3-28-2014

Effect of Aquafeed on Productivity of Red Amaranth and on Water Quality under Aquaponic Cultivation

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DOI: 10.25148/etd.FI14040848

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EFFECT OF AQUAFEED ON PRODUCTIVITY OF RED AMARANTH AND ON WATER QUALITY UNDER AQUAPONIC CULTIVATION

A thesis submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

in

ENVIRONMENTAL STUDIES

by

Miles Medina

2014

To: Dean Kenneth G. Furton
College of Arts and Sciences

This thesis, written by Miles Medina, and entitled Effect of Aquafeed on Productivity of Red Amaranth and on Water Quality under Aquaponic Cultivation, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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Date of Defense: March 28, 2014

The thesis of Miles Medina is approved.

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Florida International University, 2014

ACKNOWLEDGMENTS

I would like to thank the members of my committee for their support. I will always be grateful for the opportunities provided to me by the FIU Agroecology Program under the direction of Drs. Mahadev Bhat and Krish Jayachandran, my Co-Major Professors. Over the course of my degree program, Dr. Bhat's enthusiastic encouragement and our stimulating conversations were a source of strength. During my thesis research his help and guidance were indispensible. Dr. Jayachandran's technical knowledge and good humor helped me stay relaxed and focused as obstacles presented themselves. He provided essential lab training and the opportunity to present this work at a national conference. I am also grateful to Dr. Alok Deoraj, committee member, whose knowledge of aquaculture was most helpful in the development of the research questions and during the design the experiment.

I would also like to thank Dr. Kateel Shetty and fellow graduate student Nina de la Rosa for their assistance in the lab. I am grateful to Stephany Alvarez, Luis Garbinski, Joshua Muñoz and Edy Cicilio for their help during construction of the aquaponic units. And I am particularly grateful to undergraduate Carlos Pulido who fed the fish and collected data while I was out of town at a conference. I would also like to thank Manny García-Barbón for donating the Blue tilapia used in the study.

Finally, I wish to thank the USDA for funding under the National Institute of Food and Agriculture's Hispanic Serving Institutions Higher Education Grant # 2011-38422-30804.

ABSTRACT OF THE THESIS

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Aquaponics, the integrated production of fish and hydroponic crops in a recirculating system, is an intensive cultivation method in which metabolic fish wastes fertilize plants. This study compares the effects of two aquafeeds on Red amaranth (*Amaranthus tricolor*) productivity and on water quality under cultivation of Blue tilapia (*Oreochromis aureus*), with three aquaponic units (n=3) per treatment over a 60-day trial. The fishmeal-based control feed contains higher crude protein (40%) and phosphorus (1.12%) than the plant-based alternative feed (32% and 0.40%). The alternative feed resulted in a significantly higher amaranth crop yield (p<0.05) with significantly lower nitrate-N and TDS concentrations in the culture water over the course of the trial. Orthophosphate, TAN, pH and DO levels were not significantly different between treatments. An economic analysis revealed that an improved crop yield from a lower-input aquafeed could potentially increase total aquaponic farm revenue in spite of a reduction in fish yield.

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ABBREVIATIONS AND ACRONYMS

CP	Crude protein
DO	Dissolved oxygen
EC	Electroconductivity
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed conversion ratio
RAS	Recirculating aquaculture system
SGR	Specific growth rate
TAN	Total ammonia nitrogen
TDS	Total dissolved solids
UVI	University of the Virgin Islands

CHAPTER I

INTRODUCTION

Currently over 50% of the global population resides in urban areas, and the United Nations projects that by 2050 this figure will surpass two-thirds. This trend toward urbanization applies to both developed and developing regions, whose populations were 77.5% and 46.0% urban in 2010, respectively; by 2050, urban populations in developed and developing regions are projected to exceed 85% and 64%, respectively (UNDESA, 2011). Urban agriculture has been an important source of food security and supplemental income for the urban poor in developing countries, and it is emerging as a source of food security among the urban poor in developed countries (Cohen & Garrett, 2009). Thus, it is appropriate to further develop agricultural methods suited to modern urban and peri-urban environments considering both the challenges and opportunities that arise from this production setting.

Aquaponics, the integration of aquaculture and hydroponic (soilless) crop production, is an emerging and highly productive culture method particularly suitable for urban settings. Hydroponics liberates crop production from unfavorable soil conditions and space constraints (through vertical integration) that may be common in urban areas (Lal, 2013; Orsini, Kahane, Nono-Womdim & Gianquinto, 2013). Tank aquaculture also operates relatively independently of local environmental conditions (Losordo, Masser & Rakocy, 1992). The integration of hydroponics and tank aquaculture in aquaponic production addresses many of the negative environmental impacts typically associated with intensive fish and crop production by recycling fish wastes for use as crop fertilizer:

Agricultural runoff is virtually eliminated, water is conserved through filtration and recirculation, and the amount of land required per unit of production is reduced (Pillay, 2004; Losordo et al., 1992; Rakocy, Masser & Losordo, 2006). Further, the removal of dissolved nutrients by plants generates revenue via cultivation of a marketable crop (Bailey, Rakocy, Cole, & Shultz, n.d., Rakocy et al., 2006). A number of aquaponic farms currently operate throughout the United States and abroad.

Formulated aquafeed is a crucial input that represents a substantial operating cost for any intensive aquaculture operation (El-Sayed, 1999; El-Sayed, 2004). High protein content provides rapid fish growth and higher yields, and fishmeal has generally been the preferred source of protein in formulated feeds. In recent years, however, the supply of fishmeal has plateaued, leading to rising prices and the development of alternative protein sources (El-Sayed, 2004; Tacon, Hasan, & Metian, 2011). Based on improved understanding of fish growth and nutrition as well as concern over the environmental impacts of aquacultural waste, today's aquafeed formulations achieve higher yields with lower quantities of fishmeal and other nutrients (Cho & Bureau, 2001; Tacon et al., 2011).

In aquaponic production, aquafeed is doubly important, because it is the primary source of nutrients for both the fish and the plants: Nutrients that originate in the feed become effluent that serves as hydroponic fertilizer. Typically, the nutrient profile of fish effluent is remarkably well matched to the nutritive requirements of crops, but nutrient supplementation for aquaponic crops is not uncommon among operations that rely on standard aquafeeds (Bunting, 2013; Rakocy et al., 2006). For instance, while aquaculture effluent often contains abundant nitrogen (derived from amino acids in the feed) and

phosphorus, it is often lacking in other important plant nutrients such as potassium and chelated iron (N. Storey, personal communication, 2014; Rakocy et al., 2006; Rakocy, Shultz, Bailey & Thoman, 2004b). To date, no studies have examined the effect of aquafeed on aquaponic crop productivity.

Study objectives

The aim of the study is to explore the effects of two formulated aquafeeds on plant productivity and effluent water quality in an aquaponic setting. The control aquafeed is an industry-standard aquafeed based on fishmeal. The alternative aquafeed is specially formulated for use in aquaponic production, and it is plant-based with lower crude protein and phosphorus content than the control feed. Under the alternative treatment we expect to observe greater plant productivity with lower concentrations of nitrate and dissolved solids in the culture water. Other water quality parameters under observation include pH and dissolved oxygen levels; these are expected to be similar between treatments. Additionally, although fish growth is not the focus of the study we expect to observe greater growth under the higher-protein control treatment.

The cultured crop species is *Amaranthus tricolor*, known colloquially as Red amaranth, callaloo, or Chinese spinach. The leaves and stems of *A. tricolor* are a rich source of protein, carotenoids, vitamins, minerals and fiber and are nutritionally similar or superior to spinach (O'Brien & Price, 1983; Prakash & Pal, 1991). While its consumption is common in temperate and tropical developing regions including China, Africa and the Caribbean *A. tricolor* is considered an underexploited crop with high economic potential (Shukla, Bhargava, Chatterjee, Pandey, & Kumar, 2010). *A. tricolor*

is an exceptionally tolerant and resistant leafy vegetable crop capable of being cultivated during hot summer months when other leafy vegetables are out of season (Shukla, Bhargava, Chatterjee, Srivastava, & Singh, 2006).

The cultured fish species is Blue tilapia (*Oreochromis aureus*). Tilapia comprise the second most important group of cultivated fish worldwide (FAO, 2012A). They are widely cultured throughout the tropics and subtropics due to their ease of cultivation, high tolerance to poor water quality, rapid growth, and expanding market demand (Popma & Masser, 2009). Tilapia are also a popular choice among aquaponic farmers and hobbyists. Among tilapia species, Nile tilapia (*O. niloticus*) and Nile-Blue hybrids are the most commonly cultivated (FAO, 2011). Live possession of any tilapia species except Blue tilapia is prohibited by the State of Florida without a commercial permit. Because the focus of the study is the plant productivity response, the results are presumed to be relevant for tilapia in general.

In addition to the technical aspect of the study described above, I conduct an economic analysis to explore the effect of aquafeeds on aquaponic farm revenue. The analysis, based on production and revenue data from the literature as well as data from the technical aspect of the study, estimates the economic benefit from adoption of an alternative aquafeed that enhances crop production within the context of commercial aquaponics, as compared to a standard aquafeed that would typically be used in commercial aquaponics although it is formulated for commercial fish-only aquaculture.

CHAPTER II

LITERATURE REVIEW

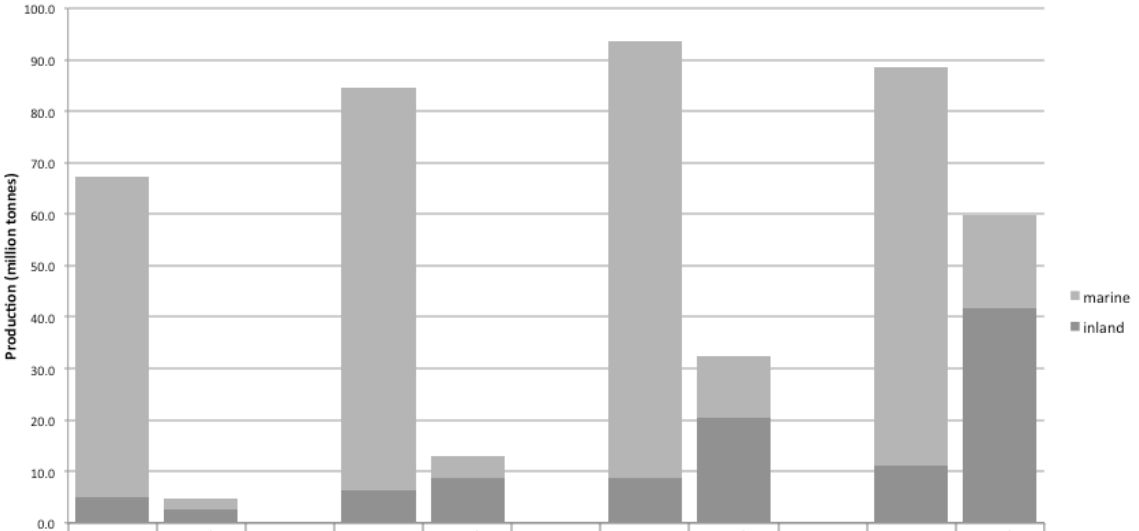
The global context of modern aquaculture

The United Nations projects that the global population will reach 8.9 million by 2050 and that 99% of this growth will occur in developing regions including Asia, Africa, and Latin America (United Nations Department of Economic and Social Affairs [UNDESA], 2004). Consumption of fish in developing regions is increasing with population and per capita income. For instance, from 1980 to 2010, consumption of fish in developing countries increased more than four-fold (from 25.0 million to 104.3 million tonnes per year) while consumption in developed countries remained relatively stable, near 30 million tonnes per year (FAO, 2012a). Because capture production is not expected to substantially increase, FAO predicts that the rising global demand for fish will have to be met entirely by aquaculture (2012b).

Aquaculture includes the cultivation of fish, crustaceans, mollusks, and aquatic plants. Since the 1980s it has emerged as the fastest growing form of agriculture worldwide. Global aquacultural production of fish and other aquatic animals grew at an average of 6.3% per year from 34.6 million tonnes in 2001 to 59.9 million tonnes in 2010, while capture production plateaued at around 90 million tonnes per year over the same period. Asia consistently leads aquacultural production, with 53.3 million tonnes representing 89.0% of global production in 2010. Global production of aquatic plants was 19.9 million tonnes in 2010, with 95.5% coming from aquaculture (FAO, 2012a).

Aquacultural operations are primarily categorized by the waters in which they occur. Marine cultivation occurs within net pens in coastal or open ocean waters. Inland cultivation occurs within pens in freshwater ecosystems (lakes and rivers) or in artificial ponds, raceways, or tanks (Lovelace, 2009; Pillay, 2004). In 2010, inland cultivation accounted for the bulk of global aquacultural production (69.6%) with 41.7 million tonnes (Figure 1) (FAO, 2012a).

