EVALUATION OF NITROGEN EXCRETION BY FISH BASED UPON DIETARY PROTEIN CONTENT AND NITRATE UPTAKE BY BASIL IN FLOATING RAFT AQUAPONIC SYSTEMS

A Thesis

by

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ABSTRACT

In aquaponic systems, nitrate production and uptake rates must be tailored to one another to prevent costly imbalances. Consequently, the objective of this research was to provide additional management guidance for floating raft aquaponic production systems growing Italian large-leaf basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR').

Experiment 1 focused on establishing ammonia excretion rates based on protein intake of tilapia (Oreochromis spp.), koi (*Cyprinus carpio haematopterus*), and hybrid striped bass (*Morone chrysops* x *M. saxatilis*). Data collected during the experiment showed respective ammonia excretion rates for tilapia, koi, and hybrid striped bass of 50.9, 64.5, and 79.0 mg TAN per gram of protein ingested, though feed digestibility potentially affected some of the measurements.

Experiment 2 attempted to identify nitrate uptake rates for individual Italian large-leaf basil plants grown hydroponically with an observed yield of 85.6 grams of fresh shoot mass for every 342.9 mg of available (NO_3^-)-N. Calculations using a maximum ammonia excretion rate of 92.2 mg TAN per gram of protein, and an ideal nitrification rate of 0.976 grams of nitrate (NO_3^-) per gram ammonia, indicated a protein input requirement of 44.4 mg per each gram of shoot material. The use of ammonia excretion measurements from Experiment 1 resulted in estimated protein input requirements of 80.6, 63.6, and 52.0 mg of protein for tilapia, koi, and hybrid striped, respectively.

Experiment 3 evaluated dissolved nitrogen availability for marketable basil culture in floating raft aquaponic systems using recommended feeding and fish stocking rates. While the feed input to the aquaponic systems ended up being even less than recommended, a surplus of nitrogen at the conclusion of the experiment indicated that feed input requirements for herb and leafy green production in aquaponic systems is much lower than recommendations. Additionally, feed input calculations using projected nitrogen production and uptake rates were even lower, with a requirement of only 16.5 grams of 32% protein feed per day to produce 3338.8 grams of fresh shoot material over 28 days. Subsequently, actual protein input requirements for basil were estimated to be between 44.4 and 67.5 mg protein per each gram of shoot mass to be produced.

DEDICATION

I would like to dedicate this thesis to my dad, Leonard Rotter Jr. I wish I had graduated sooner so you could have shared in the overwhelming joy of finally completing my Master's, but I know you'll be looking down with a grin on that long-awaited day.

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Contributors

This work was supervised by a thesis committee consisting of chair and co-chair Drs. David Reed and Daniel Leskovar of the Department of Horticultural Sciences, and Dr. Todd Sink of the Department of Rangeland, Wildlife and Fisheries Management.

All other work conducted for the thesis was completed by the student independently.

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INTRODUCTION AND LITERATURE REVIEW

Introduction

As the global population continues to increase, intensive agricultural production methods have become more commonplace to provide for the burgeoning populace’s nutritional requirements. In recent history, concentrated animal feeding operations (CAFO) and hydroponic production of food crops have become some of the most utilized methods to help meet this need. While these practices have the potential to provide an adequate amount of sustenance to satisfy global demand, they are not without significant resource requirements and ecological drawbacks (Brondizio et al., 2019, Loconto et al., 2020). Concentrated waste streams and runoff from these production methods impact environmental water quality and require costly mitigation measures (Brondizio et al., 2019, Mózner et al., 2012). Additionally, regional feasibility is often limited due to the substantial amounts of water required to operate these types of production methods.

Current Trends in Fish Production

From 1961 to 2017 global food fish consumption increased annually at an average rate of 3.1%, in comparison to all other animal proteins (meat, eggs, milk, etc.) combined which increased by 2.1% annually (FAO, 2020). As a result of increasing per capita fish consumption, fisheries and aquaculture activities have continued to shift toward more intensive harvest and production methods, with inland and marine capture fisheries seeing a collective increase of 11% in production between 1995 to 2018, while their aquaculture counterparts experienced a 451% increase (FAO, 2020). During this time, inland aquaculture production sustained the largest individual increase of 497%, while marine capture fisheries only recorded a growth rate of 5% (FAO, 2020). As of 2018, 52% of global food fish consumption was provided by aquaculture, with that share projected to increase to 59% by 2030 (FAO, 2020). This continued shift toward intensive aquaculture production is in large part due to the fraction of wild fish stocks being harvested within biologically sustainable levels decreasing from 90% in 1974 to 65.8% in 2017 (FAO, 2020).

According to the USDA Census of Aquaculture, pond culture was used as a production method by approximately 41% of aquaculture farms in the United States in 2018, down from 48% in 2013 (USDA, 2018). Standard pond production may require as much as 9347 cubic meters of water per hectare annually to replenish loss from seepage and evaporation (Rakocy et al., 2006) which limits regional feasibility to areas with ample access to suitable water sources. While pond production is currently the most common method for global inland aquaculture production, recent advances in recirculating aquaculture systems with higher water use efficiencies have begun receiving greater interest (FAO, 2020). In order to minimize water use, these recirculating aquaculture systems typically employ maximum harvest densities up to 60 kg per cubic meter, compared to harvest densities of 0.6 to 1.2 kg per cubic meter commonly seen in pond production (Rakocy et al., 2006).

These differences in stocking rates and water requirements are a direct result of each production method’s ability to maintain water quality in response to oxygen demand and feed input. Feeding rates, composition of feeds, individual species metabolic rates, and the amounts of wasted feed all impact water quality (Losordo et al., 1998). While feed composition and feeding rates are dictated by the species and age of the cultured fish stock, substandard environmental conditions resulting from excess dissolved nitrogen can lead to physical stress which affects feed intake and metabolic activity. As a result, aquaculture systems must be closely monitored to detect problems early enough to maintain adequate growth rates and prevent catastrophic loss (Beem, 2014). In indoor recirculating aquaculture systems environmental variables are regularly adjusted to suit the cultured species and ensure proper feed consumption, whereas manipulating these conditions is more difficult in pond culture due to larger water volumes and exposure to seasonal temperature changes and adverse weather (Beem, 2014). Reductions in feed intake are also not as readily observed in pond production as uneaten feed sinks below the surface, unlike recirculating aquaculture systems (RAS) where it is automatically removed from circulation and easily viewed in mechanical filtration basins.

In animal feeding operations, the term feed conversion ratio (FCR) refers to an animals' ability to convert feed into body mass. Aquatic organisms, such as fish, traditionally have much lower FCRs than land-based livestock due to some key differences in physiology and habitat. For example, tilapia (*Oreochromis spp.*) commonly have FCRs in the range of 1.4:1 to 1.8:1, which are considered some of the best in animal agriculture (DeLong et al., 2009). According to the Mississippi Agricultural & Forestry Experiment Station, cold-blooded (poikilothermic) aquatic organisms have lower FCRs than their terrestrial counterparts because they do not maintain a constant body temperature, require less energy to maintain position due to buoyancy, experience lower energy losses through gill excretions and urine, and have lower energy costs associated with ingested feed (Robinson and Li, 2015). Differences in dietary requirements between species and age classes also impact total feed inputs which account for a large portion of variable operational expenses. In the United States, feed costs for commercial pond production of catfish has historically accounted for approximately half of all variable operating costs and as of 2015 were closer to 60% (Robinson and Li, 2015). As a result, feed efficiency plays a major role in determining the economic viability of any animal farming operation.

Current Trends in Food Crop Production

Just as food animal production methods have increasingly focused on minimizing input requirements while maximizing production, so too have techniques for vegetable food crop production. Controlled environment agriculture (CEA) refers to the production of crops where the environment has been altered to make it more hospitable for plant growth. These alterations typically involve the use of greenhouses, high tunnels, hoop houses, and indoor facilities to better control the environment. Despite the additional expense of constructing and operating these structures, the resulting uniformity of crop production, protection from crop loss due to adverse weather, and decreased pest and disease pressures have made CEA an appealing alternative to field production. From 1988 to 2014 the wholesale value of controlled environment food production in the United States increased from USD$64.2 million to USD$796.7 million adjusted for inflation (AFI) (Walters et al., 2020). According to the USDA's 2019 Census of Horticultural Specialties, the total value of sales (AFI) from operations growing food crops under protection has increased 2,442% since 1979 from USD$27.7 million to USD$703.5 million with 54% of production in 2019 accounted for by hydroponic production methods (USDA, 2019).

Hydroponics is traditionally defined as the cultivation of plants in nutrient solution without the use of soil, although some soilless substrate growing methods common to potted plant production may occasionally be included (Walters et al., 2020). This method of crop production has several advantages over field agriculture, many of them derived from its ability to maintain optimum nutrient, water, and aeration levels (Khan et al., 2021). Differences in water retention and cation exchange capacities of field soil can subject crops to fluctuating water and nutrient availability and limit plant production due to resource competition. In contrast, hydroponic systems continually bathe plant roots in nutrient solution which increases crop uniformity, shortens production windows, and facilitates denser plant spacings (Khan et al., 2021, Barbosa et al., 2015). Additionally, recirculating hydroponic systems capture excess nutrient solution for reuse, reducing overall nutrient and water use to approximately 8% of the requirements for field agriculture (Barbosa et al., 2015).

In order to compensate for the continual loss of water and nutrients to leaching, soil-based crop production techniques must often fertilize and irrigate more frequently, leading to a corresponding increase in production expenses associated with the additional labor and fertilizer input requirements. Although hydroponic systems do reduce several of the variable costs commonly associated with field agriculture (e.g. labor, water consumption, nutrient use), they typically require larger fixed costs in the form of equipment and facilities. Additionally, climate control systems, supplemental lighting, and other equipment associated with hydroponics can increase energy expenses to 82 times that of field production (Barbosa et al., 2015), subsequently deterring producers from adopting these production methods.

Integrated Food Production

The most detrimental waste product generated in recirculating aquaculture systems is ammonia, a majority of which originates from fish metabolism of feed and turnover of muscular protein. Since ammonia is extremely toxic to aquatic organisms, recirculating aquaculture systems commonly use the nitrification cycle to facilitate its conversion to nitrate, which is also the preferred form of nitrogen for plant uptake. Intensive recirculating aquaculture systems usually remove and replace between 2% to 10% of their total system volume per day (Ebeling et al., 2006), but when this water exchange rate is reduced to less than 2%, these dissolved nutrients rapidly accumulate to concentrations that rival those found in hydroponic nutrient solutions (Rakocy et al., 2006). Additionally, mutual facility and equipment requirements allows expenses to be split between vegetable and fish crops (Tyson et al., 2011, Rakocy et al., 2006). Also, vegetable production in these integrated systems typically only requires 10% of the water needed for comparable field agriculture (Goddek et al., 2015). These realizations garnered integrated systems even greater interest as a sustainable alternative to recirculating aquaculture and hydroponic systems, leading more producers to begin integrating RAS and hydroponic systems in what became commonly referred to as aquaponic systems (Goddek et al., 2015, Love et al., 2015).

As recirculating aquaculture and hydroponic systems continued to be coupled together, different types of hydroponic sub-systems were discovered to be better suited for certain crops and environmental conditions with limited applicability for others. Disparities in crop suitability, and differences between systems in the complexity of their design and the associated expense for construction and operation play a major role in selecting an appropriate hydroponic sub-system for integration. According to a 2013 survey of commercial aquaponic producers (Love et al., 2015), the percentage of producers utilizing each type of hydroponic sub-system (with some producers utilizing multiple production systems) were as follows: floating rafts (77%), media beds (76%), nutrient film technique (29%), vertical towers (29%), wicking beds (6%), and Dutch buckets (5%). The same survey determined the following usage rates for several different vegetable types amongst respondents: basil (81%), salad greens (76%), non-basil herbs (73%), tomatoes (68%), head lettuce (68%), kale (56%), chard (55%), bok choi (51%), peppers (48%), and cucumbers (45%) (Love et al., 2015).

These findings were not surprising as floating raft production is a preferred sub-system for lettuce, herbs, and other leafy green vegetable crops that typically utilize a staggered crop production cycle (Rakocy et al., 2006). Also, a higher proportion of media bed use can be attributed to its suitability for cultivation of fruiting crops (e.g tomatoes, peppers, cucumbers, eggplant) where batch production cycles and the need for substrate to anchor top-heavy plants are typical (Rakocy et al., 2006). Consequently, leafy crops seem to have an advantage over fruiting crops in aquaponic production due to their relatively short production cycles from seeding to harvest, generally low nutrient requirements, and minimal nutrient assimilation for the growth of inedible plant material. Whereas the entire aboveground portion of most leafy crops is edible, a study investigating nitrate uptake rates for aquaponic tomatoes found that only 69% of nitrogen uptake could be converted into edible fruit mass (Graber and Junge, 2009). Additionally, research conducted at the University of the Virgin Islands showed that the greatest annual incomes for their commercial floating raft system was obtained through the cultivation of herbs and leafy greens, while crops like cantaloupe and okra generated minimal income (Rakocy et al., 2011).

Nitrate Production & Uptake

The accumulation of nitrogenous compounds in aquaculture systems presents one of the greatest roadblocks to increasing production, second only to dissolved oxygen (Ebeling et al., 2006). Most ammonia present in aquaculture systems originates from the catabolism of protein and subsequent excretion of ammonia and other nitrogenous byproducts by aquatic organisms (Ebeling et al., 2006, Rakocy et al., 2006). Catabolism of protein is mostly the result of feed metabolization with a smaller portion attributed to the turnover of muscular protein. Following excretion, a portion of the un-ionized ammonia (NH_3) is converted to ionized ammonium (NH_4^+) and the sum of the two is referred to as total ammonia-nitrogen (TAN).

The proportions of these two types of ammonia are determined as a function of pH, temperature, and to a much lesser degree by salinity (Emerson et al., 1975). As pH and temperature increase, the proportion of TAN accounted for by un-ionized ammonia increases, while increases in salinity cause ammonium to become more prevalent. As TAN concentrations increase, established colonies of autotrophic (self-feeding) ammonia oxidizing bacteria species utilize it as an energy source and produce nitrite (NO_2^-), with autotrophic nitrite oxidizing bacteria species ultimately converting it to nitrate, the preferred form for plant uptake (Losordo et al., 1998).

