ABSTRACT

Title of Document: EVALUATING ALTERNATIVE NUTRIENT SOURCES IN SUBSISTENCE-LEVEL AQUAPONIC SYSTEMS

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Many food production methods are both economically and environmentally unsustainable. Our project investigated aquaponics, an alternative method of agriculture that could address these issues. Aquaponics combines fish and plant crop production in a symbiotic, closed-loop system. We aimed to reduce the initial and operating costs of current aquaponic systems by utilizing alternative feeds. These improvements may allow for sustainable implementation of the system in rural or developing regions. We conducted a multi-phase process to determine the most affordable and effective feed alternatives for use in an aquaponic system. At the end of two preliminary phases, soybean meal was identified as the most effective potential feed supplement. In our final phase, we constructed and tested six full-scale aquaponic systems of our own design. Data showed that soybean meal can be used to reduce operating costs and reliance on fishmeal. However, a more targeted investigation is needed to identify the optimal formulation of alternative feed blends.

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Abbreviations and Units

ADC	Apparent Digestibility Coefficient
AMVA	American Veterinary Medical Association
ANOVA	Analysis of Variance
CF	Solution Conductivity Factor
DO	Dissolved Oxygen
FAO	Food and Agriculture Organization
FCR	Feed Conversion Rate
FDA	Food and Drug Administration
FE	Feed Efficiency
GFCI	Ground Fault Circuit Interrupter
IMF	International Monetary Fund
LDCs	Least Developed Countries
LECA	Light Expanded Clay Aggregate
LIFDC	Low Income Food Deficit Countries
MEGA	Maximizing Efficiency of Greenhouses using Aquaponics
NH_3	Ammonia
NO_2	Nitrite
NO_3	Nitrate
NOAA	National Oceanic and Atmospheric Administration
O_3	Ozone
PO_4	Phosphate
SVSU	Saginaw Valley State University
TWC	The WorldFish Center
UMD	University of Maryland
UVI	University of the Virgin Islands
VAC	Voltage, Alternating Current
WFP	World Food Programme

ºC	Degrees Celsius
cm	Centimeter
fish/L	Fish per liter
fish/gal.	Fish per gallons
ft^2	Square feet
g.	Grams
gal.	Gallons
gal./min	Gallons per minute
hp	Horsepower
in.	Inches
Kcal/100 g	Kilocalories per 100 grams
Kg	Kilograms
$Kg/(m^2 \cdot year)$	Kilograms per square meter per year
lb.	Pound
l	Liters
l/h	Liters per hour
l/min	Liters per minute
m	Meters
m^2	Square meters
mg/L	Milligrams per Liter
mg/kg	Milligrams per kilogram
mm	Millimeters
mmol photons/m^2s	Millimole photons per square meter per second
ppm	Parts per million
V	Voltage
W	Watts

Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), 842 million people, approximately one in every eight, suffered from chronic hunger, the lack of sufficient dietary energy to conduct an active life, from 2011 to 2013 (Food and Agriculture Organization (FAO), 2013). Of these 842 million, 98% live in developing countries[1] and nearly three quarters reside in rural areas (World Food Programme, n.d.). Several factors are known to contribute to the high incidence of hunger in developing regions. While the population in developing countries is increasing, available farmland is decreasing due to development, desertification[1], and drought (Ezeh, Bongaarts, & Mberu, 2012; Ramankutty, Foley, & Olejniczak, 2002). For example, areas in sub-Saharan Africa, Ethiopia, Malawi, and Cambodia with imperfect food markets subsequently lack food security because of geographic isolation (van Brakel & Ross, 2010; Ghanem, 2008; FAO, 2012; CSIS, 2010; Holden & Shiferaw, 2002; Zeller, Diagne, & Mataya, 1998).

Furthermore, there is a widespread lack of infrastructure within developing regions of the world. Globally, 1.4 billion people are without electricity, 85% of whom live in rural communities (Kaygusuz, 2012). One billion people do not have access to a clean water supply, and many areas cannot support the large amounts of water demanded by agriculture (Hunter, MacDonald, & Carter, 2010). Fisheries, an economically feasible alternative to land-based agriculture, have become critical to food security in the developing world (FAO, 2012). Fish accounts for 19.2% of animal protein intake in the diet of developing countries and an average of 24% in the 62 countries categorized as Low-Income Food Deficient Countries[1] (LIFDCs) by the

[1] For a full glossary of terms, see Appendix N. All terms with this denotation will be defined in Appendix N.

FAO (2012; 2014). In 2010, fish exports from LIFDCs accounted for $4.7 billion of the world's total trade (FAO, 2012).

Unfortunately, overfishing of the world's oceans has since reduced the viability of wild-caught fish as a stable food source. An estimated 90% of large fish such as tuna, swordfish, marlin, cod, and halibut are gone from oceans, and selectively harvesting these fish has placed evolutionary pressure on the population, resulting in slower growth and reduced body sizes (Conover, Munch, & Arnott, 2009; Myers & Worm, 2003). Wild-caught fish and natural aquatic resources are therefore unlikely to be able to satisfy the protein demands of a growing world population (van Brakel & Ross, 2010).

Despite these setbacks to wild fisheries, fish as a protein source for humans has been steadily increasing via aquaculture[1] (Larsen & Roney, 2013). Aquaculture, the farming of fish or other seafood, recently surpassed both wild-caught fish and beef production and has become the fastest growing food production sector, maintaining an average growth of 8.3% annually since the mid-1980s (Larsen & Roney, 2013; National Oceanic and Atmospheric Administration Fisheries, 2012a). Aquaculture now provides more than 50% of the seafood eaten globally; producing an all-time high of 60 million metric tons (130 billion lb.) in 2010 (NOAA Fisheries, 2012a; FAO, 2012). Recirculating[1] aquaculture systems have gained momentum in part because they can sustain both omnivorous[1] and carnivorous[1] species at a low cost while limiting disease transfer, nutrient pollution, and genetic mixing[1] that are frequently associated with traditional aquaculture (Naylor et al., 2009; Martins et al., 2010).

The rapid expansion of aquaculture, however, has raised questions about its sustainability. Most fish raised in aquaculture systems require high protein diets for faster growth, which commonly consist of fishmeal made from lower-value, wild-caught fish, such as

anchovies, mackerel, sardines, and menhaden (Larsen & Roney, 2013; Naylor et al., 2009; Pauly & Watson, 2009). Furthermore, the global distribution of aquaculture systems remains unbalanced as the least developed countries[1] (LDCs) contribute only 4.1% to world aquaculture production (FAO, 2012). This suggests that current aquaculture systems are infeasible for application in these LDCs due to cost, inaccessibility, and the depletion of fisheries, which has led to increased prices for fish feed.

Aquaponics is a novel, alternative method of fish and crop production that combines aquaculture with hydroponics[1], a method of growing plants without soil. The plants filter waste products harmful to the fish from the system by utilizing them as a nutrient source (Rakocy, Bailey, Shultz, & Thomas, 2004). This symbiotic interaction in the system can reduce the need for filters, fertilization, mechanical maintenance[1], water monitoring, and water changes as compared to aquaculture or hydroponics alone (Diver, 2006; Rakocy et al., 2004). These advantages can reduce operating costs and improve the potential for profit through increased yields (Rakocy et al., 2004).

Current aquaponic systems exist in several urban areas for educational and commercial production purposes, but they have not seen widespread use in rural settings as a means of subsistence[1]. This is partially because current aquaponic systems require large inputs of capital, electricity, and processed fish feed (Lapere, 2010; Hishamunda, Jolly, & Engle, 1998; Kassie & Zikhali, 2009). Studies conducted in South Africa, Rwanda, and Nigeria found that the lack of available resources (such as electricity, presence of markets, and access to skilled labor) in developing countries is an important factor that limits the feasibility of a cost efficient and sustainable[1] aquaponic system (Lapere, 2010; Hishamunda et al., 1998; Kassie & Zikhali, 2009). Additionally, formulated fish feeds represent one of the largest variable costs in traditional

aquaculture systems (Naylor et al., 2009). A viable aquaponic system would therefore have to grow local crops, be built using local building materials, and utilize locally available alternative feeding sources.

The primary goal of our project is to evaluate the effect of alternative feed sources on fish growth and crop production of a closed-loop subsistence-level aquaponic system. To do so, we have implemented a four phase experimental design. We define subsistence-level as a low energy system that can provide a supply of food for direct consumption by the individuals maintaining it. By using alternative nutrient sources in subsistence-level aquaponics, we hypothesize that we can produce biomass yields[1] comparable to existing systems while reducing input costs.

Literature Review

Hydroponics

Hydroponics is a broad term, but it is commonly defined as a form of soilless agriculture in which plant roots are suspended in either a static, continuously aerated nutrient solution or a continuous flow of nutrient solution (Jones, 2012). Hydroponics is optimal in situations where space is limited. Traditional crops require substantial amounts of land, labor, and other resources. Hydroponics nearly eliminates the need for soil and labor for tilling and other agriculture practices (Jones, 2012). Another issue with traditional agriculture is the loss of nutrients from the fields due to leaching. According to Wignarajah (as cited by Pessarakli, 2014) leaching is the process by which water soluble plant nutrients leave the soil through rainwater or irrigation. Leaching does not occur in hydroponics due to the lack of soil. While hydroponics does save a lot of soil management and labor time, it presents other challenges. The initial startup costs for hydroponics are high. In addition, the required knowledge on plant health, system operation, and nutrient requirements often deter amateur farmers (Jones, 2012). Despite these difficulties, hydroponic farming has grown in past years, generating revenues of $606.8 million worldwide in 2013 (Kruchkin, 2013).

Aquaculture

Aquaculture may be defined as the process of "…breeding, rearing, and harvesting of plants and animals in all types of water environments including ponds, rivers, lakes, and the ocean" (NOAA Fisheries, 2012b). Aquaculture has reduced the pressures on wild caught fisheries to fulfill increased demands by providing an alternative source of large fish (Chamberlain & Rosenthal, 1995). The difference between demand and natural fish population supply has been filled by a steady increase in fish from cage aquaculture[1] (Findlay, Podemski, &

Kasian, 2009). Despite the intriguing advantages of aquaculture systems, there are pitfalls as well. Cage aquaculture produces uneaten feed, solid nitrogenous waste, disease, antibiotics and harmful oils that can be introduced to the local ecosystem (Liu & Sumaila, 2010; Findlay et al., 2009). In addition, feed for aquaculture systems currently uses fish and fish waste as a food source (FAO, 2012). Together, these issues can increase the potential for eutrophication[1] and threaten biodiversity[1] in the surrounding aquatic communities. Recirculating aquaculture provides a more sustainable option with the recycling of water (Durham, 2010) and increased biosecurity. Unfortunately, most commercial systems to date have failed to achieve the goals of increased sustainability due to poor design, inferior management, and flawed economics (Masser, Rakocy, & Losordo, 1999, March). Commercial aquaculture systems produce 40.1% to the world total fish production with 62.7 million metric tonnes (69.1 million tons) in 2011 compared to 34.7 million metric tonnes (38.3 million tons) ten years earlier (FAO, 2011). Despite large production numbers, research continues to reveal ecological detriments of aquaculture and the demands for more sustainable methods within the production of fish remain.

Aquaponics

Aquaponics combines fish and plant crop production in a symbiotic[1], closed-loop system. Aquaponics can provide fish yields comparable to intensive aquaculture and plant yields exceeding those of conventional hydroponics. The symbiotic relationship that develops between the fish and plants can result in these increased yields while reducing costs and required inputs and maintenance (Savidov, Hutchings, & Rakocy, 2005; Wilson, 2005). In most aquaponic systems, fish are fed a high-protein diet (Rakocy, Masser, & Losordo, 2006, November; Rakocy, 2011). The fish excrete waste that is high in potentially toxic nitrogen compounds, including ammonia (NH_3), through their gills as well as in their feces. These compounds are first

processed into nitrite (NO_2), then nitrate (NO_3) by nitrifying bacteria on submerged surfaces in the system. The plants utilize the nitrate from the water for growth, serving as a biofilter[1] and thereby reducing the need for active biological or chemical filtration and water quality management. As the plants filter the water, they also reduce the need to replace water for the fish tanks, while the fish provide biologically available[1] nutrients for the plants. Together, these key processes can eliminate the need for expensive nutrient management systems employed in conventional hydroponics, and induce plant growth more effectively (Rakocy et al., 2006, November).

Overall, the closed-loop design of an aquaponic system effectively minimizes required inputs (nutrient, energy, and manpower) thereby lowering economic barriers to aquaponics as compared to conventional hydroponics and aquaculture (Rakocy et al., 2006, November).

Existing Systems

One of the most tested and documented aquaponic systems was developed at the University of the Virgin Islands (UVI) (see Appendix L for diagram). In this system, 7,800 L (2,060 gal.) fish-rearing tanks are used with flow rates of 380 L/min (100 gal./min). The water flows through degassing and filter tanks before providing water for 11,000 L (3,000 gal.) plant beds growing lettuce, basil, and several other plants. Because of the extensive filtration system for maintaining water quality, the UVI system has stocking densities of 0.077 fish/L (0.29 fish/gallon). A settling tank and clarifier also remove solids while regulating pH[1] to stay between 6.5 and 7 through the application of potassium hydroxide and calcium hydroxide. An extensive aeration[1] system is also required to keep fish alive in this aquaponic system; the test system at UVI uses 22 air diffusers in each fish-rearing tank, requiring a constant supply of power (Rakocy, 2006).

Because of all of this equipment, the system, which produces 5,000 kg (11,000 lb.) of tilapia and 37,800 heads of lettuce per year, costs over $40,000 to build, excluding labor costs ("Commercial Facility," 2009). The system was developed in a tropical environment and the plant beds were not covered or protected, though subsequent research has shown that the system adapts well to a greenhouse environment (Savidov et al., 2005). An enclosure would add to the cost if needed for non-tropical settings.

In the UVI system, a commercial fish feed with approximately 34% protein is used in a well-defined feeding regimen in order to achieve optimal growth. Nile tilapia (*Oreochromis niloticus*) fingerlings had a survival rate of 98.3% over the course of the study. 3.2 mm (0.13 in.) feed pellets are used until the fish reach a mean size of 300 g. Subsequently, 4.8 mm (0.19 in.) pellets are used. The fish are fed 2.5% of their body weight each day up to 300 g and then are given 1.25% per day. Under this feeding regimen, fish in 24.8° C water reach an average mass of 813.8 g in 24 weeks from a starting average mass of 79.2 g (Rakocy et al., 2006, November). For plants in the system, seeds are germinated in rockwool[1] and then transferred onto polystyrene rafts that float on the water circulating through the plant beds. This method of hydroponics is highly stable, requiring less maintenance than other options, which trap solid waste and must be cleaned periodically (Lennard & Leonard, 2006). In a study that adapted the UVI system for use in a greenhouse in Canada, the plants were kept in an area with air temperature between 22º and 25º C, and artificially lit at levels greater than 300 mmol photons/m^2/s for sixteen hours a day (Savidov et al., 2005).

Intensive aquaponic systems are becoming more popular and are being implemented on a larger scale. The majority of this growth has occurred in urban areas, where there are more available resources. One example is the Milwaukee, WI based Sweet Water Foundation, which

raises over 80,000 fish in a former crane factory. Another example is the Massachusetts Avenue Project in New York, an organization focusing on large urban aquaponics where one greenhouse has the ability to raise over 35,000 fish concurrently (Metcalf & Widener, 2011). These current high-density systems exist for commercial applications. However, the requirements (a large investment of capital, reliable power, and proper infrastructure) make existing systems largely infeasible for use in developing nations, particularly in rural areas. A large number of people therefore cannot utilize existing aquaponic systems, but may benefit immensely from a simplified, less resource-intensive system.

Unfortunately, research regarding the successful implementation of small-scale aquaponics is limited, and previous large-scale[1] projects in developing countries have failed. Attempts to implement large-scale tilapia production systems in rural areas have been unsuccessful, due to local government resistance and problems with economic feasibility. In South Africa, for example, farming of Nile tilapia is illegal, and there are no examples of the government making exceptions. Furthermore, financing these ventures was described as difficult (Lapere, 2010). Despite logistical concerns regarding large-scale tilapia production, there is potential for subsistence-level aquaponics. One article written by Nelson and Pade (2007) discusses the potential benefits of a small-scale simplified aquaponic system they termed "Village Aquaponics." They predicted that aquaponic systems that grow food primarily for local consumption can be a viable means of providing protein and vegetables to people in both developing and developed nations. This prediction can only be verified with further studies of subsistence-level aquaponics.

Luke's Mission is one of few documented examples of a local aquaponic system being implemented in a rural environment. This system was built in Haiti, and although a website and

a journal article on the project exist, no further updates have been documented since construction in 2004 (Perry & Rittgers, 2004). This seems to indicate that, if successful, it was an isolated instance, and the project has not expanded. This system does not have access to electrical power, and thus utilized alternative means of circulation and other required system processes.

Hughey (2005) designed a flooding system that allowed a low flow pump to be used. He predicted that an off-grid[1] aquaponic system was possible, and that it would be very beneficial. He planned to implement a pilot system in Kenya using wind power, but noted that design modifications still needed to be made. This pilot system, which he calls "barrel-ponics" due to the use of plastic barrels as plant beds and other containers, was then constructed. The program appears to be successful, but quantitative information about the system has not been collected to evaluate its efficiency relative to other aquaponic systems. However, applications of this system could be limited because it requires over 400 W (0.536 hp) of electricity to run a 2650 L (700 gal.) system and access to plastic barrels that are often used to ship food-products (Hughey, 2008).

In order to successfully utilize aquaponics as a primary means of subsistence, low energy methods of water circulation, aeration, and filtration are needed.

Fish and Plants

Nile tilapia is a common choice of fish for aquaponics. Tilapia is a freshwater fish species that grows and reproduces quickly. They are also readily accepted in the world market (Rakocy, 2011). Red and Nile tilapia (both members of the *Oreochromis niloticus* species) are regularly used, but Nile tilapia grow larger and can have a better survivability rate (Rakocy et al., 2004; Rakocy et al., 2006, November). Even in adverse conditions, Nile tilapia grow easily, making them a good choice for both high- and low-intensity[1] aquaponic systems. They consume

a primarily vegetable diet, which allows the use of nutrient options that are more sustainable and more available than conventional processed fish feed (Delong, Losordo & Rakocy, 2009, June). Ideal conditions in which tilapia grow and reproduce most quickly are as follows: Dissolved oxygen (DO) concentrations of 5.0 - 7.5 mg/L (ppm), temperatures 27 - 29º C and pH 6-9. They can survive extreme oxygen conditions of as low as 0.3 mg/L briefly, but should be maintained above 1.0 mg/L. Temperatures below 10 - 11º C are fatal, and feeding stops below 17º C (Popma & Masser, 1999, March).

There are also many varieties of plant species that can be grown in aquaponic systems. Of these plants, the most commonly grown in aquaponic systems are lettuce, basil, and tomatoes. Broccoli, a cool climate crop, is also suitable for aquaponics (Rakocy et al., 2006, November; Harston, 2007). Fruiting vegetables have a longer growing cycle and often have more pest and disease problems associated with them, but typically receive higher prices at markets (Rakocy et al., 2006, November). The exception to that is basil, which thrives in the aquaponic environment (yielding up to 42 kg/(m^2·year)) and can be sold at a high price in most regions. For this reason, basil is the most researched crop in aquaponics. Crops that can be grown in an aquaponic setting fall into three main categories based on the solution conductivity factor[1] (CF) in which the plants perform best. Group 1 comprises plants with high CF and includes tomato and eggplant. Group 2 plants have medium CF and include lettuce, basil, and cucumber. Group 3 consists of plants with low CF and includes watercress (Savidov et al., 2005). It is important to consider the requirements of the specific cultivars[1] that will be grown in an aquaponic system when determining if nutrient supplementation will be necessary. There are thirteen total nutrients that these plants need to absorb from the water: nitrogen, potassium, calcium, magnesium, phosphorus, sulfur, chlorine, iron, manganese, boron, zinc, copper, and molybdenum. The first

three are macronutrients[1], while the others are micronutrients[1]. The limiting factors[1] in the UVI system are potassium, calcium, and magnesium, which they supplement in the form of potassium hydroxide, calcium hydroxide, and dolomite ($CaMg(CO_3)_2$) in order to maintain plant growth (Rakocy et al., 2006, November).

Nutrient Sources

Once the aquaponic system is in place, the primary cost of continuing production is purchasing fingerlings and feed. Tilapia can reproduce quickly in an aquaponic system as previously noted, and therefore the cost of fingerlings can be largely avoided through husbandry practices that retain a brood stock[1]. Feed poses more of a challenge as it is expensive to purchase, and many rural areas do not have market access to purchase processed feeds (Metcalf & Widener, 2011). Therefore, alternative sources of nutrients that are widely available and more cost-effective would make a system more flexible and feasible. We searched existing literature on the subject in order to find possible alternatives to commercial fish feed.

El-Sayed (1999) conducted a study on fish feeding of farmed tilapia. He found that over 50% of operating costs in aquaculture are dedicated to dietary sources for fish feeding. The high costs are attributed to the expense of traditional commercial fish feeds, which typically consist of fish meal as the main protein source. El-Sayed's paper investigates blending fish feed with alternative substances. When blended in 1/1 ratio with fish meal, fish silage[1] provides insignificant differences to growth and digestibility of Nile tilapia. Higher ratios of fish by-products were also tested, but led to reduced performance. Tilapia growth surpassed the control fish meal feed when a 3/1 meat and bone meal[1] was used as the supplemental feed. These supplemental feeds provide possible alternatives in reducing the conventional use of fish meal (El-Sayed, 1999).

The percentage of overexploited fish stocks increased from 10% in 1974 to 26% in 1989 and has continued to rise since then. Over 29% of fish stocks are estimated to be overexploited and are in need of strict management plans to restore ecological balance. The top ten species are fully exploited and production rates therefore remain stagnant. The top ten species together account for nearly 30% of the world marine capture fisheries production and include anchoveta, blue whiting, and Alaska pollock (FAO, 2013; Conover, Munch, & Arnott, 2009). In 2012, FAO reported that the state of world marine fisheries is worsening and overexploitation must be managed to prevent further ecological damage and increase production rates (FAO, 2013).

Our literature review of potential alternative feeds led us to several potential sources that would be more accessible in our targeted regions. These nutrients include vegetable compost[1], dairy manure[1], poultry manure[1], activated sludge[1], rice bran[1], sorghum[1], and soybean meal[1]. In addition to these by-products and wastes, we looked for an intermediary plant[1] that could be used to capture nutrients dissolved in water and that would be more readily accepted by the fish as a feed. This intermediate plant is necessary because several of the nutrient sources mentioned above may not be accepted if offered directly as feed to the tilapia. Duckweed[1] was identified as the best option to serve this purpose.

Duckweed.

Duckweed is one of the fastest-growing vascular plants[1], doubling in mass every 16-48 hours. The plant contains very little fiber content and has extremely high digestibility. It can be used as a complete feed for adult tilapia, providing all of the major and minor nutrients required to promote rapid growth.

Apparent digestibility coefficient (ADC) [1] can be used as a metric to measure the proportion of a food that is digested compared to what is absorbed, expressed as a

percentage. Crude protein content is often measured by using the Kjeldahl method (Casal, Vermaat, & Wiegman, 2000). El-Shafai et al. (2004) found that duckweed diets of 20-40% protein have an ADC of 76-80%, comparable to that of conventional plant ingredients used as components of commercial fish feeds. They also found that Nile tilapia grown using feeds with duckweed had higher protein content than those grown with conventional processed feeds. The researchers concluded that, in aquaculture systems, duckweed was a viable alternative or supplement to expensive, fishmeal based commercial feeds, which are unavailable in many regions.

Duckweed can grow well under various conditions, but optimally in situations with low-flow, warm water up to 33º C, pH 6.5-7.5, ammonia concentrations above 12 mg/L, and a steady source of nitrogen and phosphorus provided by the decomposition of organic matter. In some situations, an addition of a small amount of sea salt can add beneficial trace minerals. Even in water with only trace concentrations of nutrients, duckweed grows with 15-25% crude protein content. By contrast, in ideal conditions, it typically grows with 35-43% crude protein, which meets or exceeds the concentration of protein found in commercial tilapia feed (Leng, 1995).

Compost.

In 2003, a project at Saginaw Valley State University (SVSU) indirectly used compost in their aquaponic systems. The SVSU system was housed in a pair of experimental greenhouses that were managed by a multidisciplinary team from the university. The greenhouses began as homemade hydroponic systems to maintain low-cost maintenance and energy solutions. The system later became an aquaponic system with the addition of a 570 L (150 gal.) water tank containing twelve Koi fish. A pump recirculated water between the fish tank and two 190 L (50 gal.) hydroponic grow beds, in which water intermittently floods and drains. Vermiculture, the

process of growing worms, was introduced as a means to supplementing the hydroponic plant growth. Worms were placed into vermiculture[1] beds and cultivated in a mix of SVSU's compost, which consisted of food scraps and shredded, recycled paper. As the worms cultured in the beds, excess water leached through vermicompost to buckets placed below drain pipes. This leachate would then be used to fertilize the hydroponic plants and those grown in topsoil (Jorgensen, Meisel, Schilling, Swenson, & Thomas, 2009).

There is limited literature regarding the use of compost to fertilize duckweed. However, compost is often used as a fertilizer in organic farming (Rynk, 1992). Additionally, duckweed thrives in the presence of decomposing organic matter. Thus, compost is a promising nutrient source for duckweed growth.

Dairy and Poultry Manures.

Dairy and poultry manure are routinely used as fertilizers in agriculture due to their high concentration of key nutrients, such as phosphorous and nitrogen. This makes them excellent nutrient sources for growing duckweed, if risks of pathogens are effectively managed. In a study conducted by Yao, Wu, Zhu, Sun, and Miller (2011), protein extract from dairy manure was used as a source of nitrogen to grow fungus, demonstrating its usefulness as fertilizer. Alhadhrami and Yousif (1994) used camel and cow manures in isonitrogenous[1] and isocaloric[1] diets prepared for blue tilapia. Their results indicate that pelleted feeds containing 10-20% manure provided comparable results to a control diet of commercial feed. Higher inclusions of manure show reduced growth. Another study by Green (1992) showed that chicken manure can replace up to 58% of pelleted supplemental feed without significantly affecting tilapia growth. The ubiquity of both manures in agriculture makes them excellent sources for our experiment.

Activated Sludge.

Reusing treated wastewater in agricultural settings is becoming an increasingly popular practice around the world. In a study conducted by Rojas-Valencia, de Velásquez, and Franco (2011), the efficacy of wastewater treatment through the application of ozone was examined. Treating raw wastewater for one hour with ozone at a concentration of 7.36 mg/L O_3 resulted in the removal of 87% of biological oxygen demand[1] and 93% of chemical oxygen demand[1]. This method preserved nutrients in the water, while eliminating harmful pathogenic microorganisms. In another study, the use of dried fecal sludge as a fertilizer by farmers in Ghana was examined. The research revealed that despite challenges, including smell and transportation, farmers were seeing a large increase in productivity of the soil, resulting in increased yields, profit, and food security for their families. The study concluded that with proper training about disease transmission, the use of fecal sludge is a viable and affordable alternative to the imported manufactured fertilizers that it replaces (Cofie, Kranjac-Berisavljevic, & Drechsel, 2005). Lopez Zavala, Funamizu, and Tukakuwa (2004) studied the biological activity in bio-toilets, a type of composting toilet, and the contents of the resulting composted material. They found that the low-temperature processes carried out in this specific model resulted in a dramatic reduction of ammonia nitrogen: a 93% drop. This is detrimental to its usefulness as an agricultural fertilizer, which they noted. However, they also concluded that further modifications could be made in order to increase the agronomic value. The waste is available everywhere and, if properly treated, the nutrient-rich properties could be used to stimulate the growth of duckweed, which in turn would provide a source of fish food.

Rice bran.

Rice bran is the residue left after the rice has been milled, and it has shown promise as a source of fish feed. According to one study led by Amissah, Ellis, Oduro, and Manful (2003), several different bran samples were found to contain energy levels in the area of 300 Kcal/100 g. The bran also displayed high concentrations of potassium, phosphorous, and calcium, proving its usefulness as a potential nutrient source. In a study by Veverica, Liti, Were, and Bowman (2001), rice bran was used as the primary feed in an aquaculture system. Although fish raised on a rice bran diet had the lowest average fish weight, rice bran proved more economically feasible[1] than other feeds and supported acceptable water quality.

Sorghum.

Past studies demonstrate the effectiveness[1] of sorghum as another viable alternative nutrient source in aquaponics. A study led by Guimarães, Pezzato, Barros, and Tachibana (2008) measured the apparent ADC in several feed sources to ascertain values of nutrient availability in these sources. The digestibility values of energy and dry matter in sorghum were 82.37% and 87.29%, which were the second highest results for the five ingredients tested. Sorghum displayed a high availability of the essential amino acid leucine. However, sorghum proved the least effective in terms of protein digestibility, at only 56.77%. In 2000, the global area of sorghum exceeded 50 million hectares (124 million acres) and the fastest growing sorghum producing zones were in developing countries (Okuthe, Ngesa, & Ochola, 2013).

Soybean meal.

Soybean meal is the processed portion of the soybean after its oil has been extracted. Soybeans are grown on every continent, excluding Antarctica, and are commonly used in both human and animal diets. The most common soybean products used as feed in aquaculture are

toasted soybean meals. These come in two different varieties, dehulled and hulled. We used the dehulled product, which has a protein content of roughly 49% (Brown, Kaushik, & Peres, 2008). Certain anti-nutritional[1] factors present in raw soybeans limit their effectiveness as a food source. However, these factors can be mitigated through heat treatment. In fact, studies show that up to 40% of the protein provided in standard fish meal may be replaced with soybean meal without adverse effects on the growth and body of fish (Buyukcapar & Kamalak, 2007).

