

BIOMASS PRODUCTION AND NUTRIENT DYNAMICS IN AN AQUAPONICS SYSTEM

by

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DEDICATION

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ABSTRACT

The goal of this study was to prove that aquaponic systems can produce lettuce of equal growth and quality compared to hydroponic lettuce production and to determine the stocking density of fish required for plant growth. Aquaponics is the integration of recirculating aquaculture and hydroponic plant production. The project had four objectives. The first objective was to determine the biomass of fish required for plant growth to develop a fish to plant density ratio. The second objective was to compare lettuce grown with aquaponic water and a hydroponic solution under the same environmental conditions. The third objective was to compare the quality of lettuce grown with aquaponics water plus nutrient supplementation with a hydroponic solution. The fourth objective was to determine the nitrogen dynamics in the aquaponic system and to compare the nutrient composition of lettuce grown with aquaponics water with nutrient supplementation and hydroponic solution. It was determined that under the specified environmental conditions 5 kg m^{-3} of Nile tilapia (*O. niloticus*) fed 2% of their body weight daily yields on average 4.7 kg m^{-2} of lettuce (*L. sativa* cv. Rex) in 35 days. There was no significant difference ($p \leq 0.05$) in biomass or chlorophyll concentration index in lettuce (*L. sativa* cv. Rex) grown with aquaponics water and nutrient supplements versus a hydroponic solution. The aquaponics solution generated equal biomass and chlorophyll concentration indexes compared to the hydroponic solution. Aquaponics water plus supplementation can yield *L. sativa* cv. Rex with equal biomass accumulation and chlorophyll concentration indexes compared to hydroponics lettuce. Nutrients added to the aquaponics system consisted of iron, manganese, and zinc. These nutrient concentrations became depleted in the aquaponics water over time and were not

replenished via the fish feed. Dolomite was added to the aquaponics system every two weeks to increase the buffering capacity of the water and maintain optimal pH levels. Aquaponics lettuce had similar nutrient composition to hydroponic lettuce. One head of *L. sativa* cv. Rex (176.75 ± 31.03) will assimilate approximately 5.96 grams of nitrogen (3.38% per dry gram lettuce). One kilogram of fish will yield 6.4 lettuce heads (1,128 grams) and fixate 38.13 grams of nitrogen.

INTRODUCTION

PROBLEM STATEMENT

The world population is growing fast and as of November 2009 is approximately 6.8 billion, while 308 million people reside in the United States (U.S. Census Bureau 2009). Water and land resources for agriculture are diminishing and world fisheries are at or past their maximum sustainable yields. To feed humanity for the next 40 years it is speculated that more food must be produced than all the food produced since the beginning of recorded history (Parker 2002). Extractive resources are taken from nature and then consumed in some applications for food or energy. Renewable resources such as trees and fish exist in finite quantities at any point in time but can regenerate (Rasband et al. 2004). Fisheries are an extractable renewable resource when managed properly. Recent consumption and demand for seafood primarily driven by technological fishing development and market demand (arising from recent knowledge of the health benefits from fish and increasing populations) has led to a mismanaged system. There are multiple reasons for the mismanagement of fisheries. First, increases in consumer demand has skyrocketed past what natural fish stocks can support. Second, pollution, climate change and the lack of global enforcement in fisheries management has led to severely depleted fish stocks. Some predict commercial fish stocks to disappear within the next decade or even sooner if problems are not addressed (FAO 2007). The majority of commercially fished species are at or past their maximum sustainable yields meaning that fish populations can not naturally rebound on their own. Aquaculture yields promise for sustaining fishery resources. However this can not be done by simple mass production via aquaculture. Environmental problems that arise from aquaculture include: biological

pollution (escaped fish); fish for fish feed ingredients, farm discharge pollution leading to eutrophication, habitat modification and chemical pollution from antibiotics and pesticides. Aquaculture practices must be coupled with sound environmental and fisheries policies to yield a productive and sustainable management system.

Aquaculture is the fastest growing sector of food production producing over 60 million metric tons of products in 2005 (FAO 2007). This will only continue to rise as demand for seafood is increasing and fisheries are being depleted. In aquaculture, as with livestock or poultry, water is not consumed but the biochemical nature of water is altered. In the United States aquaculture has increased in importance. The U.S. imports more seafood than it exports resulting in a \$9.2 billion seafood import deficit (DOC 2007). Seafood consumption in the U.S. has increased to over 16 pounds per person in 2005 and is now third in the world behind China and Japan respectively (FAO 2005). The recent growth of organic farming has supported the use of fish and fish byproduct fertilizers over conventional synthetically derived chemical fertilizers. Water is a precious resource and becoming a scarcity relative to human demand. Sustainability of agriculture and economic activities that require water can only be achieved through proper management practices (Postel 2002). Water is one of the essential commodities for sustaining life and producing food. Demand for food is increasing with an increase in human population. Water and land resources are decreasing and as demand for their utilization to produce food increases their value will increase. Resources used in farming of food consist primarily of water, land, and feed. All three of these resources are limited. Resources used in farming of food consist primarily of water, land, and feed. All three of these resources are limited. Agriculture accounts for approximately 70% of the global water

use (Rogers 2008). Agricultural runoff contributes to phosphorous and nitrogen pollution of surface waterways (Fitzsimmons and Posadas 1997). Elevated nitrogen and phosphorous levels have been documented to pose negative impacts on aquatic ecosystems. Farm irrigation is one of the major sinks for freshwater in the world. It is speculated that a 10% drop in farm irrigation water can save more water than water used by municipalities and public consumers (Rogers 2008).