Figure 1. World production of fish and other aquatic animals, 1980-2010 (FAO, 2012a).



	capture	aquaculture	capture	aquaculture	capture	aquaculture	capture	aquaculture
	1980		1990		2000		2010	
marine	62.1	2.1	78.2	4.3	85.0	11.9	77.4	18.1
inland	5.1	2.7	6.4	8.7	8.6	20.5	11.2	41.7

Aquacultural operations are also categorized by the intensity of management, namely, extensive, semi-intensive, or intensive. Under extensive cultivation, fish receive nutrition from naturally occurring food sources such as detritus and plankton; management efforts focus on protection from predators and competition (Baulcomb, 2013; Bunting, 2013). Semi-intensive cultivation involves some supplementation of the

natural food supply, or fertilization to increase the natural food supply. Under intensive cultivation, fish receive nutrition exclusively from formulated, high-protein aquafeeds. Greater intensity implies higher stocking densities, concentrated waste, greater risk of disease outbreak, and higher yield per unit of area (Beveridge & Little, 2002; Naylor et al., 2000). Within these categories lie a diversity of practices, but the global trend is toward intensification with formulated aquafeeds rapidly increasing in importance (Tacon et al., 2011). Aquafeed production was 29.2 million tonnes in 2008 and is expected to grow to 71.0 million tonnes by 2020 (FAO, 2012b).

Inland aquaculture: From wastewater-fed to recirculating systems

Semi-intensive wastewater-fed pond aquaculture has been practiced for centuries throughout East, South, and Southeast Asia and remains common in undeveloped areas where unpolluted freshwater is unavailable. Ponds constructed downstream from discharge sites receive wastewater (typically untreated) that acts as fertilizer to stimulate primary production. Plankton and other organisms serve as a natural food supply for the fish. The destruction of pathogens can be achieved relatively quickly by retaining wastewater in a series of stabilization ponds before it reaches the fishpond. The fishpond effluent, often of higher quality than the influent, may be used to irrigate downstream crops, trees, or pasture. Thus, the use of wastewater in aquaculture and irrigation presents an environmentally friendly and agriculturally productive alternative to mechanical wastewater treatment that is especially relevant for urban and peri-urban areas where mechanical treatment infrastructure is not economically feasible (Beveridge & Little, 2002; Edwards, 2005).

Urban wastewater-fed pond operations are generally considered very ecologically sustainable. Most are relatively small-scale operations that provide poor families with food security and income, and many are integrated fish-plant systems. Unfortunately, the urbanization on which they depend often also leads to their displacement. As industry grows in and around urban centers, toxic industrial waste mixed in with residential waste renders the wastewater unsafe for fish cultivation. Further, as a more profitable land use, growing industry often competes with farmers for land. Finally, farms are also susceptible to the loss of their nutrient source when wastewater infrastructure improvement or alteration changes the flow of the water by creating canals or moving the point of discharge (Bunting, Pretty & Edwards, 2010; Edwards, 2005; Little & Bunting, 2005). As countries like China develop and urbanize, it is likely inevitable that wastewater-fed pond culture will be replaced by higher-tech, intensive production systems.

The development of recirculating aquaculture systems (RAS) is particularly relevant for urban areas, because RAS is highly productive and can be located virtually anywhere, independent of climate and water resource availability (Little & Bunting, 2005; Timmons, 2005). Whereas flow-through production systems (such as ponds and raceways) require nearby sources of water, filtration allows RAS to recycle 90 to 99% of its culture water (Lovelace, 2009; Pillay, 2004; Timmons, 2005). Thus, RAS discharges minimal effluent, and filtered sludge can be used to generate biogas or applied as fertilizer at nearby farms or gardens (Baulcomb, 2013). And while pond systems are open and susceptible to disease and contamination, the RAS culture environment is contained and highly controlled (Bunting & Little, 2005; Timmons, 2005). Further, pond

aquaculture may not be feasible in areas where demand favors marine fish, but freshwater and marine cultures are both possible under RAS (Tal et al., 2009; Timmons, 2005). The higher cost of urban land is a constraint to urban RAS, but this may be offset by favorable policy instruments or tax breaks; reliable supply from consistent, year-round production; improved feed conversion ratios; proximity to market and reduced transportation costs; and price premiums for safe, environmentally friendly, and locally produced fish. Another constraint is the relatively high capitalization cost, but RAS lends itself to efficiencies from economies of scale (Little & Bunting, 2005; Timmons, 2005).

Aquaponics is an integrated form of RAS in which fish effluent that is rich in dissolved nutrients is used as fertilizer for hydroponic plants. The plants remove nutrients through their roots, and the filtered water returns to the fish tank. Thus, the filtration process allows for the indefinite recycling of water while producing a marketable crop. Aquaponics is a commercially viable production method capable of plant yields equal to or greater than those under traditional field production (Bailey et al., n.d.; Lewis et al., 1978; McMurtry et al., 1997; Rakocy, Bailey, Shultz, & Thoman, 2004a; Rakocy et al., 2006; Rakocy et al., 2004b).

Ecological considerations for intensive aquaculture

The sustainability of intensive aquaculture on a large scale requires consideration of the environmental resources on which it depends (inputs) and the ecological systems to which it discharges wastes (outputs). Net pen production occurs within a host ecosystem (e.g. coastal waters) with wastes from a high concentration of animals freely flowing out of the production area and into the surrounding environment (Baucomb, 2013; Bunting,

2013). Flow-through raceway production systems receive a constant supply of water from a river, spring or well and continuously discharge effluent as water leaves the farm (Lovelace, 2009; Pillay, 2004). But RAS (including aquaponics) resolves the output or pollution problems inherent in other forms of intensive aquaculture (Bunting, 2013; Pillay, 2004). As a form of tank aquaculture RAS is independent of aquatic ecosystems. This separation of production from the natural environment creates a point of intervention that, if properly managed, virtually precludes the discharge of effluents as pollutants (Baucomb, 2013; Bunting, 2013).

The question of sustainability of inputs remains relevant for RAS as it does for other forms of intensive aquaculture. Due to filtration water usage is extremely low in RAS and especially in aquaponics (Lovelace, 2009; McMurtry et al., 1997; Pillay, 2004; Timmons, 2005; Rakocy et al., 2006). However, formulated aquafeeds are an input of critical importance in terms of the sustainable growth of the aquaculture sector and the health of the world's fisheries (Deutsch et al., 2007; Naylor et al., 2000; Tacon et al., 2011).

Fish farming alleviates pressure on fisheries to the extent that demand for fish is met by aquacultural production. However, to the extent that aquaculture depends on marine-captured fish as an input (as fishmeal and fish oil for formulated feeds), it may contribute to overfishing, the degradation of marine food webs, and ultimately a limit on the productive capacity of the aquaculture industry (Deutsch et al., 2007; Naylor et al., 2000). Fishmeal is a major component of many aquafeeds, because it is easily digestible with favorable amino acid and fatty acid profiles. Formulated aquafeeds for fish at higher trophic levels (such as salmon) contain a larger proportion of fishmeal than the

cultivation of herbivorous or omnivorous fish such as carp and tilapia (Tacon et al., 2011). Demand for fishmeal has increased while supplies have declined 1.7% per year since 1995. As a result, prices have risen from 400-600 USD/tonne during the 1990s and early 2000s to over 1,200 USD/tonne in 2009 (Olsen & Hasan, 2012; Tacon et al., 2011). Much research effort, both academic and private, has focused on the development of alternative protein sources from plants, microbes, and byproducts of meat production (El-Sayed, 1999; El-Sayed, 2004; Olsen & Hasan, 2012). As a result the proportion of fishmeal used in formulated aquafeeds has declined. Considering fishmeal's decreasing supply, increasing demand and prices, and the development of substitutes, the total use of fishmeal in formulated aquafeeds is projected to decrease over the long term both in absolute terms and relative to its proportion as an aquafeed ingredient: from 3.72 Mt (or 12.8% of aquafeed by weight) in 2008 to 3.49 Mt (or 4.9% of aquafeed by weight) in 2020 (Tacon et al., 2011).

Potential ecological benefits of urban aquaponic production

Aquacultural operations can be classified according to the culture environment (marine, brackish, or freshwater) and setting (marine, coastal, or inland), intensity of management (intensive, semi-intensive, or extensive), production format (pond, tank, raceway, etc.), number and type(s) of species under cultivation, and level of integration with other agricultural processes (Baulcomb, 2013; Bunting, 2013). In an urban setting, a commercial aquaponics operation may be considered an intensive recirculating polyculture tank production system.

The recirculating aquaculture format is associated with higher operating costs (from filtration equipment and maintenance), but in aquaponics these costs are offset by revenue generated from the crop yield (Bailey et al., n.d.; Bunting, 2013; Rakocy et al., 2006). In an urban setting, integration with the urban waste stream may further increase profitability while promoting ecological sustainability: Costly formulated feed may be supplemented by duckweed, phytoplankton, algae or fodder fish produced in ponds or tanks fertilized by treated wastewater (Bunting, 2013; El-Sayed, 2004). The fish diet may also be supplemented by the on-farm production of black soldier flies that convert compostable wastes to high-protein biomass. These wastes may include those generated on-farm (e.g. filtered fish waste sludge, crop residues, and culled produce) as well as urban food scraps (from local supermarkets and restaurants) (Allen, 2013; Baulcomb, 2013; Bunting, 2013). Thus, urban aquaponics can act as a net nutrient sink by productively reducing the nutrient output to surrounding ecosystems, and address the issue of food waste by recycling it into the local food production system.

Aquaponics is particularly efficient in its use of water, only requiring replacement of water lost to evaporation and transpiration. Compared to other forms of recirculating aquaculture, aquaponics can reduce water usage by 93% or more, with a daily replacement rate as low as <1% (Lovelace, 2009; Masser et al., 1999; Rakocy, Hargreaves, & Bailey, 1993). Further, due to higher plant density water use under aquaponic crop production can be up to ten times more efficient than under irrigated field production (Al-Hafedh, Alam, & Beltagi, 2008; McMurtry et al., 1997).

Because it does not depend on soil, hydroponics is arguably the most soil-conserving method of crop production (Lal, 2013). As demand for food increases,

hydroponic production can relieve pressure to convert forested land to agriculture, just as aquaculture relieves pressure on fisheries. In urban environments, aquaponics offers the potential to return land with contaminated or infertile soil to highly productive agriculture. Space efficiencies can be achieved through the vertical orientation or arrangement of hydroponic components to multiply growing space and yield per area (N. Storey, personal communication, January 2, 2014; Rakocy et al., 2006).

In order to compare the operational energy consumption of a commercial aquaponic system with that of a fish-only RAS, we first consider a series of production trials that spanned four years and were conducted with an experimental, commercial-scale raft aquaponic system at the University of the Virgin Islands (UVI). The system's total water volume was 111,196 L; it occupied 500 m^2 of land, and its operation consumed 53.69 kWh per day. Rakocy et al. projected annual yields of approximately 4,780 kg of tilapia and 5,010 kg of basil (2004a). Thus, the operational energy consumption estimate for the UVI aquaponic system is 4.10 kWh per kg of tilapia or 3.91 kWh per kg of basil. Next, we consider the energy consumption for a hypothetical 100,000 kg yr^{-1}, fish-only RAS, estimated at 2.2 kWh per kg of tilapia by Eding et al. (2009). Of course, drawing a direct comparison between these two estimates is difficult considering the scale of the fish-only RAS (more than 20 times greater than that of the UVI aquaponic system in terms of tilapia production). Further, the comparison is complicated by the fact that energy input to the UVI system results in a crop yield in addition to the tilapia yield. For a crude comparison of energy consumption between systems in terms of tilapia yield, we may separate the energy inputs associated with fish and crop production in the UVI system and consider only those inputs associated with

fish production. Energy inputs to the UVI system provide power to the water pump (373 W), the aeration blower for the fish tank (1,119 W), and the aeration blower for hydroponic tanks (746 W). Thus, the water pump and fish tank blower consume 35.79 kWh per day, or 2.73 kWh per kg of tilapia, while the larger-scale fish-only RAS consumes 2.2 kWh per kg of tilapia. Therefore it is possible that at a large scale similar to that of the fish-only RAS, the UVI aquaponic system would achieve similar energy efficiency in terms of tilapia production.

Structure and function of aquaponic systems

The essential structural components of any aquaponic system include the fish tank, solids filter, bacterial biofilter, and hydroponic grow beds or towers. While various configurations exist, water is generally pumped from the lowest component (fish tank or sump) up to the highest component (filter) and flows gravitationally through the grow bed on its return to the fish tank (Rakocy et al., 2006).

The bacterial biofilter is the basis of plant productivity in any aquaponic system, because it converts ammonia in the fish waste to plant-available nitrate in a two-step biological nitrification process. The biofilter is composed of naturally occurring nitrifying bacteria that live on the system's submerged surfaces, and it begins to develop as soon as ammonia is present in the culture water. Ammonia, which may be highly toxic to fish, is oxidized into nitrite (NO_2^-) by *Nitrosomonas* bacteria. *Nitrospira* and *Nitrobacter* bacteria oxidize toxic nitrite into nitrate (NO_3^-), which is relatively safe for fish (Foesel et al., 2008; Keuter, Kruse, Lipski, & Spleck, 2011). The nitrification process is described by the formulas

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O + 84 \text{ kcal/mole of ammonia}$$

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- + 17.8 \text{ kcal/mole of nitrite}$$

Thus, nitrification of one mole of ammonia consumes two moles of dissolved oxygen (O_2) and yields one mole of nitrate, one mole of water (H_2O), and two moles of hydrogen ions (H^+) (Bernstein, 2011).

Two forms of hydroponic culture are common in aquaponics: deep-water culture and media culture. In deep-water culture (DWC), also known as raft aquaponics, plants are supported above a horizontally oriented trough, typically in individual net pots filled with an inert substrate (such as pearlite or coconut coir) and held in place by a rigid sheet (e.g. polystyrene). Roots are freely suspended in the culture water.