Other studies show that soybean meal could replace from 67-100% of fish meal (Shiau, Kwok, Hwang, Chen, & Lee, 1989). Many studies have produced mixed results on tilapia. As El-Sayed (1990) describes, dietary protein concentrations in soybean meal are directly correlated to tilapia growth rate. Multiple accounts blame reductions of tilapia growth on typical sulfur and phosphorus compounds found in oilseed plants. Other studies alleviate such reduction by mixing an animal protein source with the fish meal. In the case of Sadiku and Jauncey (1995), they fed soybean flour and poultry meat meal to Nile tilapia. They found that the highest feed efficiency[1] and growth rates occurred at a 75/25 blend of soybean flour to poultry meat meal.

Metrics

In examining the base of literature available on aquaponics, we found a variety of metrics that will be useful in comparing our results with existing systems. Plant yield is measured in pounds per square foot and pounds per plant (Savidov et al., 2005). These yields are then evaluated based on market value[1] in order to measure economic feasibility. Fish yields are measured in weight per volume of the tank, growth rate of the fish in grams per day, survival rate, feed conversion rate[1] or feed efficiency (FCR or FE), and are also evaluated by market value. Nile tilapia in the UVI system had a FCR of 1.7, meaning that 1.7 pounds of feed must be consumed for the fish to grow by one pound.

The water flow and turnover rates[1] are important considerations when determining the stocking density[1] and aeration capacity of an aquaponic system. DO, pH, and nitrogen compounds (ammonia, nitrite, and nitrate) are measured regularly to ensure fish survival (Rakocy et al., 2006, November). Throughout the nitrogen cycle, ammonia, nitrite, and nitrate are intermediate and final byproducts. Ammonia and nitrite have been shown to have detrimental effects on fish growth and plant stress. Un-ionized ammonia at concentrations as low as 0.07 mg/L have caused tissue damage and slowed fish growth while nitrite concentrations as low as 5 mg/L damaged root tips (Masser et al., 1999, March). Nitrate is only toxic to fish at very high concentrations (Hrubec et al., 1996). The tolerable ranges for toxic ammonia[1] and nitrite concentrations for fish are 0-2 mg/L and 0-5 mg/L respectively (Rakocy, 1989, September). More importantly, pH is essential for fish growth, plant health, and the nitrification process. The production of toxic ammonia and nitrite increased significantly at a pH of 8.5 compared to that of 6.5 (Tyson et. al, 2007).

Applications and Global Markets

High levels of undernourishment[1] are prevalent through much of the eastern hemisphere. Sub-Saharan Africa, the Caribbean, and Southern Asia carry the highest percentages of total undernourished population with 24.8, 19.3, and 16.8%, respectively. The three regions mentioned have seen the least signs of improvement in previous 20 years, and the lack thereof may be attributed to political instability and poor infrastructure (FAO, 2013). Van Brakel and Ross (2011) evaluated the food markets in Cambodia, and found that large numbers of rural residents do not have access to urban markets, contributing heavily to food insecurity. They concluded that an aquaculture strategy that improves rural access to food would benefit up to one million impoverished Cambodians.

Small-scale agriculture has a variety of benefits. Not only does it require less energy input from potentially dangerous fertilizers and pesticides, but has also been linked to healthier, more nutritious diets. One example of the widespread implementation of small-scale agriculture was post-Soviet Union Cuba. After the fall of the Soviet Union, Cuba lost access to important resources for conventional agriculture, such as oil, fertilizer and pesticides. The resultant decline in food production caused an estimated 30% reduction in the country's caloric intake during the early 1990s (Murphy, 1999) The Cuban government responded by encouraging the extensive development of urban agriculture. By 1997 there were nearly 8,000 gardens in the capital city of Havana, covering roughly 15,000 hectares (37,000 acres) (Altieri et al., 1999). The city residents overcame issues of poor quality soil and limited fertilizer by using raised plant beds and producing organic fertilizers. The result of this small-scale urban agriculture was a resounding success. In 1998, Havana produced roughly 490,000 metric tonnes (541,000 tons) of food, with several neighborhoods producing up to 30% of their subsistence needs (Murphy, 1999). These gardens also offered the benefits of diverse, highly nutritious crops that were ready for consumption without the need for refrigeration or transportation.

However, implementing a similarly sized aquaponics based program to decrease food security has many obstacles. A major challenge is funding, as the rural residents cannot afford to put forward the capital required to purchase even a relatively inexpensive system. The same issues were faced in Thailand, where a local government provided several communities with aquaculture kits so that residents could grow their own fish. The citizens were not required to directly pay back the loan, but were told to pay into a community fund so others could afford similar kits if, and only if, their own systems were successful. The communities involved responded well, with 50% growth in participation, going from 40 families to 60 families from

1996 to 2000 (Sheriff, Little, & Tantikampton, 2008). Similar approaches could be used with aquaponic systems in other regions. It is important that the local institutions become invested in the success of the program, as this involvement is needed for the project to succeed in the long-term (Perry & Rittgers, 2004).

Experiment Structure

Our research was conducted in four phases:

- Phase 1 – Evaluation of alternative nutrient sources for growing duckweed
- Phase 2 – Evaluation of alternative feed sources for tilapia, 100% replacement
- Phase 3 – Evaluation of alternative feed sources for tilapia, 50% replacement
- Phase 4 – Large scale test of 50% soybean meal replacement

Phase 1 evaluated the use of alternative nutrient sources to grow duckweed. In particular, activated sludge[1], poultry manure, dairy manure, and local compost were compared against a control of no added nutrient source in a two-week study. This phase was conducted in triplicate for a total of fifteen duckweed systems. The duckweed was then evaluated based on total protein content through lab analysis. We reached the conclusion that activated sludge as a nutrient source grew duckweed with a protein content of 40.67%, comparable to the protein content of duckweed grown under ideal conditions. Based on this, we chose to use duckweed grown with activated sludge for one of the alternative nutrient sources in Phases 2 and 3.

Phase 2 evaluated the use of alternative feed sources as a 100% feed replacement for raising tilapia and growing basil and lettuce. In particular, duckweed, sorghum, rice bran and soybean meal were compared against a control of commercial fish feed. As mentioned above, the duckweed was grown with activated sludge. This phase was conducted over the course of eleven weeks and in duplicate. Based on the plant biomass yield and tilapia mass yield, along with considerations of fish health and water chemistry, duckweed and soybean meal were chosen for the next phase.

Phase 3 evaluated the use of alternative nutrient sources as a 50% feed replacement. Once again, tilapia were raised and basil and lettuce were grown. In this phase, duckweed and

soybean meal were evaluated against a control of 100% commercial fish feed. Each of the alternative nutrient sources was blended with 50% commercial fish feed. This phase was conducted over the course of nine and a half weeks and in triplicate. Based on the same yields and considerations as Phase 2, soybean meal was chosen as the only alternative nutrient source for the final phase.

Phase 4 was the final phase in our project. This phase was conducted in a more real-world setting and with larger numbers of fish and plants per system to help model a subsistence-level system. In this phase, soybean meal was evaluated as a 50% feed replacement against a control of commercial fish feed. Once again, the soybean meal was blended with 50% commercial fish feed. This phase was conducted over the course of eight weeks and in triplicate, shown in Figure 1. Based on this phase, we were able to evaluate the use of soybean meal as an alternative nutrient source in a partial feed replacement for a subsistence-level aquaponic system.

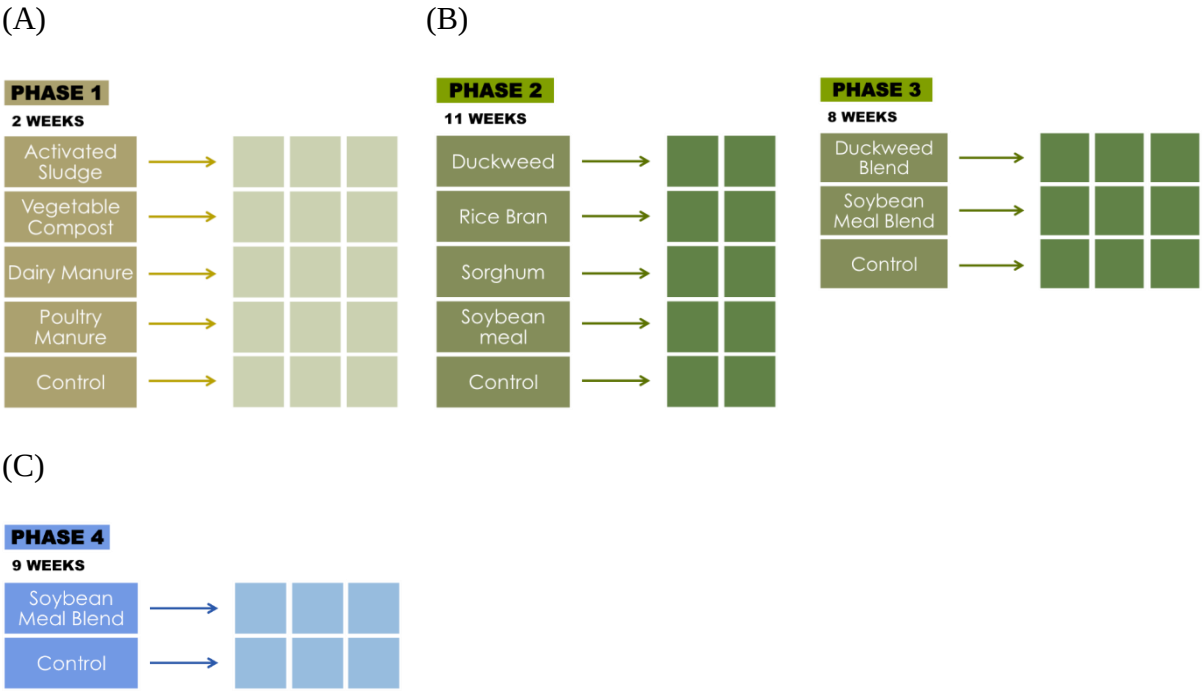


Figure 1. Schematic representation of research design.
(A) Phase 1 will determine what is the most effective nutrient source to fertilize duckweed. (B) The duckweed utilized in Phase 2 will be fertilized with the selected nutrient source from Phase

1. (C) Phase 2 will feature three subsistence-level systems utilizing the most effective alternative feed source determined by Phase 1.

Phase 1 Protein Analysis of Duckweed

Introduction

Based on protein content, digestibility, and prevalence in existing literature, four potential alternative feed sources (soybean meal, sorghum, rice bran, and duckweed) were selected for use in Phase 2. One of these alternative feed sources, duckweed, requires a protein rich nutrient source to grow. The purpose of Phase 1 was therefore to determine which of four nutrient sources grow duckweed with the highest yield and protein content. The four nutrient sources tested in Phase 1 were vegetable compost, dairy manure, poultry manure, and activated sludge. These sources were chosen because of their low cost and local availability (Cofie et al., 2005; Yao et al., 2011; Ravindran, 2013). No nutrient source was added to the control tanks. The findings from Phase 1 were implemented in Phase 2 to grow duckweed as feed for a set of small-scale aquaponic systems, described in the Research Design section of Phase 2.

Methodology

The activated sludge used in Phase 1 was purchased from Milorganite® and is a fertilizer commonly used for lawns and golf fairways. Compost was obtained from the University of Maryland's (UMD) North Campus Diner, which uses a waste-to-water composting process. The dairy manure was obtained from the UMD campus farm, managed by the Department of Animal and Avian Sciences. The poultry manure was purchased from Stutzman Environmental Products, Inc., a company specializing in organic products (see Appendix G). Samples of each of the nutrient sources were sent to A&L Eastern Laboratories to assess total Kjeldahl[1] nitrogen, the sum of organic nitrogen, ammonia and ammonium in each of the nutrient sources. The nitrogen content of each is listed in Table 1.

Table 1. *Percent nitrogen content of Milorganite® activated sludge, compost, poultry manure, and dairy manure*

Nutrient Source	Nitrogen Content (mg/L)
Activated sludge	6.02%
Vegetable compost	3.28%
Dairy manure	1.60%
Poultry manure	4.43%

Before beginning Phase 1, a pilot study was conducted to optimize the mass equivalent of nitrogen[1] to be introduced to each tank of duckweed in Phase 1. This pilot study was set up and conducted in a sealed, temperature-controlled growth chamber[1] located in the Animal Science / Agricultural Engineering building at the University of Maryland, College Park. The T12 (1½ in. diameter) fluorescent tube grow lights in the chamber, located at a constant distance of about 0.8 meters from the plant beds, were set on a timer to simulate natural light cycles (8:00 AM to 10:00 PM) (see Appendix K). Temperature in the growth chamber varied between 21.3 - 26º C daily. The humidity of the growth chamber was maintained between 71 - 77%.

The pilot study was conducted over a two week period. Each nutrient source was tested in triplicate; therefore, a total of fifteen tanks of duckweed were studied. The duckweed was grown in plastic 58.7 x 42.2 x 16 cm (23.1 x 16.6 x 6.4 in.) Sterilite® brand containers, providing a total of 0.3 m^2 (3 ft^2) of growing area. Each container was filled to a depth of 15 cm (6 in.), or 38 L (10 gal.), and was refilled to maintain water level on a daily basis. The individual tanks in the chamber were distributed to normalize fluctuations in temperature or lighting within the chamber. Ammonia concentrations were assessed every day for the first three days of the experiment to gauge standard conditions. Following this initial period, in which satisfactory growing conditions were confirmed, water chemistry was monitored every other day. The calorimetric La Motte™ Ammonia Nitrogen Test was used to track any change in Ammonia

concentrations (See Appendix F for water chemistry protocol). The ranges of ammonia measured during this pilot study are tabulated in Table 2.

Table 2. *Minimum and Maximum Ammonia Nitrogen concentrations measured during Phase 1 pilot study*

Nutrient Source	Range of Ammonia Concentration (mg/L)
Activated sludge	0.2-8
Vegetable compost	0.2-16
Poultry manure	0.2-10
Dairy manure	0.2-8
Control	0-0.2

Each day, a set amount of each nutrient source was inoculated into each tank containing 100 g of duckweed. The amount of each nutrient source used was calculated based on its nitrogen content so that each duckweed tank received equal amounts of nitrogen. On the first day, a mass equivalent of 80 mg/L of nitrogen was inoculated in each 38 L (10 gal.) tank. On day two, 7.5% of a mass equivalent of 80 mg/L of nitrogen was inoculated. On days three to fourteen, after monitoring ammonia concentrations daily, 10% of a mass equivalent of 80 mg/L of nitrogen was inoculated. From this pilot study, it was found that a threshold of 80 mg/L of nitrogen could be introduced without limiting duckweed growth over the course of two weeks.

Phase 1 was conducted in a University of Maryland Research Greenhouse space over the course of two weeks. There were three main benefits to housing it in the greenhouse: the research greenhouse received natural light, temperature could be maintained at a constant 26^{o} C day and night, and humidity was regulated at 71%. As in the pilot study, each of the four nutrient sources (compost, dairy manure, poultry manure and activated sludge) was tested in

triplicate alongside three control tanks (containing no additional nutrient source) for a total of fifteen tanks of duckweed. Because ammonia concentrations stayed within acceptable ranges during the pilot study, water chemistry was not monitored during Phase 1. Ten percent of the mass equivalent of 80 mg/L of nitrogen of each nutrient source was inoculated into each tank daily. On day one, 100 g of duckweed were introduced into each of the fifteen tanks. The first inoculation took 48 hours to cover the surface of the Sterilite® container with fronds. Based on this approximation, half the surface area of duckweed (0.14 m^2 or 1.5 ft^2) was harvested every 48 hours for the duration of Phase 1. This was accomplished by dividing each tank of duckweed in half using a plastic divider and collecting duckweed from one side of the divider using a mesh net. The collected duckweed was air dried overnight in a brown paper bag and weighed.

Results

Table 3. *Phase 1 Data: Alternative Nutrient Source Characteristics and Effect on Duckweed Production*

Nutrient Source	Nitrogen Content of Nutrient Source (%)	Average Duckweed Protein (%)	Average Duckweed Biomass (g)
Control	0.00	9.00	13.25
Std. Dev.	--	1.14	1.16
Activated Sludge	6.02	40.67	38.07
Std. Dev.	--	0.71	2.85
Vegetable Compost	3.28	32.90	18.55
Std. Dev.	--	0.30	0.719
Dairy Manure	1.60	12.47	19.99
Std. Dev.	--	0.38	0.888
Poultry Manure	4.43	34.20	33.89
Std. Dev	--	1.57	1.113

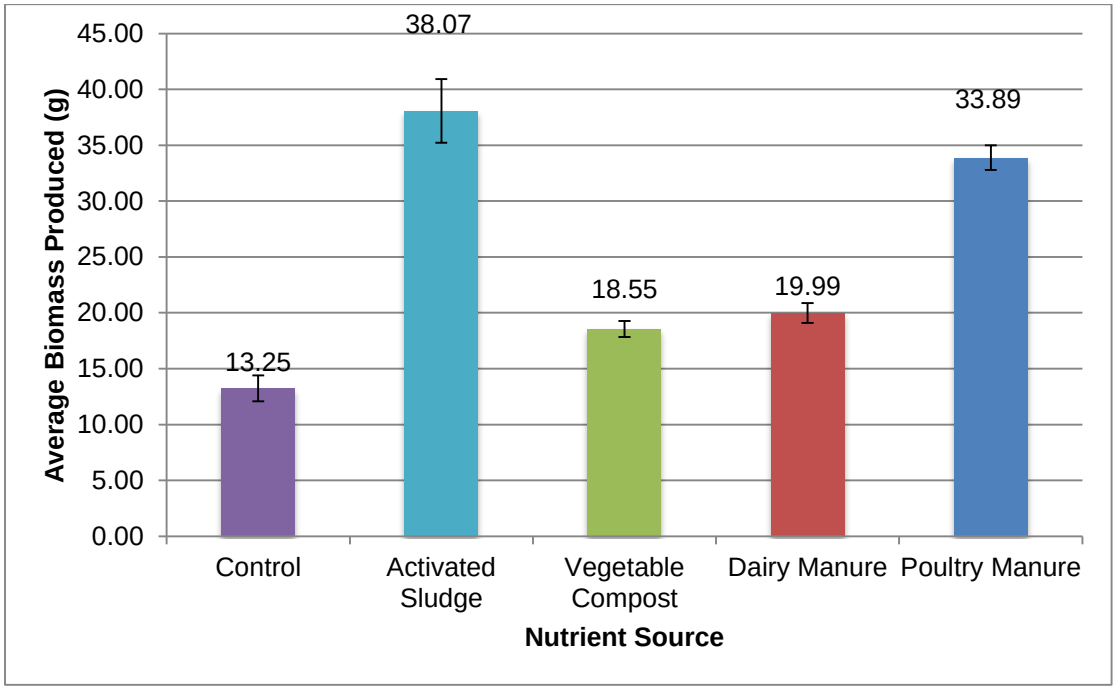


Figure 2: Phase 1 effect of nutrient sources on duckweed biomass

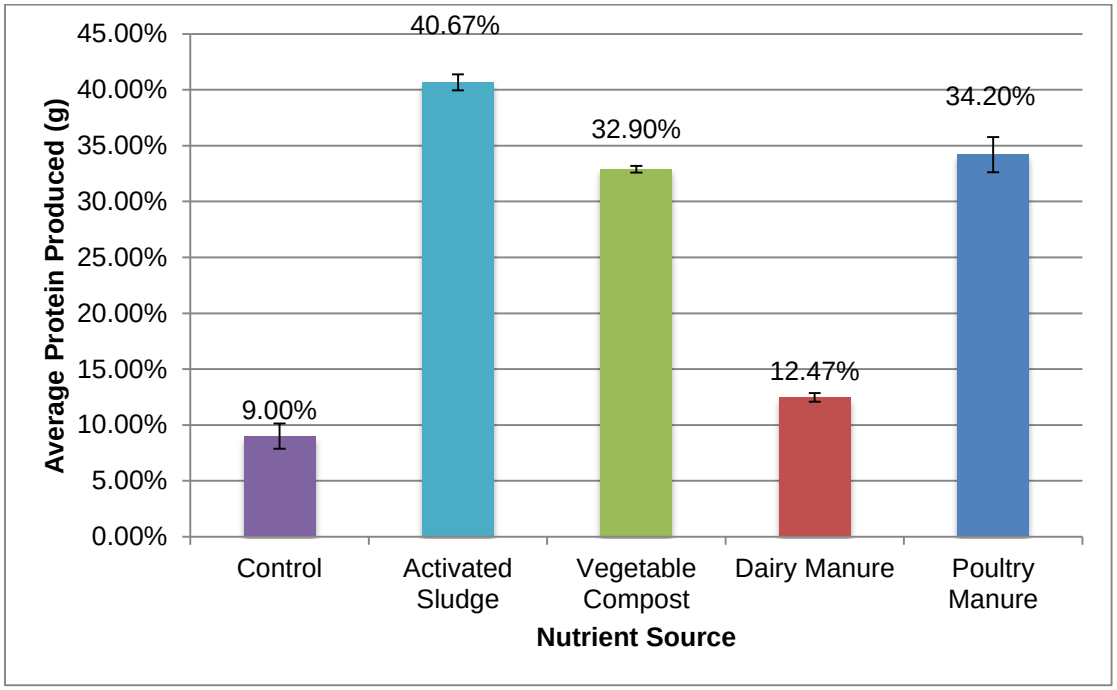


Figure 3: Phase 1 effect of nutrients sources on duckweed protein

Half the surface area of duckweed was harvested every 48 hrs. Average protein values and average biomass of duckweed harvested across three replications are shown.

In Phase 1, we determined the most effective nutrient source for growing duckweed by assessing total duckweed biomass and protein content, as shown in Table 3.

A single factor analysis of variance (ANOVA) test was conducted on the dried biomass of duckweed grown by each nutrient source treatment, producing a p-value of 0.0002 (see Appendix B). A post-hoc Tukey Multiple Comparisons Test was conducted to determine if there are significant differences in duckweed biomass when grown by different nutrient sources. All statistical analyses were conducted at an alpha level of 0.05. As seen in Figure 2, activated

sludge and poultry manure produced significantly higher biomasses than any other treatment, but activated sludge was not significantly higher than poultry manure.

According to these findings, activated sludge and poultry manure produced significantly higher biomasses of duckweed than any other treatment. Poultry manure was second to activated sludge, but only showed significantly higher biomasses when compared against control and dairy manure.

Because biomass assessment could not sufficiently determine the most effective nutrient source, we then turned to our second method of analysis: ANOVA across duckweed protein content. A Single Factor ANOVA Test determined that there were significant differences in duckweed protein content among the different treatments ($p < 0.0001$). A Tukey Multiple Comparisons Test determined that there are significant differences in crude protein content among all the treatments except poultry manure and vegetable compost (See Table 7).

Because activated sludge grew duckweed with a statistically higher protein content than any other treatment, we determined that activated sludge was the most effective nutrient source for growing duckweed.

Discussion

In Phase 1, we determined that activated sludge is the ideal nutrient source to fertilize duckweed. Activated sludge can be used to grow large amounts of duckweed with a protein content of 40.67%, comparable to the protein content of duckweed grown under ideal conditions (40-43%) (Rusoff, Blakeney, & Culley, 1980; Leng, Stambolie, & Bell, 1995). The difference between biomass yields of activated sludge and poultry manure is insignificant, as seen in Figure 2, but the protein content of activated sludge is significantly higher than the other nutrient

sources. Due to its higher protein content, activated sludge was chosen as the nutrient source for the duckweed throughout Phases 2 and 3.

There were some limitations with our Phase 1 study. Certain fertilizers tested in the pilot study, particularly activated sludge and dairy manure, contained solids that would not completely dissolve in the water. This feature led to difficulty collecting the duckweed samples without also including some fertilizer, which potentially added extra mass to the weighed sample. We resolved this issue later in Phase 1 by avoiding settled solids on the bottom of the tanks and by visually inspecting the collected duckweed before weighing. This change in collection technique reduced standard deviations between replications.

Other issues encountered during the Phase 1 study included overcrowded duckweed tanks, issues keeping tanks well mixed, and equal harvesting of each tank during each collection. A study with fewer, larger harvests in a larger container might have had fewer issues, but most issues were applicable to the whole system, not just a single treatment. Additionally, data analysis also showed very significant differences between activated sludge and the other candidates, increasing our confidence in our results.

Phase 2: Small-scale Aquaponic Analysis of Alternative Feeds

Introduction

The purpose of our initial research, Phase 1, was to determine which nutrient source grows duckweed with the highest biomass yield and protein content. We found that activated sludge was the best source according to those criteria. Using these findings, we proceeded to our second phase of research using activated sludge as our duckweed fertilizer. The purpose of Phase 2 was to determine which alternative feed would produce the highest yield of plant biomass and tilapia mass in a small-scale aquaponic system: 150 L (40 gal.) fish tank versus the 5700 L (1500 gal.) system at UVI. Specifically, we aimed to assess and compare the yields of Genovese basil, Bibb lettuce, and Nile tilapia grown with five different feed sources: duckweed (cultivated with activated sludge), sorghum, rice bran, soybean meal, and commercial fish feed as a control.

Logistics

The Phase 2 study was conducted in the same growth chamber used in the exploratory component of Phase 1. The lighting for the study was timed to mimic natural daylight so that the lights turned on at 8:00 AM and turned off at 10:00 PM (14L:10D). Phase 2 began on October 5, 2012 and concluded eleven weeks later on December 21, 2012. The temperature throughout the study ranged from 27.1- 27.7^{o} C with an average of 27.5^{o} C. The humidity throughout the study ranged from 47 - 55% with an average of 52%. For Phase 2, sorghum was obtained from Purcell Mountain Farms©, rice bran from NutraCea® (see Appendix H), soybean meal from Down To Earth Distributors, Inc., (see Appendix H) and activated sludge used to cultivate duckweed from Milorganite®. We grew duckweed using the methodology from the primary component of Phase 1 in the same research greenhouse. Genovese basil and Bibb lettuce seeds were acquired

from Home Depot® (see Appendix J). Tilapia fingerlings[1] used to stock our system were purchased from Til-Tech Aquafarms, LLC.

Research Design

Prior to initiating Phase 2 research, lettuce and basil seeds were germinated. The germination process was as follows. First, a container was filled with water to moisten Jiffy-7® peat pellets (see Appendix K). When the centers softened, the pellets were transferred atop of 2 in. diameter net pots. The net pots were prepared by filling each with light-expanded aggregate clay (LECA®) as a growing medium for the plants (see Appendix K). Without the presence of the clay, the plants would have been saturated and subject to root rot. The LECA® was positioned such that the plants would sit on top and the roots would grow through and between the clay pellets and reach the water. Next, three to four seeds were placed on the surface of each pot and covered with a layer of peat. The seeds were watered every two to three days and germinated for ten days. At that time, the most under-developed or smallest of the seedlings were removed so that only one plant remained in each pot. The peat pellet with a single seedling was then suitable to be used in the study as seen in Figure 4.

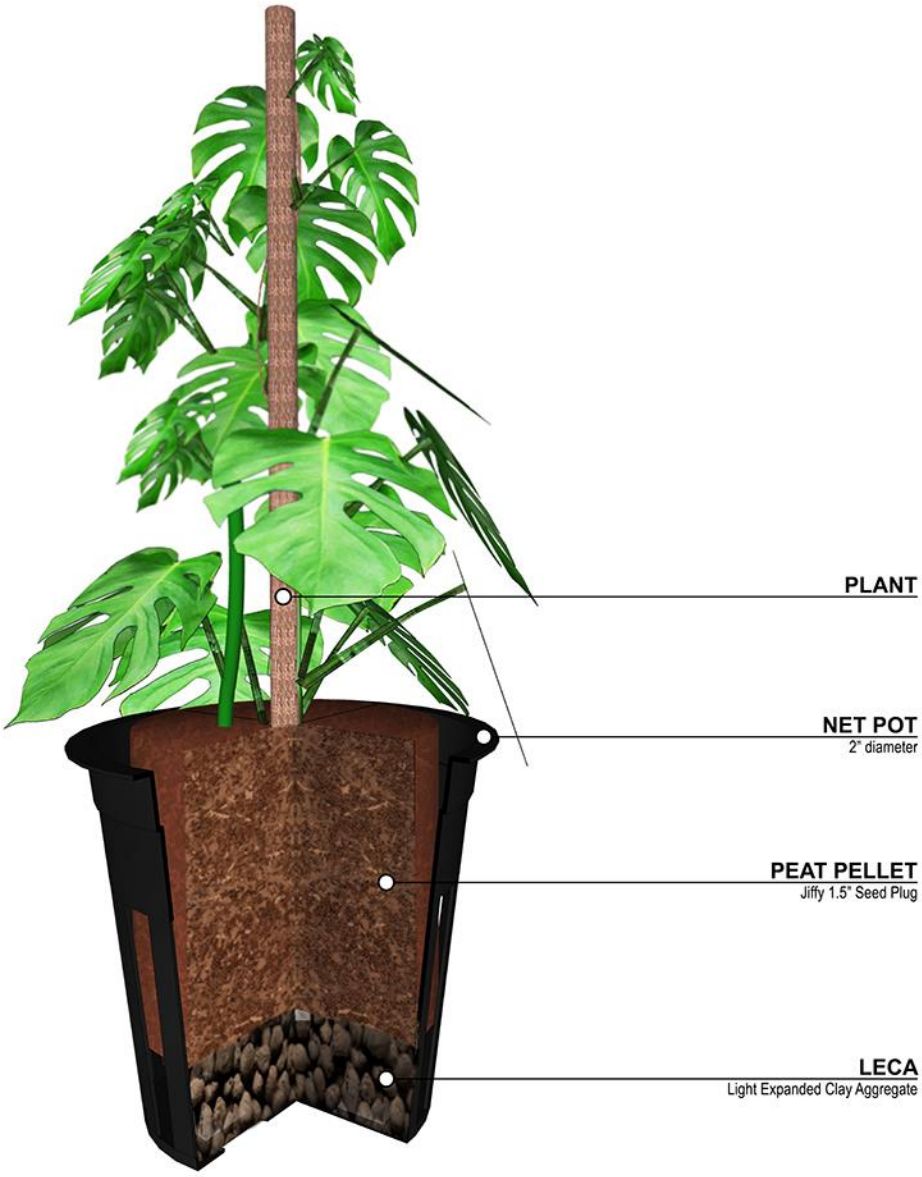


Figure 4. Peat pellet setup for germination.