Improving water use by agriculture and food production is critical in order to supply the demand for food in the future (FAO 2003). The FAO has addressed some primary issues in regards to food production and water use. Some of the target issues of the FAO are the sustainable development and management of rain fed and irrigated agriculture, the modernization of irrigation practices to demand-oriented management, improving governance of agriculture water through integrated, efficient and equitable water resources, the development of international cooperation, and the development of research in the field of food production technologies to maximize water use efficiency. Establishing proper water management practices will lead to an increase in water use efficiency and a reduction in environmental pollution. Rainwater collection and utilization could help alleviate the demand for water. However, it will take multiple efforts on all fronts to have a significant impact on water use efficiency. Farm irrigation is one of the major sinks for freshwater in the world. Some aspects of agriculture that can be changed to help conserve water are the implementation of new technologies such as drip irrigation, stringent crop management techniques, proper fertilization applications and integrating farming systems. The face of farming is changing and the future of farming resides in the sustainable production of nutritionally substantial foods.

Aquaponic systems and integrated farming practices will yield sustainable and environmentally sound farming methods.

LITERATURE REVIEW

Aquaculture

Tilapia is the fourth most consumed aquacultured product in the world (FAO 2005). Catfish and Salmon are the only two aquacultured fish consumed more than Tilapia (Kohler 2000). There are numerous types of commercial farming applications ranging from extensive systems to very intensive systems. The type of system developed for farming tilapia is dependent on the experience and knowledge of the farmer, geographic locale, resource acquisition, potential marketing and distribution as well as startup funding. Tilapias are native to Africa and the Middle East (Fitzsimmons and Posadas 1997). World wide production of Tilapia has increased from 855,000 metric tons (1.8 billion pounds) in 1990 to 1,100,000 metric tons (2.2 billion pounds) in 1994. South American countries primarily export Tilapia to the United States. Tilapia imports have increased steadily over the past decade. U.S. imports have increased from 7.5 million pounds in 1992 to 41.9 million pounds in 1996 (Fitzsimmons and Posadas 1997). In 1992 89% (0.8 million pounds) of the tilapia imports were whole frozen fish whereas in 1996 20% (8.4 million pounds) of the imports were fresh fish marketed to local fish markets (Fitzsimmons and Posadas 1997). The increase in Tilapia imports can be attributed to the decrease in wild fish populations as well as the advent of aquaculture operations. Tilapia consumption has steadily increased through 2002 to over 150,000 metric tons/year (Parker 2002). The primary marketing targets for commercially produced Tilapia are the

United States, Europe and Japan. Tilapia has been successfully marketed to tourist areas, restaurants, and fish markets and is currently in demand in the United States.

The majority of tilapia species farmed outside of Africa is of the genus *Oreochromis*. Nile tilapia (*O. niloticus*) comprise up to 90% of the species being commercially farmed (Popma and Masser 1999). Other commercially farmed tilapia species are the Blue tilapia (*O. aureus*) the Mozambique tilapia (*O. mossambicus*), and the Zanzibar tilapia (*O. urolepis hornorum*) (Popma and Masser 1999). Several species of Tilapia are raised in the United States. The most popular species are the Nile Tilapia (*O. niloticus*). Tilapia are laterally compressed, deep bodied and have an interrupted lateral line. Nile tilapias generally have prominent vertical bands while mature male Nile tilapia develops gray or pink pigment on the throat area. (Popma and Masser 1999). The Chinese have been practicing integrated fish and plant culture system for centuries. China is the leader in aquaculture production and one of the major importers of frozen and fillet tilapia into the United States. In the United States the majority of domestic aquaculture production of tilapia is sold to the live fresh fish market (Fitzsimmons 2006).