Fecal waste and uneaten feed also contribute to ammonia accumulation, but in systems with adequate solids filtering capacities this waste is quickly removed to prevent this. Usually settleable solids (>100 microns) are captured and removed from systems, though some suspended solids (<100 microns) may be allowed to remain so they can further decompose into inorganic nutrients through a process called mineralization (Rakocy et al., 2006). Although nitrification by autotrophic bacteria is the preferred mechanism for controlling ammonia in aquaponic systems, other nitrogen conversion pathways may also be present as a result of differences in solids removal efficiencies. Photoautotrophic algae that use photosynthesis to assimilate nitrogen, and heterotrophic (obligate consumer) bacteria that persist in systems with elevated carbon to nitrogen ratios may reduce the overall availability of nitrate for plant growth through competition with autotrophic bacteria for dissolved nitrogen (Ebeling et al., 2006).

The nitrate uptake rates of vegetable crops grown in aquaponic systems can differ drastically depending on species, culture method, and numerous other environmental and climatic conditions. Varying nitrogen ratios and total concentration of the solution can affect root growth (Wang and Shen, 2012) and subsequent nutrient uptake as a factor of total root surface area. Inadequate solids filtration can lead to an excessive buildup on root surfaces, reducing available oxygen and impacting plant growth (Rakocy et al., 2006). Additionally, observed nitrate uptake rates can be misleading as certain environmental conditions like reduced light intensity can inhibit nitrate assimilation and consequently increase nitrate accumulation (Wen Ke and Qi Chang, 2012). The loss of nitrate via denitrification can also lead producers to infer a greater plant requirement than actually exists. Complicating matters further, due to the relatively young age of science-based aquaponic production, studies involving hydroponic production are commonly used as production guides even though recommended nutrient solution compositions and ranges can vary greatly.

While there have been some aquaponic trials regarding plant nitrate uptake calculations, due to the specificity of the research system design and combination of fish and plant species they provide narrow applicability. Research involving nitrate uptake rates for cucumber, eggplant, and tomato were conducted in Switzerland at the Zurich University of Applied Sciences, but provided limited applicability as it involved the use of a proprietary planted trickling filter design (Graber and Junge, 2009). Another aquaponic trial attempted to determine romaine lettuce nitrate uptake, but the study experienced significantly elevated levels of denitrification, making daily nitrate uptake per plant difficult to accurately calculate (Seawright et al., 1998). Along with potentially limited system applicability, nitrate depletion trials in aquaponic formats are difficult to accurately assess as ammonia and nitrate are continually produced as a result of daily fish feeding practices, while differences in solids removal efficiencies can lead to the loss of nitrate via denitrification which might mistakenly be attributed to plant uptake.

Summary

In aquaponic systems, nitrate production and uptake rates are intrinsically linked and must be tailored to each other to prevent costly imbalances to the system. If nitrate production is not sufficient to satisfy plant requirements, then plants become nutrient deficient and can experience stunted growth and lengthened production windows. Nutrient deficiencies can also increase the risk of a total crop loss as deficient plants are more susceptible to pests and disease, while longer production windows increase the likelihood these pests and diseases have enough time to establish a damaging presence. Conversely, the cost of excessive nitrate production from feeding fish more than necessary to produce vegetable crops is expensive, wastes nutrients, and leads to decreasing water quality, an increased risk of fish death, and associated production losses.

The objective of this research was to provide additional management guidance for a floating raft aquaponic production system in Texas growing Italian large-leaf basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR'), in conjunction with tilapia, hybrid striped bass (*Morone chrysops* x *M. saxatilis*), or koi (*Cyprinus carpio haematopterus*). Studies were conducted to further evaluate plant to fish biomass ratios in relation to five different feeding regimens utilizing varying protein percentages. Plant to fish biomass

ratios were evaluated using the ammonia production measurements of each fish species/feed protein concentration pairing, in conjunction with the measured nitrate depletion rate of basil in a recirculating hydroponic floating raft system. The intended outcome of this research was the further development of practical production guidance that could be utilized by aquaponic producers to minimize excessive nutritional inputs.

CHAPTER II

MATERIALS AND METHODS

Experiment 1: Ammonia Production by Tilapia, Koi, and Hybrid Striped Bass in Response to Dietary Protein Inclusion and Feeding Rate

The objective of this experiment was to establish ammonia excretion rates for three commonly cultured fish species, when provided commercial fish feeds with varying levels of dietary protein, so that excessive nitrate level fluctuations in aquaponic systems could be mitigated through alterations to the feeding regimen. Fish from each species were initially separated by weight into 10-gram ranges (0 – 10 grams, 10 – 20 grams, 20 – 30 grams), etc.), then an equal number of fish of approximate equal weights (Table 1) were stocked into eighteen, 37.9 L tanks located indoors at the Texas A&M University Aquaculture Research and Teaching Facility (ARTF). Each tank was cleaned and sanitized with Virkon^{TM} AQUATIC (Syndel, Ferndale, Washington) disinfectant beforehand to minimize residual nitrifying bacteria that might alter ammonia measurements. All tanks were filled using well-water, and initial measurements of total alkalinity and hardness were taken. Total ammonia nitrogen (TAN) and pH of each tank, and the temperature of one tank selected at random were also measured using a portable ammonia spectrophotometer (Hanna Instruments HI96715), handheld pH meter, and digital thermometer, respectively. All subsequent measurements were taken at 24-hour intervals over a period of 96 hours, immediately prior to each day's feeding.

Table 1: The initial stocking numbers and mean weights (± standard error) of tilapia (*Oreochromis spp.*), koi (*Cyprinus carpio haematopterus*), and hybrid striped bass (*Morone chrysops* x *M. saxatilis*) used to determine ammonia excretion rates for each species

Fish Species	Number of Fish	Total Weight of Fish (g)
Tilapia	**5**	**102.42±5.24**
Koi	**6**	**153.62±10.32**
Hybrid Striped Bass	**5**	**301.51±12.79**

Six dietary treatments with differing protein inclusion rates were each randomly assigned to 3 tanks for replication. A control group received no feed (0%) to establish basal ammonia excretion from muscle protein turnover, and five commonly used commercial aquaculture feeds with protein percentages of 28%, 32%, 35%, 40%, and 44% were evaluated (Table 2). Fasted fish were included as a control since they closely represent endogenous ammonia excretion (Brett and Zala, 1975, Fromm, 1963), and dietary protein intake has been shown to be the most significant driver of ammonia excretion (Altinok and Grizzle, 2004, Yang et al., 2002, Tidwell et al., 1996). Fish were fed daily to apparent satiation over a 20-minute interval, and any uneaten feed pellets were removed and tallied following each feeding session. Total daily feed consumption per tank was determined by measuring the pre-feeding weight of each tank’s feed container, then taking post-feeding weight measurements after replacing uneaten feed pellets with an equivalent number of fresh pellets.

Table 2: The product names and ingredient composition of the five aquaculture feeds used as feed treatments to determine ammonia excretion rates of tilapia (*Oreochromis spp.*), koi (*Cyprinus carpio haematopterus*), and hybrid striped bass (*Morone chrysops* x *M. saxatilis*)

Rangen™ Product Name	Crude Protein (Min)	Crude Fat (Min)	Crude Fiber (Max)	Phosphorus (Min)	Ash (Max)	Pellet Size (inches)
Catfish 28%	**28.0%**	**5.0%**	**6.0%**	**1.0%**	**10.0%**	**1/4**
Production 32	**32.0%**	**6.0%**	**6.0%**	**0.9%**	**10.0%**	**1/8**
Production 35	**35.0%**	**6.0%**	**5.0%**	**0.9%**	**9.0%**	**5/32**
EXTR 400	**40.0%**	**9.0%**	**5.0%**	**1.0%**	**12.0%**	**1/8**
EXTR 450	**44.0%**	**15.0%**	**3.0%**	**1.0%**	**12.0%**	**3/32**

Total ammonia nitrogen (TAN) measurements from each tank were then used to calculate nitrification by autotrophic bacteria under ideal conditions using the following conversion rate:

Nitrification yields approximately 0.976 g (NO3-)-N per 1 g (NH4+)-N (Ebeling et al., 2006)
1 g = 6.022 E+23 daltons (amu)
0.976 X 6.022 E+23 (amu) = 5.8776 E+23 amu (NO3-)-N per 1 g (NH4+)-N

5.8776 E+23 amu (NO3-)-N ÷ 14.0067 amu per atom N = 4.196277496 E+22 atoms N
4.196277496 E+22 X (15.999 amu per atom O X 3) = 2.0140873 E+24 amu O
5.8776 E+23 amu (NO3-)-N + 2.01408731 E+24 amu O = 2.6018473 E+24 amu (NO3-)
2.60184731 E+24 amu (NO3-) = 4.3204687247541517792 g or 4320.47 mg (NO3-)
Therefore, nitrification yields approximately 4320.47 mg (NO3-) per 1 g (NH4+)-N

Experiment 2: Nitrate Uptake by Italian Large-Leafed Basil (Ocimum basilicum var. 'Rutgers Obsession DMR')

The objective of this experiment was to determine the nitrate uptake rate of Italian large-leaf basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR') by measuring the depletion of nitrate (NO_3^-) in five parallel floating raft recirculating hydroponic systems. The 21-day trial was conducted in a plastic high tunnel at the Texas A&M University Horticulture Teaching, Research, and Extension Center (HortTREC) from 13 May to 3 June 2021. Each hydroponic system (Figure 1) was constructed using one 56.8 L painted glass aquarium, a trickling biofilter comprised of one 18.9 L bucket housing PRO Koi Pond filter pad media (Koral Filters) and 264 Bio-Pin Balls (Lee's Aquarium & Pet Products), and one 416.4 L HDPE livestock tank as a hydroponic trough. The DWC raft material was 5.1 cm extruded polystyrene with seventeen 5.1 cm holes on a

20.3 cm staggered spacing recommended by the seed producer (Johnny's Selected Seeds; Winslow, Maine)**.** A 102-watt air pump (VIVOSUN Model 009) continuously supplied oxygen to the aquariums and hydroponic troughs in each system through Top Fin large air stones. The nutrient solution described below flowed via gravity from the aquarium into the trickling biofilter before draining into the hydroponic trough where an 800 liters per hour (LPH) water pump (Aleko Multi-Function Pump Model G2950) returned the solution to the aquarium.

Figure 1: The floating raft hydroponic system during initial setup and plumbing leak test

Each aquaponic system was filled with 416.4 L of rainwater and 246.7 g Botanicare CNS17 Grow Formula 3-1-2 for a calculated initial nitrate (NO_3^-) concentration of 75 mg/L, in addition to 3.17 mg/L of nitrate contained within the rainwater. The use of Botanicare CNS17 Grow Formula 3-1-2 for the nutrient source was recommended by Dr. Daniel Leskovar of Texas A&M University, as it had been previously utilized in trials involving the hydroponic culture of leafy greens with no adverse effects. Calculations to determine the nutrient solution mixing rate were as follows:

4.08 kg net weight (per 3.78541 L CNS17)
3% Nitrogen (2.86% as nitrate-N, 0.14% as ammoniacal-N)
4.08 kg X 0.0286 = 0.116688 kg or 116.6880 g N (as nitrate-N)
116.6880 g N = 7.0271162 E+25 daltons (amu) N
7.0271162 E+25 amu N ÷ (14.0067 amu per atom N) = 5.016967737 E+24 atoms N
5.016967737 E+24 X (15.999 amu per atom O X 3) = 2.407994005 E+26 amu O
7.0271162 E+25 amu N + 2.407994005 E+26 amu O = 3.110705625 E+26 amu NO3-
3.110705625 E+26 amu NO3- = 516.5448 g NO3- (per 3.78541 L CNS17)
416.395 L X 75 mg NO3-/L (ppm) = 31229.625 mg or 31.2296 g NO3- (per 416.395 L)
31.2296 g NO3- ÷ 516.5448 g NO3- = 0.06046 or 6.046 %
0.06046 x 4.08 kg CNS17 = 0.2466768 kg or 246.6768 g CNS17 (per 416.395 L)

An initial nutrient solution sample from all five systems, along with a rainwater sample, were submitted to the Texas A&M Soil, Water, and Forage Testing Laboratory to determine their pre-transplant measurements for conductivity (EC and TDS), pH, Na, Ca, Mg, K, CO_3^{2-}, HCO_3^-, SO_4^{2-}, Cl^-, P, B, Nitrate-N (NO_3^--N), hardness, and alkalinity. The nutrient solutions of each system were sampled daily for nitrate (NO_3^-), dissolved oxygen (DO), and pH/EC/TDS/temperature using a portable nitrate spectrophotometer (Hanna Instruments HI96786), portable dissolved oxygen meter (Milwaukee Instruments MW600), and waterproof pH/EC/TDS/temperature meter (Hanna Instruments HI98130), respectively. Final nutrient solution samples were collected and submitted to the same laboratory for comparison to the initial nutrient solution measurements. Lastly, periodic nutrient solution depth measurements were taken from the side of each DWC trough to track water consumption.

Basil seedlings were grown for 21 days in 3.8 cm rockwool cubes (Grodan A-OK Starter Plugs) and provided CNS 17 nutrient solution at a concentration of 75 mg/L nitrate (NO_3^-) every third day. Upon reaching the 6-8 leaf stage, seedlings were transplanted into 5.1 cm net pots in the floating rafts of each system on 13 May 2020 (Figure 2). Two perpendicular width measurements, and the height of each seedling was taken before transplanting. The heights, widths, and fresh shoot weights of seventeen additional basil seedlings were also taken, before placing the destructively harvested shoots in paper bags to air dry on greenhouse benches for initial dry weight measurements. Weekly height and width measurements were taken for all transplants until the conclusion of the trial.

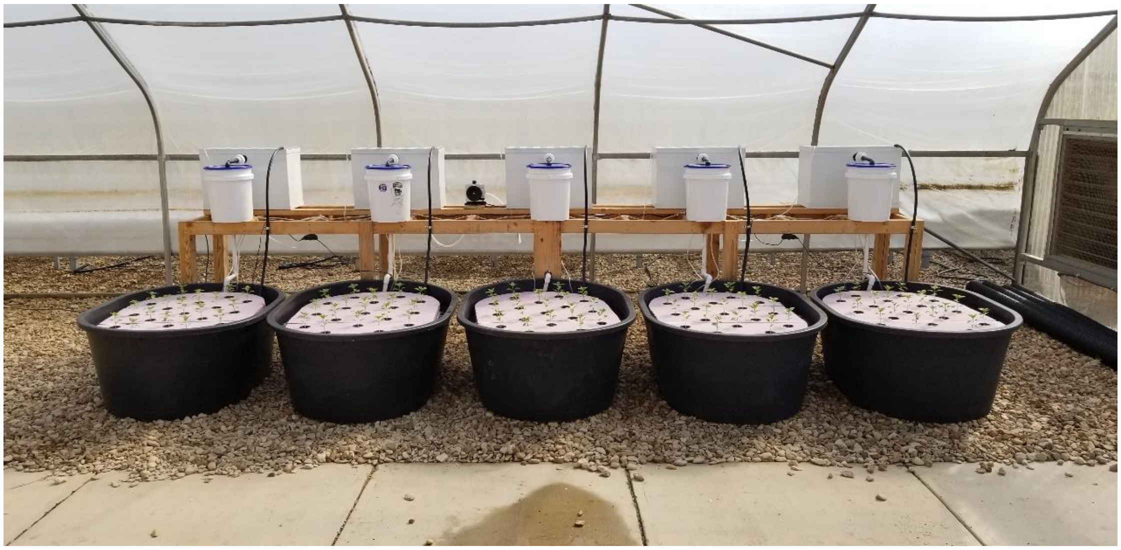

Figure 2: The floating raft hydroponic system after initial transplant of Italian Large-Leafed Basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR')

The experiment was terminated as soon as one of the hydroponic systems reached total nitrate depletion (0 mg/L NO_3^-). Final heights and widths of the shoots were collected from all basil plants, along with shoot and root fresh weights before placing all roots and shoots in paper bags to air dry on greenhouse benches for final dry weight measurements.