Plant pot set-up is shown.

Tilapia fingerlings averaged one-inch long upon arrival. The tilapia arrived in plastic bags with approximately 19 L (5 gal.) of water. The bags were inspected for any injured or dead individuals, which were removed. A 150 W (0.20 hp) aquarium heater was added to each bag, slowly raising water temperature to that of the receiving tanks. Approximately 7.5 L (2 gal.) of

water were removed from the bag and discarded. We used a drip acclimation[1] process to initially acclimate fingerlings to our system's water quality. A tube was used to slowly siphon water from the tanks into the bag. After 20 minutes, two gallons of water had been removed. Two more gallons of water were removed and discarded, and the rate of the siphon was increased slightly. After 15 minutes, another two gallons was removed, and the siphon rate increased again. After a final 10 minutes, the siphon was cut-off. After 10 minutes, the tilapia fingerlings were netted and added to the receiving tanks and observed for 30 minutes to check for signs of stress. All fingerlings were stocked in two holding tanks for the first week prior to being stocked in the experimental tanks. This was to ensure that all of the fingerlings were exposed to the same conditions prior to the start of the study, including being on the same diet.

The growth chamber housed ten individual units. Each unit consisted of one 208 L (55 gal.) tank filled to 150 L (40 gal.) and an 88.3 x 41.9 x 15.0 cm (34.8 x 16.5 x 6 in.) Sterilite® plant bed container. Each tank was filled with 150 L (40 gal.) of water for ease of maintenance and avoidance of accidental flooding. The water level in the plant bed container was maintained at a depth of 10 cm (4 in.) or 38 L (10 gal.), to allow the rafts to remain buoyant. Twelve round spaces were uniformly distributed across the surface of ½ in. thick polystyrene sheets that were fitted to each plant bed container. Air stones were used to increase dissolved oxygen concentrations inside each tank, and an Azoo 1200 11 W pump was used for water circulation (see Appendix K). The net pots containing the germinated plants were inserted into the spaces in the polystyrene sheets. Each unit contained six Genovese basil plants, six Bibb lettuce plants, and eight tilapia fingerlings. Two replicates of the trials were conducted.

General Procedure

The fish were fed twice a day, in the morning (between 9:00am and 11:00am) and evening (between 4:00pm and 6:00pm), over the course of this phase. Water temperature, dissolved oxygen concentrations, and pH were measured daily in the evening (see Appendix K for instruments). Other water chemistry measurements, specifically ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), phosphate (PO_4), total hardness, and alkalinity were measured on a weekly basis.

On a weekly basis, the water level was maintained by refilling the tanks with tap water that was dechlorinated using Reptisafe® water conditioner. Reptisafe® removes chlorines and chloramines. The liquid conditioner was stirred into a 19 L (5 gal.) bucket and added to the tank. This process was repeated until the water level reached the line at 150 L (40 gallons).

Each of the alternative feeds was pelletized so that each was uniform in size and shape for means of equal palatability. Each individual feed was blended into a powder. The powdered feed was then placed into a meat grinder and mixed with water until it began coagulating. The feed mixture was then extruded through the meat grinder as spaghetti-like strands onto the racks of a dehydrator. The feed was dehydrated for at least twelve hours so that the added moisture was removed. The dehydrated feeds were then broken up into small pellets (roughly the size of the commercial feed) so that the feed was manageable for fish consumption based on the size of the fish being fed. The commercial fish feed was already pelletized and did not have to be altered. The fish were fed 3% of their body mass for the first five weeks. This was increased to 5% afterward and maintained until the end of the study.

Data Collection Method

The edible matter of Genovese basil and Bibb lettuce were collected weekly if a majority of the treatments had harvest-ready plants. For basil, we looked for a majority of the plants to have at least one inch wide leaves. For lettuce, we looked for at least three inch long leaves. Two team members would remove the harvestable leaves using disinfected scissors and place them in marked paper bags. Two separate bags were used per unit, one for lettuce and one for basil. The collected material was then placed in a storage chamber kept at a controlled temperature of 35^{o}C and a low humidity of 12%, which allowed for the dehydration of the plant material. After two weeks, the plant matter samples were entirely dehydrated and their weights were recorded for analysis at the end of the phase. Dried biomass yields from weeks 7 and 8 were extrapolated from hydrated masses using a conversion ratio based on measurements from previous weeks.

Accurate data regarding fish growth required a careful process to weigh the fish without causing unnecessary stress. Before the weighing procedure began, the updated fish log was checked to verify the current number of fish in each tank. The tanks were weighed one at a time in the following manner. First, the fish were captured in a large net and moved to a small bucket to hold them until all the fish in the tank were collected. Then, the fish were removed from the bucket one by one and placed into a vessel resting on a scale. The weight of the vessel was recorded three times: before the fish were added, with the fish, and after the fish were removed. This measurement taken after the fish were removed was compared to the initial weight to assure that water added as the fish were transported to and from the vessel did not strongly influence the fish weight data. After the fish weight was recorded for the tank, both the bucket and vessel were rinsed before proceeding to the next tank. When turbid water caused exceptionally poor

visibility, the tanks were partially drained in order to collect the fish more efficiently and reduce the stress caused by extended periods of fish collection. After collection, the drained water was returned to the tank before moving on to the next one.

At the end of the phase, all of the fish were euthanized following procedures recommended by the 2007 AMVA guidelines. First, each fish was concussed by manually applying a blunt force to the head. Then, the fish was pithed[1], which destroys the brain by cutting into the head with a knife. Finally, the fish was decapitated. This method was chosen instead of chemical procedures to ensure that the final product could be safely consumed (Leary, 2013).

Results

In Phase 2, we determined the effectiveness of a complete alternative feed diet by assessing its effect on tilapia mass and dried plant biomass compared to a standard commercial fish feed treatment.

Tilapia Weight.

A one-way ANOVA was used to determine if our feed treatments produced significantly different yields of tilapia growth percentage. Growth percentage was calculated by dividing the difference between the original and final weights by the original weight. A p-value of 0.003 indicated that our results were statistically significant (see Appendix C). Results from our post-hoc Tukey multiple comparisons test indicate there are significant differences in tilapia growth percentages between the control treatment and each of the alternative feeds. The control commercial feed produced significantly greater fish weight. However, there are no significant differences in tilapia growth among the alternative treatments.

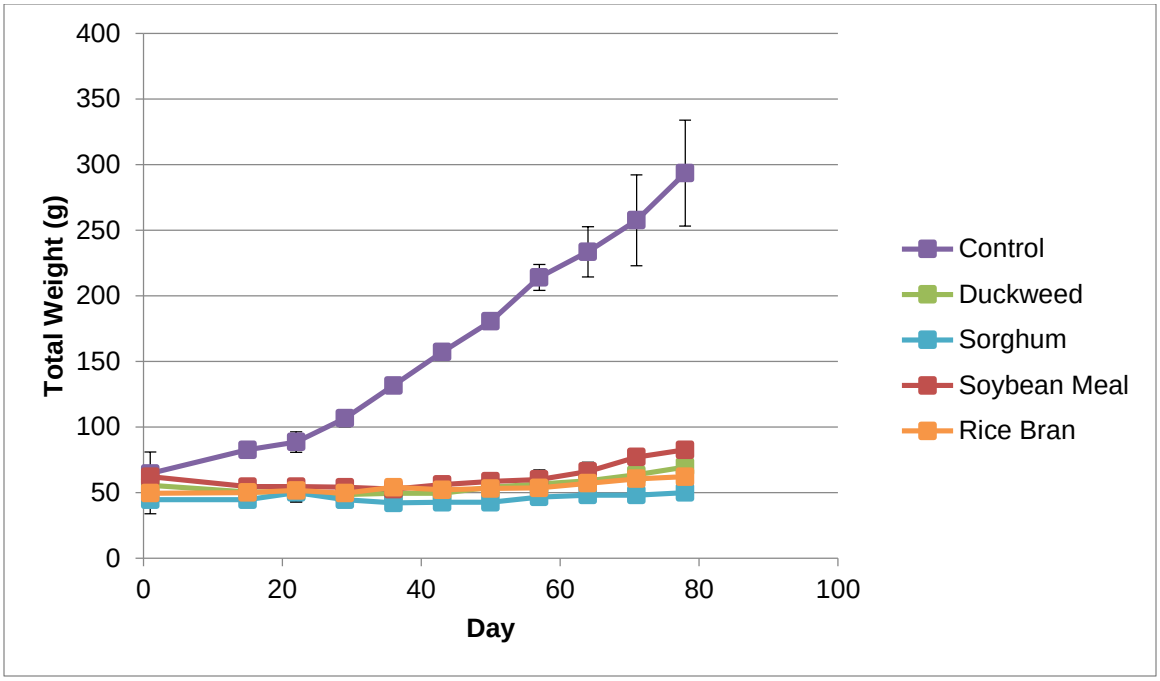


Figure 5. Average fish weight over time.

The effectiveness of the alternative feed diets (Duckweed, Sorghum, Soybean Meal and Rice bran) was assessed by its effect on tilapia weight over time in comparison to a standard commercial fish feed treatment (control).

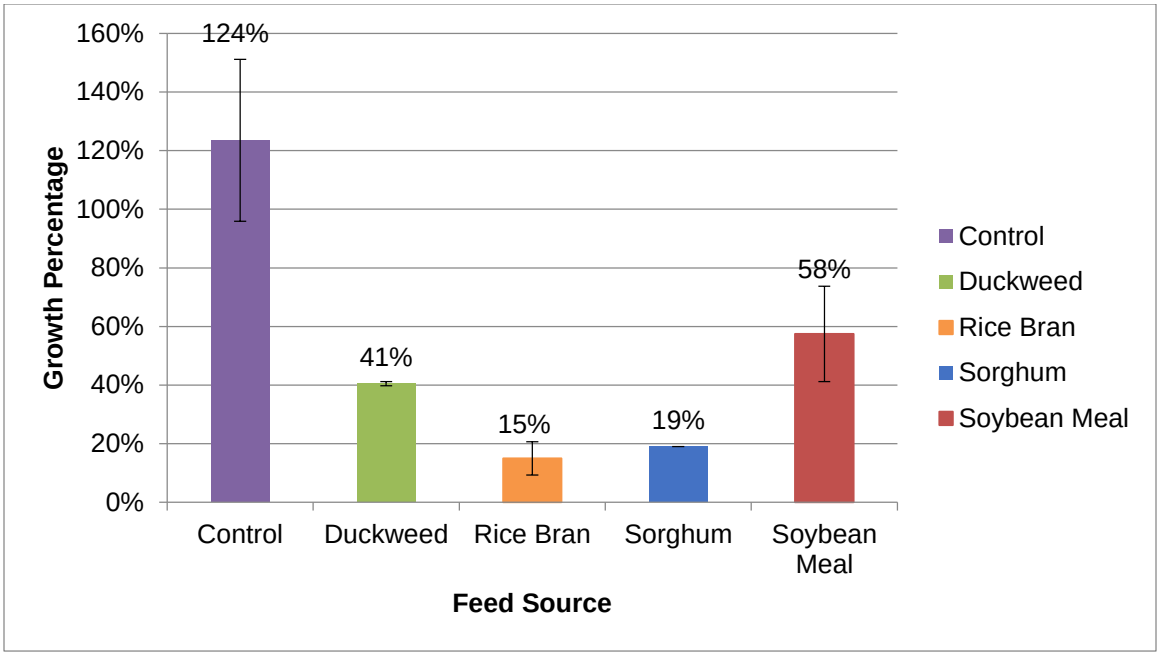


Figure 6. Phase 2 average fish growth percentages.

The effectiveness of the alternative feed diets (Duckweed, Sorghum, Soybean Meal and Rice bran) was assessed by its effect on tilapia growth in comparison to a standard commercial fish feed treatment across two replications (control) (see Appendix C).

Dried Plant Biomass.

Based on the plant biomass produced, our data indicates that the most effective feeds are commercial fish feed (control), soybean meal, and duckweed. . For visual reference, the progression of plant growth is shown in Figure 8. A two-way ANOVA and Tukey multiple comparisons test shows a significant difference in basil growth between all treatments (p-values ranged from 0.00019 to 0.00837, see Table 12 and Appendix C), except for the top three treatments (commercial fish feed, soybean meal, and duckweed). Similar results were seen for lettuce growth, although our data showed no significant difference between the rice bran and control treatments.

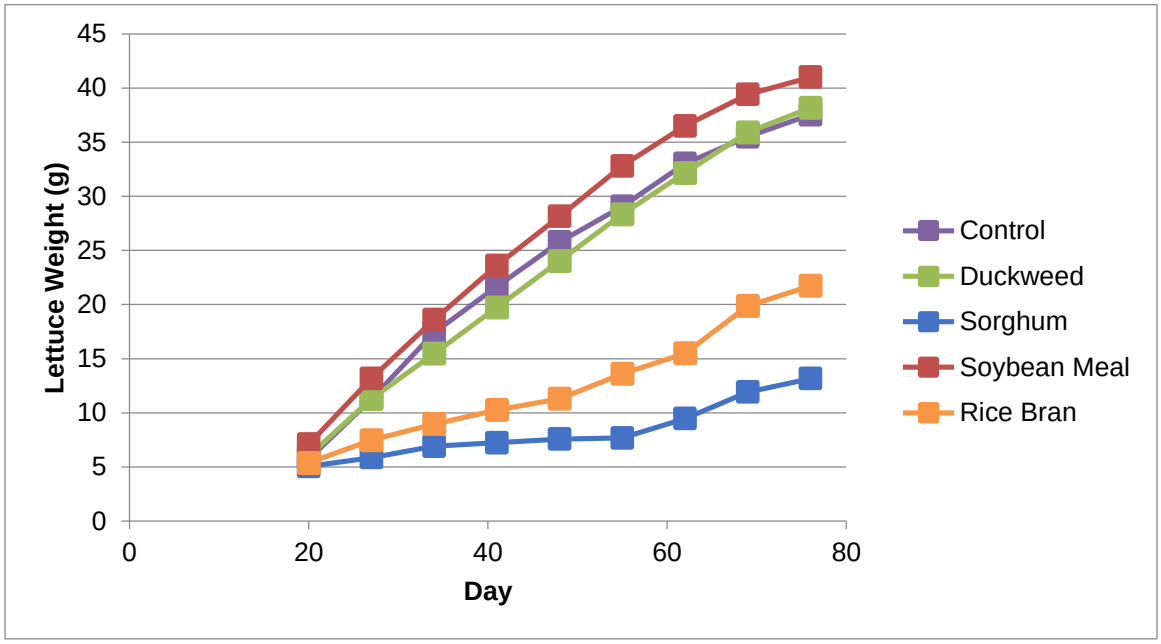


Figure 7. Phase 2 average cumulative lettuce production.

The effectiveness of the alternative feed diets (Duckweed, Sorghum, Soybean Meal and Rice bran) was assessed by average cumulative lettuce production in comparison to a standard commercial fish feed treatment across two replications (control) (see Appendix C).

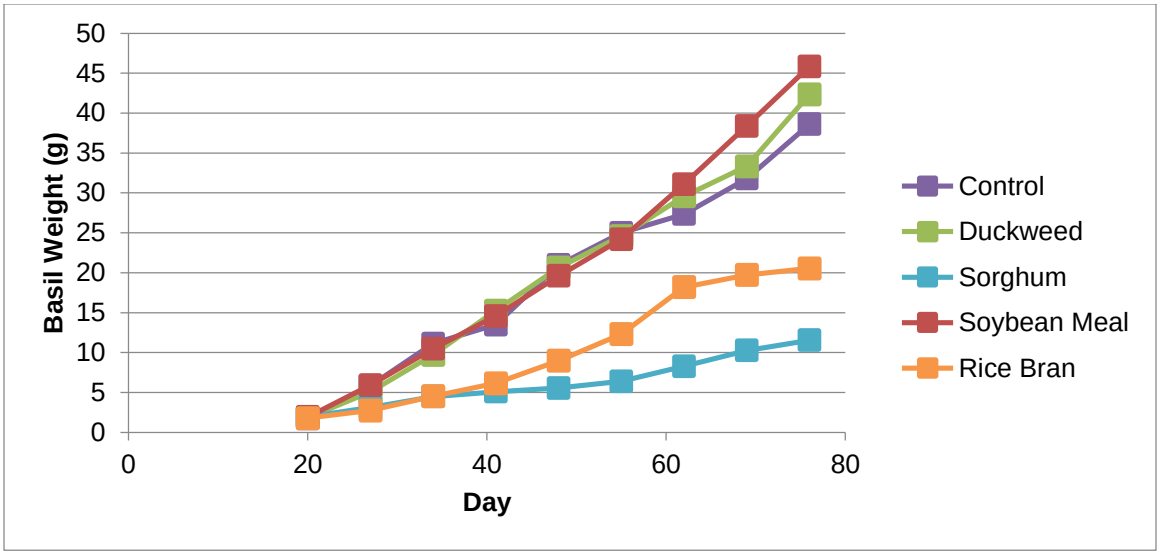


Figure 8. Phase 2 average cumulative basil production.

The effectiveness of the alternative feed diets (Duckweed, Sorghum, Soybean Meal and Rice bran) was assessed by average cumulative basil production in comparison to a standard commercial fish feed treatment across two replications (control) (see Appendix C).

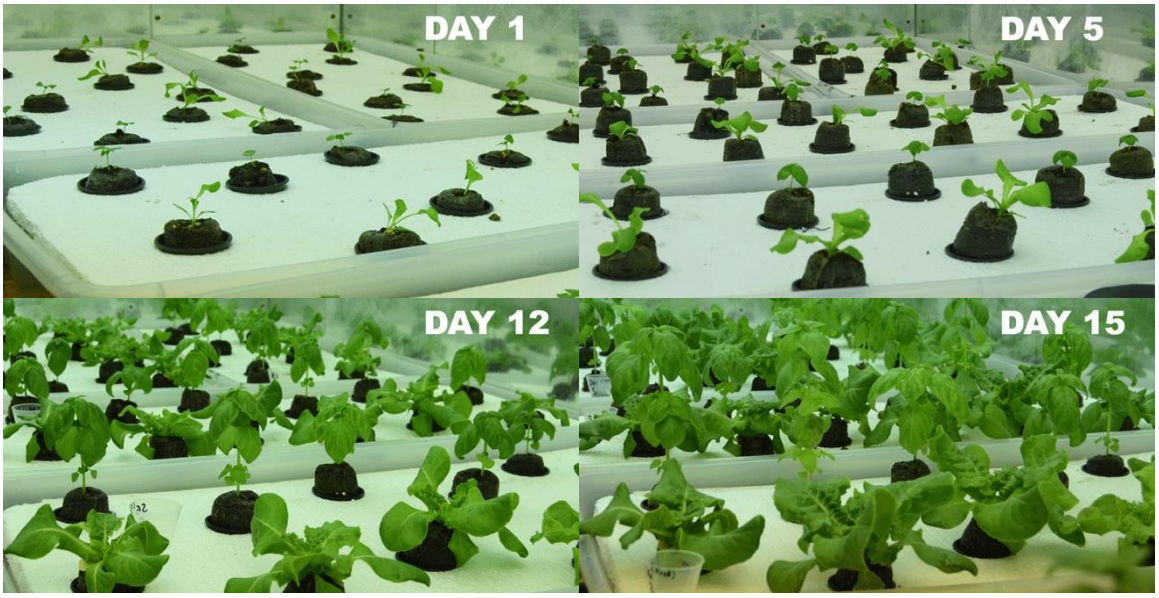


Figure 9. Basil and lettuce growth.
Phase 2, Progression of basil and lettuce growth in the course of fifteen days.

Discussion

Although there was no significant difference in tilapia mass yield among the alternative feeds, there was a difference in the biomass yield of the plants. These results led the team to choose soybean meal and duckweed as the alternative feeds in the Phase 3 testing.

Phase 2 provided important data and results for the advancement of the study into Phase 3. The difference in yields was suspected to be the result of varying nutrient content among the feeds. Due to insignificant fish growth with a complete alternative feed diet, Phase 3 tested blended feeds (50% alternative feed and 50% commercial feed) to identify differences between the two most successful alternative feeds, duckweed and soybean meal. Another study practice identified by the results of Phase 2 was the addition of a mechanism to siphon excess waste from the fish tanks. Water chemistry analysis in Phase 2 displayed elevated concentrations of ammonia. High ammonia concentrations are produced by the presence of excessive waste and uneaten feed and may lead to toxicity to fish. The team decided that removing the waste and replacing the tank water more frequently in Phase 3 would increase water quality, and therefore increase fish health and growth.

Phase 3: Small-scale Aquaponic Analysis of Blended Nutrients

Introduction

From analyzing the results of Phase 2, duckweed and soybean meal produced the highest yields of basil, lettuce, and tilapia compared to the other alternative feeds. However, the control (commercial fish feed) led to faster growth in the tilapia. We hypothesized that blending alternative feed sources with commercial fish feed would produce yields that would enable us to better distinguish between the alternative feeds. The specific aim of Phase 3 was to assess the yields of tilapia, basil, and lettuce grown with one of three feed sources: a 50% duckweed and 50% commercial feed blend (50/50 duckweed/commercial), a 50% soybean meal and 50% commercial feed blend (50/50 soybean meal/commercial), and 100% commercial fish feed as the control.

Logistics

The Phase 3 study began on March 16, 2013 and concluded nine and a half weeks later on May 22, 2013. The Phase 3 study was set up in the same manner as the Phase 2 study and in the same growth chamber. The temperature throughout the study ranged from 25.1 - 28.6^{o}C with an average of 25.8^{o}C. The humidity ranged from 50 - 62% with an average humidity throughout the study of 57.4%. We used the same sources of duckweed, soybean meal, and commercial fish feeds as in Phase 2.

Research Design

The germination protocol used in Phase 3 was similar to that used in the previous study: peat pellets housed the seeds, which were contained within net pots carrying clay pellets. In total, the chamber housed nine individual units. Each unit was identical to those used in Phase 2: a 208 L (55 gal.) barrel housing eight tilapia fingerlings and a plant bed containing six basil

plants and six lettuce plants. The trials, however, were conducted in triplicate for Phase 3, with three units for each treatment.

General Procedure

For Phase 3, some slight changes were made to the daily procedure. Tank temperature, dissolved oxygen concentrations, and pH were measured every other day. Ammonia, nitrite, nitrate, and phosphate were the only water chemistry measures that were measured on a weekly basis. Total hardness and alkalinity were measured at the beginning and the end of the phase. Feeding procedures and times remained the same.

Similar to Phase 2, conditioned tap water was used to maintain water levels in the fish tanks. However, in Phase 3 ProLine® sodium thiosulfate was used in place of Reptisafe® water conditioner (see Appendix K). Again, water was added in increments of 19 L (5 gallons). However, the sodium thiosulfate required an intermediate step where the amount to condition 19 L (5 gal.) of water was placed in a small container that was shaken vigorously to ensure proper dissolution. The dissolved sodium thiosulfate was then stirred into a 19 L (5 gal.) bucket of water that was added to the tank. This process was repeated until the water level reached 150 L (40 gallons).

Due to high concentrations of ammonia recorded in the Phase 2 study, fish waste was removed from the bottom of the tanks using a siphon every three to four days. A team member would use a small pipe with a tube attached and create a siphon. The siphon then worked as a vacuum to remove the waste from the bottom of the tank. The waste was drawn into the siphon and collected in a 19 L (5 gal.) bucket. When little to no waste remained on the bottom of the tank, the 19 L (5 gal.) bucket was emptied and the process repeated for the remaining tanks.

For Phase 3, only soybean meal and duckweed were used as alternative feeds. However, they were blended in a 50/50 mix with commercial fish feed. The commercial feed used for the control treatments was already pelletized and ready for use, as it was in Phase 2. For soybean meal, the soybean meal powder and commercial feed pellets were placed in a blender in equal parts by mass and blended until a homogeneous mixture was achieved. This powder was then pelletized using the same method as in Phase 2. For duckweed, the Phase 2 procedure was followed to create duckweed pellets. The duckweed pellets were then blended in the same manner with commercial feed pellets. The fish were fed 2% of their body mass on the first day of experimentation. This was steadily increased to 7% in response to changes in water chemistry.

Data Collection Method

For Phase 3, plant harvests and dehydration schedules and protocols remained the same from the previous phase. However, fish weighing was reduced to once every two weeks in an effort to minimize stress that may have affected fish health in Phase 2. Although there was no specific data to indicate that weighing the fish weekly was affecting fish growth, disturbing the fish often inevitably induces unwanted stress, so weighing was reduced to avoid further stress (Pankhurst & van der Kraak, 1997).

Results

We determined in Phase 2 that complete alternative feed diets did not produce adequate fish growth. Thus, Phase 3 tested 50/50 blended feeds to identify differences between duckweed and soybean meal as a partial feed source. Tilapia mass and dried plant biomass were assessed to determine which blended feed was most effective in an aquaponic system.

Tilapia Weight.

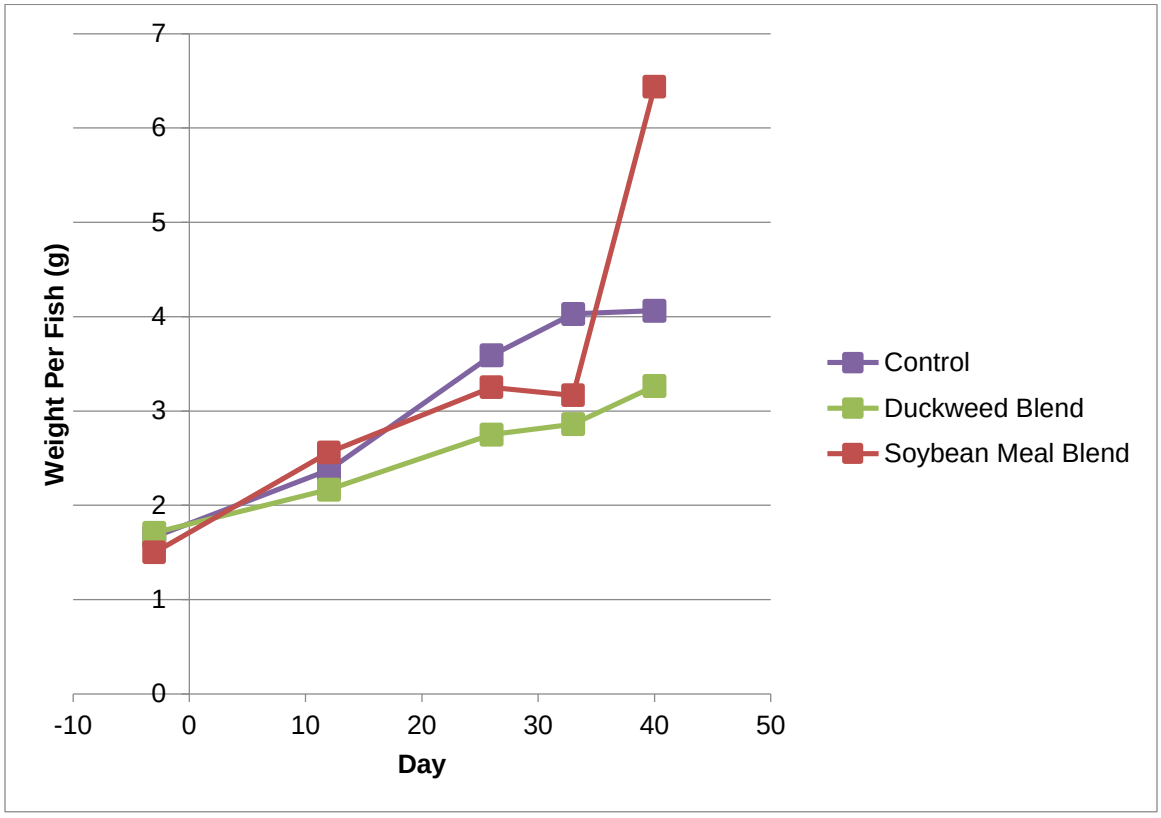


Figure 10. Phase 3 average weight per fish over time.

The effectiveness of the alternative feed diets (Duckweed Blend and Soybean Meal Blend) was assessed by average weight per fish in comparison to a standard commercial fish feed treatment across three replications (control). The first weighing took place during the acclimation period prior to Day 1, the first day of the study.