Tilapia has many favorable characteristics for aquaculture production. Tilapia can tolerate poor water quality. Wide salinity ranges, water temperature ranges, low dissolved oxygen levels, and elevated ammonia concentrations have less effect on tilapia than other fish species grown in commercial farming operations (Popma and Masser 1999). Tilapia consumption has risen annually in the U.S. and is currently one of the top five fish consumed per capita (Fitzsimmons 2006). There has been a shift in consumption from red meat to fish because it contains higher quality proteins and is low in fat. A 100 gram serving of tilapia contains 96 calories and is a good source of protein (20 g) and Vitamin

B12 that is low in total fat (2 g), sodium (52 mg), and cholesterol (50 mg) (www.nutritiondata.com). It is mild in flavor, consumed globally, absorbs seasonings well, is easy to raise and has a relatively quick turnover time compared to other species. In the U.S the majority of tilapia production is sold to live markets, or fresh on ice, to obtain a more competitive price. The physiology, behavior, nutrition and genetics of tilapia have been well studied over the years. Tilapia are a hardy fast growing fish with a low protein requirement making them a primary target for aquaponic recirculating systems. Tilapia fish are omnivorous and have a relatively low protein requirement in comparison to other carnivorous aquacultured fish (Fitzsimmons 2006). They are omnivorous consuming a wide range of organisms including phytoplankton, zooplankton, aquatic macrophytes, algae, benthic invertebrates, larval fish, detritus and decomposing material (Popma and Masser 1999).

Tilapia require the standard ten amino acids that other fish species require. Protein quantity and quality are required for sufficient and optimal growth. Most commercial tilapia feeds comprise approximately anywhere from 28-36% protein depending on life stage and age of the fish. Digestible energy of tilapia is estimated to be 8.2 to 9.4 kcal of digestible energy (DE) per gram of dietary protein (Popma and Masser 1999). Vitamin requirements are similar to that of other fish species as well. Protein is the most expensive feed ingredient in fish food and often consists of wild anchovy meals, yeast meals, and animal meals. Tilapia are able to digest plant based proteins allowing for substitution of plant based protein sources for more expensive animal based protein sources (Watanabe et al 2002). Fish aquaculture systems can provide a consistent organic

source of nutrients for the hydroponics plants. Aquaponics and farm integration can reduce the environmental impacts from agriculture and aquaculture farming.

Lettuce Hydroponics

Hydroponics is the culture of plants in a soil less media. There are numerous advantages of hydroponic food production systems over conventional agriculture. The ability to produce vegetables all year round enables growers to obtain high prices for vegetables out of season when supply is decreased. In hydroponic systems the plants roots reside in a hydroponic solution formulated for optimal plant growth (Hanan 1998). The solution can be tailored to a specific species to yield optimal growth. Plants grown in a hydroponic solution can yield greater growth rates and be of higher quality in comparison to conventional agriculture. Hydroponic systems will also produce more consistent crops. Production of hydroponic systems can be ten times greater than conventional agriculture production (Resh 2001). Another advantage of hydroponic production methods is that the risk of soilbourne viruses is reduced because the plants reside in an aquatic medium. There are many soilbourne viruses that effect crops of economic viability. Lettuce dieback is a disease caused by at least two soilbourne viruses in the family Tombusviridae: lettuce necrotic stunt virus (LNSV) and tomato bushy stunt virus (TBSV) (Obermeier et al, 2001). Symptoms include chlorosis and necrosis in older leaves and eventually plant stunting and death (Grube & Ryder 2003). Reports of lettuce dieback have been increasing in California and Arizona which account for over 95% of lettuce production in the United States (U.S. Department of Agriculture 2002). The Romaine lettuce industry has suffered from this virus and research into has not warranted

a cultivar resistant to the disease. Red and green leaf lettuce cultivars have also suffered from dieback but have not yet been documented in iceberg lettuce.

Lettuce is commonly cultured and grows well in hydroponic and aquaponic systems. Typical types of lettuce grown are bibb, looseleaf, iceberg, and romaine. Looseleaf varieties are the easiest to grow and can tolerate daytime temperature of 27°C without bolting, wilting or slow growth. Spacing can be 10 to 30 heads per square meter. Lettuce has a four to five week vegetative growth phase to harvest. A profit can be realized in short time in comparison to tilapia (4-6 weeks). Lettuce (*Lactuca sativa*) is a common plant used in hydroponic systems. It is a hardy plant that has a fast growth rate (Resh 2001). Lettuce is the first salad crop to be cultivated commercialized internationally. Lettuce has the ability to accumulate nitrogen and phosphate. The nitrogen and phosphate is assimilated from the solution medium (Resh 2001). Lettuce also has a quick growth cycle and can be harvested within four to five weeks allowing for quick realized profit and turnover of nutrients in the system (Rakocy et al. 2006). This makes lettuce a good target crop for aquaponic systems with a heavy bio-load and thus high nitrogen accumulation. Biotic (genetics, growth, and disease) and abiotic (temperature, light, water potential, nutrient availability) factors influence growth and development of plants. These factors can also influence pigment concentrations in plants. Lettuce grows best at air temperatures between 16 – 25°C and will bolt in air temperature above 25 – 28°C (Resh 2001). Day temperatures of 24°C and night temperatures of 19°C coupled with photosynthetic active radiation (PAR) levels of 17 mol/m² per day were found to produce marketable lettuce heads in 24 days after transplant (Both et al. 1994). Lettuce will bolt (flower) at high air temperatures (25-28°C) thus making it hard to grow

in warm climates and seasons (Resh 2001). Research has shown that by keeping the root zone temperatures at optimal levels even though air temperatures are high proper growth can be achieved. Iceberg lettuce was able to grow properly and form compact heads when root zone temperatures were kept at 15-17⁰C even though air temperature was 25-39⁰C (Marsic and Osvald 2002).