Experiment 3: Evaluation of Nitrate Production and Consumption Within Floating Raft Aquaponic System Utilizing Tilapia and Italian Large-Leafed Basil

This experiment was conducted to assess the adequacy of nitrogen availability for marketable basil culture in a floating raft aquaponic system using recommended tilapia stocking and feeding rates. A 28-day aquaponic trial began 16 May 2021 using four parallel floating raft recirculating aquaponic systems (Figure 3) located in a plastic high tunnel at HortTREC. Each system was constructed using one 56.8 L glass aquarium, a trickling biofilter comprised of one 18.9 L bucket housing a 400-micron screen filter (US Plastic Corp. EZ-Strainer) and 264 Bio-Pin Balls (Lee’s Aquarium & Pet Products), and one 416.4 L HDPE livestock tank as a hydroponic trough. Prior to the installation of the 400-micron screen filter, the filter pad media originally used in the trickling filter from the hydroponic system was exchanged for a 200-micron screen filter in an attempt to reduce the risk of overflow as the result of a clogged solids filter. Unfortunately, continued issues with the trickling filter overflowing ultimately led to the replacement of the 200-micron screen filter with a 400-micron screen.

The 5.1 cm extruded polystyrene floating raft material was 0.96 m^2 with seventeen 5.1 cm holes on a 20.3 cm staggered spacing recommended by the seed producer (Johnny’s Selected Seeds; Winslow, Maine). A 102-watt air pump (VIVOSUN Model 009) continuously supplied oxygen to the aquariums and hydroponic troughs in each system through Top Fin large air stones. The nutrient solution flowed via gravity from the aquarium into the trickling biofilter before draining into the hydroponic trough where an 800 LPH water pump (Aleko Multi-Function Pump Model G2950) returned the solution to the aquarium.

Figure 3: The floating raft aquaponic system after initial stocking with tilapia (*Oreochromis spp.*) and transplant of Italian Large-Leafed Basil (Ocimum basilicum var. 'Rutgers Obsession DMR')

Each aquaponic system was filled with 416.4 L of rainwater and 164.5 g Botanicare CNS17 Grow Formula 3-1-2 for a calculated initial nitrate (NO_3^-) concentration of 50 mg/L, in addition to 2.3 mg/L of nitrate contained within the rainwater. The use of Botanicare CNS17 Grow Formula 3-1-2 for the nutrient source was recommended by Dr. Daniel Leskovar of Texas A&M University, as it had been previously utilized in trials involving the hydroponic culture of leafy greens with no adverse effects. Each trickling filter was subsequently inoculated with nitrifying bacteria collected from the tilapia holding tank where fish were housed prior to stocking in the aquaponic systems. Calculations to determine the nutrient solution mixing rate were as follows:

4.08 kg net weight (per 3.78541 L CNS17)

3% Nitrogen (2.86% as nitrate-N, 0.14% as ammoniacal-N)

4.08 kg X 0.0286 = 0.116688 kg or 116.6880 g N (as nitrate-N)

116.6880 g N = 7.0271162 E+25 daltons (amu) N

7.0271162 E+25 amu N ÷ (14.0067 amu per atom N) = 5.016967737 E+24 atoms N

5.016967737 E+24 X (15.999 amu per atom O X 3) = 2.407994005 E+26 amu O

7.0271162 E+25 amu N + 2.407994005 E+26 amu O = 3.110705625 E+26 amu NO_3^-

3.110705625 E+26 amu NO_3^- = 516.5448 g NO_3^- (per 3.78541 L CNS17)

416.395 L X 50 mg NO_3^-/L (ppm) = 20819.75 mg NO_3^- or 20.8198 g NO_3^- (per 416.395 L)

20.8198 g NO_3^- ÷ 516.5448 g NO_3^- = 0.04031 or 4.031 %

0.04031 x 4.08 kg CNS17 = 0.1644648 kg or 164.4648 g CNS17 (per 416.395 L)

An initial nutrient solution sample from all four systems, along with a rainwater sample, were submitted to the Texas A&M Soil, Water, and Forage Testing Laboratory to determine their pre-transplant measurements for conductivity (EC and TDS), pH, Na, Ca, Mg, K, CO_3^{2-}, HCO_3^-, SO_4^{2-}, Cl^-, P, B, Nitrate-N (NO_3^--N), hardness, and alkalinity. The nutrient solutions of each system were sampled daily for nitrate (NO_3^-), dissolved oxygen (DO), and pH/EC/TDS/temperature using a portable nitrate spectrophotometer (Hanna Instruments HI96786), portable dissolved oxygen meter (Milwaukee Instruments MW600), and waterproof pH/EC/TDS/temperature meter (Hanna Instruments HI98130), respectively. Potassium hydroxide (KOH) and calcium hydroxide ($Ca(OH)_2$) were added as needed to maintain pH between 6 and 7, and chelated iron (Fe-EDDHA) was added upon suspected development of iron chlorosis. Weekly and final nutrient solution samples were collected and submitted to the Soil, Water, and Forage Testing Laboratory for comparison to the initial nutrient solution measurements. Lastly, daily dissolved oxygen measurements were taken from the aquariums, and periodic nutrient solution depth measurements were taken from the side of each hydroponic trough to track water consumption.

Prior to placement in the aquaponic systems, tilapia were kept in holding tanks that were slowly adjusted over 7 days by gradually adding CNS 17 until a final concentration of 50 mg/L nitrate (NO_3^-) was achieved. Each aquaponic system was stocked 15 May 2021 with 3.0 kg of tilapia for an initial density of 53.4 kg of tilapia per m^3 of aquarium volume, and the fish were allowed to acclimate for 24 hours before beginning daily feeding. Daily sporadic fish deaths were observed beginning 17 May, and comparable weights of replacement fish were used to maintain original stocking densities until ammonia measurements ranging from 21.6 to 23.4 mg/L TAN were discovered in all aquaponic systems on 25 May. Extended exposure to levels of NH_3 above 0.6 mg/L is the threshold for mortality of fish (Durborow et al., 1997).

Fish were fed to apparent satiation over a 20-minute interval using Rangen Production 32 commercial fish feed, and uneaten feed pellets were removed and tallied following each feeding session. Total daily feed consumption per tank was determined by measuring the pre-feeding weight of each tank's feed container, then taking post-feeding weight measurements after replacing uneaten feed pellets with an equivalent number of fresh pellets. Due to the elevated ammonia concentrations, feeding was also halted from 25 May until 6 June, in accordance with recommendations to temporarily reduce feed intake to allow ammonia to return to acceptable levels (Durborow et al., 1997).

Basil seedlings were grown for 28 days in 3.8 cm rockwool cubes (Grodan A-OK Starter Plugs) and provided CNS 17 nutrient solution at a concentration of 75 mg/L nitrate (NO_3^-) every third day. Upon reaching the 6-8 leaf stage, seedlings were transplanted into 5.1 cm net pots in the floating rafts of each system on 16 May 2021. Two perpendicular width measurements, and the height of each seedling was taken before transplanting. The heights, widths, and fresh shoot weights of seventeen additional basil seedlings were also taken, before placing the destructively harvested shoots in paper bags to air dry on greenhouse benches for initial dry weight measurements. Weekly height and width measurements were taken for all transplants until the conclusion of the trial. The experiment was terminated when the plants in all systems approached the recommended harvest height of 40.6 to 45.7 cm listed by the seed producer (Johnny’s Selected Seeds; Winslow, Maine). Final height, width, and mid-height widths of the shoots were collected from all basil plants, along with shoot and root fresh weights, before placing all roots and shoots in paper bags to air dry on greenhouse benches for final dry weight measurements.

CHAPTER III

RESULTS AND DISCUSSION

Experiment 1: Ammonia Production by Tilapia, Koi, and Hybrid Striped Bass in Response to Dietary Protein Inclusion and Feeding Rate

Experiment 1 attempted to determine the ammonia excretion rates of tilapia, koi, and hybrid striped bass when provided commercial feeds with varying protein percentages. Whether a fish is herbivorous, omnivorous, or carnivorous will dictate a minimally acceptable protein percentage in the feed required to maintain adequate growth and feed conversion ratios (Gatlin III, 2010). For example, tilapia and koi are both omnivorous species, while hybrid striped bass are carnivorous throughout their entire lives. Fish species whose diets are primarily herbivorous and omnivorous will require a diet that contains 25 to 35 percent crude protein, while carnivorous species may require 40 to 50 percent (Wilson, 1994). Subsequently, these differences in protein requirements directly influence ammonia excretion rates respective of protein intake.

Tilapia Feed Consumption

The tilapia in Experiment 1 consumed an average of 2.5% of their body weight (BW) in feed per day (Table 3) with no significant differences in consumption rates between treatments receiving feed. The average individual weight of tilapia used in the experiment was 20.5 grams (Table 1), with weights ranging from 11.2 to 33.8 grams and at least one fish in the 5 – 18 gram range in each tank. The observed feeding rates for all feed treatments were lower than expected as fish in the 5 – 18 gram and 18 – 75 gram ranges typically consume 5 – 10% and 3 – 5 % BW in feed per day, respectively

(DeLong et al., 2009). Since fish that are herbivorous or omnivorous have a tendency to "graze" throughout the day (DeLong et al., 2009), the reduced feed intake observed during the experiment could be the result of only providing feed once daily, rather than feeding two or more times over an extended period of several hours. Additionally, stress from crowded conditions in the tanks may have also contributed to the lower feeding rates observed in all feed treatments.

Table 3: Mean ± SE for feed intake, protein intake, and total ammonia nitrogen (TAN) measurements of tilapia (*Oreochromis spp.)* when provided a diet with varying levels of protein content

Feed Protein %	Feed Consumed (% Body Weight)^{zyx}	mg TAN per g Fish^{zyx}	mg TAN per g Feed^{zyx}	mg TAN per g Protein^{zyx}
0^{w}	**0.0 ± 0.000 a**	**0.06 ± 0.04 a**	**N/A**	**N/A**
28	**2.4 ± 0.002 b**	**0.34 ± 0.07 b**	**14.0 ± 2.9 a**	**50.0 ± 10.5 a**
32	**2.3 ± 0.004 b**	**0.45 ± 0.04 b**	**21.1 ± 2.8 a**	**65.8 ± 8.9 a**
35	**2.7 ± 0.002 b**	**0.41 ± 0.07 b**	**15.6 ± 3.0 a**	**44.6 ± 8.5 a**
40	**2.6 ± 0.003 b**	**0.49 ± 0.07 b**	**19.0 ± 2.5 a**	**47.5 ± 6.3 a**
44	**2.4 ± 0.001 b**	**0.50 ± 0.05 b**	**20.5 ± 1.8 a**	**46.7 ± 4.2 a**

^{z} Data represents means of six replicates (3 replicates x 2 days)
^{y} Data analyzed using one-way analysis of variance (ANOVA) and Tukey HSD at $p < 0.05$
^{x} Means within columns with the same letter are not significantly different as determined by Tukey HSD
^{w} Experimental control

Tilapia Ammonia Excretion

The mg of TAN excreted per gram of feed appeared to increase with the amount of protein in the feed provided, with the exception of the 32% protein feed treatment which accounted for the highest TAN excretion rate (Figure 4). Despite this observation there were no significant differences when analyzed with ANOVA and Tukey's HSD ($p < 0.05$) (Table 3). Similarly, the mg of TAN excreted per gram of fish seemed to follow increasing protein intake excluding the 32% treatment, with no significant differences found between treatments receiving feed (Table 3). Lastly, although TAN excretions per gram protein expectedly showed no significant differences between treatments receiving feed, the mg of TAN per gram protein of the 32% treatment measured 1.4 times greater than the collective average excretion rate of all other treatments (Table 3).

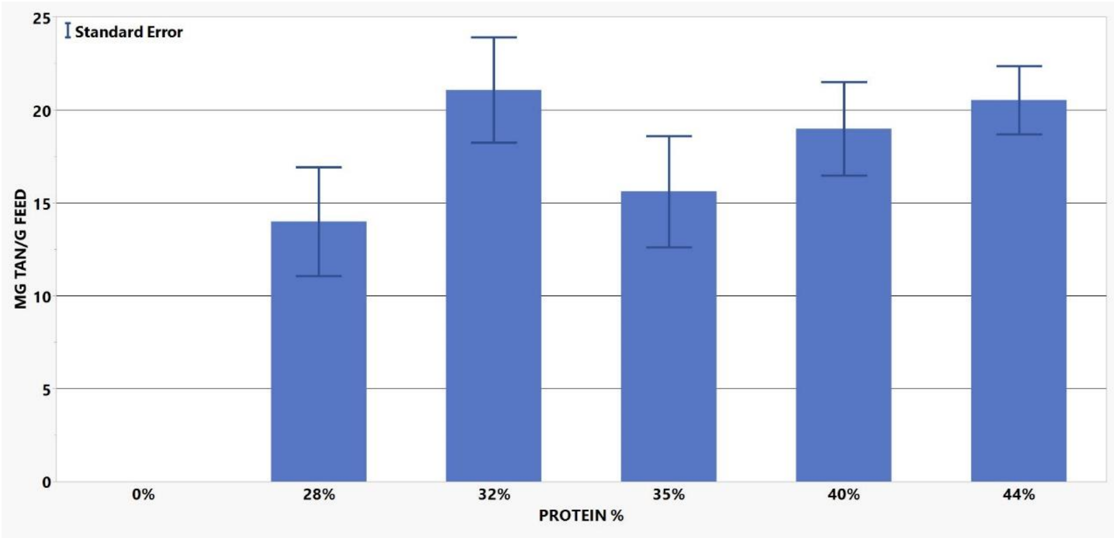

Figure 4: Mean ± SE for mg total ammonia nitrogen (TAN) excreted by tilapia (*Oreochromis spp*.) when provided commercial aquaculture feeds with varying levels of protein content

Forty-eight hours after the start of the experiment, one tank receiving 32% protein feed, and one receiving 40% protein feed experienced a single fish death. The following day, the other two tanks receiving 32% protein feed, another receiving 40%

protein feed, and one receiving 35% protein feed all experienced single fish deaths. Ultimately, even though TAN measurements from the final 48 hours of the experiment were excluded, measurements from the first 48 hours may have also been impacted. Depending on how long the dead fish remained in the tanks prior to the 48-hour measurements could have affected the amount of ammonia released as a byproduct of decomposition.