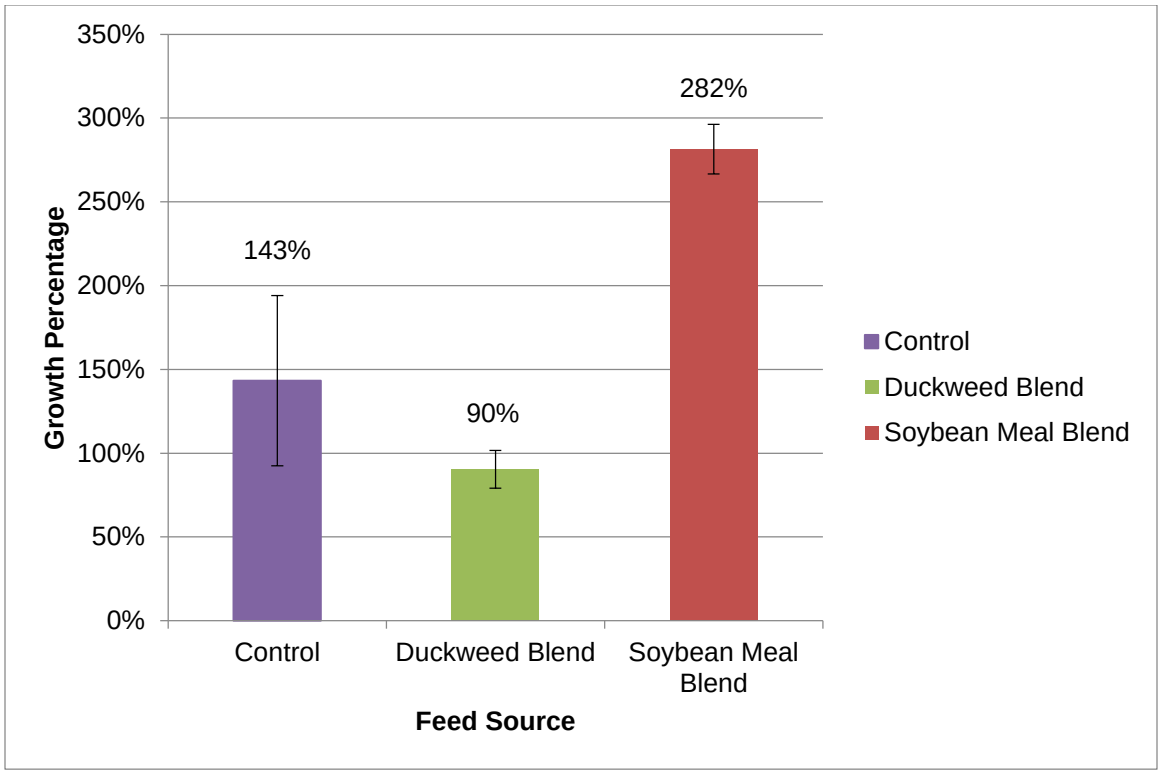


Figure 11. Phase 3 average fish growth percentages.

The effectiveness of the alternative feed diets (Duckweed Blend and Soybean Meal Blend) was assessed by average fish growth percentages in comparison to a standard commercial fish feed treatment across three replications (control).

Due to fish disease, the systems had significant fish mortality. The deaths were sporadic and did not seem to be isolated to any specific treatments. This may have influenced the reliability of the results. Instead of calculating total tilapia mass, we calculated the average weight per fish based on how many fish were in the system at the time of weighing. Deaths were believed to be the result of an unidentified disease, but necropsies yielded no insight to the cause of the disease.

A one-way ANOVA and Tukey multiple comparisons tests on tilapia growth percentages indicate there are no significant differences between soybean meal blend and control treatments,

nor duckweed blend and control treatments. These results show that in terms of tilapia growth percentage, both the soybean meal and duckweed blended treatments are comparable to that of a commercial fish feed diet.

Results from our post-hoc test of fish growth indicate a statistically significant difference between tilapia growth percentage produced by blended soybean meal and blended duckweed treatments. Because the soybean meal blend produced a higher average biomass per fish, we determined that soybean meal blend was the more effective of the two alternative feeds.

Dried Plant Biomass.

A two-way ANOVA and Tukey multiple comparisons test indicate there is no significance in plant growth between the three treatments at an alpha level of 0.05. We could make no conclusive statements about the effectiveness of our alternative feed treatments based on plant production.

The plant growth may have been limited by low nutrient concentrations found in the water due to the waste siphoning. These nutrient concentrations were lower than those measured in Phase 2 and can be found in Appendix A.

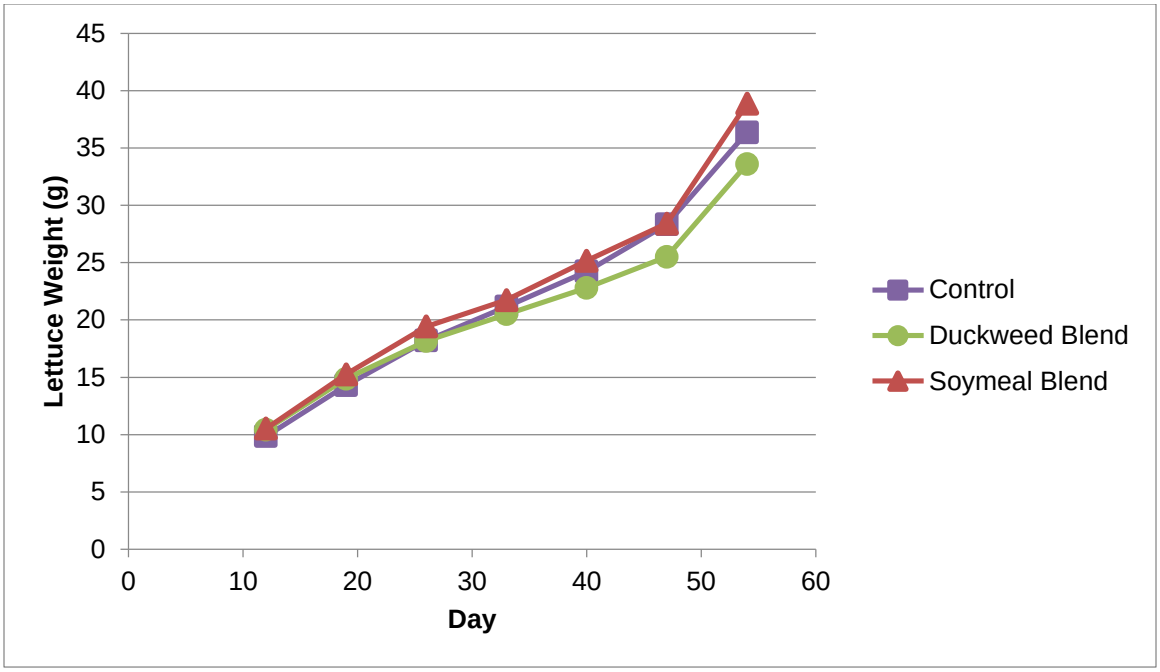


Figure 12. Phase 3 average cumulative lettuce production.

The effectiveness of the alternative feed diets (Duckweed Blend and Soybean Meal Blend) was assessed by average cumulative lettuce production in comparison to a standard commercial fish feed treatment across three replications (control).

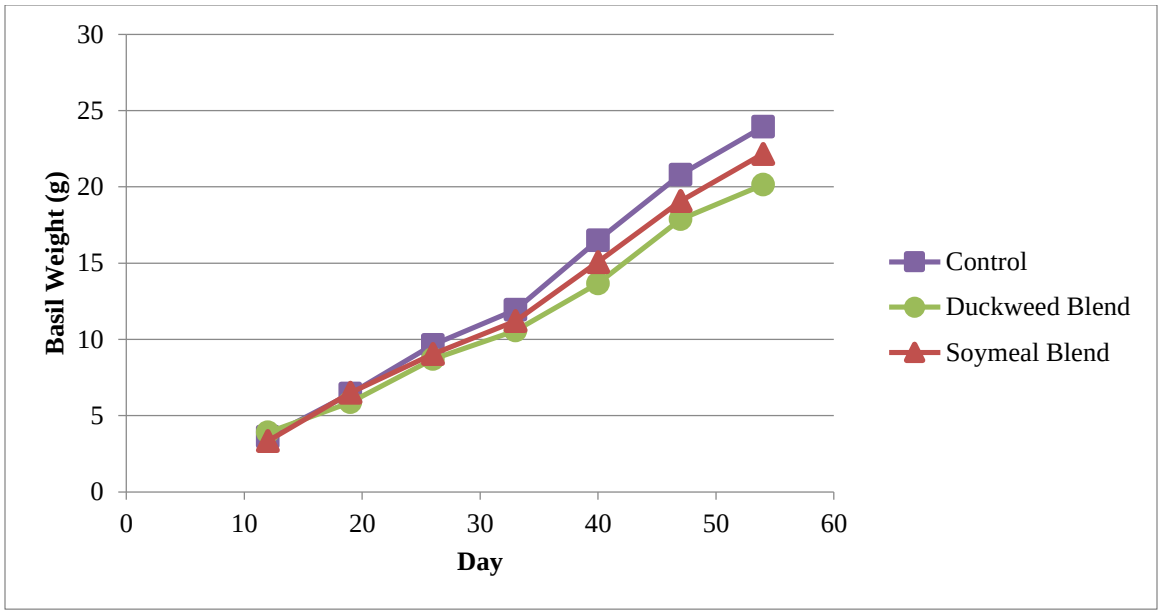


Figure 13. Phase 3 cumulative basil production.

The effectiveness of the alternative feed diets (Duckweed Blend and Soybean Meal Blend) was assessed by average cumulative basil production in comparison to a standard commercial fish feed treatment across three replications (control).

At the conclusion of Phase 3, fish tissue samples were sent to A&L Eastern Laboratories for analytical toxin testing. Fish that were fed soybean meal had the lowest concentrations of arsenic, cadmium, lead, and mercury in tissue samples compared to duckweed and control. According to the FDA's Guidance Regulation on toxins in fish, 1 mg/kg of mercury per edible portion of fish is actionable (CPG § 540.600, 2007). There does not appear to be any explicit regulation on the concentration of cadmium, lead, or arsenic. The values of mercury concentration in all our fish are well below the actionable concentration, with the lowest values found in the fish fed with blended soybean meal (Appendix D).

Discussion

Plant growth across all three tested feeds did not vary; there was no statistically significant difference between the groups. However, tilapia fed the soybean meal blend grew more significantly than tilapia fed the duckweed blend by the end of the study. Though confounding variables, particularly the unexplained fish deaths, may have influenced the results, we found soybean meal to be the best alternative nutrient source for an aquaponic system when considering both fish and plant growth.

Upon completion of the Phase 3 study, we made several conclusions that affected the Phase 4 research design. First, in an effort to reduce nitrogenous waste concentrations in Phase 3, we removed fish waste on a regular basis. However, we observed that this removal of fish waste caused the nitrogenous waste concentrations to drop significantly, which was concerning because nitrogenous compounds are necessary for plant growth. This, along with stress induced by the procedure, caused us to discontinue the waste removal process for the subsequent study.

Phase 4: Larger-scale Analysis of Soybean Meal in Aquaponic Systems

Introduction

Phase 4 was the final stage of the study. The purpose of this phase was to determine the effectiveness and economic feasibility of using soybean meal as a supplemental feed in an aquaponic system with larger water volume, more plants, and more fish than previous phases. This phase also continued to verify results obtained for the nutrient source chosen from Phase 3. The most successful alternative feed, as determined from Phase 3, was utilized in Phase 4. The systems maintained the stocking density at 0.053 fish/L (0.20 fish/gallons). Phase 4 used a 50/50 soybean meal/commercial fish feed blend and then 100% commercial fish feed for a control. The feasibility and efficacy of the feed were determined by the biomass yield of the fish and plants as well as the feed conversion ratios of each nutrient source. Thus, Phase 4 is a continuation of Phases 2 and 3, but with only one alternative feed and different, larger scale.

Logistics

The system of Phase 4 was set up on the rooftop of the University of Maryland's South Campus Diner in a 74 m^2 (800 ft^2) high tunnel[1] that we constructed. Phase 4 began on September 10, 2013 and concluded eight weeks later on November 13, 2013 due to cold weather conditions. The temperature throughout the study ranged from 11.1 - 39.8^oC with an average of 26.3^oC. Humidity in the high tunnel was often very high, leading to condensation on the polyethylene film cover. The high tunnel housed six independent aquaponic systems. Each system was allotted approximately the same area with appropriate spacing in between to allow team members to access the systems from all sides in order to check for problems and perform maintenance. Its location in an open section of the rooftop meant little obstruction to sunlight or other irregularities. The foundation of the high tunnel was reinforced with cement anchors,

while the electrical system consisted of four 120 VAC receptacles (eight outlets) rated for the six pumps, storage refrigerator, lights, air pumps, and heaters (see Appendix K). Three systems were fed with a commercial fish feed produced by Purina® AquaMax® (see Appendix I). The remaining three were fed with the experimental feed, a 50/50 blend of the Purina® AquaMax® fish feed and soybean meal.

Figure 14 shows a diagram of our aquaponic systems. Photos of the high tunnel and systems are shown in Figure 17 and Figure 18, respectively. Each unit included an approximately 3.72 m^2 (40 ft^2) plant bed and a 750 L (200 gal.) water tank. A Neiko ½ hp. water pump was used to pump water between the plant bed and the water tank (see Appendix K). The ½ hp. pump was calculated to be sufficient for achieving the required head 1.5 m (5 ft.) and flow rate of 1900 L/h (500 gallons/hour). See Appendix M for Phase 4 Fluid Dynamics Calculations.

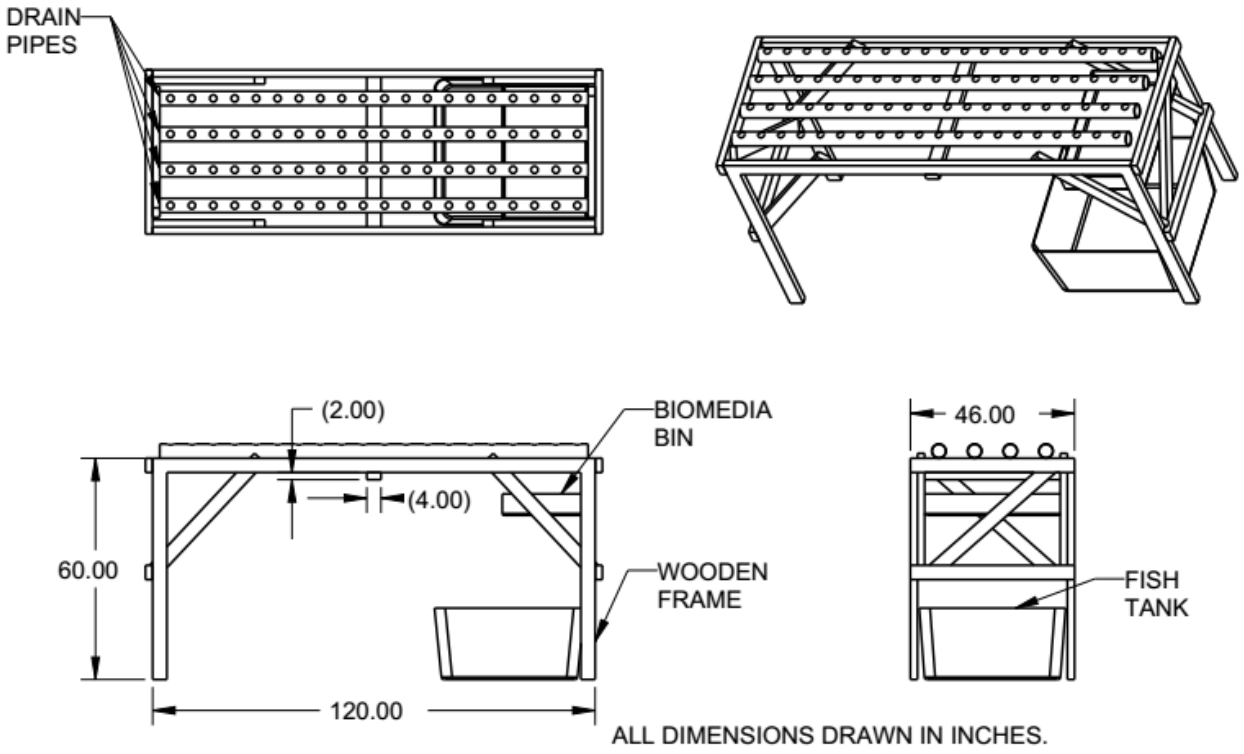


Figure 14. Top, isometric, front, and side views of the overall structure.

The tank shown below the structure and the four drain pipes are on top. The intermediate piping, biomedia containers, and pump are not shown. Units in inches.

The structural frame of the units, also shown in Figure 14 with isometric, front, and side views, was composed of pine wood. Several rows of 4 in. diameter, 10 ft. long drain pipes produced by Advanced Drainage Systems® were placed in a parallel formation above each tank to receive water from the pump, which was situated at the bottom of the system. In each system, underneath the four rows of drain pipes was the main 750 L (200 gal.) polyethylene water tank. The tanks were raised by a short metal frame that allowed for PVC piping to route from the single two inch drainage pipe at the center of the tank to the ½ hp. pump. Also, ¼ in. knotless mesh by Delta Net & Twine© was used as a cover for the tank in order to prevent fish from jumping out and other outside matter from falling into and contaminating the tank. Along the top of each pipe, we drilled 5 cm (2 in.) diameter holes, spaced 10 cm (4 in.) apart, for the placement of the net pots holding the plants. As with the previous phases, the plants acted as a biofilter for the system, with water entering the plant drain pipe at one end and exiting at the other end.

The plants were added to the systems in three stages. The first wave consisted of seedlings (basil and lettuce) purchased from a local nursery; the team desired more mature plants in order to help spur the development of a system biofilter. Once these plants had been established, additional basil and lettuce plants were installed using the same method as from Phases 2 and 3 using peat pots[1] to germinate the plants and then transferring them to the clay aggregate. The roots of these plants would grow through the peat pots and between the clay

aggregate to reach the water. A third stage of plant introduction included okra and broccoli from seed (see Appendix J), which were planted after high temperatures had subsided.

The plants used in this phase were Bibb lettuce, Genovese basil, Clemson okra, and Early Dividend broccoli. The lettuce was spaced two holes apart for a total of ten plants in a pipe and the basil, okra, and broccoli were each spaced three holes apart in a pipe for a total of seven plants in a pipe. A top and side view of an individual drain pipe is shown in Figure 15. The inclusion of lettuce and basil is a continuation from Phases 2 and 3.

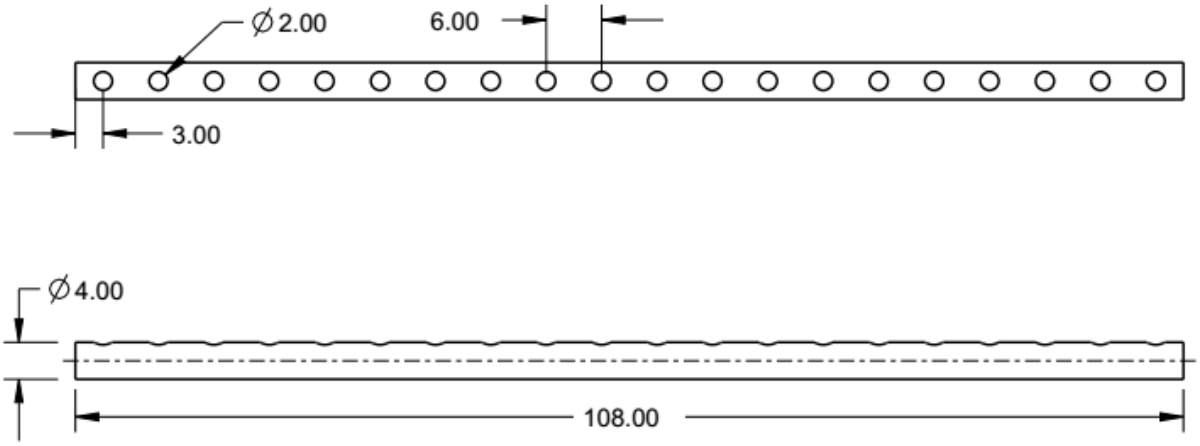


Figure 15. Top and side views of the individual drain pipe.
6 in. (15.24 cm) separate each of the 2 in. (5.08 cm) diameter plant holes. The entire 4 in. drain pipe is 10 ft. long. Lettuce was spaced two holes apart. The basil, okra, and broccoli were spaced three holes apart. Units in inches.

At the drainage end of each pipe, a plastic container held a bonded filter pad and biomedia. The pad was used as a mechanical filter and splash guard to reduce water loss. Kaldnes® biomedia was used to stimulate beneficial bacterial growth by adding surface area (see Appendix K). The water would drain into these containers from the plant beds and provide

nutrients to the bacteria on the surface of the biomedia. Unlike in Phases 2 and 3, the biomedia was no longer situated within the fish tank, but in the separate container just above it. After the water filtered through this arrangement of the filter pad and biomedia, it exited down a 2½ in. drainage pipe in the bottom of the container and flowed into fish tanks below. The air gap between the end of the drainage pipe and the surface of the water in the fish tanks allowed the flowing water to achieve turbulence and entrain air upon impact at the surface, as shown in Figure 16. This was the primary method of introducing dissolved oxygen into the fish tanks, replacing the air stones used in Phases 2 and 3.

Figure 16. Water flow in system.

Reentry of water into the main fish tank .

Figure 17. Rooftop high tunnel.

Exterior face view of the rooftop high tunnel.

Figure 18. Rooftop high tunnel interior.

Interior view of the rooftop high tunnel showing 5 of the 6 aquaponic systems.

As mentioned above, the fish were stocked at 0.05 fish/L (0.20 fish/gallons). This stocking density was based on the stocking density used by Rakocy in his aquaponic systems (Rakocy et al., 2004). However, while his design was intended for commercial use, our design is a study of sustainability, low-energy input use, and fish health, leading us to use a lower stocking density. Unlike previous phases, the fingerlings purchased for Phase 4 had an average starting length of approximately 7.62 cm (3 inches). The decision to purchase more mature fingerlings was made because of delays to the project that resulted in its September start and projected November or December finish. Because of the expected low temperatures and short duration, the purchased fingerlings had to be further along in their development in order to reach near market size. This motivation is similar to the one described above for purchasing pre-grown plants in the first wave of planting.

Additionally, a 300 W (0.4 hp.) EHEIM submersible aquarium thermostat heater was placed inside each fish tank in order to facilitate temperature stability and prevent fish death due to cold temperatures (see Appendix K). These measures would likely not be necessary in a tropical region and are a requirement only because of the local climate. While the high tunnel was able to provide a warming greenhouse effect, temperature swings during the 2013 autumn proved to be problematic, particularly at night. These thermostat heaters were set at 27.2ºC.

The greenhouse held two 208 L (55 gal.) tank used to hold spare water for refilling. The tanks of each unit were refilled with approximately 95 L (25 gal.) once every three to four days, depending on evaporation in the systems. Procedure for preparing the refill water was similar to the process from Phases 2 and 3. Water was acquired from a tap source and treated with the appropriate amount of sodium thiosulfate in order to remove chlorine harmful to the fish. Because a much larger volume of water was being treated in Phase 4, the water was allowed to sit in the 208 L (55 gal.) tanks for a few days with an air stone in order to further bubble out the chlorine and allow the temperatures to equilibrate. During cold weather, a heater set at 27.2ºC was also used to raise the temperature of the water to avoid shock to the fish during refilling.

Research Design

The research design involved six aquaponic units inside the high tunnel, three of which were fed the commercial fish feed as the control, and three of which were fed the 50/50 blend of commercial fish feed and soybean meal. The fish were weighed at the start of the phase, and then at four week intervals until the end of the study. The percentage of the feed given to the fish varied based on suggestions from a schedule as delineated in prior literature (Rakocy, 1989, September). In his studies, Rakocy fed at a rate of between 4% and 10% body mass for initial

masses of 5 to 20 grams. These masses are approximately similar to the initial measurements in our experiment.

In our design, adjustments to feed rates were made on a weekly basis and dependent on water chemistry results. The feed rates were therefore changed based on measurable characteristics of the water quality, such as dissolved oxygen, pH, ammonia, and nitrate. This schedule is outlined in Table 4. This table illustrates the week to week progression of feeding rates as a percentage of the body mass of the fish. Steady increases from 3% to 5% to 7% during the first half of the study correspond to the push to increase fish growth in coordination with acceptable water chemistry (ammonia and nitrite). We were not able to start at a large feeding rate immediately because we were implementing new systems and bacteria populations had not built up yet. The decline and plateau at 5% corresponds to higher ammonia and nitrite concentrations observed later on in the study.

Table 4. *Percentage tilapia body mass fed over time*

Week	Percentage of Body Mass Fed (%)
09/13/13	3
09/20/13	3
09/27/13	5
10/04/13	7
10/11/13	7
10/18/13	7
10/25/13	5
11/01/13	5
11/08/13	5

General Procedure

Fish were fed twice a day, morning (between 8:00 AM and 10:00 AM) and afternoon (between 4:00 PM and 6:00 PM). Air temperature and air humidity were monitored daily. Water levels, system condition and plant health were monitored daily. If the water flow was constrained, it was most likely due to algae or biomedia buildup in the pipes, so the pump would need to be shut off and the pipe blockage removed manually. During daily feedings, any overflows discovered would be countered by turning off the pump immediately and dislodging blockages. Ultimately, frequent clearing of the drain pipes was required in order to prevent consistent algal obstructions.

The feeds for the fish were stored in a small refrigerator inside the high tunnel and were divided up into small bags designated for each tank on each day of the week. At the beginning of each week, the feeds would be prepared and parceled out for the whole week. In the morning and afternoon feedings, half the contents of each bag were poured into the designated tanks. The team member would then observe the fish and record any unusual behavior or occurrences in the log book. In the afternoon, every other day, tank temperature, dissolved oxygen, and pH were recorded.

On a weekly basis, concentrations of the three chemicals involved in the nitrogen cycle (ammonia, nitrite, and nitrate) were measured. Since these three chemicals were all directly related to feed rate, they were used as a basis for feed rate decisions. Moderate nitrate concentrations are desirable as this facilitates plant growth. If ammonia and nitrite concentrations were within a safe range, feeding levels were increased to maximize fish growth. Phosphorus, hardness, and alkalinity were infrequently measured for benchmarking purposes.

Because concentrations stayed within an acceptable range, they did not require constant monitoring.

If at any point the concentrations of ammonia rose above the tolerable range thresholds, the systems would need to be supplemented with the reserve water stored in the 208 L (55 gal.) tank in order to dilute the concentration of ammonia. Additionally, daily feeding levels would be reduced. A similar procedure was utilized for high nitrite concentrations. However, since nitrite is a secondary stage in the nitrogen cycle, nitrite concentrations were slow to change, lagging behind ammonia changes.

Data Collection Method

The fish weight data, collected every four weeks, was used to calculate the required amount of feed for each tank. The process started with weighing and taring a 19 L (5 gal.) bucket of water from the tank. This container sat on the scale for the duration of the process to avoid variations in weight. The fish were collected using a net that spanned the walls of the tank. The nets crowded the fish into a small section (minimizing contact between the fish and the net itself) and allowed for smaller handheld nets to collect the fish and place them in a temporary holding bucket. Finally, the fish were individually placed in the pre-weighed container and the total weight recorded.

Plants were harvested weekly to determine the biomass yield for that time period. To determine whether the leaves were harvestable, we looked for the same criteria as Phase 2. If no leaves were large enough, the plants at that location would not be harvested for the week and no number would be recorded. The plant matter weight in each bag was recorded. Unlike Phases 2 and 3, the plants were not dried before weighing in order to obtain wet weight data used in real-world markets for our economic analysis. The dehydrated mass in Phases 2 and 3 was used for a

more academic emphasis, while the wet mass in Phase 4 was production focused. This shift mirrors the scaling up of the aquaponic systems and length of grow out for the fish.

As noted in Appendix E, the only plant biomass recorded was for the lettuce and basil wet weights. The broccoli and okra were not harvested because none of the plants had fruited and therefore no foodstuff was produced. There was non-negligible plant mass grown, but it was inedible and therefore unnecessary data for the study.

Results

The data for Phase 4 follows the same general format as Phases 2 and 3, focusing on plant biomass and fish weight over time. Like Phase 3, an emphasis was put on the average fish weight instead of the overall fish weight. This is because there were different numbers of fish stocked into each tank.

FCRs were also used as indications of how effective the feeds were in each tank. FCR is calculated by dividing total dry feed fed (g) by total wet weight gain (El-Saidy & Gaber, 2005). By finding a ratio of the two variables, a feed-to-growth efficiency rate could be calculated. These values are shown in Table 5. Additionally, a one-way ANOVA was conducted to determine whether a significant difference existed between the two kinds of feeds and their respective feed conversion ratios.

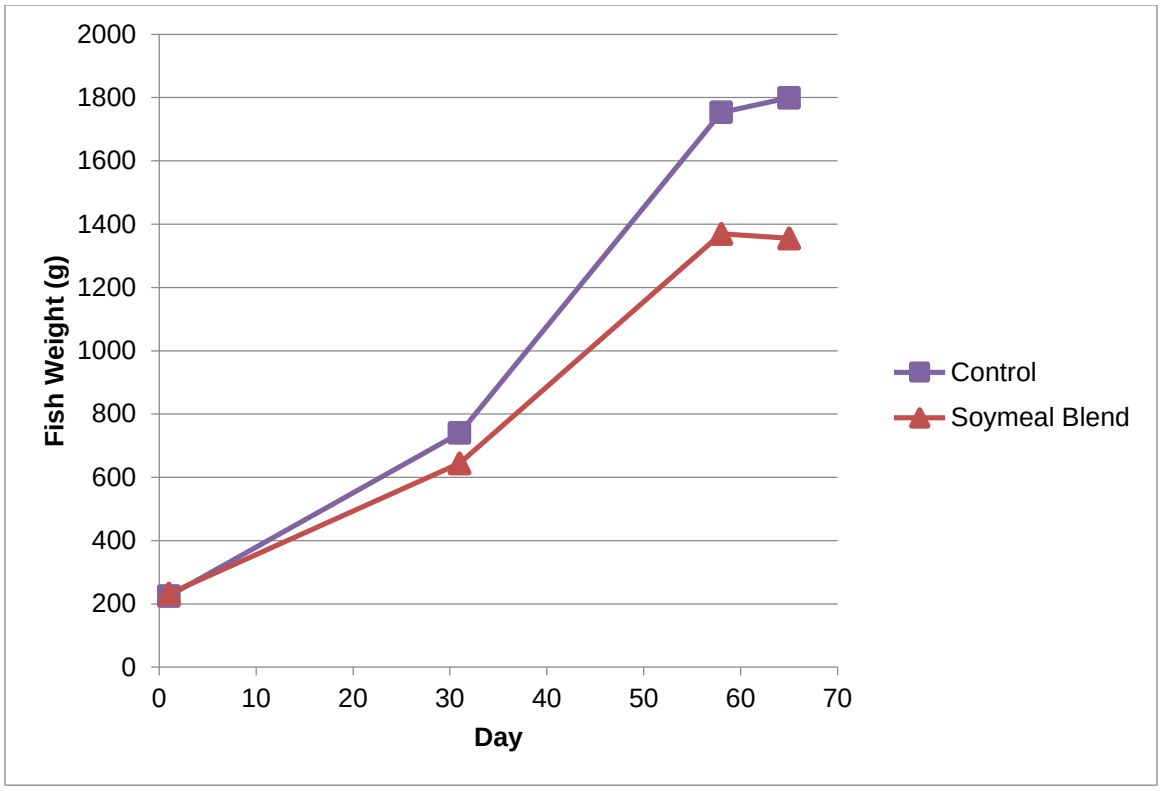


Figure 19. Average fish weight vs. time.