Temperature affects the pigment stability of lettuce. Higher temperatures lead to increased pigment degradation (Shaked-Sachray et al 2002). Coloration and nutritional value are important marketing parameters of lettuce. Coloration (intensity and uniformity) and nutritional value are linked to chlorophyll and anthocyanin levels (Simonne et al, 2002). Anthocyanin and chlorophyll concentrations in lettuce are effected by the plants genetics as well as the growing conditions (Dela et al, 2003). Genotype, temperature, and light can influence the shifting of pigment levels independently of one another or synergistically (Crozier et al, 1997). Temperature can affect anthocyanin and chlorophyll levels in lettuce negatively impacting both quality and nutrition. Day and night air temperature fluctuations (30/20⁰C) result in higher anthocyanin and chlorophyll b concentrations than a constant (30 or 20⁰C) day/night air temperature (Gazula et al, 2005). This suggests that the effects on quality and nutrition from high day air temperatures can be mitigated with low night air temperatures. Temperature affects the pigment stability. Higher temperatures lead to increased pigment degradation (Shaked-Sachray et al 2002). A marketable lettuce head weighs a minimum of 150 grams and can be achieved in 3-4 weeks after transplant under optimal growing conditions (Both et al. 1994; Resh 2001).

Nutritional requirements of plants vary at different stages of development. Nitrogen is the most essential nutrient that drives plant growth and elongation. Nitrogen is part of a large number of vital organic compounds such as amino acids, proteins, coenzymes, nucleic acids, and chlorophyll (Resh 2001). Phosphorous levels greater than 15-30 mg kg⁻¹ are sufficient for most agronomic crops (Ludwick, 2002). Lettuce has a higher phosphorous requirement for maximum growth. Phosphorous plays a vital role in the synthesis of organic compounds such as sugar phosphates, ATP, nucleic acids, phospholipids and coenzymes (Resh 2001). Phosphorous requirement thresholds range from 35 mg kg⁻¹ (Ludwick 2002) to 80 mg kg⁻¹ (McPharlin et al, 1996) for lettuce. This can vary depending on growing media and season. A deficiency in phosphorous will stunt growth and flowering or fruit set. Iron has been found to be the limited nutrient in recirculating aquaponic systems (Fitzsimmons and Posadas 1997). Therefore a recirculating aquaponics system will need iron supplementation over an extended period of time. Iron is required for chlorophyll synthesis and is an essential part of cytochromes which are electron carriers in photosynthesis and respiration (Resh 2001).

Aquaponics

Aquaponics is the integration of recirculating fish production systems with hydroponic plant production to utilize the fertilizers efficiently. The integration of these two systems leads to the removal of nutrients (primarily nitrates and phosphates) from the system omitting the need for water changes thus conserving water. However, water is needed to fill the initial system. Dissolved nutrients from fish are similar to the nutrients required for hydroponic growth of plants (Rakocy et al. 2006). Aquaponics is the most

efficient food production system in terms of amount of product produced per volume of water (Table 1). It takes approximately 500 liters of water to produce \$100 of product (fish and lettuce), whereas producing cattle takes more than 100 times as much water to produce a \$100 of product (Rakocy et al. 2004).

Aquaponic systems recirculate water to utilize nutrients efficiently thus producing food in a sustainable manner with little environmental impact. Removal of nutrients from fish effluent via plant nutrient uptake is an efficient and productive method of filtration. The production of fish and vegetables through the integration of fish aquaculture and plant production has been demonstrated (Fitzsimmons 1991; Fitzsimmons 1992; Rakocy et al. 1993; McMurtry et al. 1997; Chaves 2000; McIntosh and Fitzsimmons 2003; Sabidov 2004; Castro et al. 2006; Diver 2006). Coupling fish aquaculture with hydroponic plant culture is more sustainable than conventional agriculture systems (McMurtry et al. 1990; Fitzsimmons 1992; Rakocy et al. 1992; Rakocy and Nair 1987; Rakocy and Hargreaves 1993). Popular food crops such as lettuce, basil, tomatoes and strawberries, have been successfully cultivated with the use of fish effluent as the primary fertilizer (McMurtry et al. 1997, Takeda et al. 1997, Rakocy et al. 2004, Hanson et al. 2008). The integration of recirculating aquaculture systems (RAS) with hydroponic plant production is referred to as aquaponics. The primary resources used in animal or crop production are water, nutrients, light, and land. Intensive RAS can produce more fish per liter of water than other types of aquaculture systems (Timmons et al. 2002) therefore reducing water used. Greenhouse hydroponics production can produce from five to ten times more output compared to conventional agriculture (Resh 2001; Hannan 1998). It takes approximately 100 liters of water to raise a fish in a recirculating aquaculture