Koi Feed Consumption

The koi in Experiment 1 consumed an average of 1.4% of their body weight (BW) in feed per day (Table 4) with no significant differences in consumption rates between treatments receiving feed. The average individual weight of koi used in the experiment was 25.6 grams (Table 1), with weights ranging from 10.7 to 54.1 grams, and all but three tanks having at least one fish in the 5 – 18 gram range. The observed feeding rates for all feed treatments were lower than expected as fish in the 5 – 18 gram and 18 – 75 gram ranges typically consume 5 – 10% and 3 – 5% BW in feed per day, respectively (DeLong et al., 2009).

Table 4: Mean ± SE for feed intake, protein intake, and total ammonia nitrogen (TAN) measurements of koi (*Cyprinus carpio haematopterus)* when provided a diet with varying levels of protein content

Feed Protein %	Feed Consumed (% Body Weight)^{zyx}	mg TAN per g Fish^{zyx}	mg TAN per g Feed^{zyx}	mg TAN per g Protein^{zyx}
0^{w}	0.0 ± 0.000 a	0.06 ± 0.01 a	N/A	N/A
28	1.4 ± 0.001 b	0.25 ± 0.03 b	17.3 ± 1.3 a	61.7 ± 4.8 a
32	1.3 ± 0.001 b	0.27 ± 0.04 b	20.1 ± 1.2 ab	62.9 ± 3.9 a
35	1.5 ± 0.001 b	0.34 ± 0.03 bc	22.0 ±1.0 ab	62.7 ± 3.0 a
40	1.3 ± 0.002 b	0.33 ± 0.04 bc	25.6 ± 1.0b	64.0 ± 2.4 a
44	1.4 ± 0.001 b	0.44 ± 0.04 c	31.4 ± 2.0 c	71.4 ± 4.6 a

^{z} **Data represents means of six replicates (3 replicates x 2 days)**
^{y} **Data analyzed using one-way analysis of variance (ANOVA) and Tukey HSD at p < 0.05**
^{x} **Means within columns with the same letter are not significantly different as determined by Tukey HSD**
^{w} **Experimental control**

Koi Ammonia Excretion

The mg of TAN excreted per gram of fish increased in correlation with increasing protein availability with the exception of the 40% protein feed treatment which was lower than the excretion rate observed in the 35% treatment. Significant differences were found between the 28% and 32% treatments in comparison to the 44% treatment (Table 4) when analyzed with ANOVA and Tukey's HSD ($p < 0.05$). The 35% and 40% treatments showed no significant differences with either the 44% treatment or the 28% and 32% treatments. The mg TAN per gram of feed rose correspondingly with increasing treatment protein percentage (Figure 5), and significant differences were observed between the 28%, 40%, and 44% treatments when analyzed with ANOVA and

Tukey's HSD ($p < 0.05$). The mg TAN per gram of feed for the 32% and 35% treatments were not found to be significantly different from the 28% or 40% treatments, but were significantly different from the 44% treatment. Additionally, measurements of mg TAN per gram of protein were statistically insignificant between treatment levels (Table 4) when analyzed with ANOVA and Tukey's HSD ($p < 0.05$). Lastly, although no koi died during the experiment, only measurements from the initial 48 hours were used to minimize variation of data collection between fish species.

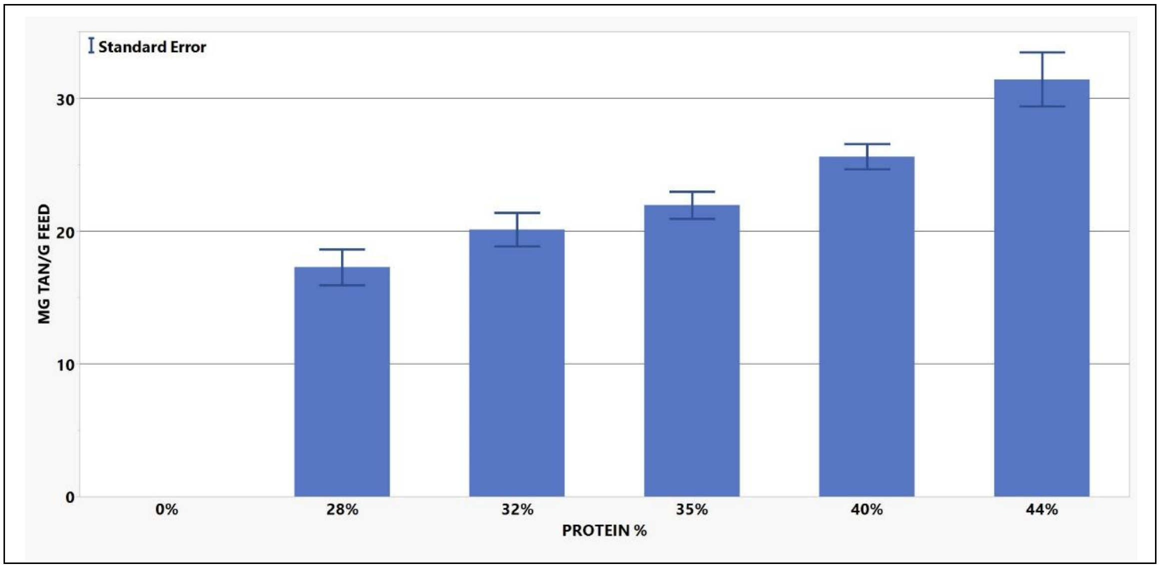

Figure 5: Mean ± SE for mg total ammonia nitrogen (TAN) excreted by koi (*Cyprinus carpio haematopterus)* when provided commercial aquaculture feeds with varying levels of protein content

Hybrid Striped Bass Feed Consumption

The hybrid striped bass in Experiment 1 consumed an average of 1.1% of their body weight (BW) in feed per day (Table 5) with no significant differences in consumption rates between feed treatments receiving feed. The average individual weight of hybrid striped bass used in the experiment was 60.3 grams, with weights ranging from 50.1 to 81.6 grams and only 3 tanks containing fish larger than the 18 - 75 gram range. The observed feeding rates for all feed treatments were lower than expected as fish in the 18 – 75 gram range typically consume 5 – 3% BW in feed per day (DeLong et al., 2009).

Table 5: Mean ± SE for feed intake, protein intake, and total ammonia nitrogen measurements of hybrid striped bass (*Morone chrysops* x *M. saxatilis)* when provided a diet with varying levels of protein content

Feed Protein %	Feed Consumed (% Body Weight)^{zyx}	mg TAN per g Fish^{zyx}	mg TAN per g Feed^{zyx}	mg TAN per g Protein^{zyx}
0^{w}	**0.0 ± 0.000 a**	**0.08 ± 0.03 a**	**N/A**	**N/A**
28	**1.1 ± 0.001 b**	**0.22 ± 0.02 b**	**21.0 ± 2.2 a**	**74.8 ± 7.9 a**
32	**1.0 ± 0.001 b**	**0.27 ± 0.02 bc**	**28.1 ± 1.6 ab**	**87.9 ± 5.1 a**
35	**1.2 ± 0.001 b**	**0.28 ± 0.03 bc**	**23.1 ± 2.0 a**	**65.9 ± 5.8 a**
40	**1.1 ± 0.001 b**	**0.32 ± 0.03 bc**	**32.3 ± 4.6 ab**	**80.7 ± 11.5 a**
44	**0.9 ± 0.001 b**	**0.35 ± 0.03 c**	**37.6 ± 3.6 b**	**85.4 ± 8.3 a**

[z] Data represents means of six replicates (3 replicates x 2 days)
[y] Data analyzed using one-way analysis of variance (ANOVA) and Tukey HSD at $p < 0.05$
[x] Means within columns with the same letter are not significantly different as determined by Tukey HSD
[w] Experimental control
[v] Calculated excluding experimental control

Hybrid Striped Bass Ammonia Excretion

The mg of TAN excreted per gram of feed appeared to increase with the amount of protein in the feed provided, with the exception of the 35% protein feed treatment

(Figure 6) which was less than that of the 32% treatment. When analyzed with ANOVA and Tukey's HSD ($p < 0.05$), the 28% and 35% treatments were found to be significantly different from the 44% treatment, while the 32% and 40% treatments were not significantly different from any other treatment receiving feed (Table 5). Additionally, the 28% and 44% treatments were the only treatments to show significant differences in mg TAN excreted per gram of fish, and TAN excretions per gram of protein expectedly showed no significant differences between treatments when analyzed with ANOVA and Tukey's HSD ($p < 0.05$) (Table 5). Although no hybrid striped bass died during the experiment, only measurements from the initial 48 hours were used to minimize variation of data collection between fish species.

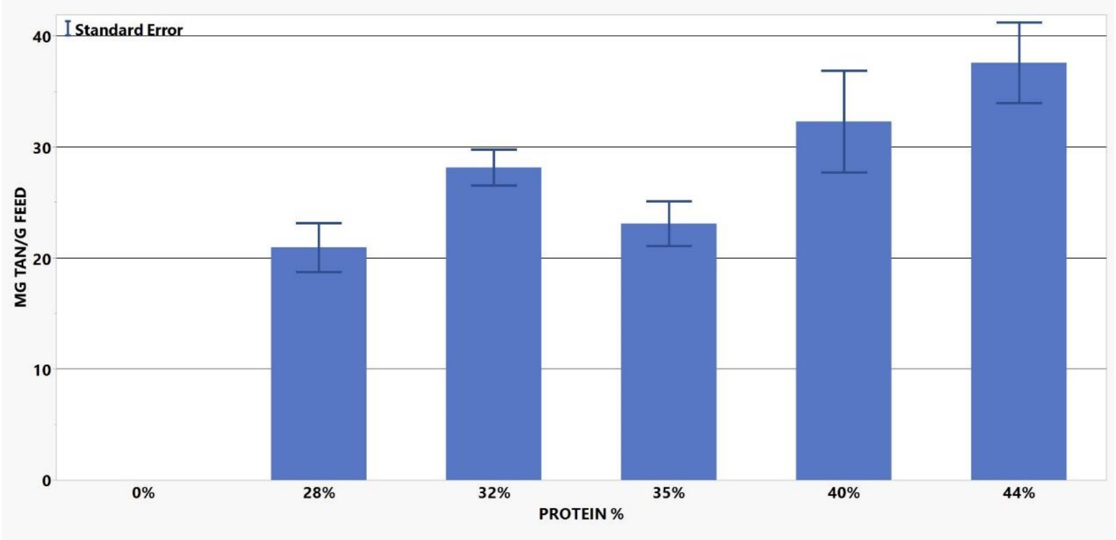

Figure 6: Mean ± SE for mg total ammonia nitrogen (TAN) excreted by hybrid striped bass (*Morone chrysops* x *M. saxatilis)* when provided commercial aquaculture feeds with varying levels of protein content

Conclusions

The mean measurements of mg TAN per g protein for all species in Experiment 1 differed from feed based estimates of TAN production found in “Recirculating Aquaculture Systems” (Timmons et al., 2002). Based on the assumption that protein is 16% nitrogen, 80% of nitrogen is assimilated by the organism, 80% of assimilated nitrogen is excreted, and 90% of nitrogen is excreted as TAN and 10% as urea (Timmons et al., 2002), an estimated 92.16 mg TAN would be generated per each g of protein consumed if feces and uneaten feed are not allowed to remain in the solution. Even though feces were not removed during the experiment, an average of 50.9, 64.5, and 79.0 mg TAN per gram of protein was respectively produced by tilapia, koi and hybrid striped bass.

The measurements of ammonia production for all species being lower than projected is likely the result of decreased feed consumption, as stressed fish are known to reduce feed intake. The capture, grading and placement of fish into smaller habitats with different lighting and water quality, and increased stocking densities are most likely the reasons reductions in feed intake were observed for all species (Kulczykowska and Sánchez Vázquez, 2010). Additionally, feeding began immediately after stocking in order to reduce the amount of time nitrifying bacteria had to multiply and impact ammonia excretion measurements, but it seems that the fish were not fully acclimated to their new environments and subsequently consumed less feed. Lastly, though tilapia experienced a death in the 32% feed treatment that likely contributed to elevated ammonia measurements in comparison to others with higher rates of protein inclusion, hybrid striped bass also recorded relatively greater ammonia excretion measurements for the 32% treatment. The presence of these anomalies in both tilapia and hybrid striped bass indicate there may have been some variability in the digestibility of ingredients (Table 6) in the commercial feeds used as treatments.

As a result of these findings, future trials should ensure sufficient fish acclimation and mitigate interference from nitrifying bacteria by replacing the water in each aquarium following the collection of daily ammonia excretion measurements, rather than using pre-sterilized static aquariums over a 96-hour period. Furthermore, an additional fishless replicate group with a pre-established concentration of ammonia should be used to monitor ammonia loss via nitrification so excretion measurements can be adjusted accordingly. The use of formulated feeds as treatments may also be desirable to eliminate outliers in ammonia production arising from variability in feed digestibility.