Total fish weight per tank over time, averaged across all three replications

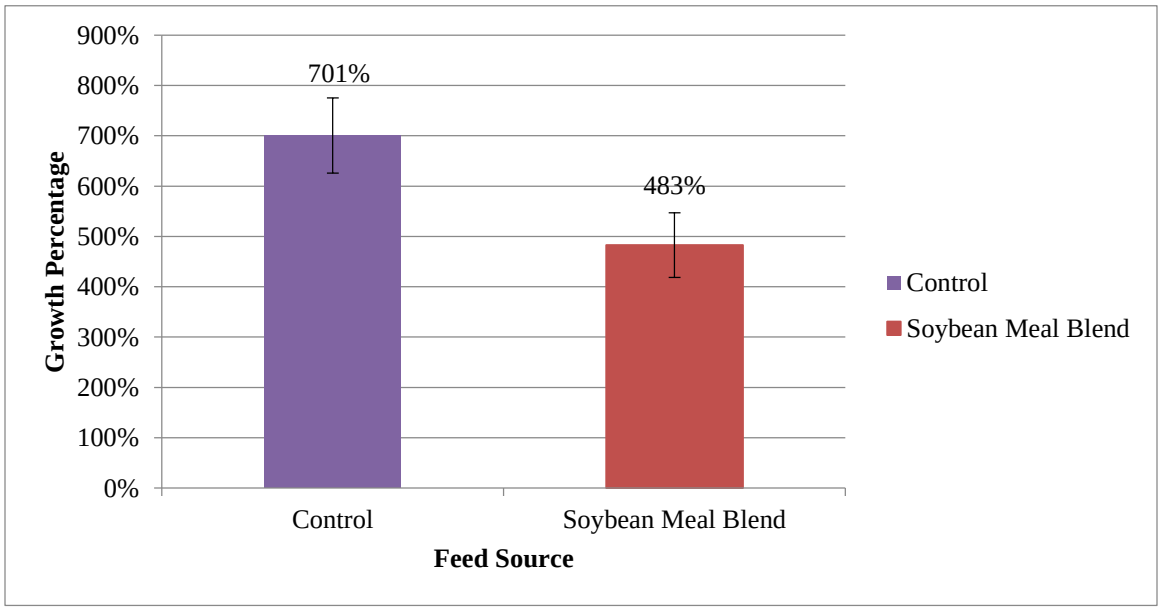


Figure 20. Average fish growth percentages.

Total fish growth during the entire phase for each treatment averaged across all three replication

Table 5. *Phase 4 Data, Average Growth, FCR, and Survival Rate*

	Avg. Growth %	Avg. FCR	Avg. Survival Rate %
Control	700.74	1.54	98.10
Std. Dev.	74.76	0.98	1.60
Soybean Meal Blend	483.36	2.08	98.10
Std. Dev.	64.25	1.45	3.20

A one-way ANOVA test indicated that there is a significant difference (p-value of 0.01863) in tilapia growth percentage between the soybean meal blended treatment and our control treatment of commercial fish feed. From these results, we cannot determine that our alternative diet was as efficient for growing tilapia as the commercial diet. However, it still produced growth, just not at a comparable rate, as can be seen in Table 5.

Plant Growth.

A two-way ANOVA test indicated that there was no significant difference in lettuce production between the two feed treatments (p-value of 0.08288). A similar conclusion was made for basil production (p-value of 0.34702).

Based on numbers alone, our data suggests that in terms of plant production a soybean meal blended treatment is comparable to that of a commercial fish feed treatment. Based on our plotted graphs (see Figure 19 and Figure 20), it is evident that the plant production did not differ significantly for the first six weeks. However, the systems receiving a full commercial diet grew more successfully from weeks six through nine, as can be seen in Figure 21 and Figure 22. Exact reasons for this are unknown. A possible explanation for this trend is that a second wave of lettuce and basil seedlings grew to harvest size by week six. These seedlings were germinated using the protocol from Phase 2 and 3, unlike the original batch which were purchased already

germinated from a local nursery. Additionally, high temperatures early in the phase caused several lettuce plants to bolt[1], reducing the harvested quantities of lettuce during that time.

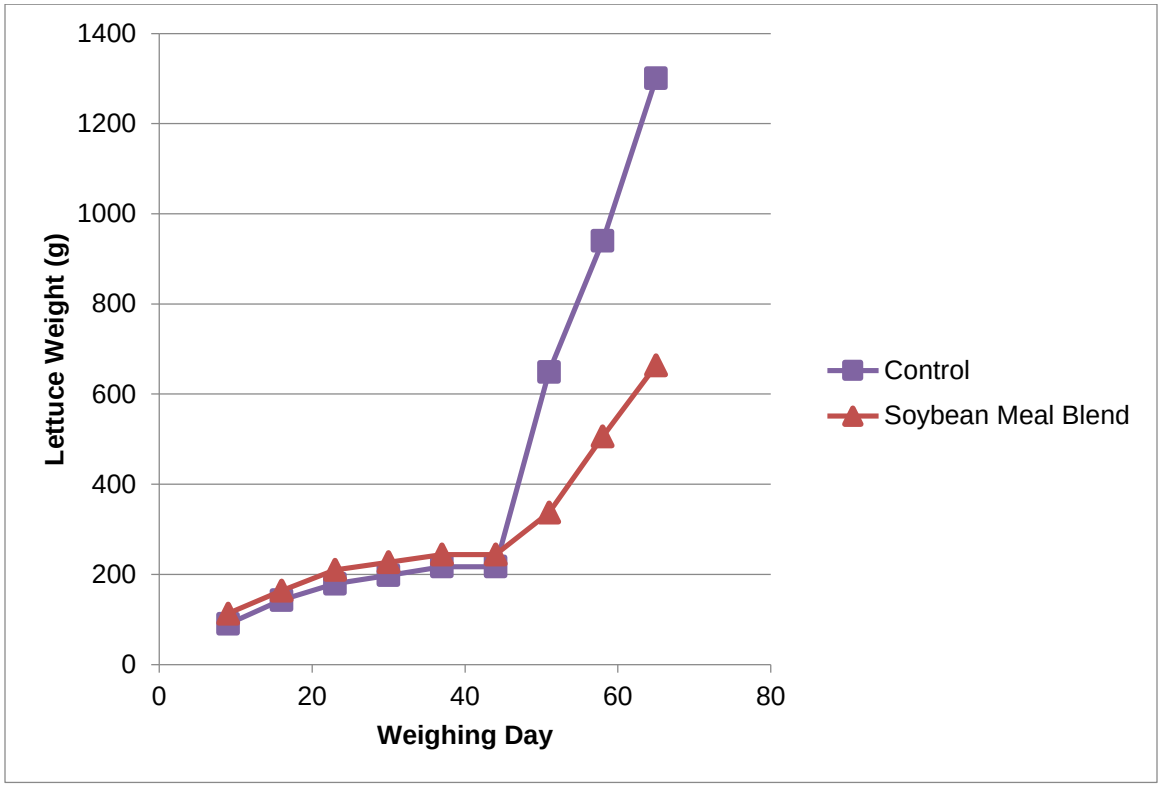


Figure 21. Phase 4 average cumulative lettuce production.

Cumulative weight of edible harvests of lettuce over the course of Phase 4, averaged across all three replications of each treatment

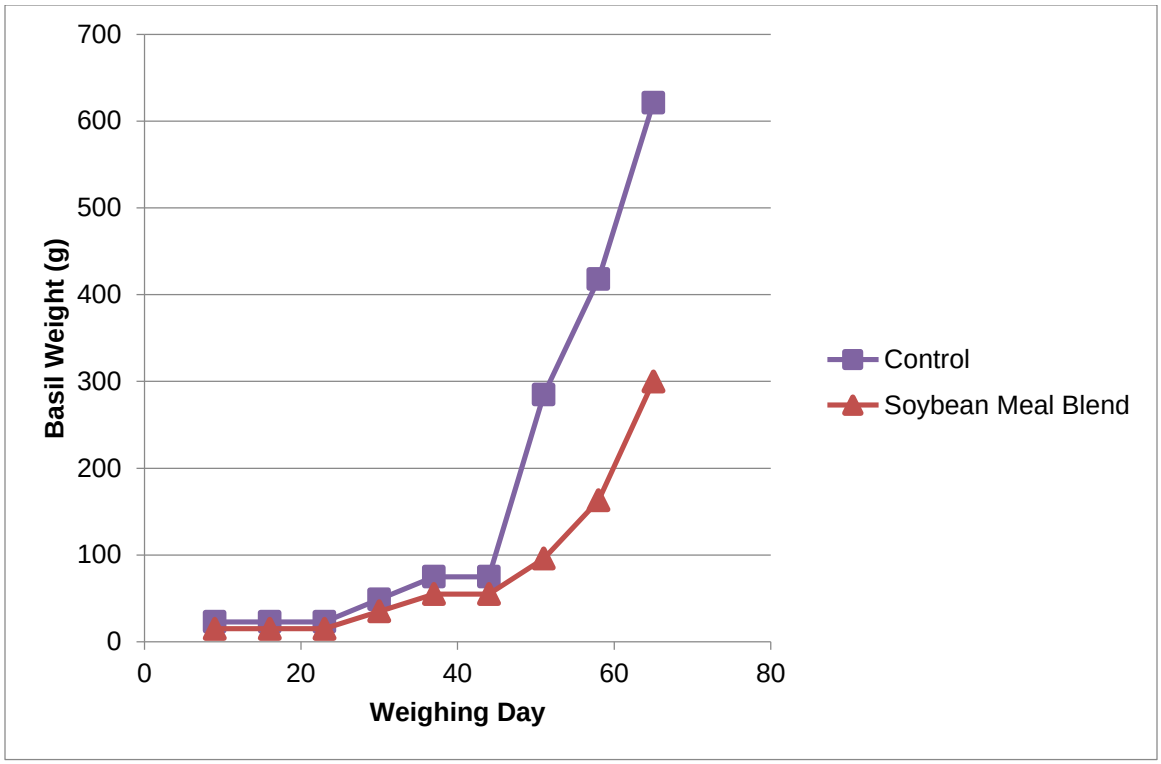


Figure 22. Phase 4 average cumulative basil production.

Cumulative weight of edible harvests of basil over the course of Phase 4, averaged across all three replications of each treatment.

Discussion

Introduction

As the project progressed, the research question expanded and shifted. Investigating the original question of what alternative feed sources can effectively and efficiently support a subsistence-level aquaponic system led to another question studied in Phase 1: Which nutrient source could grow duckweed, a potential alternative feed for tilapia, with the highest overall biomass and protein content? We used the best nutrient source from Phase 1 to grow the duckweed used in Phases 2 and 3. The results from Phase 2 caused us to change our focus from 100% feed replacements diets to 50% replacements diets. Thus, we reevaluated the overall research question, which subsequently became: Which alternative feed source can most effectively and efficiently *supplement* commercial fish feed in a subsistence-level aquaponic system?

Finally, for Phase 4, our research question evolved into: How does supplementing commercial fish feed with soybean meal in a larger subsistence-level aquaponic system affect economic viability of the system?

Phase 1

In Phase 1, we collected data to determine which nutrient source could grow duckweed with the highest overall biomass and protein content. We determined that activated sludge can be used to grow large amounts of duckweed with very high protein content of 40.67%, comparable to the protein content of duckweed grown under ideal conditions (40-43%) (Rusoff et al., 1980; Leng et al., 1995). The difference between biomass yields of activated sludge and poultry manure is insignificant (see Figure 2), but the protein content of activated sludge is

significantly higher [Figure 3]. Due to its higher protein content, activated sludge was chosen as the nutrient source for the duckweed throughout Phases 2 and 3.

There were some limitations with our Phase 1 study. Certain tested fertilizers, particularly activated sludge and dairy manure, had solids that would not completely dissolve in the water. This led to difficulty collecting the duckweed samples without also including some fertilizer, which potentially added extra mass to the weighed sample. We resolved this issue later in the phase by avoiding settled solids on the bottom of the tanks and by visually inspecting the collected duckweed before weighing. This change in collection technique reduced standard deviations between replications.

Phases 2 and 3

Phases 2 and 3 served as a preliminary evaluation of all candidate alternative feeds, with the original intent to conduct two identical replications. However, results from Phase 2 caused a reworking of our original research question. None of the alternative feeds resulted in fish growth comparable to the growth in the control tanks (see Figure 5 and Figure 6). Plant growth data, however, eliminated rice bran and sorghum from further consideration as those feeds yielded significantly less basil and lettuce than duckweed and soybean meal (see Figure 7 and Figure 8). A possible reason for this is that lower digestibility of our feed sources by the tilapia results in more nutrients in the water for the plants to absorb.

The layout of our system led to unexpected issues during Phase 2. High turbidity in the fish tanks made observation difficult. Suspended solids, in the form of uneaten feed and fish waste, led to degraded water quality and spikes in concentrations of toxic ammonia and nitrite to near dangerous concentrations. Also refer to Appendix A, which details the acceptable nitrogen

ranges for fish health. We lowered these concentrations to acceptable ranges by more frequently siphoning out settled solids and replacing tank water with clean water in Phase 3.

In Phase 2, we used a 100% replacement feed treatment for non-control tanks, with no commercial fish feed given to these tanks after acclimation. Possibly due to the reduced digestibility of the alternate sources, unsatisfactory palatability, a lack of key nutrients, or the presence of antinutrients, fish in these experimental tanks grew significantly less than fish fed commercial fish feed. The tilapia receiving alternate feed diets also showed very slow growth rates compared to the tilapia receiving commercial feed diets (see Figure 5 and Figure 6). According to (Francis, Makkar, & Becker, 2001), soybean meal can contain antinutrients including: protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins, and allergens. Their analysis showed tentatively that a majority of these are not important in a practical sense and will not impact growth at the concentrations commonly seen in fish diets.

It was brought to our attention in February 2014 that there was a product recall on the fish feed we ordered for Phase 2 (Food and Drug Administration, 2012) There were elevated concentrations of vitamin D in several lot numbers between April 2, 2012 and May 8, 2012, and our order fell within that range. All of the tilapia were initially acclimated using this commercial fish feed. We did notice some sluggish activity and lack of interest in feeding, but we cannot confirm whether it was caused by the incorrect formulation, as no literature on toxicity of vitamin D in fish is available. The behavior may also have been caused by an excess of feed in the system.

Phase 3

During Phase 3, we switched from the 100% replacement feed to 50/50 blends for the alternative feed tanks. One test feed consisted of commercial fish feed and duckweed while the

other consisted of commercial feed and soybean meal. As a result, we changed our research goal to evaluating duckweed and soybean meal as supplements instead of total replacements. The data from Phase 3 was sufficient to determine that soybean meal was the most effective alternative feed supplement. Though differences in plant growth between the two feeds was insignificant (see Figure 12 and Figure 13), fish fed with 50/50 soybean meal and commercial fish feed grew to weights comparable to those of the control fish (see Figure 10 and Figure 11). The difference in fish growth between the soybean meal and duckweed tanks was significant enough for us to conclude that soybean meal is the better candidate. Based on toxin tissue analysis, soybean meal had the lowest concentrations of arsenic, cadmium, lead, and mercury compared to duckweed and control (See Table 18). Additionally, we can only compare our resultant concentration of mercury to the concentration determined to be actionable by the FDA, as there does not appear to be any explicit regulation on the concentrations of cadmium, lead, or arsenic (CPG § 540.600, 2007). Our toxicity analysis indicated that the levels of mercury in all of our fish were below actionable concentration of 1 mg/kg, with the lowest concentration found in the fish fed with blended soybean meal (Appendix D). Although most of the toxin concentrations were detectable, they were below the concentration for accurate quantification. Thus, though we are confident that they are within a safe range, we cannot be entirely confident in quantitative values.

Phase 3 had high fish mortality in several of the replications. We examined the carcasses of individual specimens, but were unable to identify a conclusive cause of death. The pattern of deaths suggests a pathogen, as certain tanks, especially those with unexplained organic matter buildup, lost several individuals while others of the same treatment suffered no mortality (see Appendix D).

The control tanks had the highest mortality, possibly due to already elevated stress levels caused by the high concentrations of ammonia and nitrite nitrogen from the commercial fish feed. We did not consider the number of fish deaths in the final analysis because the distribution of fish deaths indicates that they were likely not caused by any specific feeds. A one-way ANOVA indicated that the fish mortalities were not attributed to a specific feed treatment and were randomly distributed among the tanks (p-value: 0.42). We corrected for the mortality by using the average weight per remaining fish in the comparison.

We observed less vigorous feeding activity with the soybean meal alternative diet. This disagrees with the findings of Robaina et al. (1995), who observed no change in feeding behavior. The combined soybean meal pellets proved to be less buoyant than the commercial diet, which means that the tilapia may have had less time to notice the food and consume it while it was in the water column. However, tilapia are typically quite willing to feed off the bottom of the tank, and slow sinking pellets are common in tilapia culture (Fitzsimmons, 2000). The observed turbidity may have had an impact, as floating feed allowed fish more time to locate the feed before it was filtered or settled out of the water column.

Our results agree with other studies conducted on tilapia and other species of fish, which identified soybean meal as a viable diet supplement at varying levels of inclusion (Venou, Alexis, Fountoulaki, & Haralabous, 2006; Webster, Tiwell, Goodgame, Yancey, & Mackey, 1992; Dabrowski, Poczyczynski, Köck, & Berger, 1989; Kikuchi, 1999; Shiau, Chuang, & Sun, 1987). Our 50% inclusion rate is higher than what was used by these studies, which replaced between 25% and 45% of the fish diet with soybean meal. We decided to push the limits of previously studied feed replacement percentages, because higher replacement rates result in higher cost savings. These studies observed possible detrimental effects beginning to appear as

the inclusion rate increased, possibly as a result of decreased starch digestibility[1] of the included soybean meal (Venou et al., 2006).

Some differences between our findings and previous literature can be attributed to differences between experimental apparatuses. We are modeling a subsistence-level aquaponic system utilizing available resources in developing countries. This includes feed replacement and less water filtration. Nitrogen concentrations were higher in our systems than would be found in the high flow-rate systems used in other studies (Rakocy et al., 2011). This poor water quality may have reduced growth and feed conversion efficiency.

Little research has been conducted towards utilizing alternative feeds in aquaponic systems. Nearly all of the existing work with soybean meal was done in recirculating aquaculture systems without any plant production component. As part of an aquaponic system, the elevated nitrogen concentrations contribute to enhanced plant growth at the cost of water quality. The parameters of the water must be maintained within the growing range of the fish while simultaneously at a concentration that provides sufficient nutrients for growth of plants. Fish growth follows a linear trend with feeding rate while water quality degrades exponentially with increased feeding (Swick, 2001). Thus, it is very important to regulate water quality in order to prevent excessive stress on the fish. Stress reduces growth, as the fish become less efficient at processing the feed (Pankhurst & van der Kraak, 1997).

Phase 4

As mentioned earlier, Phase 2 showed that complete substitution soybean meal for commercial feed entirely yielded poor fish growth. In Phase 3, fish growth in the 100% commercial and 50% soybean meal diets was very similar, with no statistically significant difference between the average fish weight across the three repetitions of each system. Thus, we

modified the hypothesis slightly; we now hypothesize that an unprocessed vegetable protein supplement can be used in the diet of tilapia to increase the viability of aquaponic systems in developing countries by reducing costs while maintaining fish growth.

The goal for Phase 4 was to evaluate the effects of soybean meal on the cost of the system and system output, but on a larger scale and over a longer period of time compared to earlier phases. Phase 4 also modeled real-world settings. Instead of being in a controlled growth chamber, Phase 4 was conducted outdoors in a rooftop high tunnel where it was subject to unpredictable weather conditions. Our Phase 4 growth study was ended prematurely by a period of cold weather that threatened to lower water temperatures below tolerable levels for tilapia (Popma & Masser, 1999, March). We collected eight weeks of data, which was enough to distinguish between the two treatments, but too short to reach harvest size for several of our plant crops. Additionally, several plants grew sporadically through the experiment, which caused some inconsistent harvests. The diminished autumn sunlight also affected plant growth.

In our study, we were modeling a system intended for a tropical region. However, we conducted our study in temperate region in mid-autumn, and thus we faced some challenges associated with the mismatched climates. We had an excessive amount of condensation in the high tunnel resulting from the temperature difference created when the tunnel was sealed in as the weather cooled. The condensation created challenges for our electrical systems, sometimes tripping ground fault circuit interrupters (GFCI) outlets and interrupting water flow. An early bout of extremely warm weather also caused several lettuce plants to bolt, thereby rendering them useless to the rest of our study. Eventually, we had to use water heaters and, later on, an air heater to keep water temperatures in a good growing range for the tilapia and to prevent freezing

air temps from damaging plants at night. These measures should not be necessary in tropical or sub-tropical climates.

The type of commercial feed used for control and blended pellets was changed before the final weighing due to an oversight. We noticed decreased appetite for the new feed across both control and soybean meal systems, which may have also been a result of the steadily decreasing temperature outside and inside of the high-tunnel. However, only the last fish weighing was affected by this switch, and the study was ended shortly after due to cold weather.

Tilapia grew well in both treatments during the early portion of the phase, while the weather was warm. To compare the growth between the two treatments, a feed conversion ratio was calculated. The lower the feed conversion ratio, the more efficient the organism is at converting feed to edible product. An aquaculture study conducted by Minufiya University in Shebin El-Kom, Egypt, showed a tilapia FCR of 2.5 (Abdelhamid, 2011), while a study by Rakocy, Bailey, Schultz, and Danaher (2011) showed a range of 1.7 (for Nile tilapia) to 1.8 (for Red Tilapia).

In the first four weeks of Phase 4, control had an average FCR of 0.844 (see Appendix E). An FCR lower than one suggests that either the tilapia were also feeding on additional matter, perhaps algae growing in the tanks, or it simply arose because of the addition of wet fish tissue from dry feed, which is common in young fish growing rapidly (Fry, 2011). Although this adds a confounding variable to our study, this finding is promising in our intended applications, since naturally growing algae can be an effective nutritional supplement for fish (Riche & Garling, 2003, August). However, later measurements of fish weight showed a significant difference between control and soybean meal treatments. Control fish displayed a statistically significant increased mass over the soybean meal fed fish.

Plant production also showed differences between the two treatments. Results from ANOVA tests conclude there is are no significant differences in lettuce or basil growth between the two groups. Although this suggests that control and blended treatments are comparable for plant production, we have reason to question these results. We recorded high standard deviations between replications that may have influenced the results and ANOVA test. We attribute these discrepancies to temperature fluctuations in the high tunnel, unequal light distribution between systems, and human error. Although these parameters were closely controlled in our previous phases (2 and 3), these factors were more difficult to control in a real-world application.
On the other hand, our ANOVA tests indicate that there was no difference in lettuce growth between the two groups. Although this suggests that control and blended treatments are comparable for lettuce growth, we are hesitant to accept these results. We recorded high standard deviations between replications that may have skewed the results and ANOVA test. We were also growing okra and broccoli in these systems, but because both crops were germinated late into the study, neither crops reached harvest over the course of our eight-week study.

Water Chemistry

Originally, we intended to use water chemistry as a parameter for feed success, since the ideal alternative nutrient feed should not significantly degrade water quality. Specifically, we focused on ammonia and nitrite, nitrogenous wastes that are poisonous to fish at high concentrations. However, we identified no difference in toxic nitrogen concentrations between the different treatments throughout all phases of research. In our studies, we used water chemistry as a general assessment of fish health. However, an idea system would not require regular chemical monitoring. Instead, fish behavior and plant health are adequate indicators of

system success. If a tank had elevated toxic nitrogen concentrations, we did not feed that day, and monitored the tank closely.

In Phase 2, we regularly observed elevated concentrations of toxic nitrogen. One possible cause was a lack of waste removal from the bottom of the barrels. When uneaten feed and fish waste degrade, they release nitrogenous wastes into the water. In preparation for Phase 3, we decided to change our methodology to address this issue.

In Phase 3, we removed fish waste from the bottom of the barrels on a weekly basis. After adopting this technique, we observed no traces of nitrogen in the water. Although the concentrations of toxic ammonia and nitrite were nearly zero, the regular waste removal process was stressful for the tilapia and limited plant growth. The plants exhibited discoloration and extreme stress from low nutrients. From our qualitative and quantitative observations of Phase 3, we decided to eliminate the waste removal process for Phase 4.

During Phase 4, dissolved oxygen concentrations as low as 0.67 mg/L were noted in two tanks. However, these measurements may have been due to a DO probe malfunction. In instances of low dissolved oxygen, the tanks with low dissolved oxygen were not fed until concentrations rose to acceptable ranges of above 5 mg/L, since digestion increases respiration rates, which decreases dissolved oxygen concentrations (Masser et al., 1999, March). These skipped feedings likely had an insignificant effect on the final fish weights as they were infrequent. Additionally, we did not experience any fish death caused by low dissolved oxygen concentrations.

System Design

In addition to utilizing alternative nutrient sources, an ideal subsistence-level aquaponic system could be built with less expensive materials, maintained without instrumentation testing,

and operated with varying electrical conditions. Our Phase 4 system consisted of thick, plastic fish tanks connected to drain pipes in a high tunnel - materials that are readily available in developed nations, but less so and at a higher cost in the developing nations such as Nigeria and Thailand (Ogunlana & Olomolaiye, 1989; Ogunlana, Promkuntong, & Jearkjirm, 1999).

There are alternative ways of constructing an aquaponic system using more accessible materials. For instance, the plastic tank could be replaced by a wooden or metal box with a liner. Increasing the surface area by making the plant beds larger could also act as a natural biofilter, replacing the foam padding we used in our system, while other methods of introducing beneficial bacteria could replace the biomedia. The exact materials used in each system depend on the materials available in a given part of the world, but an ideal subsistence-level design would be flexible enough work with different materials.

One of the biggest limitations of our Phase 4 system was its reliance on constant electricity. A power outage after the data collection stage resulted in the deaths of an entire tank of fish. Electricity in rural, developing regions of the world such as Nigeria is even less stable (Uduma & Arciszewski, 2010). Therefore, decreasing the electricity requirements of the system would be very beneficial. We investigated several solutions for keeping an aquaponic system running without electricity, such as building a manual bike pump and elevated water storage into the system or adding solar panels, but many of them hinged on designing a low-flow system. Our ½ hp. motor was more than sufficient for our system, so a subsistence-level system could use a much less energy intensive motor, particularly if the plant beds were at the same or similar elevation as the fish tanks, reducing head requirements (see Appendix M for Phase 4 Fluid Dynamics Calculations). The lower flow would also reduce surface agitation leading to a lower rate of evaporation, reducing the requirement for clean fresh water replacement. That, coupled

with a backup such as a bike pump or even batteries with solar photovoltaic, could work well in the targeted rural farming regions of the world.

Future Directions

Though our data has shown that higher inclusion rates of alternative feeds yields less fish and plant growth, there is still the potential for a simple, 100% replacement formula. Areas and communities where purchasing commercial feed is not possible or practical would need such a replacement solution to facilitate aquaponic systems. Valuable future work would be to develop this simplified alternative, perhaps using soybean meal and duckweed as a starting point. Additionally, processing the raw soybean meal prior to inclusion in fish diets may further improve its efficacy as a nutrient source. Wee and Shu (1989) noted that boiling the soybean meal inactivated up to 80% of the trypsin inhibitor[1], and that fish growth was significantly better with boiled or defatted soybean meal than with raw soybean meal. This can, however, degrade the nutritive value of the feed as well, so further work is needed to investigate the trade-offs presented when using soybean meal in tilapia diets.

For our systems, we only investigated Nile tilapia and a limited variety of crops. While tilapia are a hardy fish and a good choice for warm climates, future research should continue testing alternative diets for other species, including those that can tolerate colder water temperatures (e.g. catfish, trout, yellow perch (Buttner, Flimlin, & Webster, 1992)) to better suit local economies and tastes. Further investigation should also be conducted on different varieties of plants, again to better suit the preferences of the local culture and climate where an aquaponic system may be deployed.

We considered aspects of a model system to meet the requirements of food insecure regions of the world, but further research could be done to investigate lower flow and lower

stocking densities. The fish tanks could be made in a variety of ways from locally sourced materials (wood, stone, cinder block, pond liner, etc.). The water flow could be accomplished by using electric pumps in areas with reliable electricity, but could also be done by filling an elevated reservoir using a bicycle powered pump or by manually lifting the water in buckets and using valves to control the flow of water back through the plant beds and into the fish rearing tanks. Further work could be done to investigate other types of hydroponic media. Our system used peat pots, expanded clay pebbles and net pots that were suspended in free-flowing water. There are other possibilities for growing media that may be cheaper and easier to maintain, such as gravel beds with an ebb and flow system.

Economic Analysis

We investigated whether it would be economically beneficial to include a plant supplement in fish feed. In aquaponics, according to the WorldFish Center (TWC) (2009), purchasing feed is 50-70% of the main operating cost[1], a percentage that varies depending on farming intensity. Thus, adjusting the existing farm-made feed ingredients to include cheaper, more sustainable plant-based feeds would lower the production cost. This would benefit economically struggling areas in developing countries.

According to the International Monetary Fund (IMF)'s January 2014 report (International Monetary Fund, 2014), the commodity price[1] of soybean meal is $500 per metric ton ($0.23 per pound), which is less than one-third that of fishmeal (priced at $1600 per metric ton or $0.73 per pound). Therefore, any replacement of fishmeal with an alternative nutrient lowers operating costs, as long as growth is not sacrificed.