Commodity	Liters of Water
Rice	470,000
Cotton	160,000
Milk	147,000
Sugar	123,000
Beef Cattle	81,200
Vegetables and Fruit (soil)	37,900
Wheat	24,500
Hydroponics	600
Aquaponics (fish and lettuce)	500
Aquaponics (fish and basil)	173

Table 1. Amount of water required to produce \$100 of output of commodity (Rakocy et al. 2004).

system (Timmons et al. 2002). Water loss in fish systems occurs mainly from water changes and/or discharge or evaporation. In hydroponic lettuce production systems one liter of water will yield on average 3.5 grams of dry lettuce biomass (Gonnella et al. 2002; Gonella et al. 2001). Preliminary studies at The University of Arizona's aquaponics research greenhouse determined that it takes less than a liter of water per lettuce head, or approximately 4.2 liters of water per kilogram of head wet weight of lettuce. The integration of fish and plant systems can potentially reduce the amount of water used per kilogram of food produced. Aquaponic systems can yield similar crop production to hydroponic systems (Sabidov 2004). Water from RAS systems can be used in greenhouse hydroponics to intensify production by utilizing resources more efficiently potentially reducing water usage by 20-27% compared to conventional agriculture (Chavez et al. 2000).

Lettuce (*Lactuca sativa* cv.) is commonly cultured in hydroponic and aquaponic systems. Lettuce has a four to five week vegetative growth phase to harvest. It is a hardy plant that has a fast growth rate (Resh 2001). It was found that lettuce can deposit a large amount of nitrogen to its leaves and the nitrogen deposition can be manipulated by plant density and nitrogen availability (Seawright 1998). Tilapia can tolerate a pH from acidic to alkaline (pH 5-11) (Chervinski 1982) and a wide range of salinity concentrations (Watanabe et al. 2002). Plants grow best and uptake nutrients at a lower pH (5.5-6.5) (Resh 2001). Specifically, lettuce will grow well in a pH range of 5.5-6.5 (Resh 2001, Islam et al. 1980). Nitrifying bacteria is inhibited below a pH of 6.5, with an optimum pH of 7.8 depending on bacterial species and temperature (Antoniou et al. 1990; Tyson et al. 2007). Lettuce will exhibit normal growth at oxygen levels of the solution greater than

0.10 mM (Yoshida et al 1997). Lettuce is tolerant of lower oxygen levels in comparison to other plants. Tilapia can withstand low dissolved oxygen levels but optimal growth occurs with levels greater than 2 mg / l (Watanabe et al. 2002). Electrical conductivity levels range between 1-2 mS / cm for hydroponic lettuce production (Resh 2001), well below the levels (2000 mS / cm) that can be toxic to Nile tilapia (Timmons 2002). Optimal water temperature for Nile tilapia (*Oreochromis niloticus*) ranges between 28-35° C (Chervinski 1982) while lettuce grows best at water temperatures between 21-25°C (Resh 2001). These water parameters can influence the physiology of the organisms within the aquaponic system and must be monitored and controlled for optimal system performance.

The conceptual aspect of aquaponics is to balance the nutrients within a given system. Nutrients are delivered to the system through an input source, in this case fish feed. Protein content in the feed dictates the amount of nitrogen that is available to the plants after the fish assimilate and process the nutrients (Timmons 1996). The density of fish, protein content in the feed, and the feeding rate drive the nutrient loading of the system. Balancing the amount of nutrients produced from the fish system with the nutrient requirements of the plants can lead to optimized resource utilization and system productivity. Nitrogen is used for protein and amino acid synthesis. Proteins make up structural tissues, transport oxygen or hemoglobin, regulate reactions as hormones, and catalyze biochemical reactions as enzymes (Parker 2002; Santamaria 2002). Protein is the most costly ingredient in fish diets. Protein is the primary nutrient for fish growth and can account for more than 60 percent of fish feed cost (Hatch and Kinnucan 1993). High body fat reduces the shelf life of the frozen fish, reduces dressing percentage possibly

decreasing market quality. Increasing the protein content in the feed will improve the quality of the fish (Hatch and Kinnucan 1993). Protein has an effect on the growth of fish, quality of fish, cost of feed, and the water chemistry of the system. Aquatic animals convert feed at better rates than other terrestrial food animals (Parker 2002). Fish require less energy for body support and are cold blooded therefore expending less metabolic energy for body temperature regulation. This will yield low feed conversion ratios (FCR) thus yielding more product per feed unit. The conversion of feed to usable energy and somatic growth in fish is 25-30% (Rakocy & Hargreaves 1993). Studies by Quillere et al. (1995) revealed a 60% nitrogen recovery (fish 31% and tomato plants 28%) from an aquaponics system.