Table 6: List of ingredients for feed treatments provided to tilapia (*Oreochromis spp.)*, koi (*Cyprinus carpio haematopterus)*, and hybrid striped bass (*Morone chrysops* x *M. saxatilis)* for determination of ammonia excretion rates

Treatment	Ingredients
Catfish 28%	**Plant protein products, processed grain byproducts, grain products, animal protein products, fish oil, dicalcium phosphate, L-lysine, L-ascorbic acid phosphate (source of vitamin C), choline chloride, vitamin E supplement, niacin supplement, d-calcium pantothenate, riboflavin supplement, thiamine mononitrate, biotin, pyridoxine hydrochloride, folic acid, vitamin A supplement, vitamin D3 supplement, vitamin B12 supplement, manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, sodium selenite, potassium iodate, propionic acid (preservative)**
Production 32	**Plant protein products, grain products, processed grain byproducts, animal protein products, fish oil, soy lecithin, dicalcium phosphate, L-ascorbic acid phosphate (source of vitamin C), choline chloride, vitamin E supplement, niacin supplement, d-calcium pantothenate, riboflavin supplement, thiamine mononitrate, biotin, pyridoxine hydrochloride, folic acid, vitamin A supplement, vitamin D3 supplement, vitamin B12 supplement, manganese sulfate, copper sulfate, sodium selenite, potassium iodate, propionic acid (preservative), ethoxyquin (preservative)**
Production 35	**Plant protein products, processed grain byproducts, grain products, animal protein products, fish oil, soy lecithin, dicalcium phosphate, L-ascorbic acid phosphate (source of vitamin C), choline chloride, vitamin E supplement, niacin supplement, d-calcium pantothenate, riboflavin supplement, thiamine mononitrate, biotin, pyridoxine hydrochloride, folic acid, vitamin A supplement, vitamin D3 supplement, vitamin B12 supplement, manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, sodium selenite, potassium iodate, propionic acid (preservative)**
EXTR 400	**Animal protein products, plant protein products, grain products, processed grain byproducts, fish oil, soy lecithin, L-ascorbic acid phosphate (source of Vitamin C), choline chloride, vitamin E supplement, niacin supplement, d-calcium pantothenate, riboflavin supplement, thiamine mononitrate, biotin, pyridoxine hydrochloride, folic acid, vitamin A supplement, vitamin D3 supplement, vitamin B12 supplement, manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, sodium selenite, potassium iodate, propionic acid (preservative), ethoxyquin (preservative)**
EXTR 450	**Animal protein products, grain products, processed grain byproducts, fish oil, soy lecithin, L-ascorbic acid phosphate (source of vitamin C), choline chloride, vitamin E supplement, niacin supplement, d-calcium pantothenate, riboflavin supplement, thiamine mononitrate, biotin, pyridoxine hydrochloride, folic acid, vitamin A supplement, vitamin D3 supplement, vitamin B12 supplement, manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, sodium selenite, potassium iodate, propionic acid (preservative), ethoxyquin (preservative)**

Experiment 2: Daily Nitrate Uptake by Italian Large-Leafed Basil (Ocimum basilicum var. 'Rutgers Obsession DMR')

The objective of Experiment 2 was to determine the daily nitrate (NO_3^-) uptake rate of Italian large-leafed basil (*Ocimum basilicum* var. ‘Rutgers Obsession DMR’) in a floating raft hydroponic system by tracking the depletion of nitrate in solution. The use of a floating raft hydroponic system was based on industry preference (Love et al., 2015) as it can better regulate solution temperature, as root temperature plays a greater role in hydroponic plant production than air temperature (Rakocy et al., 2006, Genuncio et al., 2012). Additionally, as roots are continually immersed in nutrient and oxygen rich solution, floating raft systems typically experience elevated nutrient uptake rates in comparison to their nutrient film technique (NFT) and media based counterparts (Goddek et al., 2015).

As there is limited availability of information regarding total plant nitrate consumption rates for this cultivar of basil, this data should indicate the necessary nitrate output required by fish in an aquaponic system to cultivate each individual basil plant.

Plant Growth

The height and width measurements of the basil plants were uniform (Table 7) and overall growth was steady during the experiment (Figure 7 & Figure 8). Plants appeared to experience an uptick in the growth rate for width between day 17 and 21 (Figure 8), but this was likely the result of canopies widening as the support of neighboring plants was lost during harvesting. At the conclusion of the experiment, basil plants reached an average height of 29 cm and average width of 36.8 cm with no physical signs of nutrient deficiency (Figure 9). While the seed producer (Johnny’s Selected Seeds; Winslow, Maine) recommends a larger height (40.6 – 45.7 cm) for harvest than was reached during this experiment, the average width of basil plants at harvest were wider than the recommended 20.3-cm plant spacing. Fortunately, since basil is capable of being harvested multiple times a smaller harvest size may be acceptable in order to avoid additional lateral growth that could lead to increased light competition and disease as a result of decreased air flow and increased moisture.

Table 7: Mean ± SE for plant height and width measurements from Italian Large-Leafed Basil (Ocimum basilicum var. 'Rutgers Obsession DMR') grown 21 days in a floating raft hydroponic system

Hydroponic Trough	**Day 0[y]**		**Day 17**		**Day 21**	
	Height (cm)	**Width (cm)**	**Height (cm)**	**Width (cm)**	**Height (cm)**	**Width (cm)**
1[z]	**9.3 ± 0.13**	**8.3 ± 0.12**	**24.6 ± 0.24**	**24.4 ± 0.32**	**28.9 ± 0.39**	**36.7 ± 0.81**
2[z]	**9.6 ± 0.11**	**8.8 ± 0.13**	**24.5 ± 0.31**	**24.6 ± 0.29**	**29.0 ± 0.34**	**37.8 ±0.54**
3[z]	**9.5 ± 0.06**	**8.7 ± 0.09**	**23.5 ± 0.28**	**24.5 ± 0.35**	**28.8 ± 0.35**	**35.9 ±0.50**
4[z]	**9.8 ± 0.07**	**8.8 ± 0.10**	**24.4 ± 0.25**	**25.4 ± .030**	**29.1 ± 0.24**	**37.9 ± 0.51**
5[z]	**9.6 ± 0.09**	**8.9 ± 0.09**	**23.8 ± 0.29**	**24.4 ± 0.34**	**29.1 ± 0.40**	**35.6 ±0.51**
All	**9.6 ± 0.05**	**8.7 ± 0.05**	**24.2 ± 0.13**	**24.7 ± 0.15**	**29.0 ± 0.15**	**36.8 ± 0.28**

[z] Data represents mean of seventeen individual basil plant measurements
[y] Start date of 13 May 2020

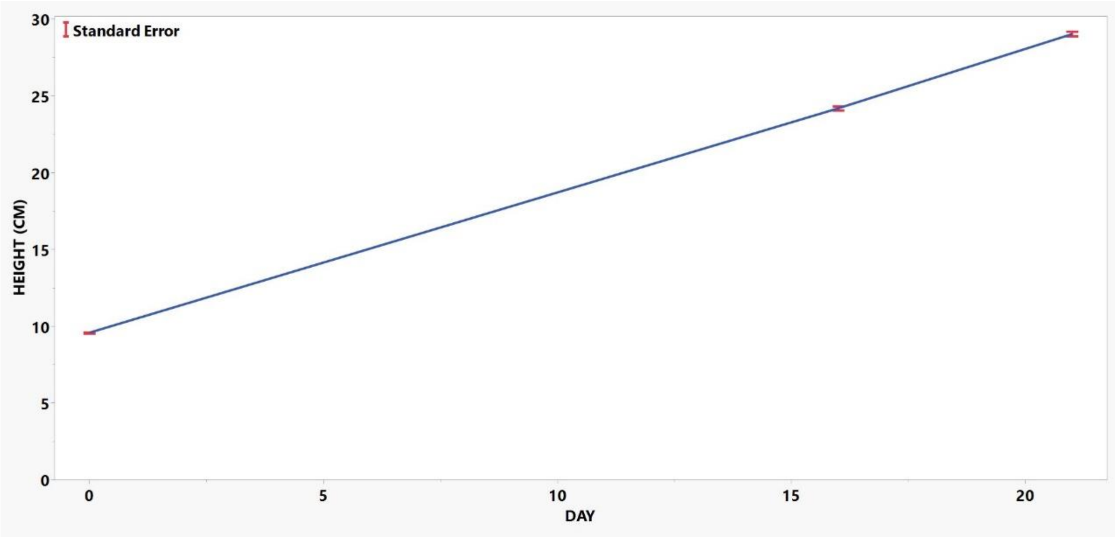

Figure 7: Mean ± SE for plant height measurements over 21 days in a floating raft hydroponic system planted with Italian large-leafed basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR')

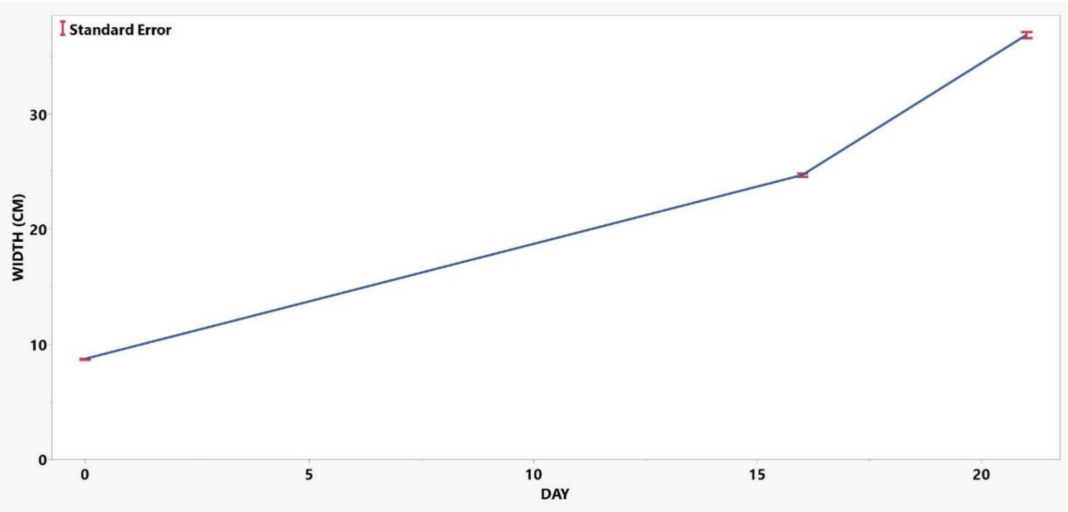

Figure 8: Mean ± SE for plant width measurements over 21 days in a floating raft hydroponic system planted with Italian large-leafed basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR')

Figure 9: The floating raft hydroponic systems before harvest of Italian Large-Leafed Basil (Ocimum basilicum var. 'Rutgers Obsession DMR')

Over the course of the experiment basil plants gained an average shoot mass of 85.6 and 20.3 grams of fresh and dry weight respectively, while fresh root mass increased by 48.7 grams (Table 8).

Table 8: Mean ± SE for fresh and dry weight measurements of shoot and root mass from Italian Large-Leafed Basil (Ocimum basilicum var. 'Rutgers Obsession DMR') grown 21 days in a floating raft hydroponic system

Hydroponic Trough	Initial Shoot Weight (g)^{zy}		Final Shoot Weight (g)^{z}		Final Root Weight (g)^{z}	
	Fresh	**Dry**	**Fresh**	**Dry**	**Fresh**	**Dry**
1	**4.1 ± 0.1**	**0.2 ± 0.0**	**90.2 ± 2.6**	**19.9 ± 0.3**	**48.7 ± 1.6**	**12.7 ± 0.1**
2			**88.9 ± 1.9**	**20.3 ± 0.3**	**47.7 ± 1.4**	**13.0 ± 0.1**
3			**89.1 ± 2.0**	**20.4 ± 0.3**	**49.2 ± 1.8**	**12.9 ± 0.2**
4			**91.3 ± 2.1**	**21.3 ± 0.3**	**50.2 ± 2.2**	**13.0 ± 0.1**
5			**88.9 ± 1.4**	**20.7 ± 0.2**	**48.0 ± 1.3**	**12.7 ± 0.1**
All			**89.7 ± 0.9**	**20.5 ± 0.1**	**48.7 ± 0.7**	**12.8 ± 0.1**

[z] Data represents mean of seventeen individual basil plant measurements
[y] Data collected at transplant from destructively harvested sample of representative basil plants

Nutrient Solution

During Experiment 2 each basil plant consumed an average of 16.3 mg of (NO_3^-)-N per day and produced an average of one gram of fresh shoot material for every 3.8 mg of (NO_3^-)-N consumed (Table 9).

Table 9: Mean ± SE for macronutrient uptake rates of Italian large-leafed basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR') grown 21 days in a floating raft hydroponic system

Macronutrient Consumption (mg)*				
Macronutrient	**Total Uptake Per Plant**	**Daily Uptake Per Plant**	**Total Uptake Per g Shoot (FW)**	**Total Uptake Per g Shoot (DW)**
(NO_3^-)-N	**342.9 ± 7.5**	**16.3 ± 0.4**	**4.0 ± 0.1**	**16.6 ± 0.5**
P	**94.3 ± 1.9**	**4.5 ± 0.1**	**1.1 ± 0.0**	**4.6 ± 0.1**
K	**331.0 ± 1.7**	**15.8 ± 0.1**	**3.9 ± 0.0**	**16.0 ± 0.2**
Ca	**234.0 ± 7.2**	**11.2 ± 0.3**	**2.7 ± 0.1**	**11.3 ± 0.3**
Mg	**100.1 ± 1.2**	**4.8 ± 0.1**	**1.2 ± 0.0**	**4.9 ± 0.1**
SO_4^-	**40.3 ± 2.5**	**1.9 ± 0.1**	**0.5 ± 0.0**	**2.0 ± 0.1**

*** Mean ± SE calculated excluding first hydroponic system due to error when recording system volume final measurement**

The experiment concluded on day 21 as concentrations of (NO_3^-)-N reached 0 mg per liter (Figure 10). Potassium was the only other macronutrient that neared depletion. During that time solution electroconductivity (EC) decreased from 0.25 to 0.09 millisiemens per centimeter (mS/cm) (Figure 11), and pH decreased from 6.7 to a measurement of 6.3 at day 10 before steadily increasing to 7.6 at the conclusion of the experiment (Figure 12). Nutrient solution dissolved oxygen (DO) ranged from 7.0 - 7.6 mg/L with an average of 7.3 mg/L (Figure 13), while the temperature averaged 26.2 degrees Celsius (Figure 14).