When conducting economic analysis, we looked at one aquaculture study located in Bangladesh. Since our research is aimed at tropical developing regions this study serves as an

appropriate model. Fish farmers experimented with alternative feed formulas, where only 10% of the feed was composed of fish feed, and up to 90% of the feed was composed of plant-based products such as rice bran and duckweed. In Bangladesh, commercial fish feed is valued at $0.35/kg ($0.16/lb.) (TWC, 2009). This replacement formula lowered the cost of feed to $0.22/kg ($0.10/lb.). However, even in the locally made alternative diet, the cost of fishmeal exceeded any other expenditure. Although fishmeal only comprised 10% of the farm alternate feed, it made up 45% of the cost. Thus, it would be advantageous to lower the ratio of fish meal to alternate nutrient as much as possible while still maintaining satisfactory fish growth. For instance, simply lowering the fishmeal quantity to 5% in the example feed would decrease production costs by approximately 23%.

In our Phase 4 study, we compared the efficiency of a 50/50 commercial fish feed/alternative feed blend with that of a complete commercial fish diet. For the fish treated with commercial fish feed, we utilized 9.6 kg of commercial fish feed and yielded 5.4 kg of fish product. Using the Price Commodity Index as provided by the International Monetary Fund, we determined that the total feed cost in order to produce this amount of product would be $15.06.

The tilapia receiving control treatments had an average FCR of 1.786, while fish receiving blended soybean meal/commercial fish feed treatments had a less effective FCR of 2.27. The lower FCR indicates that the fish are less effective at converting the 50/50 blended feed to body mass. These fish require a longer grow-out time to reach market size, and consequently will require more feed. Our next step was to determine whether the significantly lower cost of soybean meal is a worthwhile investment, despite the longer grow-out time and more overall feed required.

We calculated that we would need 12.25 kg of 50/50 soybean meal and commercial fish feed blend in order to match the production levels of our control tanks. This would cost approximately $12.60, an 8.86% decrease from $15.06, the cost for a complete commercial fish feed treatment. Thus, we conclude that it is economically beneficial to use an alternate protein supplement or replacement feed. Even though our fish raised with a blended treatment grew slower, there is a greater return on investment over time. Similarly, the reduced cost of fertilizer would lower the production costs of the plant crops as well, further increasing the potential for profit.

A potential area of further study is adjusting the ratio of replacement feed to commercial feed. A study conducted by Kikuchi (1999) found that the ideal proportion of soybean meal substitution in fish feeds is below 43%. Above this concentration, feed conversion efficiency was compromised.

Although soybean meal is projected to be an economically beneficial feed supplement, our percentage feed replacement produced insignificant results. Our results, comparing the fish production for the two treatments, are shown in Figure 23. A study conducted over a longer growing season, where fish can reach market size, would lead to more conclusive results.

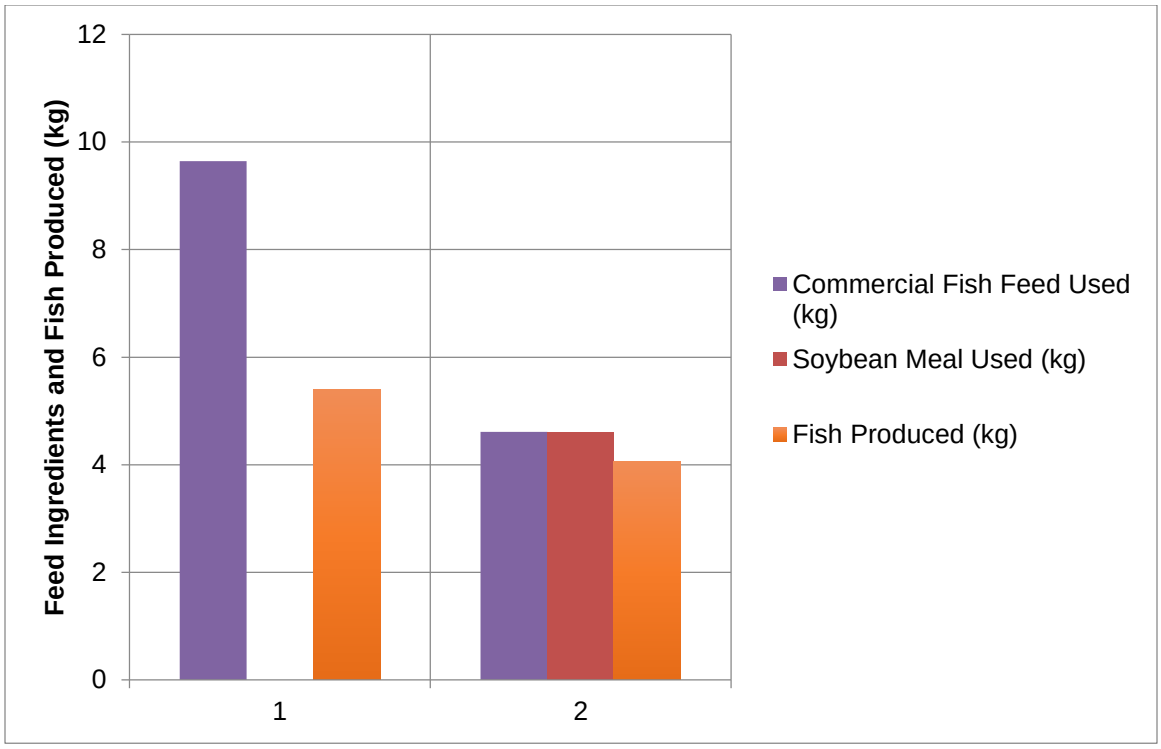


Figure 23. Comparison of feed ingredients and fish product.

Feed input and total fish produced for each treatment across all three replications

Conclusion

Team MEGA identified aquaponics as a potential method of providing access to high quality food, which remains inaccessible to a large number of people. The primary constraints limiting widespread implementation are initial and operating costs and market access. To address these challenges, we began investigating widely available, low-cost alternative nutrient sources that could be used to supplement fish diets, reducing operating costs and the need for market access. We decided to evaluate these nutrient sources in a way that modeled the intended application. Nutritional studies to this point have typically been conducted in fish-only systems. By using aquaponic systems, we were able to observe and quantify the effects of feed source on fish and plant growth.

Research Summary

To begin, we identified four alternative feeds sources: duckweed, soybean meal, rice bran, and sorghum. Each option is high in protein, is lower in cost than fishmeal, and is commonly found or grown in many regions worldwide. Duckweed has been used as an alternative feed in practice, but very little empirical data was available. We designed and conducted Phase 1 of our study to determine what fertilizer would produce the most duckweed with the highest protein content. We tested dairy manure, poultry manure, vegetable compost, and activated sewage sludge in a two-week controlled growth study and found that activated sewage sludge produced a large amount of duckweed with very high protein content (40%).

Using activated sludge, we grew duckweed and tested this feed against the other three alternatives with 100% replacement of the control commercial diet (Phase 2). After eleven weeks, we found that though there was a clear difference between the plant growth of several of the treatments, the 100% replacement diets were not producing significant growth in the tilapia.

We eliminated sorghum and rice bran due to lower plant yields, and repeated the study with 50% replacement for duckweed and soybean meal (Phase 3). At the conclusion of this trial, we determined that soybean meal yielded better fish growth.

To further study the results found in Phase 3, and to obtain data more relevant to the intended final application, we conducted a larger scale growth study in a less-controlled environment. In a rooftop high-tunnel, Phase 4 compared the fish and plant growth of three replications each of soybean meal and a commercial diet over eight weeks. Despite the short growing season due to cold weather, we obtained usable growth data, and conducted an economic analysis based on the values. We found that despite reduced growth, with the 50% soybean meal replacement the cost of feed would be reduced by just over 8%. The large-scale study also enabled us to explore some of the challenges that must be overcome in order to successfully implement a robust, low-cost, low-energy aquaponic system.

Using existing literature, which found that soybean meal replacement did not lower growth significantly, we extrapolated on an economic case study in Bangladesh. We found that by replacing 50% of the fishmeal in a farm-made diet (10% fishmeal), the cost of the total diet could be reduced by as much as 22%.

Potential Implications

Our findings have implications for a number of aspects of aquaculture and aquaponics, particularly in low-density and subsistence-level applications. We tested a range of waste products that can be used to grow duckweed as a supplemental feed source. Our data indicates that using processed human waste as a fertilizer yields high growth rates and protein content. Since this is a ubiquitous waste stream, it can be used worldwide. Growing duckweed would also serve as a means of removing excess nutrients from wastewater, reducing environmental

impact, and recycling nutrients for food production. This duckweed was tested against other alternatives as a feed replacement in aquaponic systems, with promising results. By growing the feed source for tilapia, farmers could dramatically reduce costs without a large drop in fish or plant yields. Testing feed sources in aquaponic systems is a novel research approach, and one that provides valuable insight into the effects of feeds on both fish and plant yields. Our growth data for soybean meal suggests that soybean meal and possibly other alternative feeds could displace a larger portion of fishmeal in fish diets, reducing costs and fishing pressure on wild fisheries.

Future Work

To make the alternatives more viable options, further work needs to be done to determine what combinations are most effective, what replacement rates provide the best compromise between growth and cost, and the regions in which each is available. Further investigation is also needed to identify methods of reducing the energy requirements and allow for low-cost water movement, aeration, and filtration. As mentioned, current systems require a constant supply of electricity, which is not available in many regions of the world. We considered several options to reduce the need for electricity throughout our project, but did not conduct any tests. Potential solutions include manual water movement using bicycle pumps or buckets and electric pumps powered by solar photovoltaic (though cost is an issue). These options could be used with elevated water storage to eliminate the need for a constant energy supply, whether electrical or manual labor. For example, an elevated reservoir could be filled using buckets or a pump, and then a valve could control the flow over the next six hours, until the reservoir would be filled again.

To reduce costs and enable systems to be constructed in a variety of locations, further work needs to be done to identify materials and methods that utilize locally available, low-cost materials. The fish tanks, plant beds, and substrate all need to be found locally. The fish tanks can be made from a range of materials including plywood, gravel, concrete block, or they can be dug into the ground and lined with a pond liner. Plant beds can be made similarly, and barrels cut in half can also be used. Gravel can be an effective plant substrate, and is commonly available. Floating rafts can also be used where gravel is not present.

Final Words

We see aquaponics as a means to enable people around the world to grow high quality food on a small-scale, alleviating hunger and food insecurity. There are several challenges that must be overcome in order to make aquaponics economically viable for this purpose, but through the use of lower cost feed supplements and other methods detailed above, it has tremendous potential. With increasing costs of fossil fuels, it is unlikely that conventional agriculture will continue to be able to provide food to many regions of the world. Therefore, a range of new methods needs to be developed and implemented, and we hope that aquaponics will be part of that portfolio.

Appendix A: Acceptable Nitrogen Concentration Ranges for Tilapia Health

Table 6. *Acceptable Nitrogen Concentration Ranges for Tilapia* (Rakocy, 1989, September)

Name	Chemical Formula	Acceptable Range
Ammonia	NH_3 - N	0 – 2 ppm
Nitrite	NO_3 – N	0 – 5 ppm

Appendix B: Phase 1 Data Tables

Table 7. *Phase 1 Data: Tukey Multiple Comparisons Test for Total Duckweed Biomass and Protein Content*

	Biomass	Protein
Groups	p-value	p-value
Vegetable Compost-Activated Sludge	0.0031257*	0.000012*
Control-Activated Sludge	0.0004914*	0*
Dairy Manure-Activated Sludge	0.005382*	0*
Poultry Manure-Activated Sludge	0.8029669	0.000062*
Control-Vegetable Compost	0.6441074	0*
Dairy Manure-Vegetable Compost	0.9948887	0*
Poultry Manure-Vegetable Compost	0.0157963*	0.4869878
Dairy Manure-Control	0.4370417	0.0081312*
Poultry Manure-Control	0.0020727*	0*
Poultry Manure-Dairy Manure	0.0282972*	0*

*Indicates significant value at an alpha level of 0.05

Appendix C: Phase 2 Data Tables

Table 8. *Phase 2 Data, Tilapia Weights and Final Growth Percentage*

Date	10/5/12	10/19/12	10/26/12	11/2/12	11/9/12	11/16/12	11/23/12	11/30/12	12/7/12	12/14/12	12/21/12	Growth %
Day	1	15	22	29	36	43	50	57	64	71	78	78
Control	64.50	82.50	88.50	106.50	131.50	157.00	180.50	214.00	233.50	257.50	293.50	123.19%
Std. Dev.	16.26	3.54	7.78	6.36	2.12	4.24	3.54	9.90	19.09	34.65	40.31	27.05%
Sorghum	44.50	44.50	50.00	44.50	42.00	42.50	42.50	46.50	48.00	48.00	50.00	19.05%
Std. Dev.	10.61	2.12	7.07	2.12	0.00	2.12	2.12	2.12	0.00	0.00	0.00	0.00%
Soymeal	62.00	54.50	54.50	54.00	52.50	56.00	58.50	60.00	66.00	77.00	82.50	57.14%
Std. Dev.	0.00	2.12	2.12	0.00	4.95	4.24	2.12	5.66	7.07	2.83	0.71	16.23%
Rice Bran	49.50	50.00	51.50	49.50	54.00	52.00	53.00	53.50	57.00	60.50	62.00	14.81%
Std. Dev.	6.36	0.00	2.12	0.71	2.83	2.83	1.41	3.54	4.24	2.12	0.00	6.02%
Duckweed	55.50	50.50	50.50	48.50	49.50	49.50	54.00	56.50	59.00	63.50	69.50	40.40%
Std. Dev.	13.44	3.54	7.78	7.78	7.78	9.19	7.07	10.61	7.07	10.61	10.61	0.64%

Table 9. *Phase 2 Data, Tukey Multiple Comparisons Test for Tilapia Growth Percentage*

Groups	p-value
Duckweed/Control	0.0120178*
Rice Bran/Control	0.0036537*
Sorghum/Control	0.0043352*
Soybean Meal/Control	0.0310616*
Rice Bran/Duckweed	0.4819033
Sorghum/Duckweed	0.6133975
Soybean Meal/Duckweed	0.7685347
Sorghum/Rice Bran	0.9982951
Soybean Meal/Rice Bran	0.1467364
Soybean Meal/Sorghum	0.1952919

*Indicates significant value at an alpha level of 0.05

Table 10. *Phase 2 Data, Average Cumulative Lettuce Production*

Date	10/24/12	10/31/12	11/7/12	11/14/12	11/21/12	11/28/12	12/5/08	12/12/12	12/19/13
Day	20	27	34	41	48	55	62	69	76
Control	5.69	11.27	17.41	21.66	25.78	29.07	33.03	35.49	37.53
Std. Dev.	0.70	0.29	0.88	2.67	2.58	2.61	3.43	2.12	0.08
Sorghum	5.05	5.86	6.91	7.24	7.57	7.68	9.47	11.93	13.17
Std. Dev.	1.57	1.37	1.50	1.70	1.91	1.84	2.55	0.23	1.26
Soymeal	7.10	13.18	18.60	23.61	28.14	32.80	36.50	39.41	41.00
Std. Dev.	0.25	1.72	2.84	3.26	3.49	2.35	2.78	3.46	4.20

Rice Bran	5.37	7.48	8.97	10.27	11.30	13.61	15.50	19.87	21.74
Std. Dev.	0.90	0.50	0.60	0.35	0.63	2.47	2.21	3.24	3.16
Duckweed	6.01	11.28	15.47	19.74	24.02	28.35	32.15	35.90	38.16
Std. Dev.	1.64	0.79	0.46	0.79	0.64	0.07	1.14	2.33	3.04

Table 11. *Phase 2 Data, Average Cumulative Basil Production*

Date	10/24/12	10/31/12	11/7/12	11/14/12	11/21/12	11/28/12	12/5/12	12/12/12	12/19/12
Day	20	27	34	41	48	55	62	69	76
Control (g)	1.73	5.85	11.12	13.50	20.97	25.01	27.33	31.78	38.61
Std. Dev.	0.21	0.45	1.64	3.49	2.92	2.78	4.39	2.33	5.27
Sorghum (g)	1.93	3.10	4.48	5.09	5.54	6.39	8.28	10.26	11.55
Std. Dev.	0.01	0.21	0.02	0.21	0.41	1.31	0.55	0.99	2.36
Soymeal (g)	1.93	5.90	10.47	14.57	19.61	24.19	31.09	38.39	45.84
Std. Dev.	0.28	0.18	0.61	0.68	1.36	0.40	1.45	0.17	0.53
Rice Bran (g)	1.77	2.74	4.54	6.16	8.98	12.31	18.19	19.73	20.55
Std. Dev.	0.42	0.95	1.48	1.03	0.70	0.34	1.46	1.54	0.76
Duckweed (g)	1.81	5.08	9.73	15.24	20.64	24.60	29.59	33.34	42.34
Std. Dev.	0.16	0.23	1.58	3.69	2.00	1.52	2.04	3.61	5.69

Table 12. *Phase 2 Data, Tukey Multiple Comparisons Test for Dried Plant Biomass*

	Basil	Lettuce
Groups	p-value	p-value
Control/Sorghum	0.00033*	0.00274*
Control/Soybean Meal	0.26646	0.86368
Control/Rice Bran	0.00837*	0.08402
Control/Duckweed	0.81631	0.99596
Sorghum/Soybean Meal	0.00016*	0.00082*
Sorghum/Rice Bran	0.12201	0.2227
Sorghum/Duckweed	0.00019*	0.00172*
Soybean Meal/Rice Bran	0.00053*	0.01872*
Soybean Meal/Duckweed	0.80611	0.97021
Rice Bran/Duckweed	0.00189*	0.04836*

*Indicates significant value at an alpha level of 0.05

Appendix D: Phase 3 Data Tables

Table 13. *Phase 3 Data, Tukey Multiple Comparisons Test on Tilapia Growth Percentage*

Groups	p-value
Duckweed/Control	0.2239944
Soybean Meal/Control	0.0145218
Soybean Meal/Duckweed	0.0036117*

*Indicates significant value at an alpha level of 0.01

Table 14. *Phase 3 Data, Distribution of Fish Mortality Among Replications*

Tank	Total Dead
1 - Control1	1
2 - Duckweed1	0
3 - Soymeal2	0
4 - Control2	5
5 - Duckweed2	0
6 - Soymeal2	0
7 - Control3	6
8 - Duckweed3	2
9 - Soymeal3	7

Figure 24: Phase 3 Diagram, Distribution of Fish Mortality Among Replications

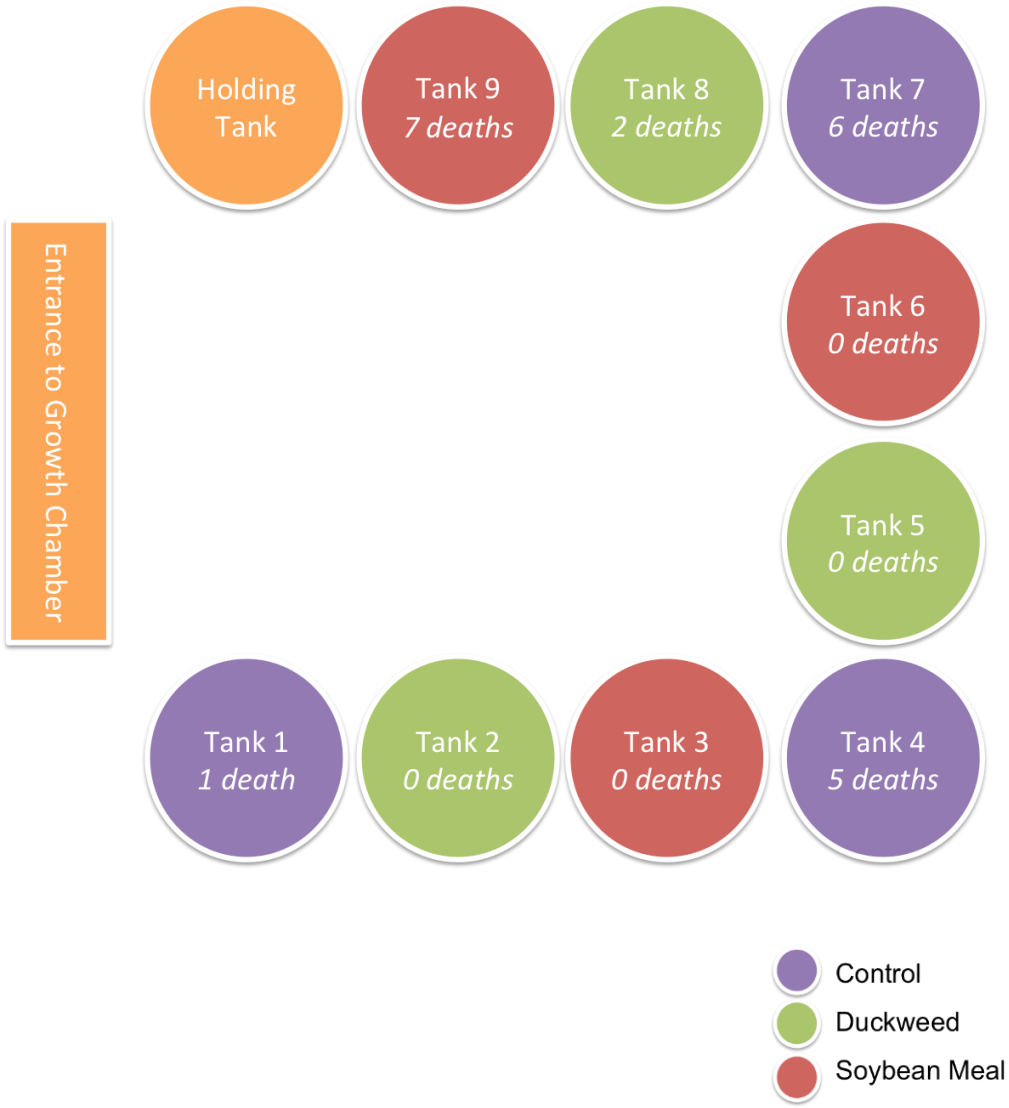


Table 15. *Phase 3 Data, Average Cumulative Lettuce Production*

Date	4/10/13	4/17/13	4/26/13	5/1/13	5/8/13	5/15/13	5/17/13
Day	12	19	26	33	40	47	54
Control	9.87	14.30	18.20	21.17	24.23	28.33	36.37
Std. Dev.	1.50	1.05	2.91	4.25	5.11	6.21	7.70
Duckweed Blend	10.43	14.87	18.17	20.50	22.80	25.50	33.60
Std. Dev.	1.91	2.82	2.86	3.03	2.79	2.34	4.60
Soybean Meal Blend	10.53	15.27	19.43	21.73	25.17	28.40	38.87
Std. Dev.	3.97	4.26	5.18	5.42	4.43	3.21	7.32

Table 16. *Phase 3 Data, Average Cumulative Basil Production*

Date	4/10/13	4/17/13	4/26/13	5/1/13	5/8/13	5/15/13	5/17/13
Day	12	19	26	33	40	47	54
Control (g)	3.60	6.43	9.63	11.93	16.50	20.80	23.93
Std. Dev.	0.70	0.12	1.12	2.26	2.75	4.04	5.95
Duckweed Blend (g)	3.90	5.87	8.70	10.57	13.67	17.87	20.13
Std. Dev.	0.98	0.78	0.95	0.68	0.91	0.85	0.76
Soybean Meal Blend (g)	3.33	6.50	9.03	11.20	15.07	19.07	22.17
Std. Dev.	0.81	1.30	2.25	3.05	4.13	6.83	8.52

Table 17. *Phase 3 Data, Tukey Multiple Comparisons Test for Dried Plant Weight*

	Basil	Lettuce
Groups	p-level	p-level
Control/Duckweed	0.75003	0.85703
Control/Soybean Meal	0.93859	0.88136
Duckweed/Soybean Meal	0.9196	0.58248

Table 18. *Toxin Analysis for Tissue Samples from Phase 3*

	Arsenic (ppm)	Cadmium (ppm)	Lead (ppm)	Mercury (ppm)
Control	0.15	0.048*	0.021*	0.025
Duckweed	0.15	0.015*	0.024*	0.033
Soybean Meal	0.11	0.018*	0.013*	0.016*

** - Estimated concentration above the adjusted method detection limit and below the adjusted reporting limit*

Appendix E: Phase 4 Data

Table 19. *Phase 4 Tilapia Weights and Final Growth Percentage*

Date	9/10	10/10	11/6	11/13	Growth %
Day	1	31	58	65	
Control (g)	224.67	740.00	1753.33	1799.00	700.74%
Std. Dev.	8.96	20.66	109.26	177.97	74.76%
Soymeal Blend (g)	232.33	644.33	1369.67	1355.33	483.36%
Std. Dev.	10.41	7.77	102.69	171.93	64.25%

Table 20. *Phase 4 Data, Average Cumulative Lettuce Production*

Date	9/18/13	9/25/13	10/2/13	10/9/13	10/16/13	10/23/13	10/30/13	11/6/13	11/13/13
Day	9	16	23	30	37	44	51	58	65
Control (g)	90	143	179	198	217	217	649	940	1301
Std. Dev.	12.49	19.09	20.65	23.64	26.65	26.65	95.69	157.51	241.86
Soybean Meal Blend (g)	113	164	210	227	244	244	337	506	664
Std. Dev.	5.86	5.51	7.55	6.51	5.69	5.69	32.39	81.38	85.64

Table 21. *Phase 4 Data, Average Cumulatice Basil Production*

Date	9/18/13	9/25/13	10/2/13	10/9/13	10/16/13	10/23/13	10/30/13	11/6/13	11/13/13
Day	9	16	23	30	37	44	51	58	65
Control (g)	23	23	23	49	75	75	285	418	621
Std. Dev.	0.58	0.58	0.58	0.58	1.00	1.00	27.22	34.12	21.52
Soybean Meal Blend (g)	15	15	15	35	55	55	96	163	300
Std. Dev.	2.65	2.65	2.65	5.77	8.96	8.96	15.62	40.50	50.69

Appendix F: Water Chemistry Protocols

Procedure for testing for presence and concentration of ammonia nitrogen in a water sample.

LaMotte

AMMONIA NITROGEN TEST KIT

OCTA-SLIDE METHOD

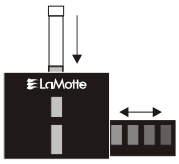
MODEL SL-NH • CODE 3351-01

QUANTITY	CONTENTS	CODE
30 mL	Ammonia Nitrogen Reagent #1	4797WT-G
30 mL	*Ammonia Nitrogen Reagent #2	*4798WT-G
2	Test Tubes, 2.5-5.0-10.0 mL, plastic, w/caps	0106
1	Ammonia Nitrogen Octa-Slide Bar, 0.2-3.0 ppm	3438
1	Octa-Slide Viewer	1100

***WARNING:** Reagents marked with an * are considered to be potential health hazards. To view or print a Material Safety Data Sheet (MSDS) for these reagents go to www.lamotte.com. To obtain a printed copy, contact LaMotte by e-mail, phone or fax.

To order individual reagents or test kit components, use the specified code number.

USE OF THE OCTA-SLIDE VIEWER



The Octa-Slide Viewer should be held so non-direct light enters through the back of the viewer. With sample tube inserted at top, slide the Octa-Slide bar through the viewer and match with color standard.

WARNING! This set contains chemicals that may be harmful if misused. Read cautions on individual containers carefully. Not to be used by children except under adult supervision

PROCEDURE

1. Fill test tube (0106) to the 5 mL line with sample water.
2. Add 4 drops of Ammonia Nitrogen Reagent #1 (4797WT). Cap and mix. Wait 1 minute.

   NOTE: When testing salt (sea) water, increase the amount of Ammonia Nitrogen Reagent #1 to 8 drops.
3. Add 12 drops of *Ammonia Nitrogen Reagent #2 (4798WT). Cap and mix. Wait five minutes.

   NOTE: When testing salt water, the reading should be taken after 1 minute to prevent precipitation.
4. Insert the Ammonia Nitrogen Octa-Slide (3438) into the Octa-Slide Viewer (1100). Insert test tube into the top of the viewer. Match sample color to a color standard. Record as ppm Ammonia Nitrogen (NH_3-N).

