14				
	Fish 2	15				
	Fish 3	9				
	Fish 4	15				
			13.25	6	14.5	2.87
3	Fish 1	7				
	Fish 2	19				
	Fish 3	15				
	Fish 4	10				
	Fish 5	13				
			12.8	12	13	4.60
4	Fish 1	8				
	Fish 2	19				
	Fish 3	11				
	Fish 4	14				
			13	11	12.5	4.69

Table 8: Duckweed (DW) consumption calculations for each trial.



Trial #	DW Type [-]	Time of Exposure (min)	DW Added to Aquarium (g)	DW Consumed (g)	DW Consumed (%) by DW mass	Tank #	Avg Fish Weight (g)	DW consumed per avg fish weight (g/g)
Trial 1	Sun	15	1.476	0.259	17.56%	1	11.75	0.022
	40	20	1.524	0.608	39.88%	3	12.8	0.047
	60	10	1.574	0.556	35.35%	2	13.25	0.042
Trial 2	Sun	10	1.514	0.217	14.33%	2	13.25	0.016
	40	15	1.502	0.6087	40.52%	4	13	0.047
	60	20	1.557	0.6432	41.30%	1	11.75	0.055
Trial 3	Sun	20	1.549	0.1918	12.38%	4	13	0.015
	40	10	1.574	0.5773	36.68%	3	12.8	0.045
	60	15	1.573	0.5479	34.83%	2	13.25	0.041
Trial 4	Sun	10	1.488	0.2524	16.96%	1	11.75	0.021
	40	15	1.598	0.6396	40.04%	2	13.25	0.048
	60	20	1.662	0.6757	40.65%	3	12.8	0.053
Trial 5	Sun	15	1.542	0.2068	13.41%	3	12.8	0.016
	40	20	1.538	0.6171	40.12%	4	13	0.047
	60	10	1.664	0.6642	39.92%	1	11.75	0.057
Trial 6	Sun	20	1.631	0.2794	17.13%	2	13.25	0.021
	40	10	1.574	0.6166	39.18%	1	11.75	0.052
	60	15	1.584	0.6145	38.79%	4	13	0.047

Table 9: Manly's alpha statistical calculations for each preparation type of duckweed. Note the four columns at the right-most side of the spreadsheet: these columns contain the calculation for the overall Manly's alpha for all three preparation types of duckweed.



Trial	m	e	n	p	log p	Manly Alpha	1/m; m = 3	Preference?	Type	Avg Manly Alpha	Standard Deviation	1/m; m = 3	Preference?
#	# of prey types	prey at end (g)	prey at start (g)	e/n [-]	[-]	[-]	[-]	Y/N	[-]	[-]	[-]	[-]	Y/N
Trial 1	3	1.217	1.476	0.824	-0.084	0.17	0.33	N	Sun	0.14	0.02	0.33	N
	3	0.917	1.524	0.601	-0.221	0.45	0.33	Y	40	0.43	0.01	0.33	Y
	3	1.018	1.574	0.647	-0.189	0.38	0.33	Y	60	0.42	0.02	0.33	Y
Trial 2	3	1.297	1.514	0.857	-0.067	0.13	0.33	N					
	3	0.894	1.502	0.595	-0.226	0.43	0.33	Y					
	3	0.914	1.557	0.587	-0.231	0.44	0.33	Y					
Trial 3	3	1.357	1.549	0.876	-0.057	0.13	0.33	N					
	3	0.997	1.574	0.633	-0.198	0.45	0.33	Y					
	3	1.025	1.573	0.652	-0.186	0.42	0.33	Y					
Trial 4	3	1.236	1.488	0.830	-0.081	0.15	0.33	N					
	3	0.958	1.598	0.600	-0.222	0.42	0.33	Y					
	3	0.987	1.662	0.593	-0.227	0.43	0.33	Y					
Trial 5	3	1.335	1.542	0.866	-0.063	0.12	0.33	N					
	3	0.921	1.538	0.599	-0.223	0.44	0.33	Y					
	3	1.000	1.664	0.601	-0.221	0.44	0.33	Y					
Trial 6	3	1.352	1.631	0.829	-0.082	0.16	0.33	N					
	3	0.957	1.574	0.608	-0.216	0.42	0.33	Y					
	3	0.970	1.584	0.612	-0.213	0.42	0.33	Y					

Appendix D: Pictures

Figure 15: Duckweed collection materials: the green aquarium net is used for collecting the duckweed out of the trial tanks, the plastic bag is used to transport the duckweed to the lab, and the (credit card sized) card on the right is used for size reference.