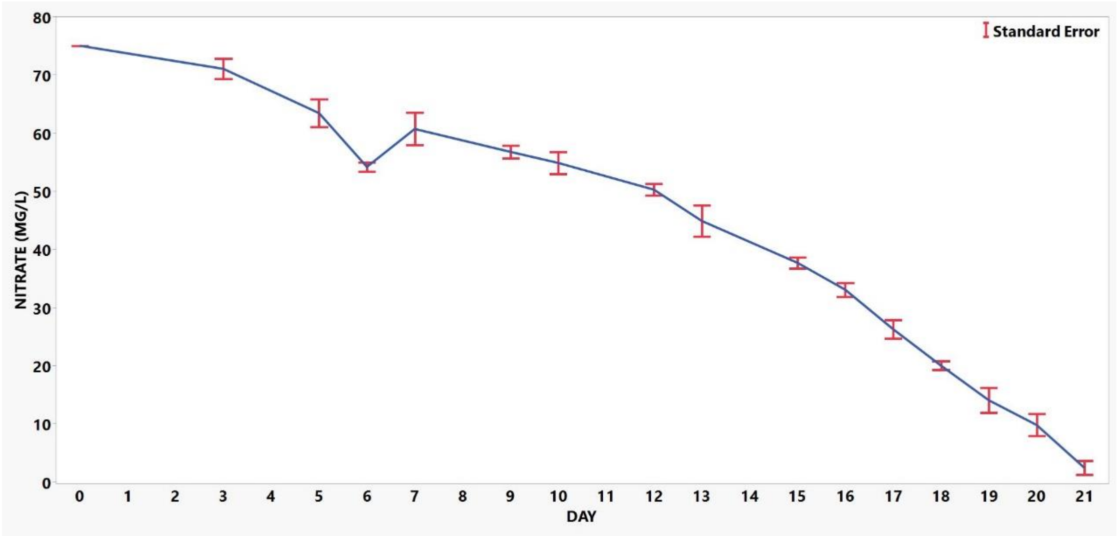

Figure 10: Mean ± SE for depletion rate of nitrate (NO_3^-) over 21 days in a floating raft hydroponic system planted with Italian large-leafed basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR')

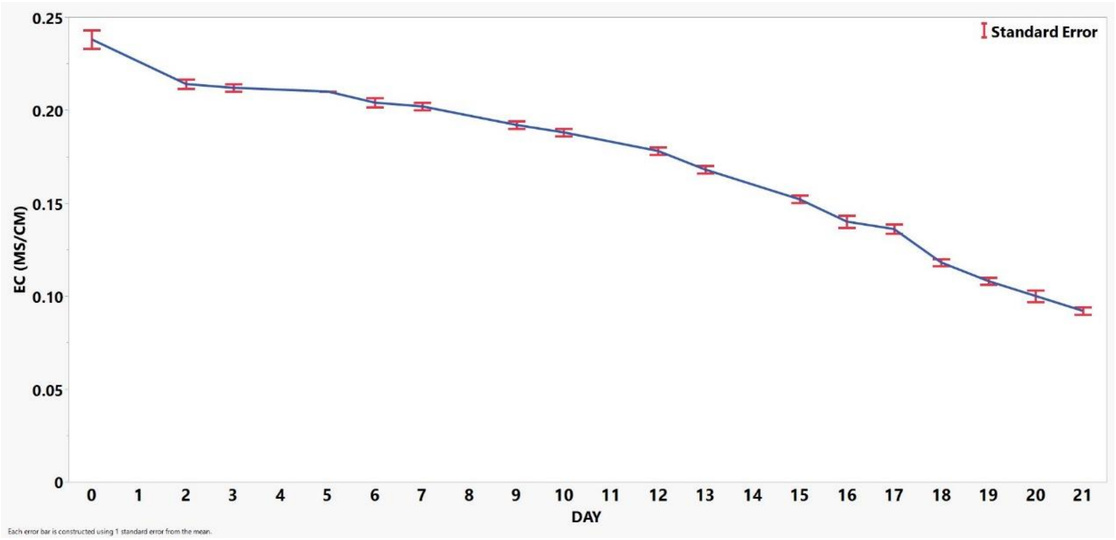

Figure 11: Mean ± SE for solution electroconductivity (EC) measurements over 21 days in a floating raft hydroponic system planted with Italian large-leafed basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR')

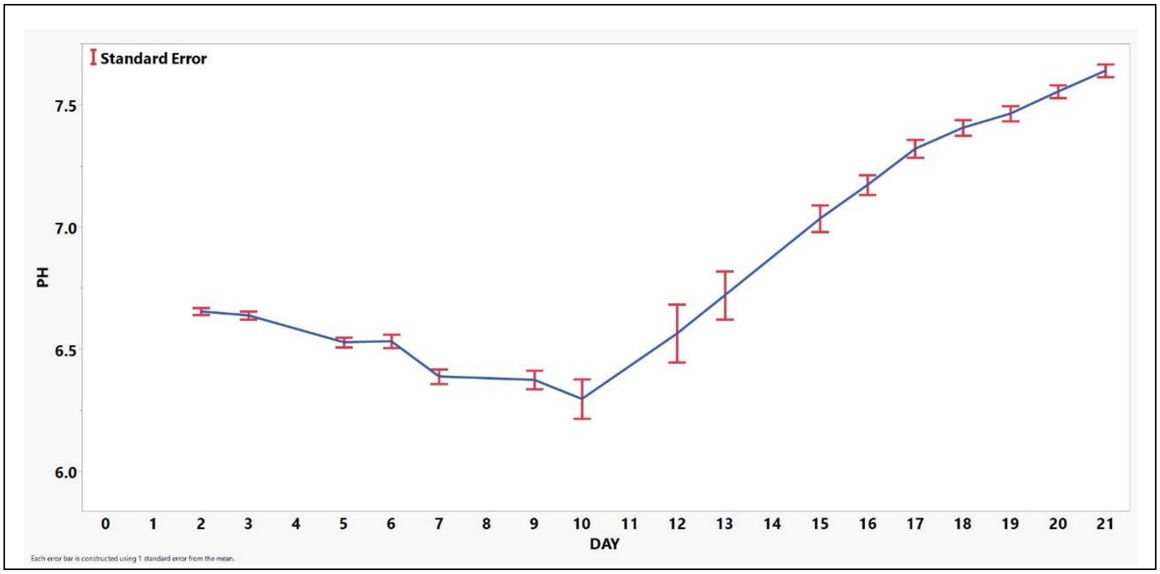

Figure 12: Mean ± SE for solution pH measurements over 21 days in a floating raft hydroponic system planted with Italian large-leafed basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR')

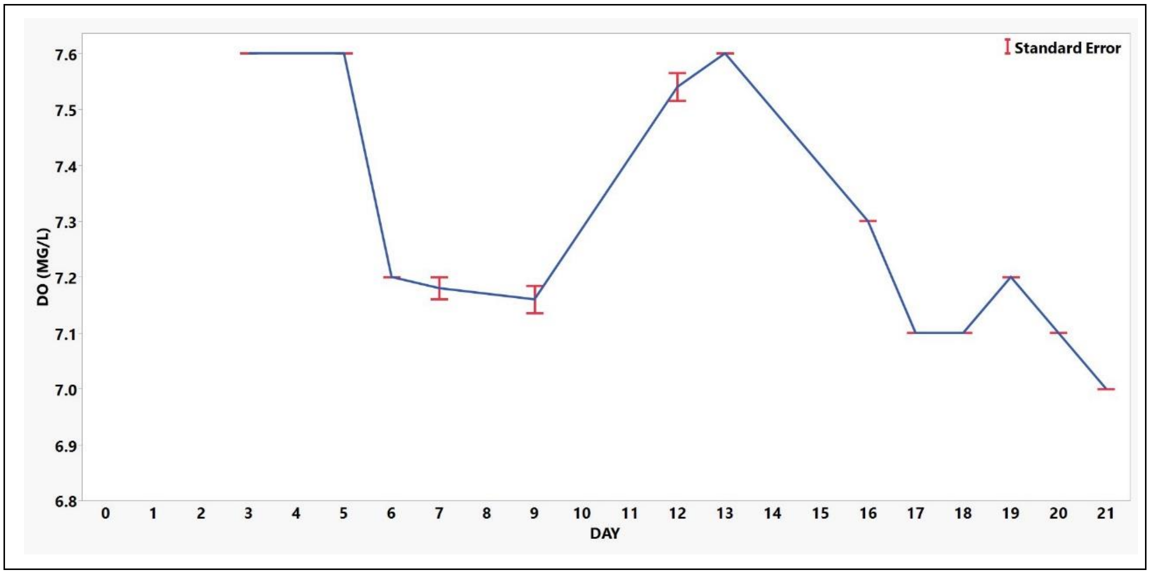

Figure 13: Mean ± SE for solution dissolved oxygen (DO) measurements over 21 days in a floating raft hydroponic system planted with Italian large-leafed basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR')

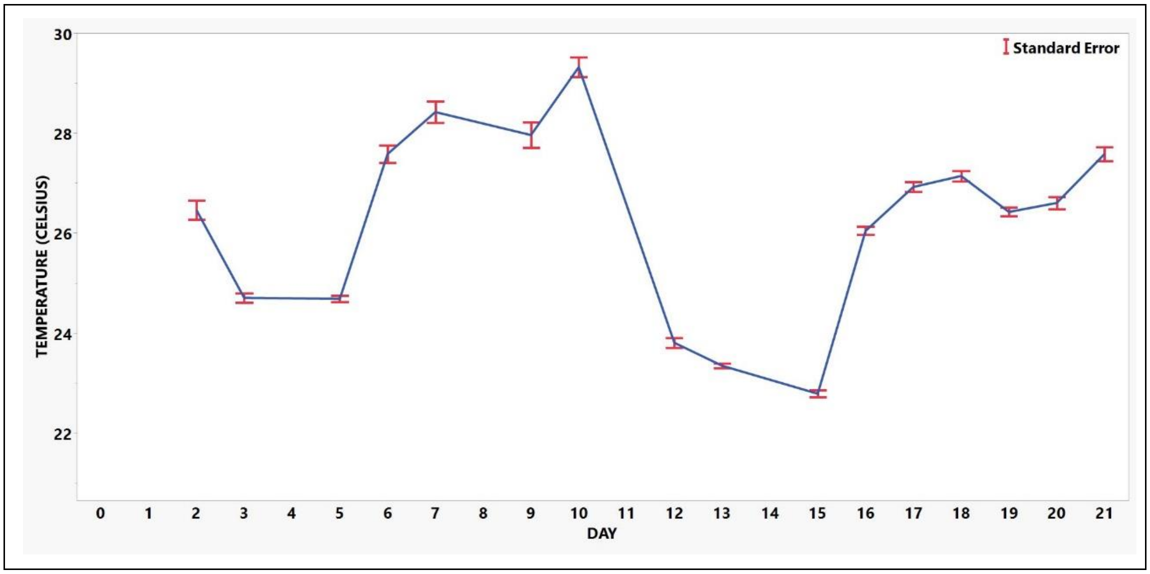

Figure 14: Mean ± SE for nutrient solution temperature measurements over 21 days in a floating raft hydroponic system planted with Italian large-leafed basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR')

Conclusions

Based on the average nitrate uptake rate measurements collected during this experiment, a post-transplant gain of 85.6 grams of fresh shoot mass would require 342.9 mg of (NO_3^-)-N generated by fish, assuming nitrate from fish and inorganic fertilizers have similar growth responses. With a 2.4% loss of nitrogen to autotrophic bacterial assimilation during nitrification, every gram of TAN excreted by fish will result in 0.976 g of (NO_3^-)-N (Ebeling et al., 2006). Using the estimate of 92.16 mg TAN generated per g of protein consumed (Timmons et al., 2002), each gram of protein will ultimately provide 89.9 mg (NO_3^-)-N in an aquaponic system containing only autotrophic bacteria. Table 10 provides a calculation of the total grams of feed required to produce an identical amount of basil over 21 days in an aquaponic format, considering 3.8 grams of protein are required to produce 85.6 grams of fresh shoot mass.

Table 10: Estimated feeding requirements for production of 1524.9 grams (FW) of Italian Large-Leafed Basil (Ocimum basilicum var. 'Rutgers Obsession DMR') over 21 days in a floating raft aquaponic system

Protein %	g Feed per Plant	# Plants	Total g Feed	# Days	Total g Feed per Day
28	13.6	17	231.6	21	11.1
32	11.9	17	202.6	21	9.6
35	10.9	17	185.3	21	8.8
40	9.5	17	162.1	21	7.7
44	8.7	17	147.4	21	7.0

Daily feeding rates found in the literature of 60 – 100 grams per square meter for lettuce (Rakocy et al., 2006), 57 grams per square meter for lettuce (Rakocy, 1988), and 15 – 42 grams per square meter for water spinach (Enduta et al., 2011) show feed inputs that are much greater than predicted in Table 9, though none of the recommended rates take feed protein content into account so elucidating actual nitrogen availability is impossible. Additionally, differences in the vegetable crops cultured and hydroponic subsystem utilized (Enduta et al., 2011) also play a role in determining appropriate feeding rates. For instance, lettuce is considered a heavy nitrogen feeder, whereas basil and other herbs are considered light feeders. Lastly, physiological differences in cultivars within plant species can cause spacing requirements to vary and subsequently affect the number of plants in each system consuming nutrients. Due to these key differences, it is understandable that the projected feed input in Table 10 is as low as it is.

Experiment 3: Evaluation of Nitrate Production and Consumption Within Floating Raft Aquaponic System Utilizing Tilapia and Italian Large-Leafed Basil

The data collected during the first and second experiments indicated that dissolved nitrogen excreted from fish would satisfy the requirements for aquaponic production of basil. Experiment 3 was conducted to test this hypothesis by evaluating the adequacy of dissolved nitrogen availability for marketable basil culture in a floating raft aquaponic system using recommended fish stocking and feeding rates. The initial stocking densities of the aquaponic systems fell within the recommended range of 30 – 60 (72) kg/m^3 for tank production of tilapia without the use of supplemental oxygen (DeLong et al., 2009, Rakocy et al., 2006), and the 0.96 square meters of plant production area meant each aquaponic system was capable of handling an estimated 57.6 to 96 grams of feed per day (Rakocy et al., 2006). Additionally, the size of tilapia that were stocked typically consume 3 – 1.5% of their body weight in feed per day (DeLong et al., 2009), which meant the projected total daily amount of feed should not exceed the estimated nitrogen removal capacity of the plant production area (Rakocy et al., 2006).

Plant Growth

Overall plant growth in the four floating raft aquaponic systems tested was uniform from transplant (Figure 15) to harvest (Figure 16) with no significant differences in height (Table 11) or width measurements (Table 12) when analyzed with ANOVA and Tukey’s HSD ($p < 0.05$). Height measurements increased steadily throughout the experiment (Figure 17), with an average final height of 47.1 cm (Table 11), which was just above the recommended harvest height range of 40.6 - 45.7 cm recommended by the seed producer (Johnny's Selected Seeds; Winslow, Maine). Width measurements increased steadily throughout the experiment until a sharp increase of approximately 219% was observed between measurements taken on day 21 and harvest (Figure 18). By day 14 average plant widths (21.0 cm) had already surpassed the 20.3-cm plant spacing in the floating rafts, so it is likely that plants experienced etiolation in response to increasing light competition. As a result, when plants were harvested from the rafts the canopies of the remaining plants likely widened without the support from neighboring plants and subsequently caused the drastic increase seen in width measurements (Figure 18). As a result, width measurements taken during harvest are not considered to be truly representative of final plant widths.