   To express results as Unionized Ammonia (NH_3):

   ppm Unionized Ammonia (NH_3) = ppm Ammonia Nitrogen (NH_3–N) x 1.2

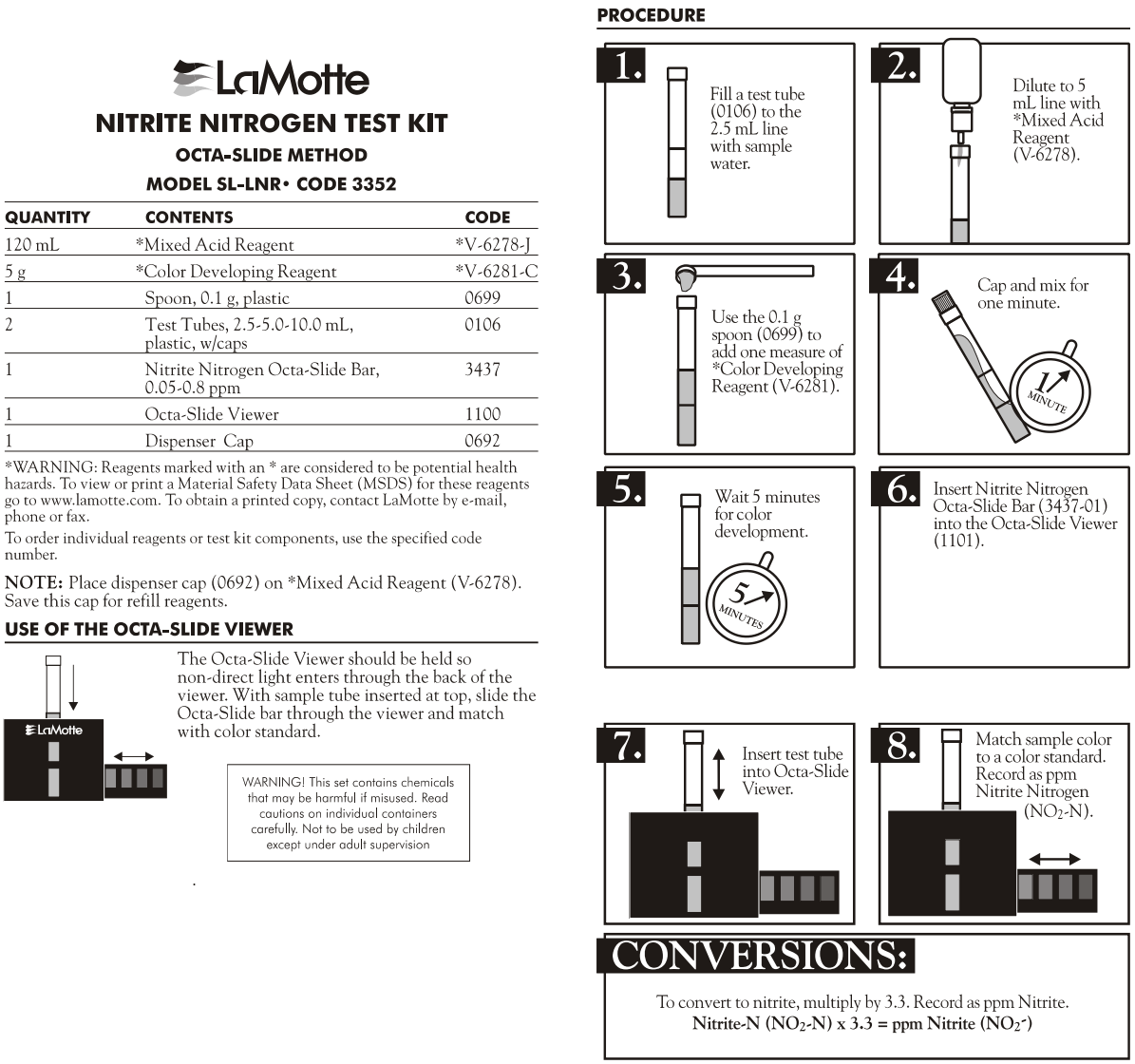
   To express results as Ionized Ammonia (NH_4^+):

   ppm Ionized Ammonia (NH_4^+) = ppm Ammonia Nitrogen (NH_3–N) x 1.3

Ammonia in water occurs in two forms: toxic unionized ammonia (NH_3) and the relatively non-toxic ionized form, ammonium ion (NH_4^+). This test method measures both forms as ammonia nitrogen (NH_3–N) to give the total ammonia-nitrogen concentration in water. The actual proportion of each compound depends on temperature, salinity, and pH. A greater concentration of unionized ammonia is present when the pH value and salinity increase.

1. Consult the table to find the percentage that corresponds to the temperature, pH, and salinity of the sample.
2. To express the test result as ppm Unionized Ammonia Nitrogen (NH_3–N), multiply the total ammonia nitrogen test result by the percentage from the table.
3. To express the test result as ppm Ionized Ammonia Nitrogen (NH_4^+–N), subtract the unionized ammonia-nitrogen determined in step 2 from the total ammonia nitrogen.

Figure 25. La Motte Ammonia Nitrogen Test Kit Protocol.



LaMotte

NITRITE NITROGEN TEST KIT

OCTA-SLIDE METHOD

MODEL SL-LNR• CODE 3352

QUANTITY	CONTENTS	CODE
120 mL	*Mixed Acid Reagent	*V-6278-J
5 g	*Color Developing Reagent	*V-6281-C
1	Spoon, 0.1 g, plastic	0699
2	Test Tubes, 2.5-5.0-10.0 mL, plastic, w/caps	0106
1	Nitrite Nitrogen Octa-Slide Bar, 0.05-0.8 ppm	3437
1	Octa-Slide Viewer	1100
1	Dispenser Cap	0692

*WARNING: Reagents marked with an * are considered to be potential health hazards. To view or print a Material Safety Data Sheet (MSDS) for these reagents go to www.lamotte.com. To obtain a printed copy, contact LaMotte by e-mail, phone or fax.

To order individual reagents or test kit components, use the specified code number.

NOTE: Place dispenser cap (0692) on *Mixed Acid Reagent (V-6278). Save this cap for refill reagents.

USE OF THE OCTA-SLIDE VIEWER

The Octa-Slide Viewer should be held so non-direct light enters through the back of the viewer. With sample tube inserted at top, slide the Octa-Slide bar through the viewer and match with color standard.

WARNING! This set contains chemicals that may be harmful if misused. Read cautions on individual containers carefully. Not to be used by children except under adult supervision

PROCEDURE

1. Fill a test tube (0106) to the 2.5 mL line with sample water.
2. Dilute to 5 mL line with *Mixed Acid Reagent (V-6278).
3. Use the 0.1 g spoon (0699) to add one measure of *Color Developing Reagent (V-6281).
4. Cap and mix for one minute. 1 MINUTE
5. Wait 5 minutes for color development. 5 MINUTES
6. Insert Nitrite Nitrogen Octa-Slide Bar (3437-01) into the Octa-Slide Viewer (1101).
7. Insert test tube into Octa-Slide Viewer.
8. Match sample color to a color standard. Record as ppm Nitrite Nitrogen (NO_2-N).

CONVERSIONS:

To convert to nitrite, multiply by 3.3. Record as ppm Nitrite.
Nitrite-N (NO_2-N) x 3.3 = ppm Nitrite (NO_2^-)

Figure 26. La Motte Nitrite Nitrogen Test Kit Protocol.

Procedure for testing for presence and concentration of nitrite nitrogen in a water sample.

LaMotte

NITRATE IN WATER TEST KIT

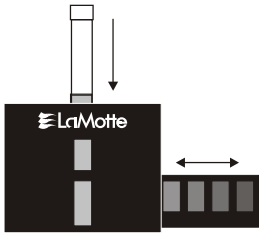
MODEL SL-NCR • CODE 3319

QUANTITY	CONTENTS	CODE
2 x 60 mL	*Mixed Acid Reagent	*V-6278-H
5 g	*Nitrate Reducing Reagent	*V-6279-C
1	Spoon, 0.1 g, plastic	0699
2	Test Tubes, plastic, w/caps	0106
1	Bottle, Water Sample	0688
1	Dispenser Cap	0692
1	Octa-Slide Viewer	1100
1	Nitrate Nitrogen Octa-Slide Bar, 0.25-10.0 ppm	3413

WARNING: Reagents marked with a * are considered to be potential health hazards. To view or print a Material Safety Data Sheet (MSDS) for these reagents see MSDS CD or www.lamotte.com. To obtain a printed copy, contact LaMotte by e-mail, phone or fax.

To order individual reagents or test kit components, use the specified code number.

USE OF THE OCTA-SLIDE VIEWER



The Octa-Slide Viewer should be held so non-direct light enters through the back of the comparator. With sample tube inserted at top, slide the Octa-Slide bar through the viewer and match with color standard.

WARNING! This set contains chemicals that may be harmful if misused. Read cautions on individual containers carefully. Not to be used by children except under adult supervision

NOTES:

A. Nitrites will cause interference in this nitrate test. If present, determine the nitrites level separately and compensate in these test results.

B. The best results are obtained when solution temperatures are close to 23°C.

C. Place Dispenser Cap (0692) on *Mixed Acid Reagent (V-6278). Save this cap for refill reagents.

PROCEDURE

1. Fill sample bottle (0688) with sample water.
2. Fill a test tube (0106) to 2.5 mL line with water from sample bottle.
3. Attach dispenser cap to a bottle of *Mixed Acid Reagent (6278). Dilute to 5 mL line with *Mixed Acid Reagent (6278). Cap and mix. Wait two minutes.
4. Use the 0.1 g spoon (0699) to add one level measure of *Nitrate Reducing Reagent (6279). Cap and invert 50-60 times in one minute. Wait ten minutes.
5. Mix sample one time and insert test tube into Octa-Slide Viewer. Insert Nitrate-Nitrogen Octa-Slide Bar (3413) into the Octa-Slide Viewer (1100). Match sample color to a color standard. Record as ppm Nitrate Nitrogen (NO_3-N). To convert to nitrate (NO_3), multiply result by 4.4. Record as ppm Nitrate.

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2.11

Figure 27. La Motte Nitrate Nitrogen Test Kit Protocol.

Procedure for testing for presence and concentration of nitrate nitrogen in a water sample.

LaMotte

LOW RANGE PHOSPHATE IN WATER TEST KIT

ASCORBIC ACID REDUCTION METHOD

MODEL PAL • CODE 3121-01

QUANTITY	CONTENTS	CODE
2 x 30 mL	*Phosphate Acid Reagent	*V-6282-G
5 g	*Phosphate Reducing Reagent	*V-6283-C
3	Test Tubes, 10 mL, glass, w/caps	0843
1	Pipet, 1.0 mL, plastic	0354
1	Spoon, 0.1 g, plastic	0699
1	Distilled Water Ampoule, 5 mL	2748
1	Phosphate Comparator, 0.0-2.0 ppm	3122
1	Axial Reader	2071

*WARNING: Reagents marked with an * are considered to be potential health hazards. To view or print a Material Safety Data Sheet (MSDS) for these reagents go t www.lamotte.com. To obtain a printed copy, contact LaMotte by e-mail, phone or fax

To order individual reagents or test kit components, use the specified code number.

Read Axial Reader Instruction Manual (35048) before proceeding.

NOTES:

This test determines levels of orthophosphates only.

This test should be run on clear samples only. Filter the sample if necessary.

Best results are obtained when solution temperatures are 23-25°C.

WARNING! This set contains chemicals that may be harmful if misused. Read cautions on individual containers carefully. Not to be used by children except under adult supervision

PROCEDURE

1. Fill test tube (0843) to 10 mL line with sample water.
2. Use 1.0 mL pipet (0354) to add 1.0 mL of *Phosphate Acid Reagent (V-6282). Cap and mix.
3. Use 0.1 g spoon (0699) to add one level measure of *Phosphate Reducing Reagent (V-6283). Cap and mix until dissolved. Wait 5 minutes.
4. Remove cap from test tube. Place tube in Phosphate Comparator (3122) with Axial Reader (2071). Read Axial Reader Instruction Manual before proceeding. Fill two test tubes (0843) to the 10 mL line with sample water. Place in Axial Reader. Match sample color to a color standard. Record as ppm Orthophosphate.

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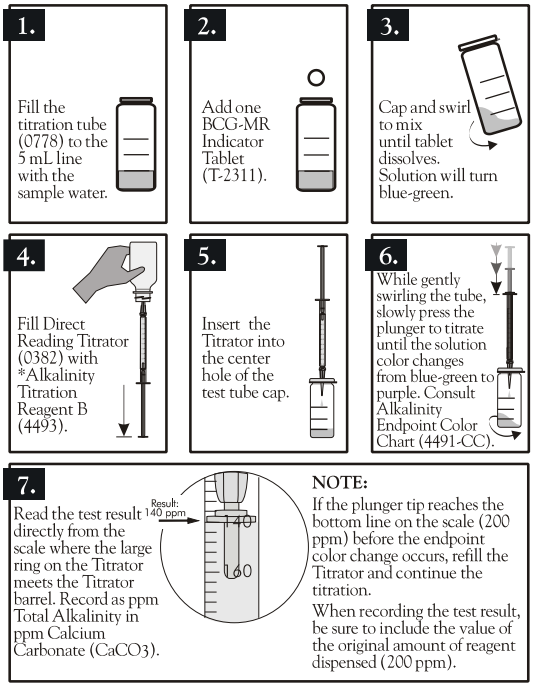
Figure 28. La Motte Low Range Phosphate Test Kit Protocol.

Procedure for testing for presence and concentration of phosphate in a water sample.

ALKALINITY TEST PROCEDURE



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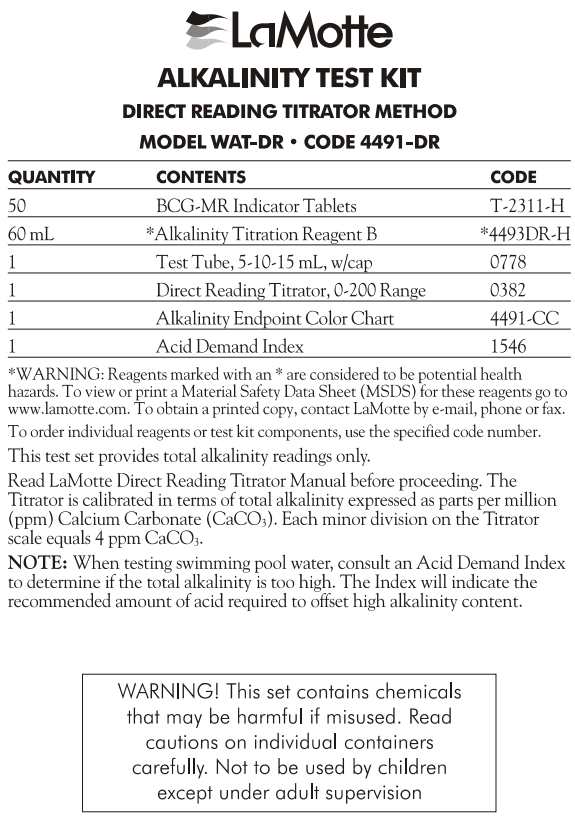

LaMotte

ALKALINITY TEST KIT

DIRECT READING TITRATOR METHOD

MODEL WAT-DR • CODE 4491-DR

QUANTITY	CONTENTS	CODE
50	BCG-MR Indicator Tablets	T-2311-H
60 mL	*Alkalinity Titration Reagent B	*4493DR-H
1	Test Tube, 5-10-15 mL, w/cap	0778
1	Direct Reading Titrator, 0-200 Range	0382
1	Alkalinity Endpoint Color Chart	4491-CC
1	Acid Demand Index	1546

*WARNING: Reagents marked with an * are considered to be potential health hazards. To view or print a Material Safety Data Sheet (MSDS) for these reagents go to www.lamotte.com. To obtain a printed copy, contact LaMotte by e-mail, phone or fax.

To order individual reagents or test kit components, use the specified code number.

This test set provides total alkalinity readings only.

Read LaMotte Direct Reading Titrator Manual before proceeding. The Titrator is calibrated in terms of total alkalinity expressed as parts per million (ppm) Calcium Carbonate ($CaCO_3$). Each minor division on the Titrator scale equals 4 ppm $CaCO_3$.

NOTE: When testing swimming pool water, consult an Acid Demand Index to determine if the total alkalinity is too high. The Index will indicate the recommended amount of acid required to offset high alkalinity content.

WARNING! This set contains chemicals that may be harmful if misused. Read cautions on individual containers carefully. Not to be used by children except under adult supervision

Figure 29. La Motte Alkalinity Test Kit Protocol.

Procedure for testing for alkalinity concentration in a water sample.

LaMotte

TOTAL HARDNESS TEST KIT

DIRECT READING TITRATOR METHOD

MODEL PHT-DR-LI • CODE 4482-DR-LI

QUANTITY	CONTENTS	CODE
15 mL	*Hardness Reagent # 5	*4483-E
15 mL	*Hardness Reagent # 6 Solution	*4485-E
60 mL	Hardness Reagent # 7	4487DR-H
1	Test Tube, 5-10-12.9-15-20-25 mL, glass, w/cap	0608
1	Direct Reading Titrator, 0-200 Range	0382
1	Pipet. 0.5 mL	0353

***WARNING:** Reagents marked with an * are considered to be potential health hazards. To view or print a Material Safety Data Sheet (MSDS) for these reagents go to lamotte.com. To obtain a printed copy, contact LaMotte by e-mail, phone or fax. To order individual reagents or test kit components, use the specified code number.

NOTE: Carefully read the instruction manual for the LaMotte Direct Reading Titrator before performing the titration described below. The titrator is calibrated in terms of Total Hardness expressed as parts per million (ppm) Calcium Carbonate $CaCO_3$. Each minor division on the titrator scale equals 4 ppm $CaCO_3$.

PROCEDURE

1. Fill the test tube (0608) to the 12.9 mL line with the water sample.
2. Add five drops of *Hardness Reagent #5 (4483) and mix.
3. Add five drops of *Hardness Reagent #6 Solution (4485) and mix. A red color will develop.
4. Fill the Direct Reading Titrator (0382) with Hardness Reagent #7 (4487DR) in the manner described in the instruction manual. Insert the titrator in the center hole of the test tube cap.
5. While gently swirling the tube, slowly press the plunger to titrate the sample until the red color changes to blue. Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. The result is expressed as Total Hardness in ppm $CaCO_3$.

   EXAMPLE: Plunger tip is 3 minor divisions below line 80. Test result is 80 plus (3 divisions x 4) equals 92 ppm.
6. If the plunger tip reaches the bottom line on the titrator scale (200 ppm) before the endpoint color change occurs, refill the titrator and continue the titration. When recording the test result, be sure to include the value of the original amount of reagent dispensed (200 ppm).
7. Parts per million $CaCO_3$ test results may be converted to grains per gallon (gpg) $CaCO_3$ by means of the following formula:

   $$\text{gpg } CaCO_3 = \text{ppm } CaCO_3 \times 0.058$$

ANALYSIS OF HARDNESS IN SALT WATER

When sea and estuarine waters containing very high levels of mineral salts are to be tested, the sample must be diluted to fall within the range of the test kit. This test set is supplied with a calibrated pipet for performing the simple, convenient dilution described below.

1. Use the 0.5 mL pipet (0353) to transfer 0.5 mL of the salt water to be tested to the test tube (0608).
2. Fill the test tube to the 12.9 mL line with distilled water (1:25.8 dilution).
3. Follow Steps 2 through 6. Multiply the resulting titrator reading by 25.8 to obtain the test result expressed as Total Hardness in ppm $CaCO_3$.

LaMOTTE COMPANY
Helping People Solve Analytical Challenges®
PO Box 329 • Chestertown • Maryland • 21620 • USA
800-344-3100 • 410-778-3100 (Outside U.S.A.)
Visit us on the web at www.lamotte.com

7.10

*Figure 30.*La Motte Total Hardness Test Kit Protocol.

Procedure for testing for hardness in a water sample.

Appendix G: Nutrient Source Product Information

Guaranteed Analysis

Total Nitrogen (N).....3.00%
2% Water Insoluble Nitrogen
1% Water Soluble nitrogen
Available Phosphate ($P_2 0_5$).....2.00%
Soluble Potash ($K_2 0$).....2.00%

Figure 31. Poultry manure product information.

Stutzman Farms SUP'R GREEN 3-2-2 poultry manure product ingredient analysis.

Section 2: COMPOSITION/INFORMATION ON INGREDIENTS

Chemical Name/Synonym(s)		CAS No.
Activated Sewage Sludge (biosolids, dried microbes)	86.8 – 90.8% by weight	8049-99-8
Iron chloride*	1-3% Iron (Fe) by weight	7705-08-0
Iron sulfate*	1-3% Iron (Fe) by weight	10028-22-5
*Total Iron (Fe) 4% by weight		
Water	4-8% by weight	7732-18-5
Calcium Carbonate	1.2% by weight	471-34-1
Polymerization agent(s)	<0.01% by weight	Varies
Fecal coliform	<0.22 MPN/g TS	NA

Trace metals and volatile organics can be detected in quantities less that 1.0%, most less than 0.1%. These components and pathogenic agents are of a low quantity to allow this product to meet US EPA 40CFR Part 503 Class A Exceptional Quality biosolid requirements.

(California Only-Proposition 65 Warning:)
This product contains detectable quantities of chemicals known cause cancer, birth defects or other reproductive harm. This notice in no way implies that we have any evidence or experience to indicate that any genuine hazard of cancer, birth defects, or reproductive harm results from the normal, proper handling described on our labels and related literature."

ACGIH TLV/OSHA PEL
ACGIG Nuisance dust limit of 10mg/M^3 (inhalable) and 3mg/M^3 (respirable) may apply to this product.

Figure 32. Activated sludge product information.

Milorganite® 5-2-0 fertilizer composition/information on ingredients

Appendix H: Alternative Feed Source Product Information

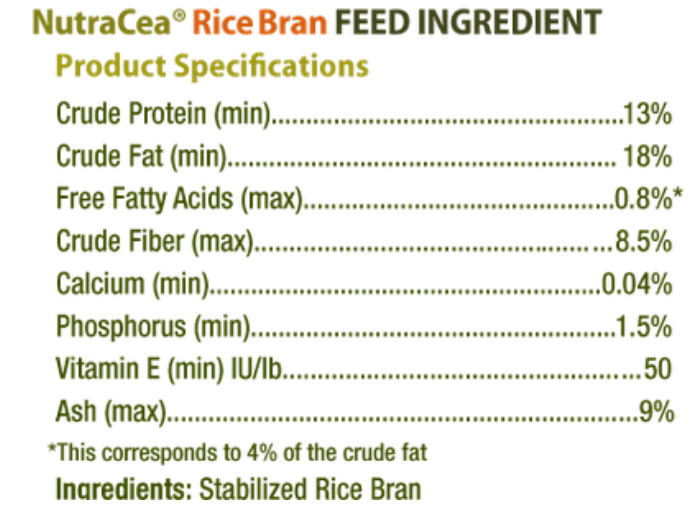

Figure 33. Rice bran product information.

NutraCea® Rice Bran product ingredient specifications.

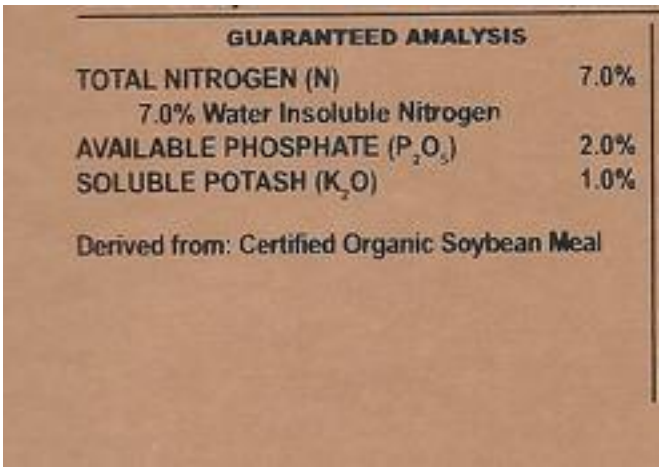

Figure 34. Soybean meal product information.

Certified organic soybean meal product ingredient analysis.

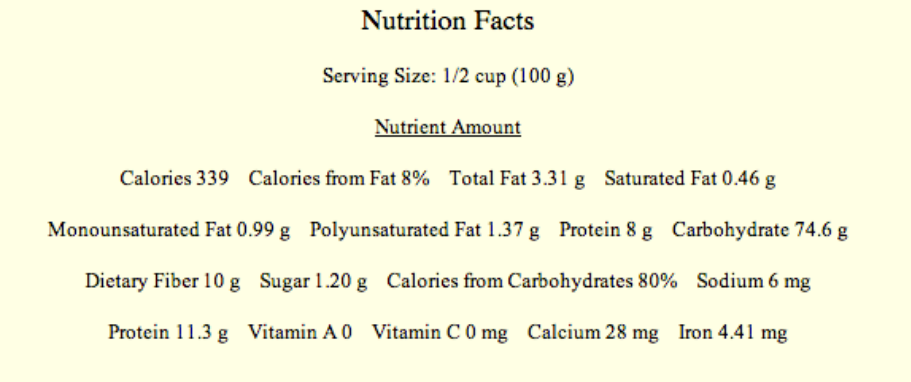

Figure 35. Sorghum product information.

Sorghum product ingredient analysis.

Appendix I: Fish Feed Product Information

*Figure 36.*Commercial fish feed product information.

Purina® AquaMax® Fingerling Starter 300 product ingredient specifications.

Appendix J: Product Information

ENVIRONMENTAL PREFERENCES

Light:	Sunny, tolerates shade; prefers shade where summers are hot.
Soil:	Well-drained, loose loam.
Fertility:	Rich.
pH:	6.0 to 7.0
Temp:	Cool (60 to 70° F).
Moisture:	Moist, but not waterlogged; frequent, light waterings.

Figure 37. Lettuce product information.

Environmental preferences for Bibb Lettuce purchased from Ferry Morse.

Assembled Depth (in.)	.01 in	Assembled Height (in.)	5.5 in
Assembled Width (in.)	3.5 in	Landscape Supply Type	Seeds
Mature Height	20	Organic	Yes
Plant / Seed Spacing (in.)	24.0 in	Returnable	90-Day
Seed Type	Herb	Sold as	Packet

Figure 38. Basil product information.

Genovese Basil purchased from Seeds of Change.

Okra, Clemson Spineless

The most popular okra on the market.

This 1939 All-America Selections winner is still the most popular variety on the market. The vigorous, 4-ft. high plants produce an abundance of dark green, grooved pods without spines. Best picked when 2.5 to 3" long. GARDEN HINTS: Soak seed in warm water overnight to speed germination. Pick pods young, while still tender. Pods are excellent for use in soups, stews and relishes.

Sun: Full Sun
Sowing Method: Direct Sow
Days to Maturity: 56 days
Height: 3-4 feet
Spread: 16 inches
Thinning: 6 inches

Figure 39. Okra product information.

Clemson Spineless Okra purchased from Burpee®.

Early Dividend Hybrid

BROCCOLI

Choose Early Dividend for improved flavor and higher yields. Large 4-5 inch, deep-green heads develop quickly followed by large side shoots. Harvest in 45 days from transplanting. Packet plants 20 ft. row.

Light	Row Spacing	Plant Spacing	Planting Depth	Days to Germination	Plant Height
Full Sun	3 ft.	1½-2 in.	½ in.	10-14	2½ ft.

Gardener's Notes: Cool weather crop. Sow seeds outdoors 2 weeks before last spring frost or start indoors 6-8 weeks before the last frost. Transplant when plants are 5 inches tall. Harvest heads before buds begin to open by cutting through stem with a sharp knife. Allow smaller side shoots to develop for extended harvest. Plant a second crop in late summer or early fall. In mild climates plant fall through early spring.

Figure 40. Broccoli product information.

Early Dividend Hybrid Broccoli purchased from Cornucopia Seed

Appendix K: Product Information

YSI Environmental

EcoSense®

DO200 Dissolved Oxygen/Temperature

Accurate, economical, handheld measurements

The EcoSense DO200 is available with a 4- or 10- meter cable.

YSI's EcoSense® line of compact, handheld instruments provides the most accurate data in the most affordable format. The instruments feature an easy-to-use interface, one-hand operation, water resistant case, and low cost of ownership over the life of the product. The DO200 simultaneously measures dissolved oxygen (% air saturation and ppm) and temperature with the following features:

- **Watertight housing**
- **Automatic temperature compensation**
- **4- and 10-meter cables available**
- **Manual salinity compensation**
- **Low battery indicator**
- **Screw-on cap membranes with low stirring/fast response**
- **Manual pressure compensation**

The DO200 is designed for quick, accurate results in an economical platform. The ability to measure DO and temperature in a simple, compact instrument allows the instrument to be used across multiple application sampling strategies including laboratory BOD testing with the optional BOD probe. With a one-year instrument and electrode warranty, along with other features, the EcoSense DO200 will fit your needs.

Pure Data for a Healthy Planet.®

Compact design and affordable price

www.YSIEcoSense.com

To order, or for more information, contact YSI Environmental.

+1 937 767 7241

800 897 4151 (US)

www.ysiecosense.com

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Fax +1 937 767 9353
environmental@ysi.com

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+1 508 748 0366
Fax +1 508 748 2543
systems@ysi.com

SonTek/YSI
+1 858 546 8327
Fax +1 858 546 8150
inquiry@sontek.com

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europe@ysi.com

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halsalem@ysi.com

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hongkong@ysi.com

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Fax +86 532 571 0101
ysiqd@ysiqd.com.cn

YSI Nanotech (Japan)
+81 44 222 0009
Fax +81 44 221 1102
nanotech@ysi.com

ISO 9001
ISO 14001

CE

YSI incorporated
Who's Minding the Planet?®

YSI Environmental

DO200 System Specifications (Instrument, Probe, and Cable)

Temperature	Range	-6 to +46°C
	Resolution	0.1°C
	Accuracy	±0.3°C ±1 digit
DO % Air Saturation	Range	0 to 200%
	Resolution	0.1% air saturation
	Accuracy	±2% of the reading or ±2% air saturation, whichever is greater
DO ppm (mg/L)	Range	0 to 20 ppm
	Resolution	0.01 ppm
	Accuracy	±2% of the reading or ±0.2 ppm, whichever is greater

Additional Technical Specifications

Operating range:	
Temperature:	0° to 50° C
Relative Humidity:	up to 95%
Water resistance:	IP-65 water resistant, splash resistant
Size:	70 mm width x 186 mm length (2.8 in. x 7.3 in.)
Weight with battery:	350 grams (0.75 lb.)
ATC Probe:	Thermistor, 10kΩ / 25° C
Battery:	One 9 volt included with purchase
Salinity compensation:	0.0 to 40.0 ppt
Pressure compensation:	600 to 1100 millibar (450 to 825 mmHg)
Calibration back-up:	Yes
Audio feedback:	Yes, all keys

DO200 Ordering Information

Accessories (order cable separately)	
DO200	DO200 instrument (°C and mbars units)
DO200US	DO200US Instrument (°F and inHg units)
200-4	4-meter probe and cable assembly
200-10	10-meter probe and cable assembly
200-BOD	Self-stirring laboratory BOD probe, includes external power adapter
280	DO instrument carrying case (molded plastic with foam insert)
5908	Membrane Kit, 1.25 PE membrane with probe solution
480	Soft, black carrying case with shoulder strap

The DO200US version instrument is also available. This instrument provides all the great features of the DO200. The DO200US has temperature units in Fahrenheit only and the pressure compensation units in inHg. Please use part number DO200US when ordering this item.