Wastewater from aquaculture farms primarily discharge water composed of nutrients and organic matter. This water is suitable for plant growth. Non-point source pollution from agriculture comprises nitrogen and causes environmental degradation (Fitzsimmons 1992). Reducing the amount of nitrogen in agriculture and aquaculture discharge water can reduce the environmental impact from both point source and non point source pollution. This can be done through proper fertilizer application rates, reducing water discharge by recirculating water, and using biological organisms to remediate the water. Water from intensive recirculating fish culture systems can have total ammonia levels up to 19.2 mg / L, nitrate levels of over 500 mg / L, phosphate levels up to 53 mg / L and potassium levels up to 150 mg / L (Fitzsimmons 1992). Phosphorous accumulation in soils occurs from heavy fertilization but does not necessarily cause agronomic problems. However agricultural runoff attributes to phosphorous pollution of

surface waterways. Elevated nitrogen and phosphorous levels have been documented to pose negative impacts on the eutrophication of water ecosystems (Sims 1998).

Fish waste and accumulated feed builds up in the system. Nitrogen, phosphorous, and organic matter accumulate in high quantities in aquaculture systems. Nitrogenous wastes are produced when nitrogen in the form of ammonia is excreted by the fish. Ammonia is the byproduct of protein synthesis by the fish. Nutrient levels from fish aquaculture are suitable for plant growth and can be manipulated by increasing fish biomass and feed rate or by increasing the protein levels in the feed. It was found that lettuce can deposit a large amount of nitrogen to its leaves and the nitrogen deposition can be manipulated by plant density and nitrogen availability (Al-Hafedh et al 2004). These levels are increased dramatically in recirculating systems and can lead to secondary effects (Timmons et al. 2002). Phytoplankton blooms can effect the oxygen concentration of the system, the nutrient dynamics, disease, and promote bacterial problems that can lead to off-flavors (Fitzsimmons 2006).

Plants use ammonia and nitrates for growth (Marschner 1995). Nitrate is taken up by the plant at better rates than ammonia nitrite can be toxic to plants (Britto and Konzucker 2002). Ammonia concentrations at elevated levels can inhibit nutrient uptake in plants by altering the ionic capacity of the water medium. Depending on plant species sensitivity symptoms of ammonia toxicity appear with external ammonia concentrations above 0.1 - 0.5 $\mu\text{mol/L}$ (Britto and Kronsucker 2002). Plants can uptake nitrate build up in fish systems serving as a natural filter that can generate profits simultaneously with the fish. Ammonia ($\text{NH}_3\text{--NH}_4^+$) is oxidized by *Nitrosomonas spp.* to nitrite (NO_2^-) (Timmons et al. 2002). Ammonia nitrogen assumes two forms dependent on the pH of the water. A

higher pH will shift ammonia concentration to the ionized form (NH_4^+) which is more toxic to fish (Timmons et al. 2002). Nitrite is then oxidized by *Nitrobacter spp.* to nitrate (NO_3^-) which is taken up by the lettuce plants for growth (Broadly et al. 2003). A mechanical filter is used to remove large particulate matter from the fish wastes prior to being processed by the biological filter. Removal of large particulate matter prior to biological filtration will reduce heterotrophic bacteria populations (Losordo 1999), thus reducing competition with nitrifying bacteria and promoting conversion of ammonia to nitrate (Timmons et al. 2002). The nitrogen is removed from the system when the plants are harvested. The nitrification process is an acid forming process which releases H^+ in turn increases the pH of the water (Timmons et al. 2002). Calcium hydroxide and potassium hydroxide, are used for pH stabilization of aquaponic systems (Rakocy et al 2006). Optimum pH for nitrifying bacteria is between 7.0-7.8 (Tyson et al. 2007). Dissolved oxygen levels should not go below 2.0 mg/L for optimal performance. One gram of $\text{NH}_3\text{-N}$ requires 4.57 grams of oxygen (Parker 2002). Temperature can also regulate the performance of nitrifying bacteria with optimum ranges from 20-30°C. The rate of nitrification (grams nitrogen per m^2 per day) for a trickling filter in relation to temperature can be expressed by the following equation $R = 140 + 8.5 * T^{\circ}\text{C}$ (Parker 2002) for nitrogen digestion and conversion. The fish component of an aquaponics system provides the water with nutrients via feeding and excretion. Nutrient byproducts from fish production systems can and have been utilized as a natural fertilizer for hydroponic plant production (Lewis 1978).

The nutrients in the system can achieve a steady state with given inputs (feed) and outputs (fish, plants, particulate matter). The concept of aquaponic systems is to balance

the nutrients within a given system so that there is no excess accumulation of nutrients, nitrogen in particular. Nutrients are delivered to the system via feed which is consumed, assimilated and excreted by the fish. Balancing the amount of nutrients produced from the fish system with the nutrient requirements of the plants can lead to optimized resource utilization and system productivity. Excess nutrients can decrease water clarity thus initiating algal, fungal or bacterial growth (Timmons 2002, Losordo et al. 1999). In aquaponics systems, dissolved waste nutrients from the aquaculture system are retained within the aquaponics system and absorbed by the plants (Rakocy et al. 2006). Therefore, it is necessary to develop an optimal ratio of fish density to plant density for an aquaponics system that can be scaled up to a commercial level. The filtration components are the pillar of which the aquaponics system is built on. Determining the nutrient inputs, the biological processes within the system, and outputs will help elucidate the efficiency and performance of an aquaponics system.