Figure 15: The floating raft aquaponic systems following the transplant of seventeen Italian large-leafed basil seedlings (Ocimum basilicum var. 'Rutgers Obsession DMR') into each replicate

Figure 16: The floating raft aquaponic systems immediately before the harvest of seventeen Italian large-leafed basil plants (Ocimum basilicum var. 'Rutgers Obsession DMR') from each replicate

Table 11: Mean ± SE for plant height measurements of Italian Large-Leafed Basil (Ocimum basilicum var. 'Rutgers Obsession DMR') grown 28 days in a floating raft aquaponic system

Aquaponic System	Height (cm)^{yx}				
	Day 0^{w}	Day 7	Day 14	Day 21	Day 28
1^{z}	7.2 ± 0.2 a	10.6 ± 0.2 a	19.6 ± 0.4 a	30.4 ± 0.4 a	46.8 ± 0.4 a
2^{z}	7.1 ± 0.2 a	10.4 ± 0.3 a	18.7 ± 0.4 a	30.5 ± 0.4 a	46.5 ± 0.6 a
3^{z}	7.3 ± 0.2 a	10.9 ± 0.2 a	19.3 ± 0.4 a	30.8 ± 0.7 a	47.2 ± 0.9 a
4^{z}	7.2 ± 0.1a	10.6 ± 0.2 a	19.2 ± 0.4 a	30.4 ± 0.6 a	47.8 ± 0.6 a
All	7.2 ± 0.1	10.6 ± 0.1	19.2 ± 0.2	30.5 ± 0.3	47.1 ± 0.3

[z] Data represents mean of seventeen individual basil plant measurements

[y] Data analyzed using one-way analysis of variance (ANOVA) and Tukey HSD at $p < 0.05$

[x] Means within columns with the same letter are not significantly different as determined by Tukey HSD

[w] Start date of 16 May 2021

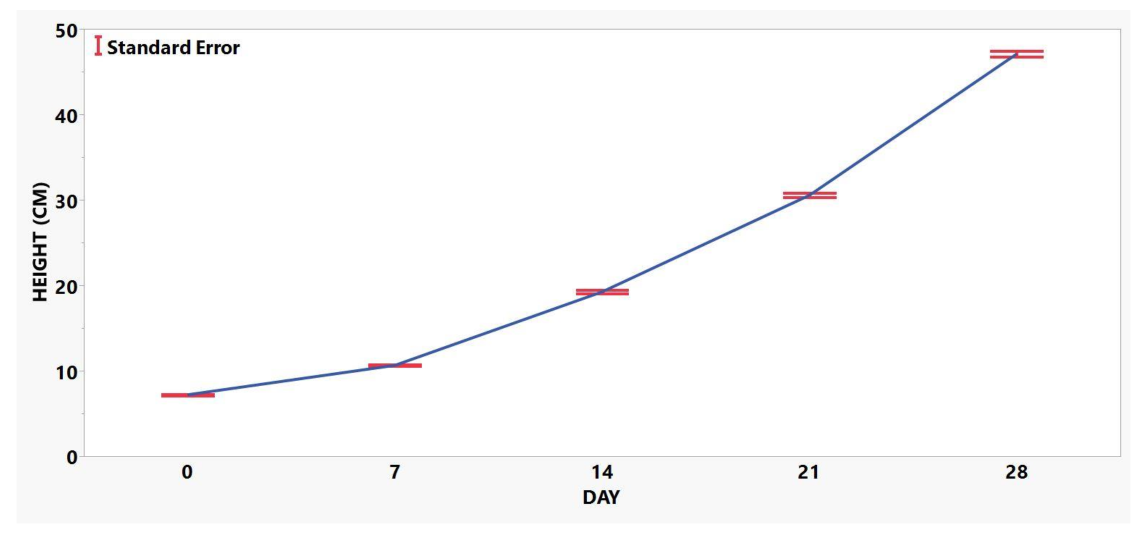

Figure 17: Mean ± SE for plant height measurements over 28 days in a floating raft aquaponic system planted with Italian large-leafed basil (Ocimum basilicum var. 'Rutgers Obsession DMR')

Table 12: Mean ± SE for plant width measurements of Italian Large-Leafed Basil (Ocimum basilicum var. 'Rutgers Obsession DMR') grown 28 days in a floating raft aquaponic system

Aquaponic System	Width (cm)^{yx}				
	Day 0^{w}	Day 7	Day 14	Day 21	Day 28
1^{z}	7.3 ± 0.1 a	14.7 ± 0.2 a	20.9 ± 0.2 a	28.1 ± 0.7 a	62.8 ± 1.3 a
2^{z}	7.2 ± 0.1 a	14.1 ± 0.2 a	20.6 ± 0.3 a	27.9 ± 0.9 a	60.3 ± 1.3 a
3^{z}	7.4 ± 0.1 a	14.5 ± 0.2 a	21.5 ± 0.3 a	28.2 ± 0.6 a	61.7 ± 1.7 a
4^{z}	7.3 ± 0.1 a	14.5 ± 0.2 a	20.8 ± 0.3 a	27.5 ± 0.7 a	60.1 ± 1.3 a
All	7.3 ± 0.0	14.5 ± 0.1	21.0 ± 0.1	27.9 ± 0.4	61.2 ± 0.7

[z] Data represents mean of seventeen individual basil plant measurements

[y] Data analyzed using one-way analysis of variance (ANOVA) and Tukey HSD at $p < 0.05$

[x] Means within columns with the same letter are not significantly different as determined by Tukey HSD

[w] Start date of 16 May 2021

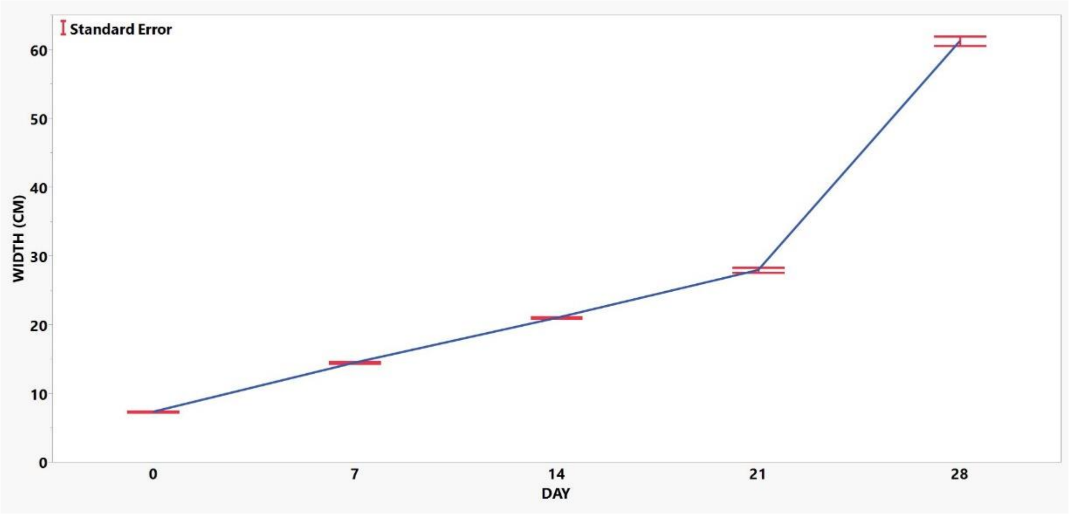

Figure 18: Mean ± SE for plant width measurements over 28 days in a floating raft aquaponic system planted with Italian large-leafed basil (Ocimum basilicum var. 'Rutgers Obsession DMR')

Over the course of the experiment basil plants gained an average of 196.4 and 18.7 grams of fresh and dry shoot weight respectively, while fresh root mass increased by 96.2 grams (Table 13). All weight measurements for shoots and roots showed no significant differences when analyzed with ANOVA and Tukey's HSD ($p < 0.05$).

Table 13: Mean ± SE for fresh and dry weight measurements of shoot and root mass from Italian Large-Leafed Basil (Ocimum basilicum var. 'Rutgers Obsession DMR') grown 28 days in a floating raft aquaponic system

Aquaponic System	Initial Shoot Weight (g)^{zyxw}		Final Shoot Weight (g)^{zxw}		Final Root Weight (g)^{zxw}	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
1	0.9 ± 0.1	0.1 ± 0.0	197.8 ± 9.2 a	18.8 ± 1.1 a	95.6 ± 6.6 a	4.9 ± 0.3 a
2			188.4 ± 10.0 a	18.2 ± 1.1 a	91.1 ± 7.0 a	4.6 ± 0.4 a
3			203.6 ± 8.5 a	19.7 ± 1.0 a	99.2 ± 6.3 a	4.8 ± 0.3 a
4			199.5 ± 9.6 a	18.5 ± 1.1 a	98.8 ± 5.8 a	4.7 ± 0.3 a
All			197.3 ± 4.6	18.8 ± 0.5	96.2 ± 3.2	4.7 ± 0.2

[z] Data represents mean of seventeen individual basil plant measurements
[y] Data collected at transplant from destructively harvested sample of representative basil plants
[x] Data analyzed using one-way analysis of variance (ANOVA) and Tukey HSD at $p < 0.05$
[w] Means within columns with the same letter are not significantly different as determined by Tukey HSD

Nutrient Solution

At the conclusion of the experiment, final mass measurements for all primary and secondary macronutrients showed net gains over initial measurements (Table 14). The total mg of (NO_3^-)-N in the system increased by 241.5% over 28 days, while phosphorus and potassium showed respective increases of 111.8% and 221.2%. The secondary macronutrients all increased as well, with calcium growing by 301.5%, and magnesium and sulfate realizing corresponding gains of 140.9% and 185.5%. These increases in macronutrients show that uptake by the plants in the aquaponic systems was less than the rate of additions made through feeding and nutrient supplementation.

Each aquaponic system received an average of 25.2 grams of feed per day, which was approximately 26.3 – 43.8% of production recommendations of 60 – 100 grams per square meter per day (Rakocy et al., 2006). Although, the amount of feed added to each aquaponic system was much less than recommended, the amount of feed each system received was 152.7% of the requirements calculated in Experiment 2. Based on those calculations, the average amount of 32% protein feed required to produce 3338.8 grams of fresh shoot material in an aquaponic system should have been 16.5 grams per day over 28 days. In conjunction with elevated feed inputs, poor digestibility of the 32% protein feed, fish deaths, and inadequate solids filtration may have also contributed to the amount of excess nitrogen observed in all aquaponic systems.

Table 14: Mean ± SE for macronutrient balance following culture of Italian large-leafed basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR') for 28 days in a floating raft aquaponic system stocked with tilapia consuming 704.7 grams of Rangen Production 32 commercial aquaculture feed

Macronutrient	Concentration^{z} (mg/L)		Total in System^{zy} (mg)		
	Initial	Final	Initial	Final	Δ
(NO_3^-)-N	11.8 ± 0.1	33.7 ± 3.6	4914.9 ± 46.2	11868.4 ± 1344.7	6953.5 ± 1382.9
P	4.1 ± 0.0	5.4 ± 0.5	1694.7 ± 6.4	1894.8 ± 201.0	200.0 ± 195.5
K	13.0 ± 0.0	34.0 ± 2.1	5413.2 ± 0.0	11973.5 ± 797.2	6560.3 ± 797.2
Ca	13.0 ± 0.0	46.3 ± 3.3	5413.2 ± 0.0	16322.6 ± 1286.0	10909.4 ± 1286.0
Mg	3.0 ± 0.0	5.0 ± 0.0	1249.2 ± 0.0	1759.8 ± 14.7	510.6 ± 14.7
SO_4^-	12.0 ± 0.0	26.3 ± 0.3	4996.8 ± 0.0	9269.9 ± 183.5	4273.1 ± 183.5

[z] Averages calculated excluding measurements from the fourth aquaponic system due to substantial water loss from overflow
[y] Calculated as concentration multiplied by system volume

In aquaponic systems, settleable solids greater than 100 microns are typically removed in less than 24 hours through the use of a clarifier, with a residence time of 3 to 7 days for suspended solids (Rakocy et al., 2006). In this experiment a 400-micron screen filter was used to capture and remove settleable solids, but instead it likely acted as a sieve that transformed settleable solids into suspended solids and allowed them to remain in circulation where microbial decomposition led to the drastic increase in ammonia (Figure 19) seen in all aquaponic systems (DeLong et al., 2009).

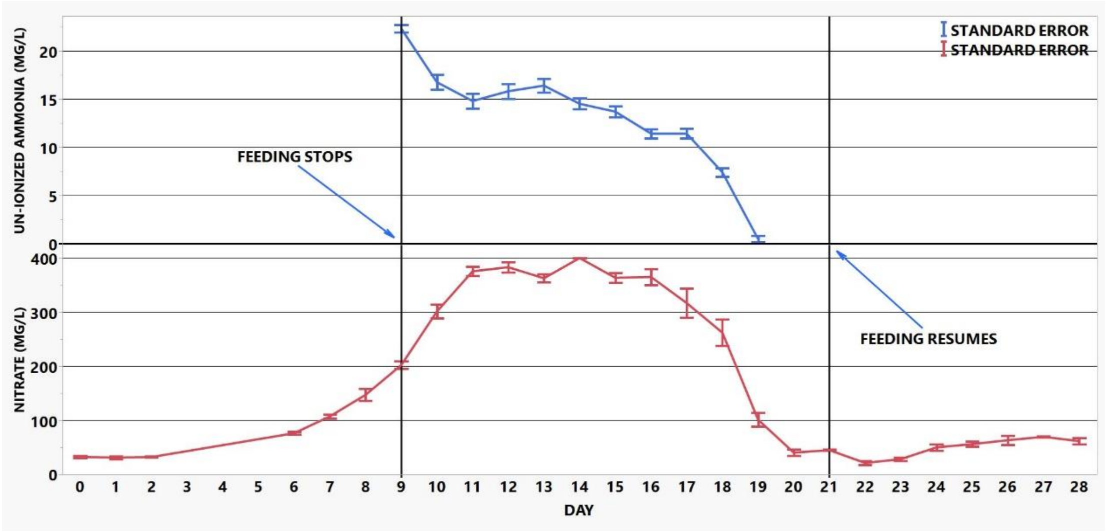

Figure 19: Mean ± SE for measurements of nitrate (NO_3^-) and un-ionized ammonia (NH_3) over 28 days in a floating raft aquaponic system planted with Italian large-leafed basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR')

This line of reasoning is supported by the observed decreases in both dissolved oxygen (Figure 20) and pH (Figure 21) levels in all aquaponic systems as microbial decomposition of organic matter reduces dissolved oxygen levels by increasing biological oxygen demand (BOD) (Rakocy et al., 2006), and lowers pH as carbonic acid is formed as a result of carbon dioxide being released into solution (Tucker and D'Abramo, 2008). Although pH did initially increase it was likely due to plant uptake and aeration removing carbon dioxide from solution (Tucker and D'Abramo, 2008) faster than it was being generated, until around day 7 when it appears the generation of dissolved carbon dioxide began to outpace its removal.