In media culture, the grow bed is filled with a soil substitute that may be composed of an aggregate material (such as pea gravel or expanded clay pellets) or a synthetic fibrous material (N. Storey, personal communication, January 2, 2014). The media provides structural support for the plants as well as habitat for nitrifying bacteria. Under a constant flow regimen, a steady volume of water is maintained in the grow bed. Under a reciprocating flow regimen, a timer or siphon mechanism periodically floods and drains the grow bed.

An advantage of media culture over DWC is that the grow bed doubles as a biofilter, while DWC requires a separate nitrification component. Lennard and Leonard observed significantly higher yields of Green Oak lettuce (*Lactuca sativa*) under media culture than under DWC during a 21-day trial (2006). Further, in a separate 21-day trial, a significantly higher yield of the lettuce, greater pH buffering capacity, and higher levels

of dissolved oxygen were observed under the constant flow regimen vs. the reciprocating flow regimen (Lennard & Leonard, 2004).

Maintenance of water quality in aquaponic production

Water quality parameters including temperature, nitrogen levels, dissolved oxygen (DO), and pH must be monitored and maintained for the proper functioning of an aquaponic system. Water temperature is determined by the ambient temperature, and smaller systems lose heat more quickly than larger systems. Fish are cold-blooded, and each species has evolved to tolerate a certain range of temperatures. Within the wider range that a species can tolerate, there is a narrower optimal range to promote growth and health. Therefore, local climate is an important factor to consider when deciding which fish species to cultivate (Bernstein, 2011). Because bacteria and most crops prefer warmer water temperatures, greenhouse production is common in northern latitudes. A common low-tech method of retaining heat in the greenhouse is the use of a heat sink (e.g. a deep tank of water that warms up during the day and releases heat at night). Another innovative approach involves composting organic wastes directly outside the greenhouse (along and against the exterior walls) so that the heat generated by decomposition is transferred inside (Allen, 2013). Other options include the use of a wood furnace connected to a heat exchanger (N. Storey, personal communication, January 2, 2014) or an electrical heater.

As indicated earlier, a well-functioning biofilter efficiently converts the ammonia in fish waste to nitrate and keeps the concentration of ammonia near 0 mg/L. This nitrification process is crucial, because ammonia in the un-ionized form (NH_3) is toxic to

fish at low concentrations while nitrate is relatively non-toxic. Ionized ammonium (NH_4^+) is also relatively non-toxic. In water the relative proportions of ionized and un-ionized ammonia depend upon pH and temperature such that the concentration of toxic NH_3 rises with pH and with temperature. For example, given a water temperature of 20°C, 0.40% and 1.24% of total ammonia is un-ionized at a pH of 7.0 and 7.5, respectively. At 25°C, these values increase to 0.57% and 1.77%, respectively. Nitrite (NO_2^-), the intermediate form of nitrogen in the nitrification process, is also toxic to many fish at low concentrations. Nitrite poisoning or "brown blood" disease interferes with the transport of oxygen by hemoglobin in the blood (Popma & Masser, 1999).

Filtration of solids is required to prevent the accumulation of organic matter (e.g. solid fish waste, uneaten food, dead fish or plant material) that consumes oxygen when it decomposes. A small amount of suspended solids can be beneficial, because bacteria mineralize the nutrients contained therein and make them available to plants. But an excess of solids may lead to anaerobic decomposition and the production of toxic methane and hydrogen sulfide (Rakocy et al., 2006). Earthworms are a relatively common means of degrading solids in media cultures, because they slowly convert organic wastes to plant-available nutrients (N. Storey, personal communication, January 2, 2014). Removal of solids can be achieved through mechanical filtration components such as conical settling tanks or mesh filters (Rakocy et al., 2006).

A high level of dissolved oxygen (6 mg/L or more) is required for the health of the fish, plants, and bacteria that each consumes oxygen through aerobic respiration. The saturation capacity of oxygen in water is inversely related to altitude, temperature, and salinity (Bernstein, 2011). For example, at sea level fresh water at 30°C can dissolve up

to 7.53 mg O_2/L, while fresh water at 20°C can dissolve up to 8.84 mg O_2/L (Masser, Rakocy, & Losordo, 1999). Oxygen is dissolved into a body of water whenever the water's surface is disturbed (through the mixing of atmospheric air with the water). In aquaponics, maintenance of a high DO concentration is often easily achieved without supplemental oxygen, and it is virtually impossible for the system to contain DO in excess (Bernstein, 2011). A constant flow regimen generates the highest concentrations of dissolved oxygen, because the constant movement of water continuously adds oxygen to the system (Lennard & Leonard, 2004).

A near-neutral pH (6.5 to 7.5) is recommended for the health of the fish, plants, and bacteria and for the optimal availability of nutrients to plants. Because nitrification gradually acidifies the water by adding H^+ ions, periodic supplements of a base (such as potassium hydroxide or calcium carbonate) may be necessary to maintain the desired pH. When plants uptake nitrate, however, their roots release hydroxide (OH^-) or bicarbonate (HCO_3^-) ions that offset acidification from nitrification (Lennard & Leonard, 2004). As an aquaponic system matures, the development of buffering capacity has a stabilizing effect on pH (Bernstein, 2011).

Environmental tolerances of tilapia

Tilapia are hardy warm-water fish with wider tolerance limits than most other cultured freshwater fish. All tilapia tolerate brackish water, and Blue tilapia grows well in salinities up to 20 parts per thousand. Prolonged exposure to concentrations of un-ionized ammonia (NH_3) above 0.2 mg/L can cause mortality, but tilapia can survive short-term exposure (3 or 4 days) to concentrations up to 3.0 mg/L. Tilapia are exceptionally tolerant

of nitrite; toxicity occurs at concentrations of 27 mg/L or greater. Tilapia tolerate a pH between 5 and 10 with an optimal pH range of 6 to 9. Blue tilapia tolerate temperatures between 8° and 41°C and stop feeding at temperatures below 17°C; the optimal range is 29°–31°C. While a dissolved oxygen (DO) concentration of 1.0 mg/L is acceptable, a concentration of at least 2.0 mg/L is optimal for growth (Popma & Masser, 1999).

CHAPTER III

METHODOLOGY

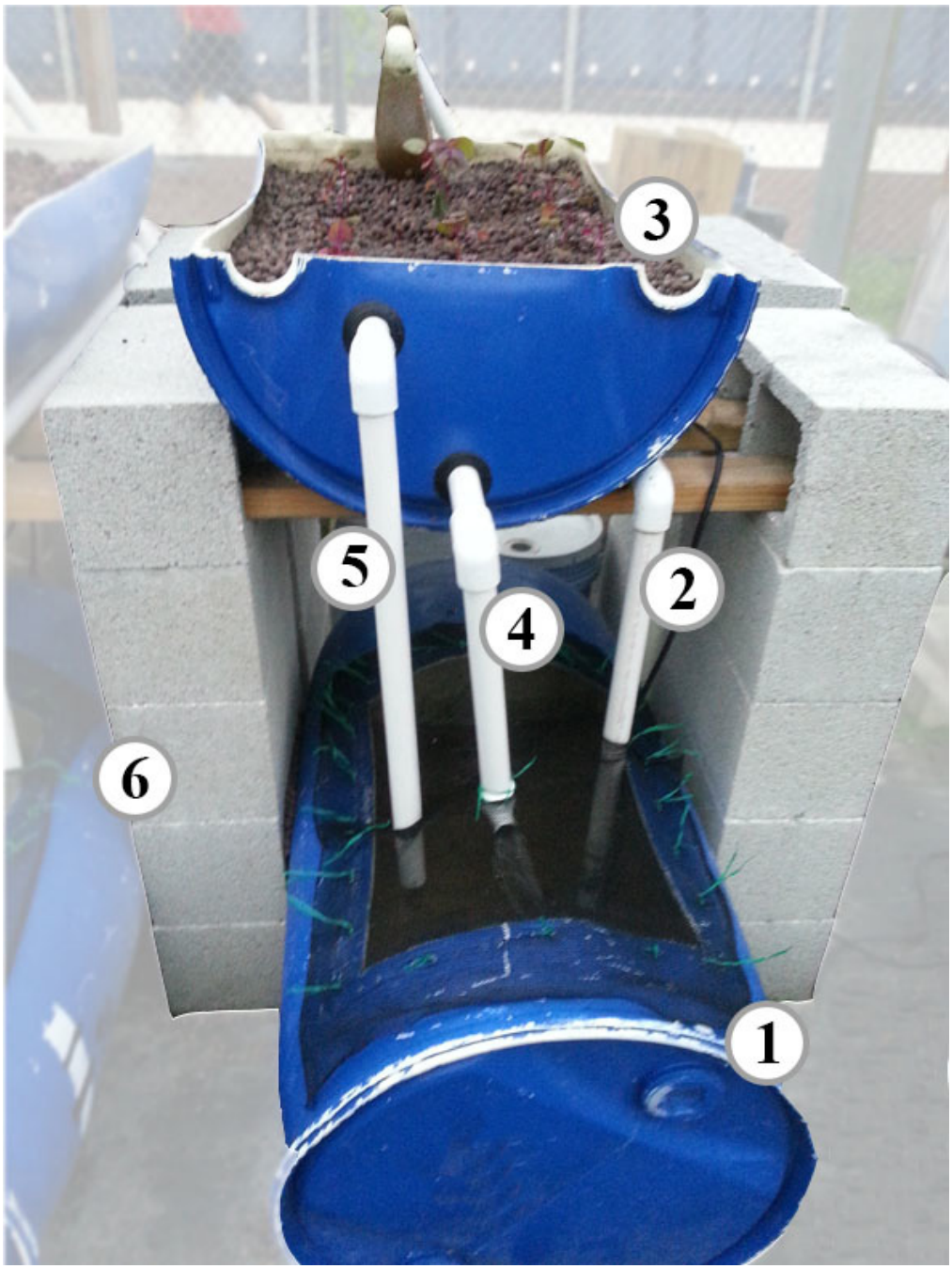
Design and setting of the aquaponic test units

Six aquaponic production units and a protective rain barrier were constructed and established inside the shade house at the Florida International University's Organic Garden (Modesto Maidique campus). Each aquaponic unit consists of one fish tank situated directly below one hydroponic grow bed containing Hydroton™ expanded-clay gravel media. Total water volume per unit is approximately 150 L. Water lost to evaporation is replaced by refilling the fish tank to a pre-measured 150 L mark in order to maintain water volume. A submersible fountain pump (1 hp) inside the fish tank continuously moves water through 1" PVC plumbing up to the grow bed. A nylon mesh attached to the outlet of the hydroponic supply pipe collects solid wastes that are routinely removed from the system. Water in the grow bed returns to the fish tank gravitationally via a 1" PVC drain pipe and an overflow pipe (Figure 2).

Each fish tank is a 55-gallon plastic rain barrel (approximately 60cm x 90cm) laid lengthwise and kept in place with concrete blocks. A rectangular opening (approximately 35 cm by 45 cm) cut into the barrel facilitates feeding of fish and allows adequate space for maintenance and for inflow and outflow pipes. An appropriately sized and secured piece of shade cloth covers each of the fish tank openings in order to prevent entry by predators, insects and other animals.

Each hydroponic grow bed is constructed from one-half of a rain barrel (cut and laid lengthwise) and contains approximately 100 l of expanded clay gravel media. Grow

Figure 2. Diagram of one of the six aquaponic test units.
A submersible pump (not shown) moves water from the fish tank (1) through the hydroponic supply pipe (2) and into the grow bed (3) that is filled with gravel media. Water flows out of the grow bed by gravity through the drain pipe (4) and the overflow pipe (5). System components are supported by a simple structure composed of concrete blocks and lumber (6).



bed surface area per replicate is approximately 0.5 m^2. The grow beds are situated above the fish tanks and are supported by a simple structure composed of 2.5 cm x 7.5 cm lumber and concrete blocks. To summarize, the experimental aquaponics units can be characterized as small-scale gravel media systems under a constant-flow regimen. The aquaponic units were placed adjacent to each other and aligned in a single row along an approximately north-south axis.

Blue tilapia (*O. aureus*) fingerlings were stocked to a density of approximately 2.40 kg of biomass (at harvest) per 100 L of water. Harvest weight of Blue tilapia is approximately 450 g. Therefore, eight tilapia fingerlings are stocked into each 150 l system. The initial masses of fingerlings per replicate (live weight) were 73.0 g, 65.0 g, 63.0 g, 65.0 g, 70.0 g, 68.0 g for Replicates 1 through 6, respectively.

Red amaranth (*Amaranthus tricolor*) seeds were allowed to germinate in moistened rockwool media plugs outside of the aquaponic systems prior to the beginning of the experimental trial. Fifteen seedlings were transplanted into each replicate. Initial seedling mass per replicate was nominal (<1g). Plants were harvested 60 days after transplantation.

Experimental treatments

Each aquaponic unit was assigned one of two experimental treatments in an alternating sequence: Odd-numbered units (1, 3 and 5) received the control treatment, and even-numbered units (2, 4, and 6) received the alternative treatment. Thus, each treatment group consisted of three replicates. The treatments consisted of two commercially available pelleted aquafeeds: Replicates in the control group received a common

fishmeal-based feed (Zeigler Finfish Silver feed), and replicates in the alternative group received a plant-based feed (Advanced Biological Concepts Organic Fish Food). Crude protein content of the control and alternative feeds were 40% and 32%, respectively. Further details on the nutrient content of the feeds are included in Appendix I.