Figure 41. Dissolved oxygen meter product information.

EcoSense® DO200 Dissolved Oxygen/Temperature specifications.

a xylem brand

SPECIFICATIONS

DOCUMENT #W26-06

YSI pH10A pH/Temperature

Accurate, Economical Pen-Style Measurement

The EcoSense ® pH10A pen-style instrument provides an ultimate feature set over similar competitive models. The pH10A features an easy-to-use graphic interface, simple one-hand operation, memory, and low cost of ownership over the life of the product. The easy, user-replaceable electrode ensures the instrument is always ready for use. The pH10A measures pH and temperature with the following features:

- IP-67 waterproof housing
- 1-year instrument warranty
- Automatic temperature compensation
- User-replaceable single- or double-junction electrodes
- Clear, easy-to-read graphic display with on-screen instructions
- Automatic calibration and buffer recognition
- 1, 2, or 3-point calibration
- GLP functionality (saves and displays last calibration data)
- "Hold" feature locks readings on display
- 50 data-set memory
- >200 hour battery life; low battery indicator
- CE compliance

Parameters:

pH

Temperature

Applications:

- Aquaculture/aquariums
- Swimming pools/spas
- Hydroponics/aquaponics
- Agriculture
- Surface water
- Wastewater

The pH10A is designed for quick, accurate results in an economical platform. With a one-year instrument warranty and six-month electrode warranty, the pH10A will fit your needs for an easy-to-use pH instrument for any sampling application.

The pH10A is a reusable pen-style instrument. The electrode cap is easy to replace while keeping the instrument. No throw-away instrument here!

YSI.com/pH10A

YSI pH10A System Specifications

pH	Range	0.00 to 14.00 units
	Resolution	0.01 units
	Accuracy	±0.1 unit within 10 °C of calibration, ±0.2 unit within 20 °C
Temperature	Range	0.0 to 100.0 °C (32.0 to 212 °F)
	Resolution	0.1 °C (0.2 °F)
	Accuracy	±0.3 °C (±0.6 °F)
Operating Range	0.0 to 50.0 °C (32.0 to 122.0 °F)	
Water Resistance	IP-67 waterproof case	
Weight with Batteries	105 grams (3.7 ounces)	
Battery	Four LR44 alkalines included with purchase	
Battery Life	200 hours or greater (low battery indicator)	
Warranty	One year instrument and six months electrode	
Auto Power	Powers off after 10 minutes of inactivity	
pH Offset Recognition	±90 mV at pH 7.00 or +98.3 mV / -81.7 mV at pH 6.86	
pH Slope Recognition	±30% at pH 4.00, 4.01, 9.18, or 10.01	
pH Temp Compensation	Auto 0.0 to 100.0 °C (32.0 to 212 °F)	
pH Buffer Recognition	USA (4.01, 7.00, & 10.01) or NIST (4.01, 6.86, & 9.18)	
pH Calibration Temp	0.0 to 60 °C (32.0 to 140.0 °F)	
Temp Sensor	Thermistor, 10k ohms, at 25 °C	
Memory	Non-volatile; 50 sets (absolute or relative pH, temperature, date and time stamp); erase all data function	

YSI pH10A Ordering Information (order items separately)

pH10A	pH10A instrument (includes 606110 electrode, batteries, instruction manual)
606110	Single-junction pH electrode (replacement)
605116	Double-junction pH electrode
605129	Soft, black carrying case with shoulder strap
3824	Assorted pH buffers case (6 pints - 2 of each buffer 4, 7 10)
606118	Replacement battery kit (4 LR44 alkaline batteries; battery cover)

YSI
1725 Brannum Lane, Yellow Springs, OH 45387
Tel +1 937.767.7241 800.897.4151 (US)
environmental@ysi.com
YSI.com

Figure 42. pH meter product information.

YSI pH 10A pH/Temperature specifications.

Jiffy-7®

Increased Returns

Jiffy-7 offers quicker rooting due to the air pruning that stimulates fibrous root development within the plug. This can decrease crop cycle up to 25% to produce stronger, more compact plants.

Unlike other plugs, Jiffy-7 is supplied in a dried compressed form allowing the grower to store un-used plugs until the next crop cycle. Efficient storage is a benefit of the Jiffy-7 while also occupying less than one quarter of the space of regular plugs. Jiffy pellets are easily re-hydrated by hand or during the normal irrigation cycle.

Convenience

All Jiffy-7 sizes feature a pre-formed soft centre for easy insertion of even the smallest cuttings. The fully enclosed capillary net allows easy handling of the plugs without loss of substrate while extending the transplant window.

Jiffy-7 plugs contain a specially formulated fertilizer to provide the best start for your young plants.

Polyroll offers unrivalled convenience by providing plugs that are pre-spaced on perforated polythene or capillary fleece underlay. Plugs are spaced to the growers' requirements. The length and width of the under lay can be made to fit individual benches ensuring maximum use of bed space. All pellet-pack trays feature a picture label slot to allow secure attachment of a label without damaging a plug.

Extensive Range

Six plug sizes are available from 18mm to 44mm diameter that are either loose in cartons or pre-loaded in a range of growing trays. Tray options range from 25 cell strips to 144 strips all in full size growing trays. Plugs can also be pre-spaced in the growing trays ensuring there is a Jiffy-7 solution to suit all crops. All trays feature the unique Jiffy air-prune design, which makes certain the plug is held firmly within the cell while allowing free air movement around the plug. This minimizes root diseases and maximizes root development.

Environment

Jiffy-7 Pellets are manufactured from sphagnum peat (and Coir fibers) harvested from carefully selected bogs which are subjected to stringent internal and governmental inspection. Peat is taken from sources where the re-generation rate is greater than the harvest rate, and the local ecology is not adversely affected. The addition of 25% coir fibers makes Jiffy-7 suitable for reduced peat production.

For further information or to arrange a trial of Jiffy-7 Plugs please contact your area manager or use the contact options below

Jiffy-7 now with increased air porosity for easier water management and faster rooting. Jiffy bio-products are earth-friendly, non-toxic and provide producers of horticultural products economical alternatives to plastic and other more expensive bio-like products.

Jiffy Products International BV
Tel.: +31 168 41 35 55
E-mail: sales@jiffygroup.com

www.jiffygroup.com

Jiffy®
It's all about the roots

Jiffy Products of America Inc.
Toll Free 1-800-323-1047 (North America only)
E-mail: prosales@jiffygroup.com

www.jiffygroup.com

Figure 43. Jiffy-7® plugs product information.

Jiffy-7® plugs usage specifications.

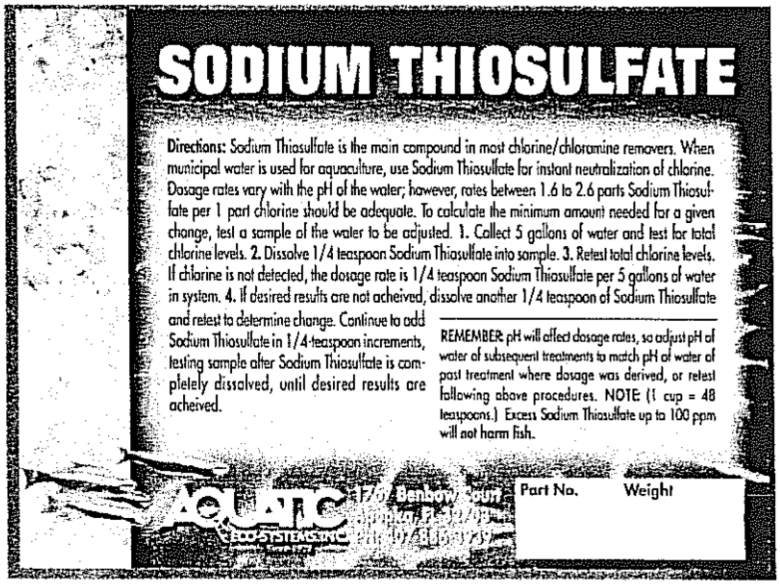


Figure 44. Sodium thiosulfate product information.

Procedure for use of sodium thiosulfate purchased from Aquatic Ecosystems Inc.

ActiveAqua Commercial Air Pump 6 outlets (eco-5064)

Commercial Air Pump with 6 outlets, 45 lt per minute

ActiveAqua™ Commercial Air Pumps

- Perfect to run several Waterfarms or multiple air stones at once
- Electrical magnetic air compressor in a high quality aluminum alloy case, wear and
- tear resistant material for cylinder and piston
- High pressure and high output. Comes with multi-outlet divider
- (6, 8, and 12) that can be individually opened or shut
- 20w
- 45 lpm
- Pressure >0.02 Mpa

Click to enlarge

ActiveAqua™ Commercial Air Pumps are perfect to run several Waterfarms or multiple air stones at once. They are a electrical magnetic air compressor in a high quality aluminum alloy case, with wear and tear resistant material for the cylinder and piston. They boast high pressure and high output featuring the included multi-outlet divider [6, 8 and 12] that can be individually opened or shut.

SKU: AAPA45L (previously PU45L)
Weight: 3.2 lbs.
Package Dimensions: 6.5L x 4.3W x 4.9H
Suggested Retail: $48.95

Figure 45. Air pump product information.

ActiveAqua™ Commercial Air Pump with 6 outlets (eco-5064) specifications.

EHEIM thermocontrol 300

EAN	4011708361238
Article No.	3619010
UPC	(-)
Min. temperature	18.00 C
Max. temperature	34.00 C
Performance at 50 Hz	300.00 watt
For aquariums of about.	600.00 l
For aquariums up to approx.	1,000.00 l
Height	506.00 mm
Dimensions in	36.00 mm
Freshwater	yes
Sea water	yes
voltage	230 volt
Standard power plug	EUR
Packing	6 Part (s)
Packing dimensions(Width)	6.50 cm
Packing dimensions(Height)	58.50 cm
Packing dimensions(Depth)	5.00 cm

Figure 47. Water heater product information.

EHEIM thermocontrol 300 specifications.

KALDNES® MEDIA

These patented biofilm carrier elements are real Kaldnes® K1 media. They are ideal f
applications. With a surface area of 259 ft2, this polyethylene biomedia is positively bu
applications, use a blower to continuously circulate the elements, while simultaneousl
and stripping the CO2. The self-cleaning action allows for the exfoliation of the older, l
and eliminates the need for backwashing.

The elements are 7 mm (5/16") long and 10 mm (7/16") in diameter. Sold by the cubic
lbs/cu.ft.

Figure 46. Biomedia product information.

Kaldnes® media specifications.

1/2 HP Clear Water Pump H.D. Farm, Home

by Neiko

50 customer reviews | 5 answered questions

Price: $24.80 + $12.20 shipping

In Stock.

Ships from and sold by eToolscity.

- 1/2 HP, Hmax 18m
- Suct.Hmax 8m
- 0.37KW 110v 60 Hz, 3400 RPM
- Qmax 24L
- Nozzle Dimension Inlet/Outlet 1" x 1"

› See more product details

7 new from $24.80

Part Number	RIDGE50635
Item Weight	9.8 pounds
Product Dimensions	11 x 6.4 x 5.3 inches
Material	Plastic, Metal
Power Source	corded-electric
Voltage	110 volts
Item Package Quantity	1

Figure 48. Phase 4 water pump product information.

½ HP clear water pump specifications.

Figure 49. Phase 2 and 3 water pump product information.

Azoo Powerheads specifications.

TECHNICAL DETAILS			
Product Type:	LAMP / BULB	Technology:	FLUORESCENT
ELECTRICAL PROPERTIES			
Watts:	185W	Amps / MA:	1.5A
PHYSICAL CHARACTERISTICS			
Shape:	T12	Base:	R17D / RDC,RECESSED DOUBLE CONTACT
Type:	LINEAR	Overall Length (mm/"):	96"
Diameter (mm/"):	1.5"		
LIGHT OUTPUT MEASUREMENT			
Initial Lumens:	13000L	Mean Lumens:	9000L
Output Type:	VHO / VERY HIGH OUTPUT		
COLOR CHARACTERISTICS			
Color:	COOL WHITE	CC Temp / Kelvin:	4100K
CRI:	62CRI	Phosphor:	641
LIFE			
Life:	12000H		
PART NUMBERS & ORDERING INFORMATION			
BT Part#:	PHF96T12CWVHOEW	BT Description:	PH F96T12/CW/VHO/EW #342329
BT Ordering Code:	0014490	Mfg:	PHILIPS
Mfg Prod Description:	F96T12/CW/VHO/EW	Mfg Ordering Code:	34232-9
Country of Origin:	MEXICO	Tariff Code :	8539.31.00.70

Figure 50. Lamp/Bulb product information.

Specifications for T12 fluorescent bulb used in growth chamber.

Leca is initial letters for : **L**ight **E**xpanded **C**lay **A**ggregate.

LECA consists of small, lightweight, bloated particles of burnt clay. The thousands of small, air-filled cavities give LECA its strength and thermal insulation properties. The base material is plastic clay which is extensively pretreated and then heated and expanded in a rotary kiln. Finally, the product is burned at about 1100 °C to form the finished LECA product.

Light expanded clay aggregate is producing in more than 20 countries with different brands name, Some countries which produce aggregate with almost the same industrial way are as:

Italy, Denmark, switzerland, Norvey, Germany, Finland, Portugal, U.K, and Iran with brand of Leca.

Russia, Poland, Sweden,China, with the brand of Keramzite. South Africa with the brand of Argex. Spain with the brand of liapour.

Entirely Natural Product

LECA is an environment-friendly, entirely natural product incorporating the same benefits as tile in brick form LECA is indestructible, non-combustible, and impervious to attack by dry-rot, wet-rot and insects.

Natural Building Material

LECA is a natural material and a LECA building is a healthy building, so that is Leca has been used in competition projects for allergy-friendly, healthy homes.

Used as a thermal-insulation material in houses, LECA has been saving energy for more than 50 years, and is now a standard component in low-energy development projects all over the world.

Figure 51. Clay aggregate product information.

Light expanded clay aggregate[1] product description.

DESCRIPTION | DIRECTIONS | INGREDIENTS & G.A.

Directions For Use: Terrarium Water Bowls: Add two drops of ReptiSafe per 8 ounces (1 cup) of water. Add as above every time you change water (i.e. daily). Aquatic Turtle/Amphibian Water Environments: Add three teaspoons per gallon of water.

Ingredients:

Purified water, herbal extracts, organic colloids, organic chelating agents, electolytes, essential vitamins and minerals.

Figure 52. Water conditioner product information.

Reptisafe™ terrarium water conditioner procedure and specifications.

Appendix L: Diagram of University of Virgin Islands Aquaponic System

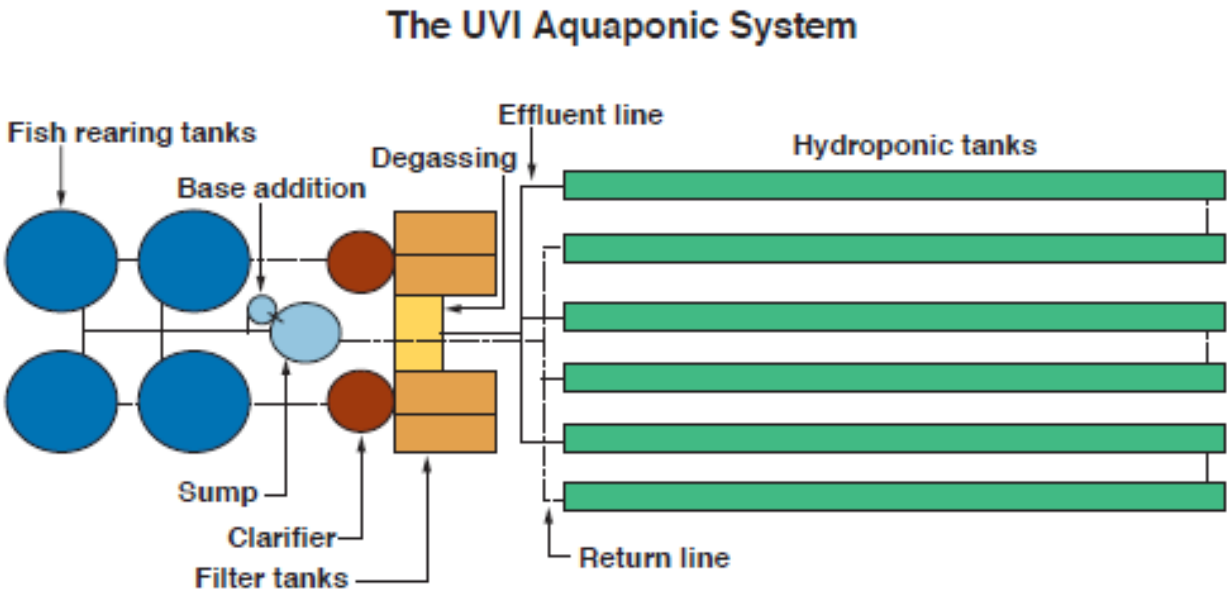


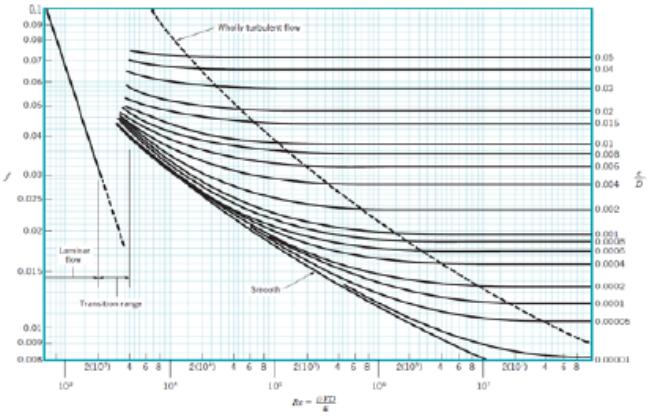
Figure 53. Aquaponic system diagram.

The University of Virgin Islands aquaponic system setup.

Appendix M: Phase 4 Fluid Dynamics Calculations

Tube Diameter (D)	0.355 in.	Cross-Sect	0.000687 ft^2
Volumetric Flow Rate (AU)	500 gal/hr	Velocity	27.01167 ft/s
PVC Surface Roughness (ε)	5.00E-06 ft		
Density (ρ)	1.934 slugs/ft3	*Pump Power*: $\dot{W} = (AU)\Delta P$	
Gravity (g)	32.174 ft/s2	Ẇ	273.2561 ft-lb/s
Specific Weight (γ_w)	62.22452 lb/ft3		0.496829 hp
Dynamic Viscosity (μ)	1.79E-05 lb-s/ft2		
Total Length (l_{tubing})	25 ft		

Tube Diameter (in.)	Velocity (ft/s)	Pressure Difference (lb/ft^2)	Pump Power (hp)
5/16	34.9	27053	0.913
0.355	27	14717	0.5
3/8	24.2	11362	0.384
1/2	13.6	3079	0.104
1	3.4	413	0.014



Assumptions
1. Incompressible fluid
2. Steady state
3. Velocity is the same between entrance and exit

Method to Calculate Pressure Differential

1) Calculate Reynold's Number [f(ρ,U,D,μ)]

Reynold's Number: $Re_D = \frac{\rho U D}{\mu}$

Re_D 8.63E+04 *(unitless)*

2) Determine relative roughness [f(ε/D, Re)]

Moody Diagram | Colebrook Equation

$$\frac{1}{\sqrt{f}} = -1.8 \log\left[\frac{\varepsilon/D}{3.7} + \frac{2.51}{Re\sqrt{f}}\right]$$

0.023082 for 5/16 *(MANUALLY UPDATE*
0.023452 for 0.355 *IF DIAMETER IS CHANGED)*
0.023628 for 3/8"
0.024725 for .5"
0.028316 for 1"

f 0.023452 *(unitless)*

3) Solve for pressure differential using Bernoulli's Equation [f(f,D,K$_L$,l$_{tubing}$)]

Bernoulli's Equation: $\frac{P_1}{\gamma_w} + \frac{U_1}{2g} + z_1 = \frac{P_2}{\gamma_w} + \frac{U_2}{2g} + z_2 + losses$

$$\Delta P = \gamma_w(\Delta z) + \gamma_w(H_{major} + H_{minor})$$

$$H_{major} = f\frac{1}{D}\frac{U^2}{2g}\sum l_{tubing}$$

$$H_{minor} = \frac{U^2}{2g}\sum K_L$$

$$\Delta P = \gamma_w(\Delta z + (\frac{U^2}{2g})(\frac{f}{D}\sum l_{tubing} + \sum K_L)$$

Iteration Scheme

f	1/sqrt(f)	RHS	Difference
0.02345	6.530231	6.529981	0.000249988
0.023451	6.530092	6.529995	9.73189E-05
0.023452	6.529953	6.530008	-5.53409E-05
0.023453	6.529814	6.530022	-0.000207991
0.023454	6.529675	6.530035	-0.000360632
0.023455	6.529535	6.530049	-0.000513263
0.023456	6.529396	6.530062	-0.000665885
0.023457	6.529257	6.530076	-0.000818497
0.023458	6.529118	6.530089	-0.0009711
0.023459	6.528979	6.530102	-0.001123693

$K_{L,tee}$	0.2
No. Fittings (Wye, 180°)	3
ΔP	14717.51187

Figure 54. Phase 4 fluid dynamics calculations.

Calculations for water circulation in Phase 4.

Appendix N: Glossary of Terms

Acclimation: a period of time after the delivery of new fish in which they are gradually adjusted to a new aquatic environment

Activated sludge: in the context of this research, it will be defined as a pathogen-free pelletized solid fertilizer made from processed human waste.

Aeration: the process used to increase oxygen content in the water of the aquaponic system by circulating air through the liquid.

ANOVA: the analysis of variance, or more briefly ANOVA, refers broadly to a collection of experimental situations and statistical procedures for the analysis of variance within an experiment.

Anti-nutritional: biologically active compounds that interrupt nutrient absorption.

Apparent digestibility coefficient (ADC): the specific measurement of a feed or diet, which can be digested by the species of tilapia.

Aquaculture: the method of cultivating marine or freshwater fish or other sea-life under controlled conditions.

Biodiversity: briefly, the variety of life, including but not limited to genetic, species, and ecosystem diversity.

Biofilter: device using living microorganisms to capture and break down organic pollutants. It is used to process wastewater by capturing harmful chemicals.

Biologically available: the extent to which an active ingredient (nutrient source) is available to the given region and user of the system.

Biological oxygen demand: the amount of dissolved oxygen needed by aerobic biological organisms in a body of water to break down organic material present in a given water sample.

Biomass yield: the ratio of the amount of biomass produced to the amount of substrate consumed, where biomass is the given weight or quantity of an organism in a given area or volume.

Bolt: the premature growth of a flowering stem, often resulting from non-ideal growing conditions such as high temperatures. This can halt the growth of harvestable material in crops like lettuce and basil.

Bonemeal: a mixture of ground bone and other slaughterhouse waste products sometimes used as a fertilizer and feed supplement.

Brood stock: a group of mature individuals used for breeding purposes.

Cage aquaculture: the farming of fish or other seafood in an enclosure within an existing body of water.

Carnivorous: deriving all or most of its nutrition from the consumption of animal tissue.

Chemical oxygen demand: a common water-quality measure used to determine the amount of organic compounds in a sample.

Commodity price index: a weighted average of selected commodity prices.

Conductivity factor: the measurement of a solutions ability to conduct electricity.

Cultivar: a variety or species of plant that is created or intentionally selected and maintained through cultivation.

Dairy manure: the phosphorous rich excrement of dairy cattle, often used as fertilizer.

Developing nations: a country with a low-level of material well-being.

Desertification: a type of land degradation in which relatively dry regions become increasingly arid over time.

Duckweed: a fast-growing group of simple floating aquatic plants living in slow-moving bodies of water. We will be referring primarily to the specific type of duckweed Lemna minor.

Economic feasibility: degree to which a system is able to be profitable after accounting for construction and upkeep over a reasonable time period (1-2 years)

Effectiveness: will be determined differently in each phase of research. Within Phase 0, effectiveness will be measured by protein content greater than 25%. In Phases I and II, effectiveness will be measured by water concentrations of nitrate, nitrite, and ammonia.

Efficiency: will be determined differently in each phase of research. Phase 0 will measure efficiency by the measurement of feed cost/mass of duckweed ($/g). Phase I will measure biomass yield and cost (feed). In Phase II, efficiency will be measured by biomass yield, market yield, and cost (construction, maintenance, feed).

Eutrophication: the process by which increased nutrients in a water body influence the ecosystem. Changes include increased algae blooms, decreased dissolved oxygen, and more.

Feed conversion rate: the measured ratio of feed consumed to net weight gain.

Fingerling: a young or small fish.

Fish silage: a liquid product made from whole fish or parts of fish that are liquefied by the action of enzymes in the presence of an added acid

Genetic mixing: the exchange of genetic information within and between populations.

Growth chamber: a growing chamber with controlled conditions (ex. temperature, light, humidity) used to study plant growth.

High-intensity/high energy: referring to the resulting aquaponic system consisting of high-technology engineering innovations that require more resources and manual labor to operate; higher stocking density

High tunnel: a commercial greenhouse used in agriculture applications to maintain a more reliable and uniform temperature and humidity and to protect plants from external variables (e.g. wind, etc.)

Hydroponics: the process of growing plants in sand, gravel, or liquid with added nutrients but without the use of soil.

Intermediary plant: a plant that grows from a feed that would not otherwise be accepted by a fish population as a direct source of food. Instead, the fish will feed off the intermediary plant.

Isocaloric: normalized to have the same energy content.

Isonitrogenous: normalized to have the same nitrogen or protein content.

Kjeldahl method: an analytical chemistry method used to quantitatively determine the amount of nitrogen in a chemical substance.

Large vs. small scale: manufactured for the purpose of commercial production as opposed to a smaller subsistence-level system.

Least developed countries (LDC): a country, defined by the United Nations as having the lowest indicators of socio-economic development, based on poverty, human resource weakness and economic vulnerability.

Light Expanded Clay Aggregate (LECA): a low-density, porous clay product used as a hydroponic substrate for growing plants.

Limiting factors: factors that limit the growth of the plant and is used as a supplement in the system.

Low Income Food Deficient Countries (LIFDC): a country defined by FAO as having a low per capita Gross National Income, weak food trade (Imports vs. Exports), and have not specifically requested being left off the list. There are currently 62 LIFDC.

Low-intensity/low energy: referring to the resulting aquaponic system consisting of low-technology engineering innovations that require minimal resources and manual labor to operate; lower stocking density.

Macronutrients: a nutrient source that is required in higher concentrations to sufficiently feed the plant (such as carbohydrates, proteins, and fats).

Market value: the monetary amount for which something can be sold in a given market. As it pertains to the project, the market value will be utilized in comparing the system's efficiency.

Mass equivalent of nitrogen: a measure of the amount of nitrogen inoculated into duckweed tanks in Phase 1; calculated by dividing the desired nitrogen quantity by the nitrogen content of one gram of nutrient source.

Mechanical maintenance: the maintenance required on a system's components in order to allow continuously smooth operation

Micronutrients: a nutrient source that requires less concentration to sufficiently feed the plant (such as vitamins and minerals)

Off-grid: operates independently of municipal electricity, water, and other utility services, but most often referred to for electricity.

Omnivorous: capable of eating and deriving nutrients from a varied diet of animals, plants, algae, and fungi. Often referring to opportunistic feeders.

Operating costs: costs included in addition to the costs of construction and initial materials. These include maintenance costs, water replacement, fish replenishment.

Peat pots: a cylinder of peat material enclosed in a fine mesh used to cultivate plants.

Pithing: the euthanizing technique by which a blunt needle is thrust into the vertebral canal resulting in the destruction of the brain and spinal cord. This method of euthanasia is considered humane and approved.

pH: the measure of acidity or basicity of an aqueous solution. The scale ranges from 0 to 14 where a range of 0 to 7 results in an acidic solution while 7 to 14 results in a basic solution. Water (pH 7) is considered neutral.

Poultry litter: a by-product of the poultry industry consisting of a mixture of chicken or turkey feces, bedding material, spilled feed, and feathers.

Recirculating: in the context of aquaculture, aquaponics, and agriculture, a system designed to reuse all water; water only leaves the system through unintended mechanisms such as leaks and evaporation. Contrast with open-loop.

Rice bran: a by-product of the rice milling process.

Rockwool: an inorganic, sterile material made from molten rock used as a growing medium.

Solution conductivity factor (CF): a measure of a solution's ability to conduct electricity, as a function of dissolved salts.

Soybean meal: a solid residue of the production of soybean oil often used as filler in animal feeds.

Sorghum: a type of grass crop grown in hot, arid regions for the grain which is used for food, beverage, and fuel production.

Starch digestibility: the amount of the starch that can be converted into useful energy and nutrients.

Stocking density (high vs. low): amount of fish per unit area, high stocking density requires high water turnover rate in order for fish to adequately grow. 0.29 fish/gal for UVI system vs. 0.145 fish/gal for Phase II.

Subsistence: self-sufficiency farming in which the farmer focuses on growing enough food to feed their families.

Sustainable: able to maintain at a certain rate or level while conserving a balance by avoiding depletion of natural resources.

Symbiotic: a close, prolonged association between two or more different organisms of a different
species that may, but does not necessarily, benefit each member.

Toxic ammonia: the unionized form of ammonia (NH_3), more of which is present at higher temperatures and pH values.

Trypsin inhibitor: chemicals that reduce the availability of biologically active trypsin, an enzyme vital to nutrition in animals.

Turnover rate: indicates the flow rate of the system relative to the capacity of fish tanks. Often expressed as a factor such as 10x. This indicates the flow rate of the system per hour is ten times the capacity of the fish tank.

Undernourished: the condition of having too little food for a good level of health and condition.

Vascular plants: any plant in which the phloem transports sugar and the xylem transports water and salts.

Vegetable compost: organic matter, in this case waste from food preparation that has been decomposed and recycled for use as soil or fertilizer.

Vermiculture: the management of worms, often used for the purpose of composting organic matter.

Appendix O: Reference List

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