PROJECT GOALS AND OBJECTIVES

Aquaponics systems conserve water, land, and protein needed to produce an edible product. Conservation of resources (primarily water) will be essential in the future in order to produce the amount of food required to supply the world food demand. In the United States the implementation and support from government agencies to develop environmentally sound food production practices will help develop and establish the industry and potentially free up water for other uses other than food production. The goal of this study was to prove that aquaponic systems can produce lettuce of equal growth and quality compared to hydroponic lettuce production and determine the stocking

density ratios of fish and plants. The project had four objectives. The first objective was to determine the biomass production (fish and plants) that can be achieved per unit of feed to develop fish to plant density ratios. Prior research has focused on fish to plant ratios in systems that grow multiple crops, or systems that are not in controlled environments. This research will elucidate the fish to plant ratios of lettuce and determine an optimal protocol for production. A design ratio for scaling up the aquaponic system was developed. The concentration of ammonia and nitrate nitrogen in the water was analyzed to determine when the level of feed input to the system reached a steady state with a given density of fish and plants. It is important to determine the individual

A



B



C



Figure 1. Picture of the University of Arizona's controlled environment aquaponics greenhouse. The greenhouse structure (A), the fish bay (B), and the hydroponic plant bay (C).

cropping requirements to establish an efficient use of resources and produce optimal quality produce.

A state of the art controlled environment aquaponics research greenhouse was designed and engineered for intensive fish and vegetable production at The University of Arizona (Figure 1). A controlled environment aquaponics greenhouse was constructed to run experiments on the growth and quality of lettuce. The aquaponics system design was based on the fish to plant ratios developed in the first experiment. A Computer system was utilized for monitoring and control of environmental parameters. Environmental monitoring and control consists of air temperature, relative humidity and photosynthetic active radiation (PAR). Water temperature, pH, electrical conductivity, and dissolved oxygen are monitored and controlled. The complete water chemistry of the system was analyzed on a weekly basis. An aquaponics greenhouse system to allows for analysis of fish and plant growth under consistent and optimal environmental conditions. A biological and mechanical filtration system processed the fish excretion (Figure 2).

The second objective of the study was to compare lettuce grown with aquaponic water and hydroponic solution under the same environmental conditions. A comparison of lettuce growth was conducted to determine if there is a difference in growth in lettuce grown with aquaponic fish water and hydroponic solution. The water chemistry was analyzed throughout the trials. The nutrient depletion in the aquaponics system was observed. A supplementation protocol was developed to increase the targeted nutrient concentrations in the aquaponics water to levels required for optimal plant growth. The third objective of the study was to compare the quality of the lettuce grown with aquaponics water and hydroponic solution. A comparison of the chlorophyll



Figure 2. The filtration system for the University of Arizona controlled environment aquaponics greenhouse. A Polygeyser™ PG7-PR filter from International Filter Solutions was used to remove particulate matter from the aquaponics water. A biological filter was constructed to supplement the PG7 filter. The sludge from the aquaponics system was collected.

concentration index (CCI%) was conducted to determine if there was a difference in CCI% in the leaves of lettuce grown with aquaponics water with supplementation and hydroponic lettuce solution. The fourth objective was to determine the nitrogen partitioning in the aquaponic system and to compare the nutrient composition of lettuce grown with aquaponics water with supplementation and hydroponic solution. The nitrogen in the aquaponic system was introduced through the fish feed then converted by the fish, consumed by bacteria, retained in the fish sludge, and converted by plants. Each component utilizes the nitrogen in the system. Prior research has focused on the nitrogen conversion of fish, bacteria, and plants. However, the nitrogen partitioning in an aquaponic system has not been established and the amount of nitrogen remediation that can be achieved in an aquaponic system had not been outlined.

The information gathered throughout this study can be applied for agricultural business development and environmental remediation. The greenhouse and aquaculture industries can utilize information on aquaponics and farm integration to develop farms of the future that will have to be able to produce food in less land with less available resources. The information from nitrogen partitioning in an aquaponic system can be used to determine the amount of plants that would be needed to bring residual ammonia and nitrate-nitrogen concentrations down from current aquaculture effluents to accepted Environmental Protection Agency (EPA) standards, adhere to Best Management Practices (BMP), and recirculate water to reduce water usage. Currently, diminishing fresh water resources and increased food demand from increasing populations has put pressure on the agriculture industry. Fish effluent is a sustainable and efficient nutrient source for plant production and plant uptake and deposition of nutrients is an

environmentally friendly means of filtration. Aquaponics systems have the potential to produce fish and vegetables in a sustainable manner with minimal environmental impact. This information will lead to more conservative agricultural practices that maximum production. Benefits from the development and optimization of aquaponic and integrated systems include the conservation and efficient use of resources, and reduced environmental impacts.