Table 1. Feeding rate schedule.

Trial days	Feeding rate	Number of days
1-7	0.10	7
8-14	0.09	7
15-21	0.08	7
22-30	0.07	9
31-35	0.06	5
36-42	0.05	7
43-60	0.04	18

Fish were fed 6 days per week during the 60-day trial period, with the exception of the first week during which the fish were fed every day. The mass of feed applied to replicates on a given day was calculated as the product of a feeding rate and an estimate of fish mass per replicate on that day according to the equations

$$b_t = b_1 + r_G \cdot (t - 1)$$

$$f_t = r_F \cdot b_t$$

where b is fish biomass (g rep^{-1}); t is the trial day: [1-60]; b_t is the estimated fish biomass per replicate on trial day t; b_1 is the observed initial fish biomass per replicate (t=1); r_G is the fish growth rate (assumed to be 0.5 g fish^{-1} day^{-1}, or 4 g rep^{-1} day^{-1}); f_t is the mass of

feed to be applied to a replicate (g rep^{-1}) on trial day t; and r_F is the feeding rate (g g^{-1}). The feeding rate schedule is shown in Table 1.

Data collection

The data are grouped into three categories: fish, plants, and water quality. Fish data are based on the initial and final masses of live fish per replicate, measured on an Ohaus Scout Pro digital balance. Plant data include initial and final masses of root, stem and leaf biomass (wet weight) per replicate measured on an Ohaus Scout Pro digital balance, the total heights of plants per replicate, and the number of leaves per replicate.

Water quality data include measurements of concentrations of total ammonia-N, nitrate-N, and orthophosphate measured with the AQ2 Discrete Analyzer (according to EPA methods EPA-129-A Rev. 8 for Ammonia-N, EPA-114-A Rev. 9 for Nitrate-N + Nitrite-N, and EPA-146-A Rev. 0 for o-Phosphate-P). For each observation, samples were taken from water entering the grow bed via the supply pipe. All samples were promptly returned to the lab and run in the AQ2 instrument for analysis without the addition of preservatives. Thirteen observations of total ammonia-N concentrations, 12 observations of nitrite-N and nitrate-N concentrations, and 11 observations of orthophosphate concentrations were made over the course of the trial.

Other water quality data include measurements of temperature (°C), pH, dissolved oxygen (DO, measured in parts per million and percent saturation), total dissolved solids (TDS, measured in parts per million), and electroconductivity (EC, measured in micro Siemens per centimeter or μS/cm). These measurements were taken on site in the fish tank with the Thermo Scientific Orion Star A329 Portable Multiparameter Meter.

Fourteen observations of pH, 13 observations of TDS and EC, and 12 observations of DO were made over the course of the trial.

Statistical analysis

All statistical analyses were conducted using SPSS v21.0 software. One-way ANOVA was used to test for significant differences in fish and plant growth parameters between treatment groups. In addition, one-way ANCOVA was used to test for significant differences in plant growth parameters to control for a plumbing problem that occurred during the trial. Repeated-measures ANOVA (rANOVA) was used to test for significant differences in water quality parameters between treatment groups over the course of the trial. Where appropriate and as noted in the results, data sets were normalized to adjust for the effect of different quantities of feed applied to replicates.

Economic analysis

The economic analysis begins with the consideration of two commercial aquaponic production scenarios based on the literature. The Bright Agrotech greenhouse aquaponic system yields greens and herbs including lettuce and basil that are grown in proprietary vertical grow towers. The tilapia that grow in the system are not harvested. Production data for the high-value basil crop (Scenario 1a) and the low-value lettuce crop (Scenario 1b) are considered for analysis (Storey, n.d.). The University of the Virgin Islands (UVI) outdoor aquaponic system was an experimental commercial aquaponic system that yielded both produce and tilapia (Scenario 2) (Bailey et al., n.d.). For each scenario, six levels of increased crop production resulting from the adoption of an

alternative feed vs. a standard feed are considered: 0%, 10%, 20%, 30%, 40% and 50%. Under Scenario 2, we also consider five levels of reduction in fish yield from the alternative aquafeed: 80%, 60%, 40%, 20% and 0%.

For Scenario 2, whose total revenue involves interaction between changes in crop and fish yields as a result of the alternative feed, we calculate the increase in crop yield that would be required to compensate for the loss of revenue at each level of reduced fish yield. Finally, results from the technical aspect of the study are applied to Scenario 2 to determine the effects on revenue.

Limitations of the study

The dissolved nutrients under consideration include ammonia, nitrite and nitrate, and orthophosphate. Concentrations of other nutrients such as potassium, calcium, magnesium, and iron were not observed. Therefore, the impact of deficiencies or overabundance of these nutrients on plant growth and water quality is not included in the analysis.

CHAPTER IV

RESULTS AND DISCUSSION

Throughout this chapter, the fishmeal-based feed is referred to as the control treatment, while the plant-based feed is referred to as the alternative treatment. Odd-numbered replicates (1, 3, and 5) received the control treatment, and even-numbered replicates (2, 4, and 6) received the alternative treatment.

The amount of daily feed applied to each replicate was based on the initial mass of the replicate, with more feed applied to replicates with greater initial mass. For example, on any given day Replicate 1 with an initial fish mass of 73 g received more feed than Replicate 2 with an initial fish mass of 65 g. In order to account for these differences in the amount of fish feed applied to replicates, we analyze the significance of the statistics based on normalized values where appropriate.

Over the course of the trial, observations of water temperature across all replicates ranged from 22.0°C to 27.3°C with a mean of 25.5°C.

Fish growth and feed conversion

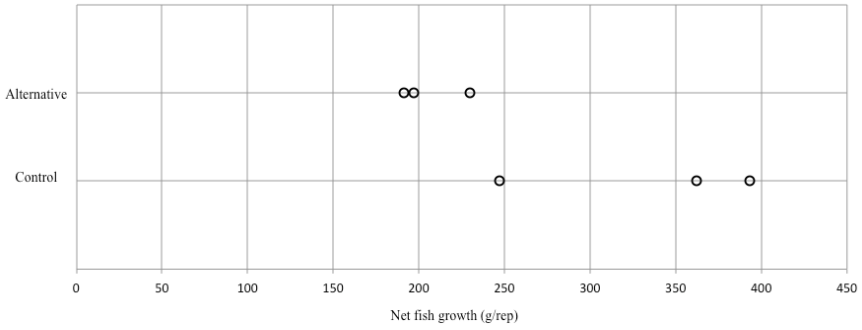
There was zero fish mortality during the trial period. The observed fish growth parameters include net growth, specific growth rate, and feed conversion ratio (Table 2). Net growth per replicate is calculated as the difference between final and initial mass (live weight) per replicate. Based on source data, the mean (±*SD*) net growth values for the control and alternative treatment groups were 334.0 g (± 76.9 g) and 206.0 g (± 21.0 g), respectively. Higher net growth occurred in all control replicates despite greater

variability within the control group than the alternative group (Figure 3). At the 0.05 level of significance, based on the source data the net fish growth was significantly higher under the control treatment (p=0.0497).

Table 2. Fish biomass data per replicate (source data).

Replicate	1	2	3	4	5	6
Initial mass (g)	73	65	63	65	70	68
Final mass (g)	320	295	456	256	432	265
Net growth (g)	247	230	393	191	362	197
Specific growth rate (% day^{-1})	2.50	2.56	3.35	2.32	3.08	2.31
Feed conversion ratio	2.29	2.34	1.36	2.83	1.54	2.79

Figure 3. Net fish growth per treatment group (source data).



Based on the normalized data, the mean (±*SD*) net fish growth values for the control and alternative treatment groups were 347.5 (±91.2) g and 217.6 (±23.5) g, respectively. At the 0.05 significance level, based on the normalized data no significant difference in net fish growth was observed between treatments (p=0.075). Statistical power for this test was reduced by normalization of the data, to 0.445. The likelihood of a Type II error is considerable.

Specific growth rate (SGR) is calculated as

$$SGR = \frac{\ln(m_2) - \ln(m_1)}{t_2 - t_1} \cdot 100$$

where m_1 and m_2 are initial and final fish biomass per replicate (g rep^{-1}), respectively, and t_1 and t_2 are trial days 1 and 60, respectively. Based on the source data, the mean (±*SD*) SGR values for the control and alternative treatment groups were 2.98 (±0.43)% day^{-1} and 2.40 (±0.14)% day^{-1}, respectively. No significant difference in specific growth rate was observed between treatments (p=0.092>0.05). Based on the normalized data, the mean (±*SD*) SGR values for the control and alternative treatment groups were 3.10 (±0.56)% day^{-1} and 2.53 (±0.17)% day^{-1}, respectively. Based on the normalized data, no significant difference in SGR was observed between treatments (p=0.173>0.05). However, statistical power was low (0.249 and 0.394 for tests based on normalized and source data, respectively), and the likelihood of a Type II error is high.

The feed conversion ratio (FCR) is the ratio of the total mass of feed applied (g rep^{-1}) to the net growth of fish mass (g rep^{-1}). Based on the source data for net fish growth, the mean (±*SD*) FCR values for the control and alternative treatments groups were 1.73 (±0.50) and 2.66 (±0.27), respectively. At the 0.05 level of significance the mean FCR of the control group was significantly less than the mean FCR of the alternative group (p=0.046). Based on the normalized net growth data, the mean (±*SD*) FCR values for the control and alternative treatments groups were 1.68 (±0.54) and 2.52 (±0.27), respectively, with no significant difference in FCRs observed (p=.075).

Plant growth and yield

Several plant growth and yield parameters were observed: the heights of plants at harvest, the number of leaves on each plant at harvest, and biomass (including measurements of roots, stems and leaves) of each plant at harvest. Table 3 summarizes the data per replicate. The alternative treatment group experienced higher mean plant growth in terms of all observed parameters except for shoot to root ratio (Table 4). However, significant differences between treatments were not observed for any parameters due to high variability within treatment groups.

Due to plumbing problems in Replicates 5 and 6 early in the experiment, the water levels in the hydroponic grow beds of these replicates was substantially lower than the water levels in the other four grow beds during much of the trial. As a result, the roots of plants in Replicates 5 and 6 did not reach the culture solution as quickly as did the plants in the other replicates, and they experienced delayed growth. By the end of the trial, plants in Replicates 5 and 6 were of substantially smaller size than plants in other replicates. Also, herbivory was observed among plants in Replicates 5 and 6, with two plants in Replicate 5 not surviving to the end of the trial. With regard to the ANOVA tests for significance, the variability within treatment groups due to the plumbing problem increased the error term and reduced the power of each statistical test to less than 0.20 (Table 5). Thus, it is highly likely that the lack of significance in the ANOVA results was due to Type II error.

In order to estimate and analyze the effect of the plumbing problem on plant growth, we may consider Replicates 1 through 4 as one treatment group (“high water level”) and Replicates 5 and 6 a second treatment group (“low water level”). At the 0.05

significance level, Normalized values for both total plant biomass (p=0.027) and plant height (p=0.020) are significantly higher under the "high water level" treatment. Therefore, it is reasonable to conclude that the lack of significance in the plant growth results was likely due to the plumbing problem.

Table 3. Normalized plant growth data per replicate.

Replicate	1	2	3	4	5	6
Combined height (cm)	532.8	621.8	498.1	589.7	245.7	400.2
Number of leaves	310.0	365.0	263.0	404.3	138.7	243.7
Root biomass (g)	115.59	122.09	103.93	168.87	24.33	85.11
Stem biomass (g)	211.92	274.49	191.91	291.36	36.58	117.34
Leaf biomass (g)	232.37	282.90	190.66	306.48	56.72	139.27
Shoot biomass (g)	444.29	557.39	382.57	597.84	93.30	256.61
Total biomass (g)	559.88	679.48	486.49	766.70	117.63	341.72
Shoot to root ratio	3.84	4.57	3.68	3.54	3.83	3.02

Table 4. Normalized mean plant growth data per treatment group.

Plant growth parameter	Control group ($M \pm SD$)	Alternative group ($M \pm SD$)
Height (cm rep^{-1})	425.54 ±156.72	537.24 ±119.80
Number of leaves per rep	237.24 ±88.51	337.67 ±83.72
Leaf biomass (g rep^{-1})	159.92 ±91.77	242.88 ±90.50
Shoot biomass (g rep^{-1})	306.72 ±187.38	470.61 ±186.43
Total biomass (g rep^{-1})	388.00 ±237.00	595.97 ±224.47
Shoot to root ratio	3.787 ±0.091	3.707 ±0.789

Table 5. P-values and statistical power given by one-way ANOVA, based on normalized plant growth data.