Figure 16: Aquarium setup for Nile tilapia feeding trials (College of Agricultural Science, Greenhouse D. Section 10, Penn State University).

Appendix E: Observations of Possible Errors During this Study

Several errors are suspected to have occurred throughout this study based on observations made by the researcher during the feeding trials.

- The timing of when the control food is given to the control fish tank matters greatly. The fish have a line of sight to one another and when one tank begins their frenzy the other tanks begin their frenzy as well. Overall, the fish are influenced by the other tanks around them. For complete isolation, veils could be used to keep the vision of one tank away from the vision of the other tank.
- When collecting the uneaten duckweed out of the tank, it was observed that fish feces had risen to the top and inevitably scooped up into the net along with the uneaten duckweed. This fact could underestimate the amount of duckweed consumed at any given tank.
- The waterfall filter had to be turned off prior to each trial so that the duckweed did not become lodged in the filtration system and jeopardize the health of the fish. This change in scenery could stress the fish out and cause them to eat much less feed.
- Another group of students would feed the fish in the morning several hours before I would conduct my afternoon trial. It is a possibility that the morning group of students could either under or over feed the fish which could influence how the fish feed in the afternoon.
- Attempts were made, but not perfected, at minimizing the feed inclusion lag when switching between tanks. Starting the timer as well as feeding all of the tanks at the same time was not possible. Between ten and thirty seconds would pass as the feeder switched between tanks.

- The exact time of the feed trial varied by about an hour and a half at the most day-by-day due to scheduled classroom commitments. The relative hunger of the fish could fluctuate slightly from the inconsistency of feeding time.
- The sporadic movement of the researcher around the tanks during the feeding may have caused certain levels of stress that affect the fish.
- On several occasions, all of the duckweed from the day before could not be logistically harvested. Although small, the leftover duckweed from the day before could alter the collection mass or even the hunger of the fish (assuming the fish have been nibbling on the duckweed.)
- Stress may also be a by-product of the duckweed recollection after the allotted feeding time. The swirling around of a net in their environment may cause the fish to become stressed and alter their normal feeding behavior.

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Academic Vita

Cody J. Campolong cjc5575@gmail.com

Education:

The Pennsylvania State University ***Expected Graduation May 2016***

- Bachelor of Science in Civil Engineering
- Minor in **Environmental Engineering**
- **Schreyer Honors College** 2013-Present
- Technical Skills: CAD, C++, Microsoft Office, Creo Parametric, PSpice, HEC-RAS

Penn State Activities:

Chi Epsilon Member	2014-Present
The National Society of Leadership and Success	2014-Present
ASCE Member	2014-Present
Behrend Honors Program	2012-2014
Kanzius Cancer Research Foundation Fundraiser	2013-2014
Lambda Sigma Vice President of Advertising	2014

Penn State Relevant Courses:

- Acid Mine Drainage Design Capstone
- Environmental Microbiology in Engineering
- **Water and Wastewater Treatment**
- Solid and Hazardous Waste/Land Based Waste Disposal
- **Open Channel Hydraulics**

Work Experience:

Penn State College of Engineering Teaching Intern Program **August 2015-December 2015**

- Collaborated with Environmental Engineering Professor in Lecture
- Developed problem set tutorials via pencasts
- Held weekly troubleshoot and Q&A review sessions

Nicholson Construction Internship (2 Summers) **May 2014-August 2015**

- Initiated Scoping and Quantification (Takeoffs)
- Conducted Jet Grouting Design, and Soil Boring Analysis (SELA 26)
- Extensive Soil Profile and Micro-pile Design Experience
- Networked with companies in order to receive price quotes in a timely fashion
- Deep Soil Mixing data compilation, design, and lab testing
- Passed Required 10-hr Safety OSHA Course Exam

CONSOL Energy Internship **May 2013-August 2013**

- Passed Required 80-hr Safety MSHA Course Exam
- Utilized Engineering and Business education to develop time studies to analyze the business component
- Hands on labor, safety, and regulatory experience in an underground coal mine (Enlow)

Penn State Awards:

John Deere Scholarship	2015-Present
Schumacher Scholarship	2015-Present
Walter J. Kinsey Scholarship	2014-Present
Dean’s List	2012-2015
Schreyer Gateway Scholarship	2013-2015
Pennsylvania American Water Scholarship	2012