Additionally, although nitrification also contributes to decreasing solution pH, the rapid decline in nitrate (NO_3^-) concentrations (Figure 19) beginning around day 14, accompanied by continued decreases in pH levels were evidence of the persistent acidification of the nutrient solution as a result of microbial degradation of organic matter. This continued drop in pH necessitated the repeated addition of potassium and calcium hydroxide beginning day 13 to keep pH levels from falling below 6 (Rakocy et al., 2006).

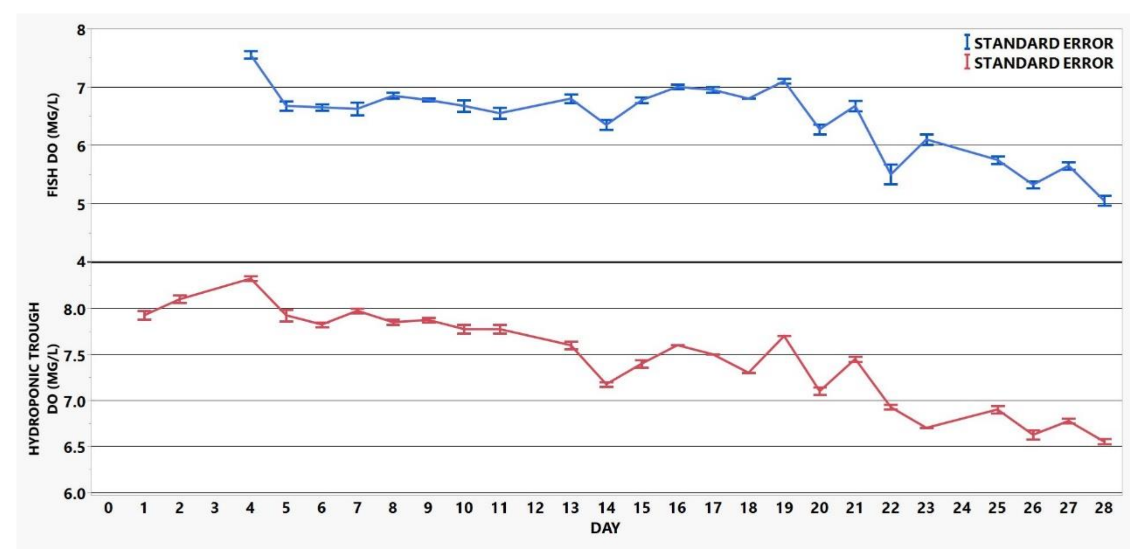

Figure 20: Mean ± SE for measurements of dissolved oxygen (DO) over 28 days in a floating raft aquaponic system planted with Italian large-leafed basil (Ocimum basilicum var. 'Rutgers Obsession DMR')

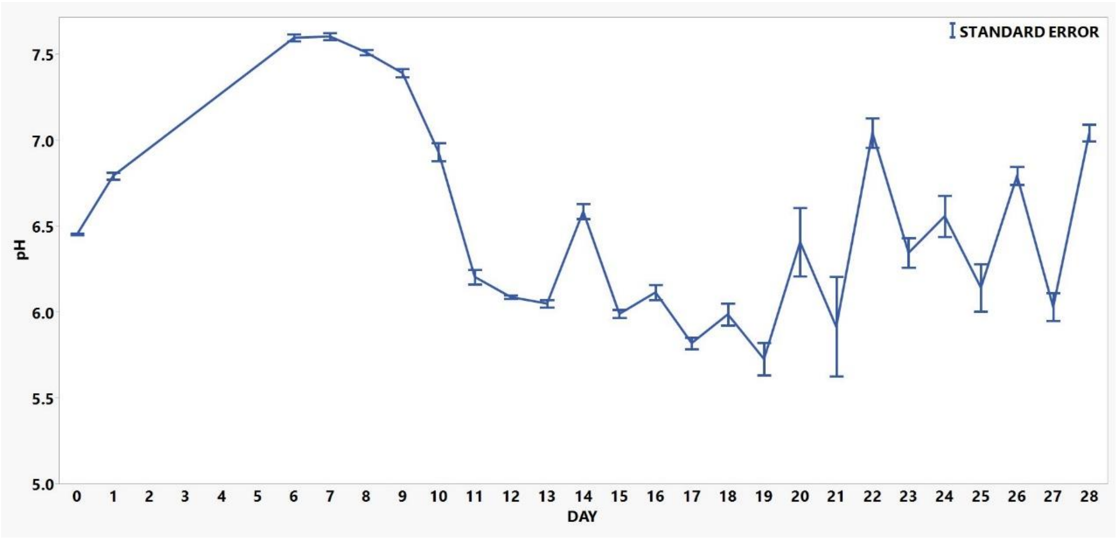

Figure 21: Mean ± SE for measurements of pH over 28 days in a floating raft aquaponic system planted with Italian large-leafed basil (Ocimum basilicum var. 'Rutgers Obsession DMR')

Daily EC measurements in all tanks continued to increase over the course of the experiment (Figure 22) as levels of dissolved nutrients increased faster than plants could remove them, while dissolved oxygen continued to decline as inadequate solids filtering led to increasing biological oxygen demand as microbial decomposition of accumulated solids deposits occurred.

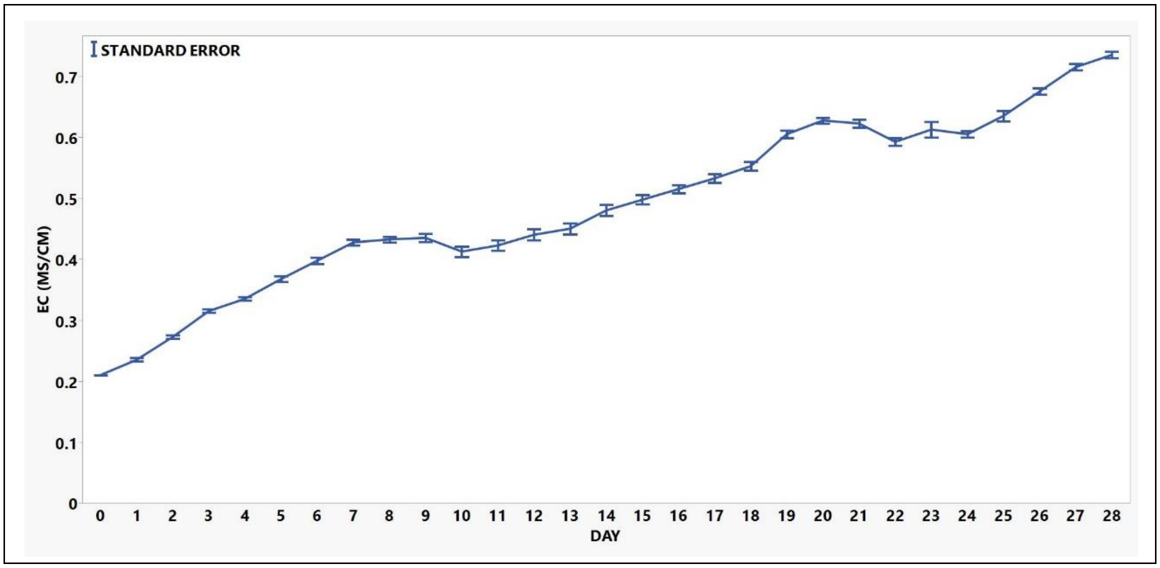

Figure 22: Mean ± SE for measurements of EC over 28 days in a floating raft aquaponic system planted with Italian large-leafed basil (*Ocimum basilicum* var. 'Rutgers Obsession DMR')

Conclusions

In Experiment 3 each aquaponic system received a daily average of 25.2 grams of feed per square meter, which was approximately 26.3 – 43.8% of production recommendations of 60 – 100 grams per square meter per day (Rakocy et al., 2006). While the amount of feed added to each aquaponic system was much less than recommended, calculations from Experiment 2 for feed input requirements were even lower with an estimated daily input requirement of 16.5 grams of 32% protein feed to produce 3338.8 grams of fresh shoot material over 28 days. Unfortunately, recommendations for feed input based on total surface area used for crop production fail to account for differences in nitrogen uptake rates and plant spacing requirements between species. To address these shortcomings, calculations for nitrogen input requirements in aquaponic systems should rather be based on the total amount of nitrogen required for each plant to reach a suitable harvest size.

Consequently, feed input recommendations for aquaponic production of Italian large-leafed basil calculated from this experiment were determined by the milligrams of protein required to produce the desired grams of fresh shoot mass. Using a generalized calculation for nitrification yield under ideal conditions and the observed nitrate uptake rates from Experiment 2 provided an estimate of 44.4 mg protein required per gram of fresh shoot mass. Conversely, the use of ammonia excretion measurements from tilapia in Experiment 1 resulted in an estimated requirement of 80.6 mg protein per gram of fresh shoot mass. Meanwhile, the data from Experiment 3 showed an average input of 67.5 mg protein per gram of fresh shoot mass produced, but large amounts of residual nitrate in each system indicated a greater input than was required by the basil.

This variation in projected feed input requirements is likely the result of several confounding variables commonly found in aquaponic systems. Differences in solids filtering efficiencies can lead to varying nitrate availabilities between systems depending on the amount of feed particles and fecal matter allowed to remain in circulation. Ideally, aquaponic systems only contain autotrophic nitrifying bacteria, but systems with even relatively modest C/N ratios will see faster growing heterotrophic bacteria outperform nitrifiers and significantly inhibit nitrification (Ebeling et al., 2006). Accordingly, the estimated input requirement of 44.4 mg protein per gram of shoot fresh weight is probably less than actually required, as nitrogen consumption by heterotrophic bacteria will ultimately reduce the amount of nitrogen available for plant uptake.

The presence of residual nitrogen at the close of Experiment 3, even with losses to heterotrophic bacterial consumption, indicated that 67.5 mg protein was greater than required to produce each gram of shoot mass. It is likely that some of the residual nitrogen originated from inadequate solids removal and subsequent fish deaths, which means that improved solids filtration and the resulting minimization of fish mortality should reduce the amount of excess nitrogen produced. As a result, the actual protein input requirement for basil is likely somewhere between 44.4 and 67.5 mg protein per each gram of shoot mass desired, and future experiments attempting to further clarify appropriate protein inputs will need to ensure that solids filtering is adequate enough to prevent accumulation of excessive un-ionized ammonia and fish mortality.

Finally, the comparison of pre-transplant and post-harvest nitrate availability is the best method for determining whether nitrogen input via feeding practices is appropriate as no two aquaponic systems will operate identically. If there is no net change in nitrogen availability from the start to the end of a production cycle, then fish stocking and feeding rates should be considered balanced with plant uptake. Additionally, any recommendations of protein input rates will always require some adjusting to account for differences in feed formulations and ingredient digestibility.

CHAPTER IV

SUMMARY

The aquaponic industry as a whole has languished under the lack of information available for growers to use when estimating system and crop production requirements. As water supplies become increasingly scarce and interest in sustainable farming practices continues to garner interest, additional guidance is vital in order for aquaponic vegetable production to satisfy that desire. While past studies have attempted to address this lack of information, they have been somewhat limited in scope regarding the fish and vegetable combinations studied. The aim of these collective experiments was to help establish a better understanding of balancing ammonia output of fish with the nitrate uptake of plants in raft aquaponic systems for a commonly utilized plant and fish pairing.

The first experiment focused on establishing a calculatable ammonia excretion rate based on the protein intake of tilapia, koi, and hybrid striped bass with the goal of providing aquaponic producers information that could allow them to change feeds intermittently in response to unforeseen changes in nitrogen requirements of the vegetable crop. Ideally, this would allow aquaponic producers that are newer to the industry to suffer less production setbacks while they become familiarized with the requirements of their individual aquaponic system. Data collected during this experiment showed respective ammonia excretion rates for tilapia, koi, and hybrid striped bass of 50.9, 64.5, and 79.0 mg TAN per gram of protein ingested, though feed digestibility concerns may need to be addressed in future trials through the use of formulated feeds.

Experiment 2 attempted to identify an individual nitrate uptake rate for Italian large-leafed basil (*Ocimum basilicum* var. ‘Rutgers Obsession DMR’) grown hydroponically. Based on the data collected, it was established that a yield of 85.6 grams of fresh shoot mass could be expected for every 342.9 mg of (NO_3^-)-N generated by fish, assuming that nitrate from fish and inorganic fertilizers have similar growth responses. A generalized ammonia excretion rate of 92.2 mg TAN per gram of protein and nitrification rate of 0.976 grams of nitrate (NO_3^-) per gram available ammonia (Ebeling et al., 2006) were used to estimate a protein input requirement of 44.4 mg per each gram of shoot material. Using ammonia excretion data collected in Experiment 1, resulted in respective protein input requirements for tilapia, koi, and hybrid striped bass of 80.6, 63.6, and 52.0 mg of protein per each gram of shoot mass.

In Experiment 3 the adequacy of dissolved nitrogen availability for marketable basil culture in a floating raft aquaponic system using recommended fish stocking and feeding rates was evaluated. A nitrogen surplus observed at the end of the experiment indicated feed input requirements in the aquaponic systems used for production of Italian large-leafed basil (*Ocimum basilicum* var. ‘Rutgers Obsession DMR’) were lower than generalized recommendations for the cultivation of herbs and leafy greens. While the amount of feed added to each aquaponic system was much less than recommended, calculations from Experiment 2 for feed input requirements were even lower with an estimated daily input requirement of 16.5 grams of 32% protein feed to produce 3338.8 grams of fresh shoot material over 28 days. As a result, actual protein input requirements for basil were estimated to be between 44.4 and 67.5 mg protein per each gram of shoot mass desired.

In conclusion, the general stocking and feeding rates used for leafy green production in aquaponic systems will lead to excessive dissolved nitrogen accumulation if utilized in the production of basil. As a result, greatly reduced feed input should be utilized in order to reduce the costly production of excessive dissolved nitrogen and minimize the need for frequent water exchanges to maintain suitable nutrient concentrations. Unfortunately, inadequate solids filtration, variations in bacterial communities occurring in aquaponic systems, and potential direct uptake of ammonia by plants make it difficult to determine the exact degree of excessive nitrogen produced. Therefore, future experiments attempting to further clarify appropriate protein inputs will need to ensure that solids filtering is adequate enough to prevent accumulation of excessive un-ionized ammonia and fish mortality.

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