Plant growth parameter	*p*	power
Height (cm rep^{-1})	0.382	0.119
Number of leaves per rep	0.227	0.198
Leaf biomass (g rep^{-1})	0.326	0.141
Shoot biomass (g rep^{-1})	0.343	0.133
Total biomass (g rep^{-1})	0.332	0.138
Shoot to root ratio	0.870	0.052

Further, if we substitute plant growth data for Replicates 5 and 6 based on the data for Replicates 1 through 4, we may estimate the significance level and statistical power that might have been observed if the plumbing problem had not occurred. Analysis of this altered data set indicates that, if not for the plumbing problem, high statistical power may have been achieved and a significant difference between treatments may have been observed for all plant growth parameters. For example, in terms of total plant biomass we may substitute the average normalized values of Replicates 1 and 3 (523.19 g) for Replicate 5 and the average normalized values of Replicates 2 and 4 (723.09 g) for Replicate 6. With this substitution, the one-way ANOVA test achieves a statistical power of 0.992 and shows strong evidence for a significant difference in total plant biomass in favor of the alternative treatment (p=0.004). Further, for a more conservative consideration we may substitute the higher normalized value of Replicates 1 and 3 (559.88 g) for Replicate 5 and the lower normalized value of Replicates 2 and 4 (679.48 g) for Replicate 6. With this substitution, the one-way ANOVA test achieves a power of 0.918 and shows strong evidence for a significant difference in total plant biomass in

favor of the alternative treatment (p=0.010). Similar results are achieved for plant height and number of leaves.

Finally, a one-way ANCOVA (analysis of covariance) analysis was conducted (using normalized data values) to determine the significance of the aquafeed treatment effect while controlling for the error caused by the plumbing problem: Replicates 1 through 4 were assigned to a "high water level" covariate group, and Replicates 5 and 6 were assigned to a "low water level" covariate group. At the 0.05 level of significance, the ANCOVA results showed significantly higher plant height, number of leaves, leaf biomass, shoot biomass and total biomass under the alternative aquafeed treatment. Confidence intervals and p-values are summarized in Table 6. It should be noted that the distinction between the "high water level" and "low water level" covariate groups is based solely on informal observation. No data were collected to quantify the differences in water levels between replicates.

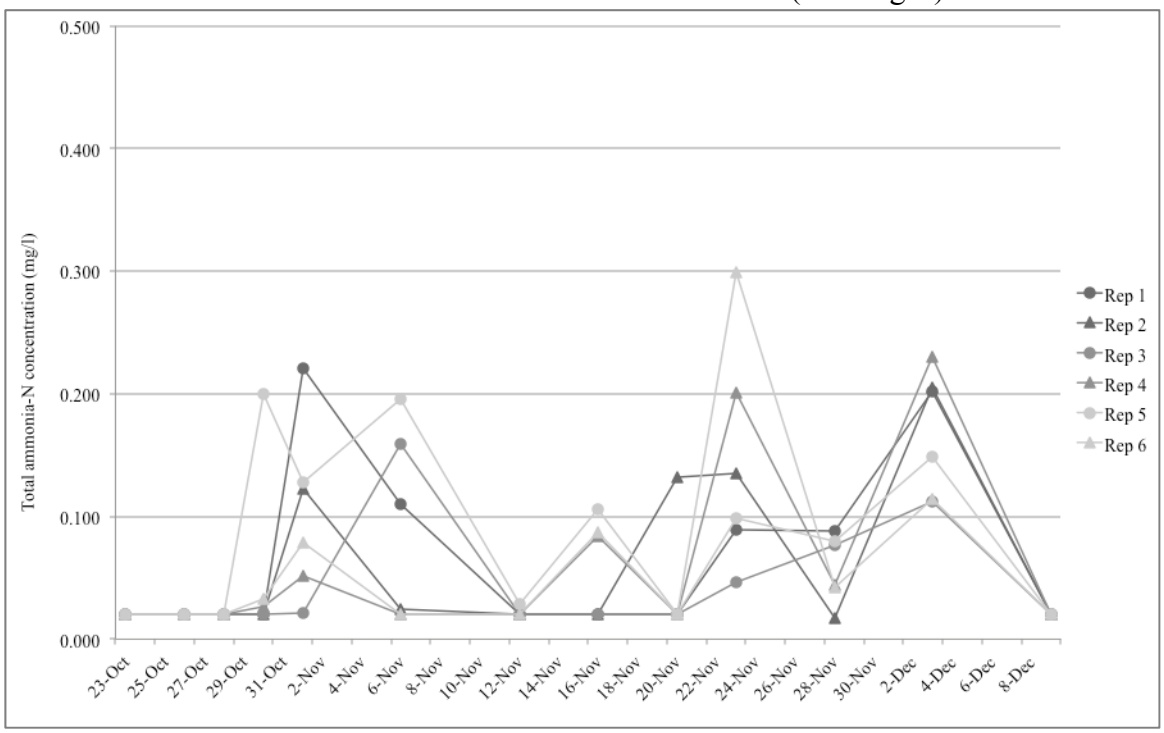
Table 6. Confidence intervals and p-values given by one-way ANCOVA, based on normalized plant growth data.

Plant growth parameter	95% confidence intervals per aquafeed treatment group:		p
	Control group	Alternative group	
Height (cm rep^{-1})	(372.62, 478.46)	(484.33, 590.16)	0.018
Number of leaves per rep	(191.11, 283.38)	(291.53, 383.80)	0.016
Leaf biomass (g rep^{-1})	(123.97, 195.86)	(206.94, 278.83)	0.014
Shoot biomass (g rep^{-1})	(251.37, 362.08)	(415.26, 525.97)	0.007
Total biomass (g rep^{-1})	(301.23, 474.78)	(509.19, 682.74)	0.012

Across all replicates, seedlings developed and grew slowly during the first 4 weeks of the trial and experienced very rapid growth during the final 3 to 4 weeks of the trial (Appendix II). Seedlings had been transplanted before the appearance of true leaves. Premature transplantation was likely the cause of the delayed growth observed during the first 4 weeks.

Total ammonia-N (TAN) concentration

Figure 4. TAN concentration per replicate (source data).
Observations below the detection limit are shown at the limit (0.02 mg/L).



Minute fluctuations in the concentration of total ammonia nitrogen (TAN) are to be expected during normal operation of any aquaponic system. However, the concentration should remain near zero. A concentration greater than 0.50 mg/L may indicate a problem with the biological filter responsible for nitrification and may pose a

risk to the fish. Throughout the course of the trial, TAN concentrations remained very low (below 0.300 mg/L) in all replicates (Figure 4). Often, TAN concentrations were below the detection limit of the test (<0.02 mg/L). These results indicate that biological nitrification functioned consistently well in each replicate. A statistically significant difference in TAN concentrations was not observed between treatment groups (p>0.05).

Nitrate-N concentration

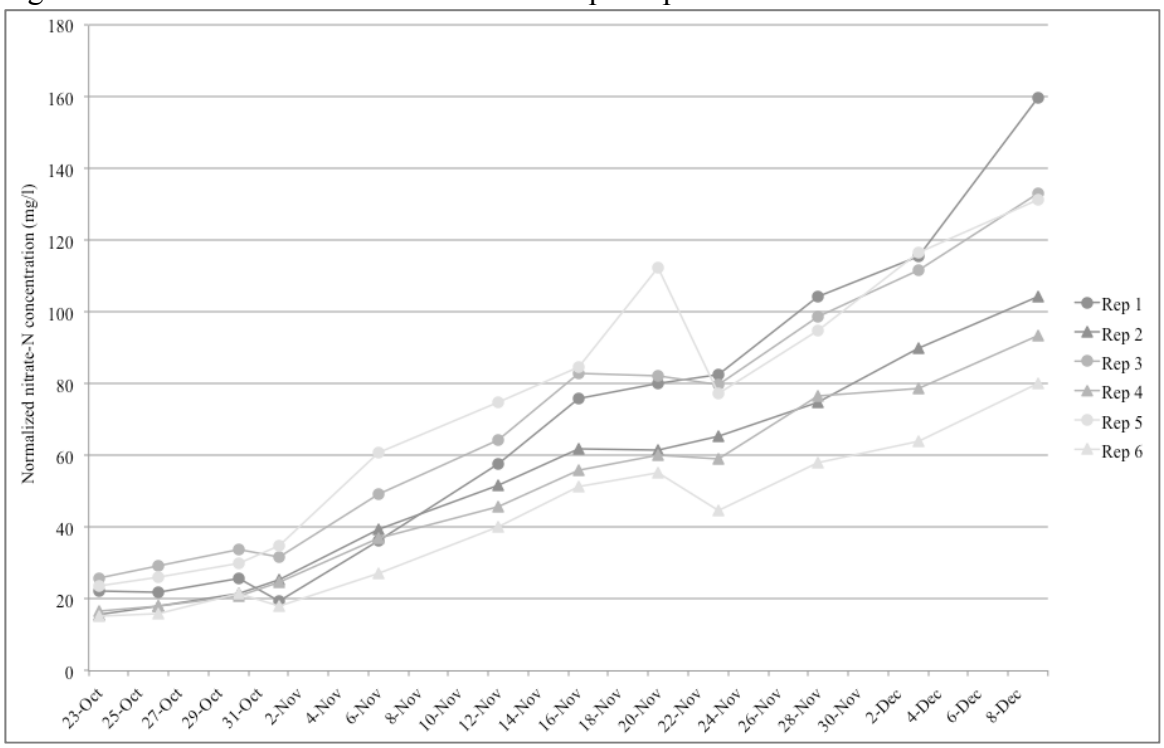
The detection method (EPA-114-A Rev. 9) detects both nitrate-N and nitrite-N, and the resulting concentration includes both compounds. Nitrite-N concentrations are assumed to be negligible, and the concentration results are attributed solely to nitrate-N.

The source data and normalized data for nitrate-N concentrations were each tested for a difference between treatments. At the 0.05 level of significance, the nitrate-N concentration was significantly higher under the fishmeal-based treatment (by an average 21.79 and 21.98 mg/L per observation based on the normalized and source data, respectively) than under the alternative treatment (p=0.004 for each data set). Considering the substantial difference in crude protein content between aquafeeds (40% and 32% CP for the control and alternative feeds, respectively), the result is not surprising.

Overall, concentrations of nitrate-N showed an increasing trend over the course of the trial (Figure 5). The accumulation of nitrate implies that the amount of plants in each system was inadequate to remove nitrate from the culture water given the amount of feed applied per replicate. In order to stabilize the nitrate concentration, one would either

provide less feed to the fish or add more plants to the system. In the interest of maintaining the experimental protocol, these adjustments were not made.

Figure 5. Normalized nitrate-N concentration per replicate.



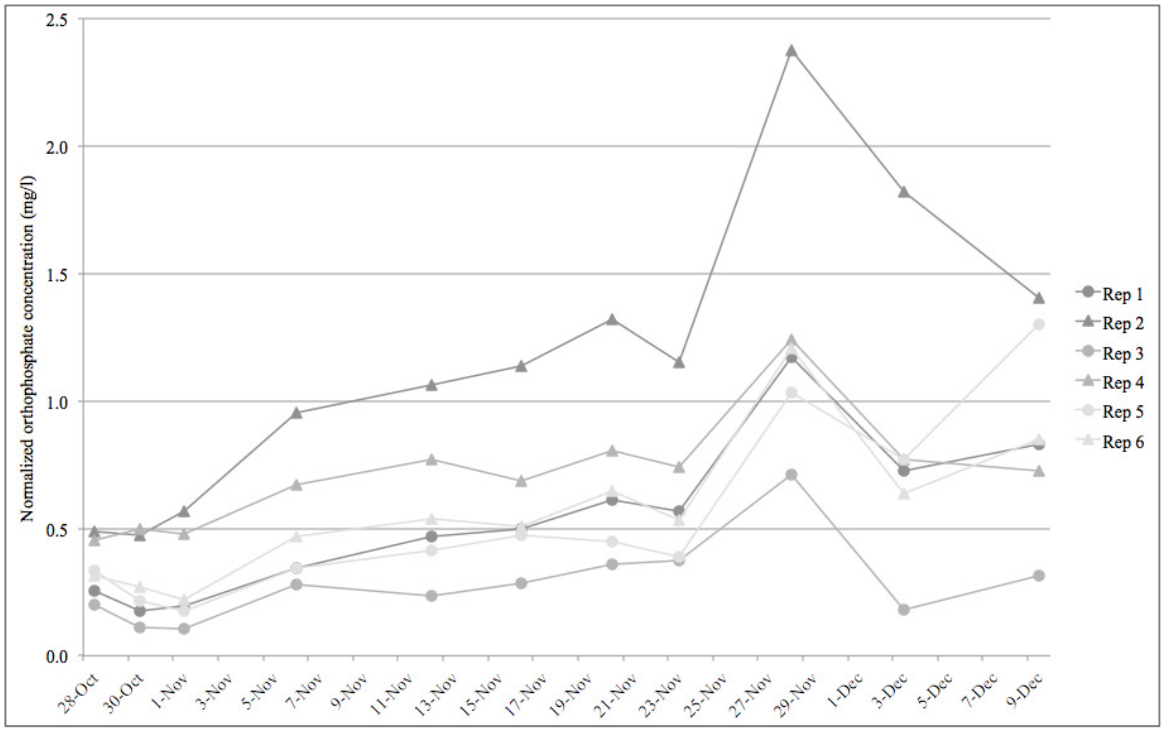
Replicates 2 and 4 (under alternative treatment) each had greater plant productivity than Replicates 1 and 3 (under control treatment), despite lower nitrate concentrations in the culture water. This would seem to indicate that nitrate levels, though lower, were adequate in these alternative replicates, and that some other limiting factor was responsible for the inferior plant productivity observed in the control replicates.

The relatively small decreases in nitrate concentrations observed in Replicates 1, 3 and 6 on Day 22 (November 1) were caused by substantial overflows and subsequent

replacements of water on Day 21 (October 31) that resulted from clogs in the main drainage pipes of these replicates. This did not seem to have affected the overall results. Of greater interest is the relative stabilization or decrease in nitrate concentrations observed in all replicates between Days 37 and 45 (November 16-24). Because this effect seems to have coincided with a period of rapid plant growth in all replicates, higher nitrate removal by growing plants offers the simplest explanation. Since observations of plant growth parameters were taken only on Days 1 and 60 of the trial, there is no data to support this claim. However, the photos in Appendix II provide evidence of rapid plant growth between Days 37 and 45, relative to growth during Days 1 through 36.

Orthophosphate concentration

Figure 6. Normalized orthophosphate concentration per replicate.



The source data and normalized data for orthophosphate concentrations were each tested for a difference between treatments. The mean of observed orthophosphate concentrations over the course of the trial is greater in the alternative treatment group than in the control group (by an average 0.341 and 0.361 mg/L based on the source data normalized data, respectively). However, there is insufficient evidence to claim that these differences in orthophosphate concentrations are significant at the 0.05 level (p=0.142 and p=0.150 for the source data and normalized data, respectively). Over the course of the trial, orthophosphate gradually accumulated in each replicate, with some minute fluctuations (Figure 6).

Total dissolved solids (TDS) concentration and electroconductivity (EC)

The source data and normalized data for concentrations of TDS were each tested for a difference between treatments. At the 0.05 level of significance, the TDS concentration was significantly higher under the fishmeal-based treatment (by an average 86.8 and 83.3 mg/L per observation based on the source and normalized data, respectively) than under the alternative treatment (p=0.011 and p=0.007 for source data and normalized data, respectively).

Dissolved solids generally accumulated in each replicate over the course of the trial, and the concentrations exhibited a clear divergence between groups as the trial period progressed (Figure 7). The first observation indicates that Replicate 1 started with a higher TDS concentration than each of the other replicates. It is likely that the storage of the tilapia fingerlings (approximately 60) in Replicate 1 for one week leading up to Day 1 of the trial led to an initial accumulation of dissolved solids. Interestingly, the TDS

Figure 7. Normalized TDS concentration per replicate.

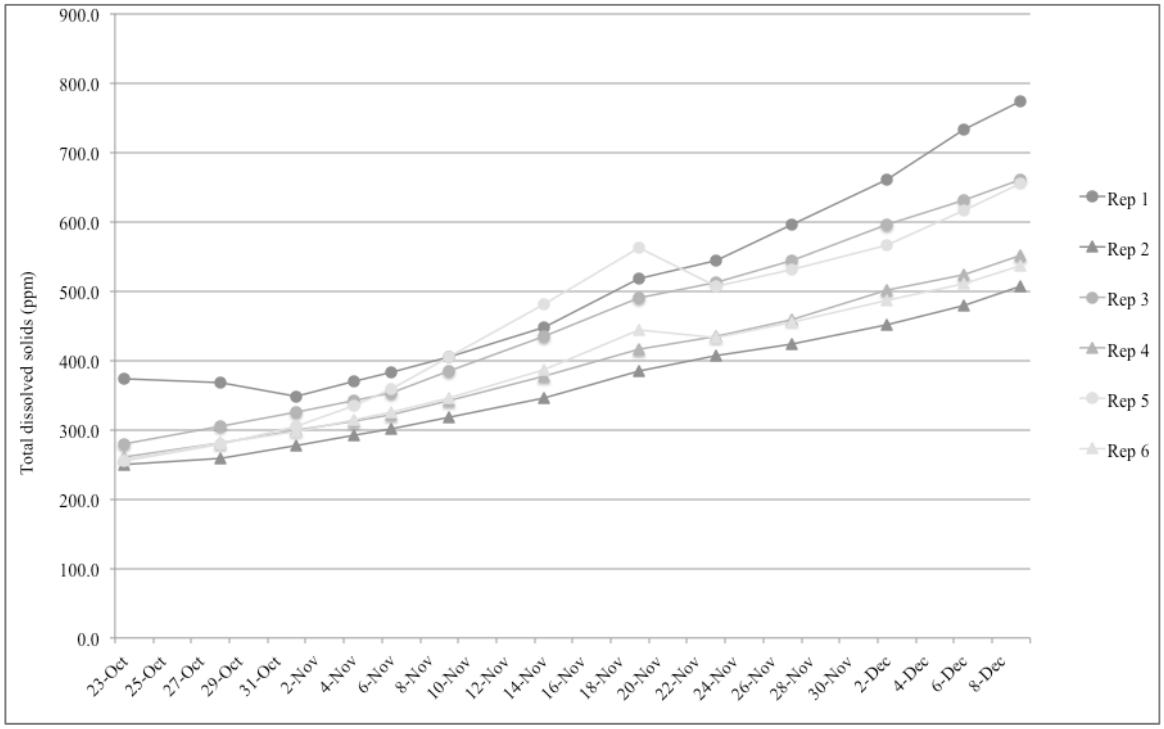
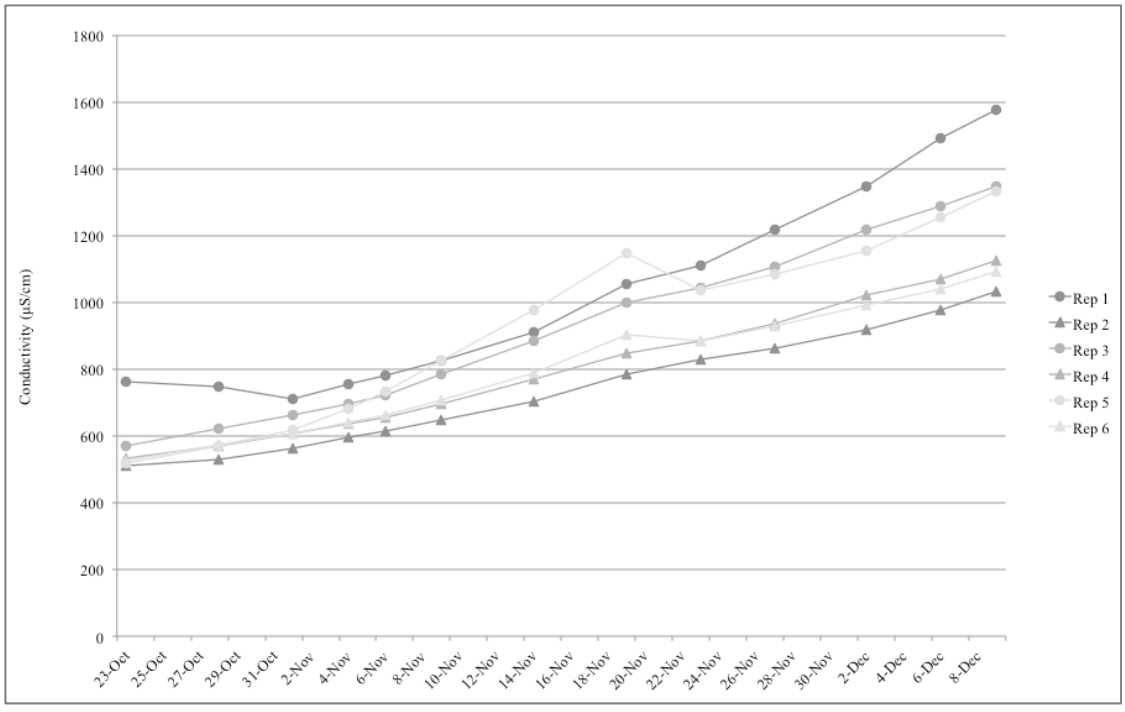


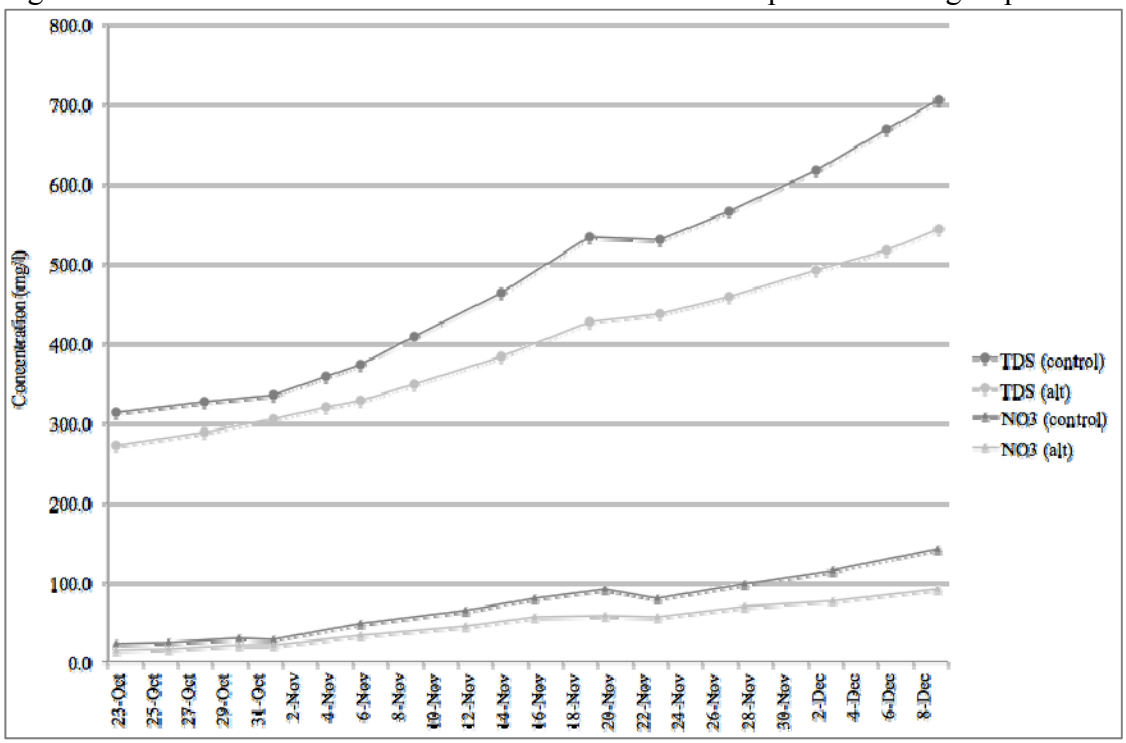
Figure 8. Electroconductivity per replicate (source data).



concentration in Replicate 1 eventually converged with those of the other control replicates. A significance test based on the normalized data, beginning from the first observed point of convergence on November 1, agrees with the above conclusion that the TDS concentration was significantly higher under the control treatment (p=0.003).

Electroconductivity levels show a similar trajectory to TDS concentrations (Figure 8). At the 0.05 significance level, the conductivity level was significantly higher under the control treatment (by an average 177.2 µS/cm per observation, based on source data) than under the alternative treatment (p=0.011).

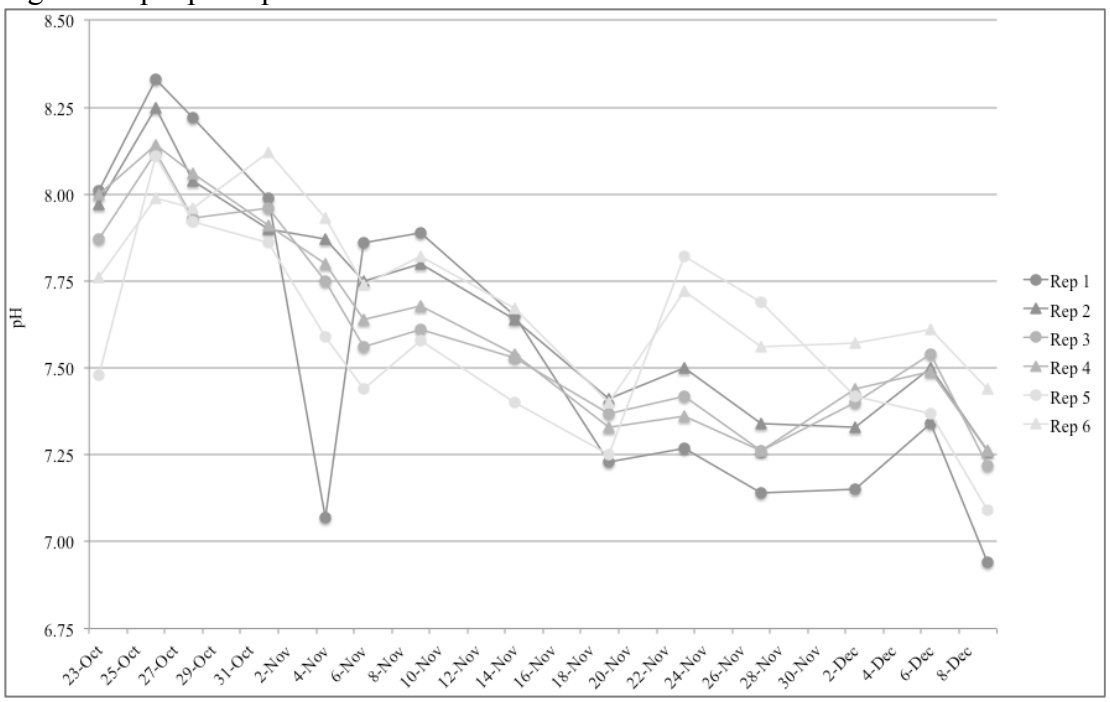
Figure 9. Normalized mean TDS and nitrate concentrations per treatment group.



The accumulation of dissolved solids other than nitrate is illustrated in Figure 9. On average the proportion of nitrate in TDS increased throughout the trial, to 20% and 17% on Day 60 in the control and alternative replicates, respectively.

pH

Figure 10. pH per replicate.



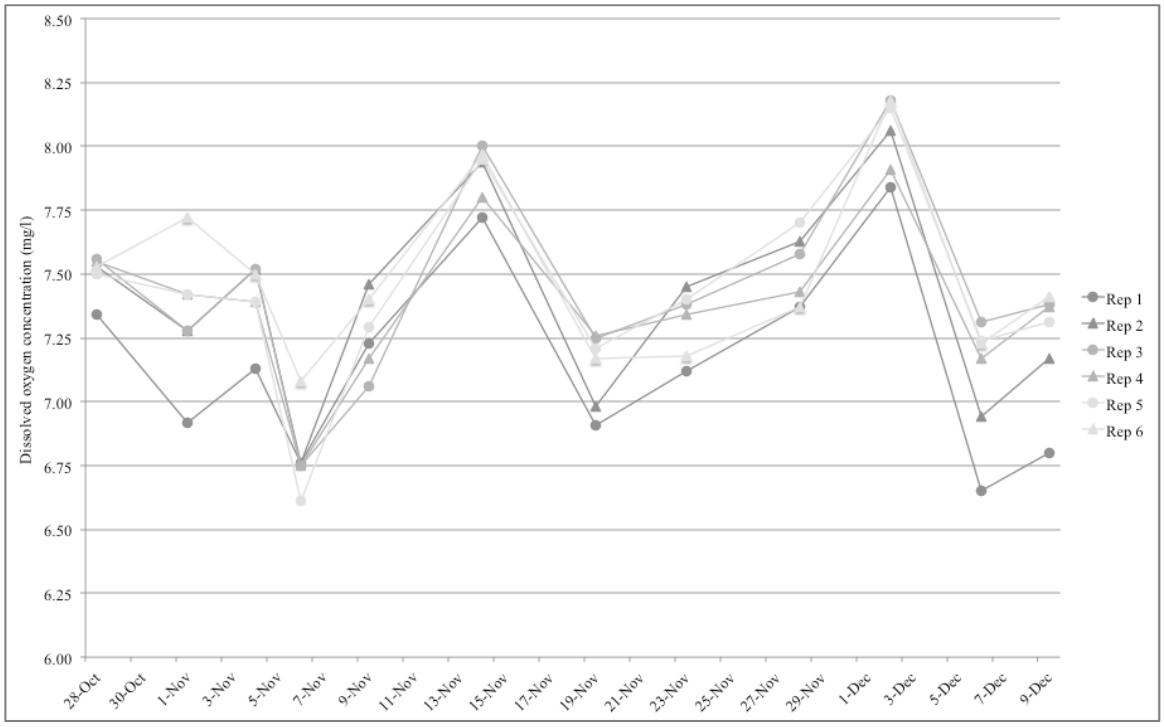
An overall decline in the pH was observed in each replicate, representing a gradual acidification of the culture water (Figure 10). The magnitude of the declining pH was small: The pH values across replicates declined from initially slightly alkaline values (ranging from 7.99 to 8.33) to near-neutral values (ranging from 6.94 to 7.44). This drop in pH values across replicates is an expected effect of nitrification, and the small magnitude of this effect is attributed to the buffering capacity associated with high calcium carbonate content of the water. The pH ranges were acceptable for the uptake of nutrients and for the health of plants, fish, and bacteria.

In order to test for a difference between treatments, the pH data were transformed into hydronium ion concentrations (mol/L) via the inverse log function. No significant

difference in hydronium ion concentrations was observed between treatments over the course of the trial (p=0.097>0.05).

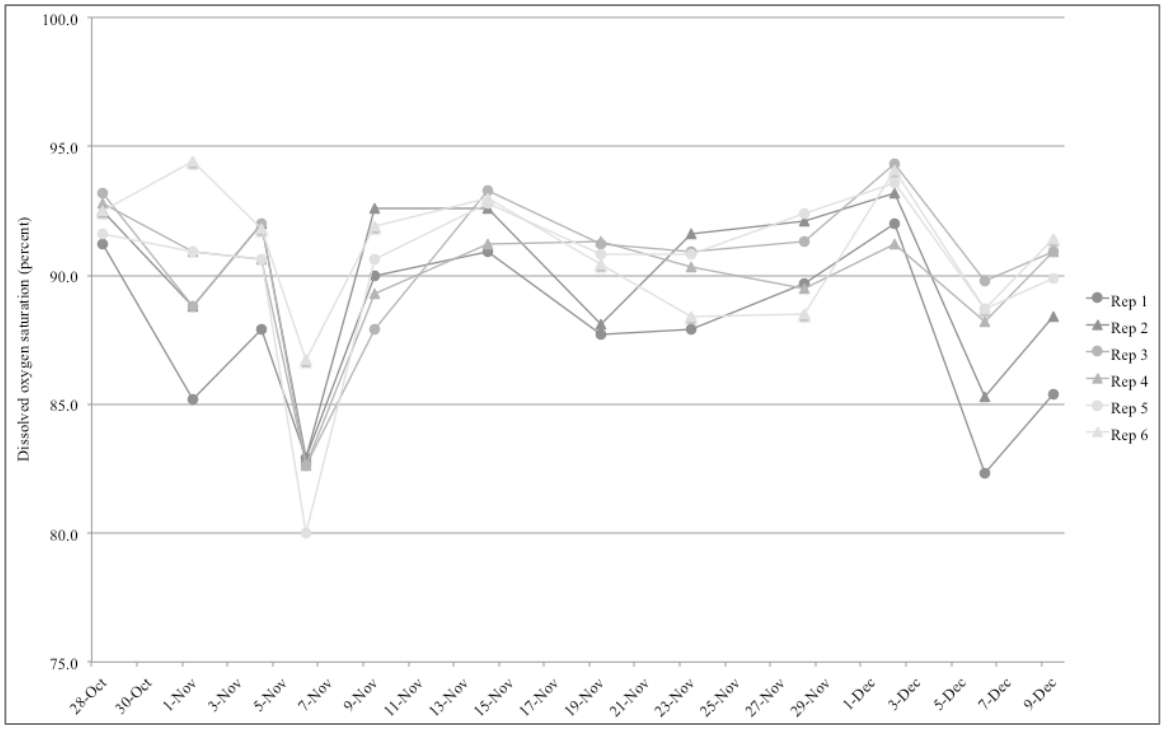
Dissolved oxygen (DO) concentration and saturation level

Figure 11. Dissolved oxygen concentration per replicate.



The concentration of dissolved oxygen remained high in all replicates throughout the trial, varying between 6.61 mg/L and 8.18 mg/L in terms of concentration and between 80.0% and 94.4% in terms of saturation (Figures 11 and 12). Abundant oxygen levels were maintained by the falling action of water with no need for supplementary aeration. Based on source data, significant differences in DO levels were not observed between treatments (p=0.48 and p=0.44 for concentration and saturation, respectively).

Figure 12. Dissolved oxygen saturation level per replicate.



Economic benefit from alternative aquafeeds

The economic analysis explores the effect of an alternative aquafeed on aquaponic farm revenue. The alternative aquafeed is assumed to result in equal or greater crop yield and equal or lower fish yield as compared to a standard aquafeed, such as the fishmeal-based control aquafeed in the technical aspect of the study. The standard aquafeed is one that would typically be used in a commercial aquaponic operation although it is formulated for fish-only aquaculture.

First we consider Scenarios 1a and 1b that represent a commercial aquaponic farm generating revenue solely from the sale of crops, with no harvest of fish. The percentage increase in revenue generated through the adoption of the alternative aquafeed is equal to the percentage increase in crop yield (Table 7). For example, Storey (n.d.) reports that

each grow tower at the Bright Agrotech aquaponic farm generates 60 pounds of basil or 62.4 pounds of lettuce per year. Under Scenario 1a we assume a price of $3 per ounce for basil (high-value crop) and annual revenue of $2,880 per grow tower planted in basil. Similarly, under Scenario 1b we assume a price of $3 per pound for lettuce (low-value crop) and annual revenue of $187.20 per grow tower planted in lettuce. It is clear that for a commercial aquaponic farm whose revenue relies solely on crop sales, an aquafeed that improves crop yield will proportionally improve crop revenue.

Table 7. Additional revenue generated from improved crop yields from adoption of an alternative aquafeed, under Scenarios 1a and 1b.

Change in crop yield	*0%*	*+10%*	*+20%*	*+30%*	*+40%*	*+50%*
Additional revenue:						
Scenario 1a	$0	$288	$576	$864	$1,152	$1,440
Scenario 1b	$0	$18.72	$37.44	$56.16	$74.88	$93.60
Change in revenue	0%	+10%	+20%	+30%	+40%	+50%

Next we consider Scenario 2, a slightly more complex situation based on data from the experimental commercial aquaponic facility at the University of the Virgin Islands (UVI). Bailey et al. (n.d.) estimate that each UVI unit generates $17,056.00 and $36,400.00 in revenue from tilapia and lettuce production, respectively, per year. Thus, tilapia and lettuce production contribute 31.91% and 68.09%, respectively, to the total gross annual revenue per unit ($53,456.00). For this analysis, we consider the possibility that adoption of the alternative aquafeed reduces fish yield while increasing crop yield as compared to the standard aquafeed. The change in total revenue is given by

$$\Delta \mathrm{TR} = \Delta \mathrm{Y_C} \cdot \frac{R_C}{\mathrm{TR}} + \Delta \mathrm{Y_F} \cdot \frac{R_F}{\mathrm{TR}}$$

where TR is total revenue; Y_C and Y_F are yields of crops and fish, respectively; and R_C and R_F are revenues from crops and fish, respectively. Thus, assuming no changes in prices for fish and crops, the change in total revenue is calculated as the sum of the products of the relative contribution of each source of revenue to the total and the corresponding change in yield from adoption of the improved feed.

Table 8. Percentage change in total revenue as a function of changes in crop and fish yields from adoption of an alternative aquafeed, under Scenario 2.

Change in crop yield (ΔY_C)	*0%*	*+10%*	*+20%*	*+30%*	*+40%*	*+50%*
Change in fish yield (ΔY_F)						
-80%	-25.5%	-18.7%	-11.9%	-5.1%	1.7%	8.5%
-60%	-19.1%	-12.3%	-5.5%	1.3%	8.1%	14.9%
-40%	-12.8%	-6.0%	0.9%	7.7%	14.5%	21.3%
-20%	-6.4%	0.4%	7.2%	14.0%	20.9%	27.7%
0%	0.0%	6.8%	13.6%	20.4%	27.2%	34.0%

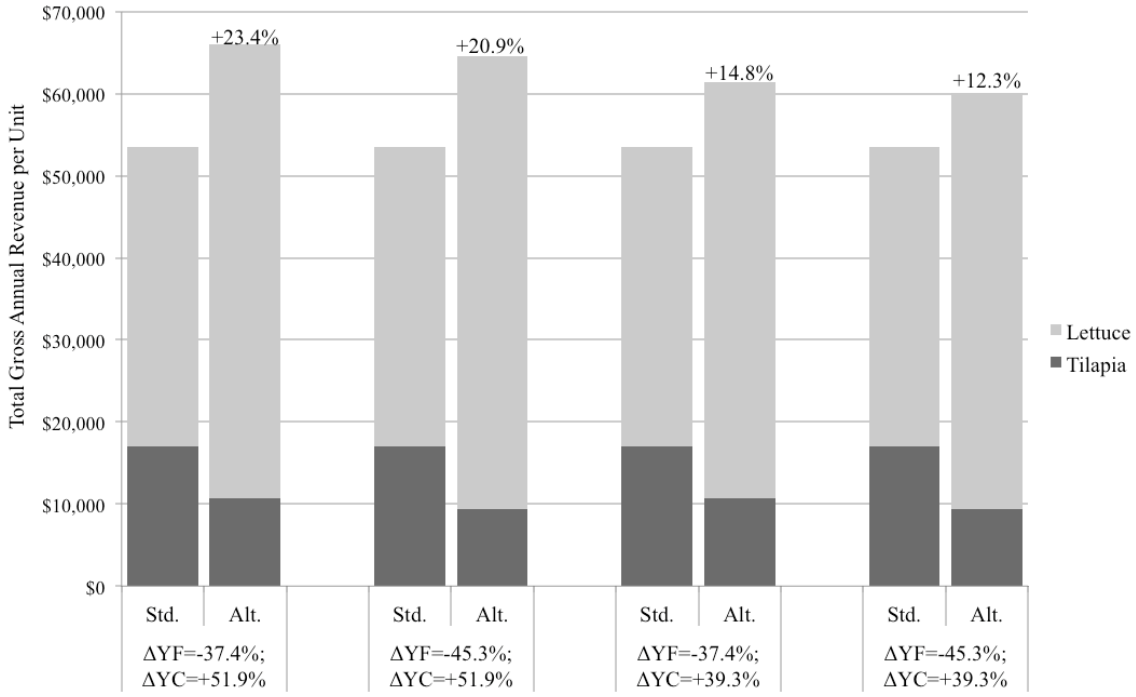
Based on the UVI model, the percentage change in total annual revenue at various levels of change in fish and crop yields from adoption of the alternative aquafeed is presented in Table 8. Under the least favorable condition, with no improvement in crop yield and 80% reduction in fish yield, total revenue decreases by 25.5%; under the most favorable condition, with no change in fish yield and 50% improvement in crop yield, total revenue increases by 34.0%. Given reductions in fish yield of 80%, 60%, 40% and 20%, the percentage increases in crop yield required to maintain total revenue in the UVI model are 37.49%, 28.11%, 18.74% and 9.37%, respectively.

Finally, we apply fish and crop yield results from the technical aspect of the study to the UVI model, in which 31.91% and 68.09% of revenue is derived from fish and crop sales, respectively. Given a 37.4% reduction in fish yield and a 51.9% increase in crop yield (based on means of normalized net fish growth observations and normalized leaf biomass observations during the experimental trial), the UVI model would see a $6,378.94 decrease in revenue from tilapia sales per unit and an $18,891.60 increase in revenue from lettuce sales per unit. Therefore, total revenue would increase by 23.4%, or $12,512.66 per unit, by adopting the alternative feed. By removing experimental "outlier" observations, we may consider more conservative estimates of changes to fish and crop yields: 45.3% reduction in fish yield and 39.3% increase in crop yield. Combinations of each of these percentage-change values and their effects on total revenue are presented in Figure 13. Under the most conservative estimate (ΔY_F=-45.3%; ΔY_C=+39.3%) based on the experimental results, total gross annual revenue for the UVI model increases by 12.3%, or $6,578.83 per unit.

Although aquafeed cost is not included in this analysis, we consider it likely that an alternative feed could be produced at a lower cost than standard feeds, assuming similar scales of production, since fishmeal is substantially more expensive than many alternative protein sources (El-Sayed, 1999). A lower feed cost would further enhance aquaponic farm profit by lowering feed costs. The results of this analysis suggest that the development of aquafeeds specifically formulated to enhance crop yields at commercial aquaponic farms would be worthwhile once the scale of commercial aquaponics is adequate. The economic benefit from an alternative, crop-enhancing feed would likely be shared between aquafeed producers (in the form of a higher price for aquafeed) and

aquaponic farmers (in the form of higher revenue from crop yields), depending on the price elasticity of supply and demand for the feed.

Figure 13. Change in total revenue under Scenario 2, based on experimental results.



CHAPTER V

CONCLUSION

The leaves and stems of *Amaranthus tricolor* provide an inexpensive and rich source of protein, minerals, vitamins and fiber to people in Asia, Africa and the Caribbean (O'Brien & Price, 1983; Prakash & Pal, 1991). The trial demonstrates that aquaponic cultivation of *A. tricolor*, like many other leafy crops, is possible and potentially highly productive. After a 60-day growing period, Replicates 1 through 4 yielded 1803.1 g of shoot biomass (stems and leaves) in approximately 2 m^2 of growing area, which translates to 901.5 g m^{-2} or 3648.2 kg $acre^{-1}$. Data on *A. tricolor* yields under field cultivation are extremely limited. However, Shukla et al. (2006) achieved a mean yield of 1.80 kg m^{-2} under intensive field cultivation in subtropical India, with leaf cuttings harvested every 15 days during a season of unspecified length (likely 4 months or more). This suggests that yield from aquaponic cultivation of *A. tricolor* may meet or exceed yield from field cultivation.

Total ammonia-N concentration, dissolved oxygen concentration, and pH consistently remained within acceptable ranges with no significant differences observed between treatments. Abundant oxygen levels were supplied by the falling action of water with no need for supplementary aeration. TAN was often undetectable and remained below 0.30 mg/L during the trial, indicating quick and effective biological nitrification by the biofilter. The pH level decreased over the course of the trial but remained near neutral. Over a longer term, periodic supplementation with a base such as potassium hydroxide may have become necessary to raise the pH level.

Concentrations of nitrate and TDS in the culture water were significantly higher under the control treatment (fishmeal-based aquafeed), and no significant difference in orthophosphate concentrations was observed between treatments. The results suggest that higher concentrations of nitrate and TDS in the culture water are not necessarily associated with higher plant productivity and imply that the effluent produced from the fishmeal-based aquafeed contains some unobserved limiting nutrient. Because fish add nutrients to the culture water and plants remove nutrients from the culture water, a more precise study of the nutrient dynamics would require additional replicates in which no plants are cultivated. Thus the accumulation of nutrients under each feed treatment would serve as a basis for comparison without the confounding effect of nutrient uptake by plants.

Due to unanticipated plumbing problems in Replicates 5 and 6, plants in these two replicates were substantially smaller than plants in the other four replicates by the end of the trial. In order to remove the statistical error caused by these plumbing problems, Replicates 5 and 6 were assigned to a separate covariate group. After this statistical adjustment, amaranth crop yield was found to be significantly higher ($p<0.05$) under the plant-based aquafeed treatment in terms of plant height, number of leaves, leaf biomass, shoot biomass and total biomass. It should be noted, however, that the assignment of replicates to covariate groups was based on informal observation of hydroponic water levels without data to quantify the differences. Nonetheless, the results demonstrate that a plant-based aquafeed can achieve similar, if not superior, aquaponic crop yields with lower inputs of N and P as compared to a high-protein, fishmeal-based aquafeed.

The adoption of a lower-cost, low-protein aquafeed that improves crop yield would clearly benefit aquaponic farmers who focus on crop production. However, aquaponic farmers who depend upon fish revenue would face a trade-off if the low-protein aquafeed extends the fish grow-out period or otherwise reduces fish yield. Based on revenue projections from the experimental aquaponic facility at UVI, adoption of the plant-based aquafeed tested in this study would increase total revenue by up to 23.4% through improved crop yields that would more than compensate for the loss of revenue from reduced fish yields.

Further research into the effect of aquafeed on aquaponic crop productivity could provide insight into the nutritive quality of aquaponic crops by testing for differences in the concentrations of vitamins, minerals and other nutrients in cultured crops between feed treatments. These results could also be compared to nutrient levels in crops from field and hydroponic cultivation.

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APPENDICES

APPENDIX I

Nutrient content of fishmeal-based feed (control treatment):

Crude protein: Minimum 40%
Crude fat: Minimum 10%
Crude fiber: Maximum 4%
Moisture: Maximum 12%
Ash: Maximum 8%
Phosphorus: Maximum 1.12%

Ingredients include marine protein and oil products, processed grain and vegetable products, processed poultry by-products, vitamins (including stable vitamin-C), minerals and amino acids.

(Source: Zeigler web site, http://www.zeiglerfeed.com/product_literature/aquaculture%20literature_finfish/Finfish%20Silver.pdf)

Nutrient content of plant-based feed (alternative treatment):

Crude protein: Minimum 32%
Crude fat: Minimum 4.68%
Crude fiber: Maximum 8.70%
Moisture: Maximum 8.0%
Lysine: Minimum 1.7%
Calcium: Minimum 0.70%, Maximum 1.20%
Phosphorus: Minimum 0.40%

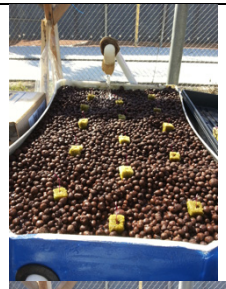
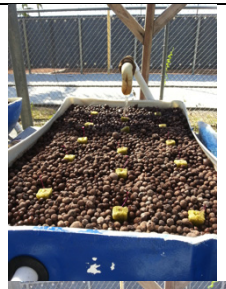
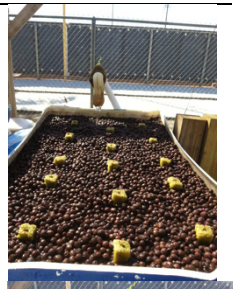
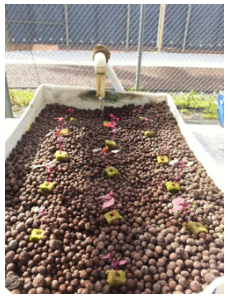
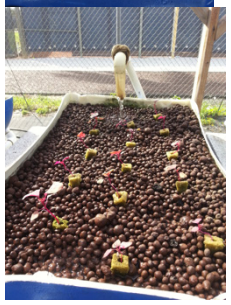
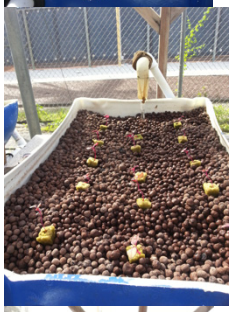
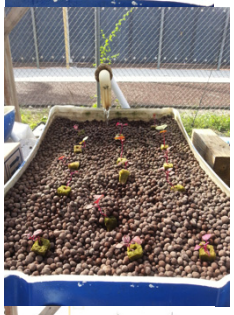
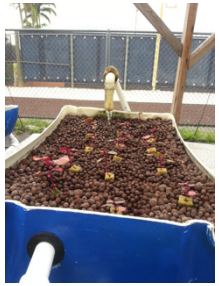
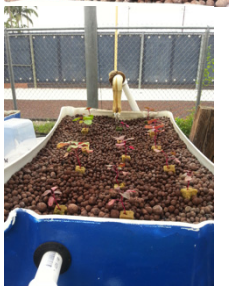
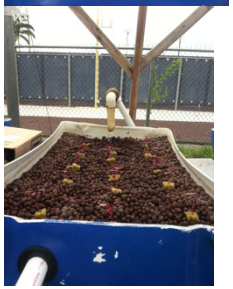
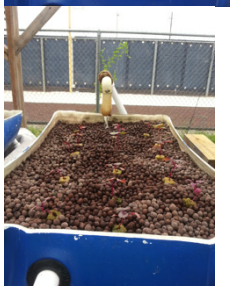
Ingredients: Organic Fabaceae, Organic Poaceae, Organic Rice Bran, Organic Canola Meal, Organic Corn, Organic Flax Meal, Dicalcium Phosphate, Lactobacillus Acidophilus Fermentation Product, Reed Sedge Peat, Monosodium Phosphate, Magnesium Oxide, Sodium Sulfate, Manganous Oxide, Folic Acid, Nacin, Choline Chloride, Biotin, Riboflavin, Vitamin A ACETATE, Vitamin B12, Vitamin D3, Vitamin E, Calcium Pantothenate, Ethylenediamine Dihydriodide, Beta Carotene, Pyridoxine Hydrochloride, Ascorbic Acid, Yeast Culture, Thiamine Mononitrate, Ferric Choline Citrate Complex, Organic Dried Kelp, Zinc Amino Acid Complex, Cobalt Choline Citrate Complex, Salt, Copper Choline Citrate Complex, Manganese Amino Acid Complex, Potassium Chloride, Attapulgite Clay, Organic Grape Seed Extract, Organic Lecithin, Enzyme Product, Organic Aloe Vera Juice, Calcium Carbonate, Sodium Selenite, Citric Acid, Calcium Hydroxide, Copper Sulfate Pentahydrate, Zinc Sulfate Monohydrate, Manganese Sulfate, Organic Garlic, Diatomaceous Earth, Organic

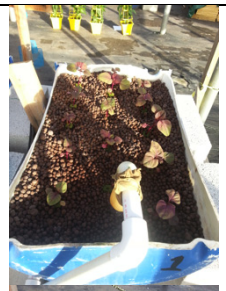
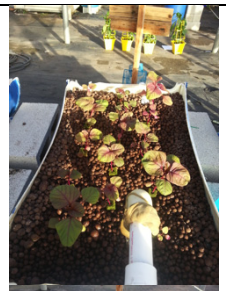
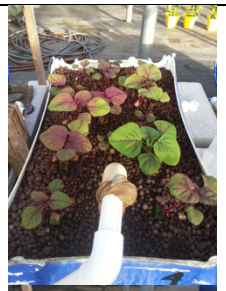
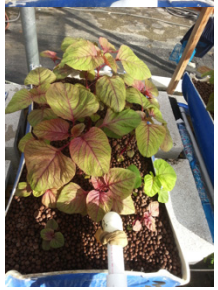
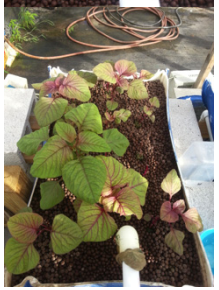
Dried Whole Milk, Organic Sugar, Potassium Citrate, Calcium Sulfate, Magnesium Sulfate, Activia Natural Source Mg, Fe, K, Organic Dry Whole Egg, Organic Tomato Powder, Organic Sources used (Orange Peel Powder, Cayenne Pepper, Dandelion Root, Dandelion, Cloves, Sage, Peppermint, Fennel, Hops, Parsley, Thyme, Lemon Grass, Elder Flowers, Chamomile Flowers, Licorice, Basil, and Ginger) for water extracts using Fermentation Acids, Organic Oat Groats, Yucca Schidigera Whole Plant Product, Organic Gelatin, Zinc Sulfate, Yeast, Granite Dust, Perfect Food Raw, Ferrous Sulfate, Zinc Oxide, Sulfur, Organic Rice Protein, Copper Sulfate, Cobalt Carbonate.

(Source: The Aquaponic Store web site, http://www.theaquaponicstore.com/AquaOrganic-Fish-Feed-5-Pounds-p/affab001.htm)

APPENDIX II

Photos of replicates during trial.

Replicate	1	2	3	4	5	6
Day 8 (Oct 18)						
Day 23 (Nov 2)						
Day 29 (Nov 8)						

Replicate	1	2	3	4	5	6
Day 37 (Nov 16)						
Day 43 (Nov 22)						
Day 53 (Dec 2)						

Day 60

(Dec 9)