Aquaponics Research Greenhouse Design

The aquaponics research greenhouse was constructed at The University of Arizona's Controlled Environment Agriculture Center. A schematic of the hardware system for the fish (Figure 3) and lettuce (Figure 4) are provided. The University of Arizona Aquaponics Greenhouse (UAAG) structure was developed and operated by the UA-CEAC and is a free standing steel A-frame greenhouse with a double wall polycarbonate glazing. The greenhouse is oriented in a north-south configuration. The height to the gutter is 2 meters and height to the ridge is 4 meters. The greenhouse is divided into three separate 7.6 meter by 7.4 meter compartments, each with individual environmental controls. Double wall polycarbonate panels are used to divide the greenhouse. The north section houses the fish aquaculture and water filtration systems and the two south bays house the hydroponic beds.

The aquaculture component of the UAAG is comprised of four 1,300 liter growout aquaculture tanks, four 190 liter fry and fingerling tanks, and a 3,200 liter gallon water collection tank. The aquaculture component consists of a total of 9,160 liters. The

tanks were designed and fabricated by GSE Incorporated (Houston, TX USA). The water from the fish aquaculture and plant hydroponic systems drains into the collection tank. A

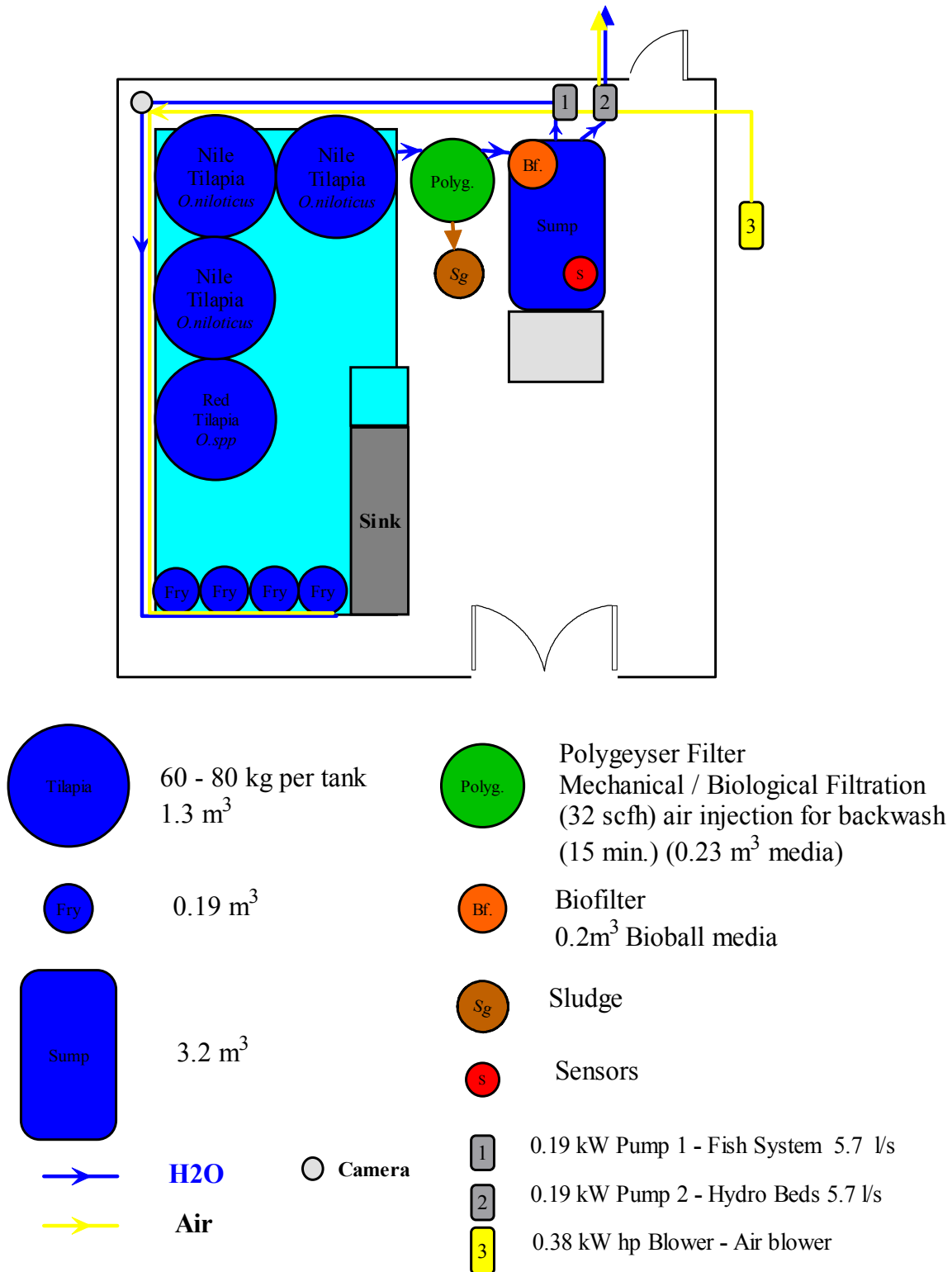
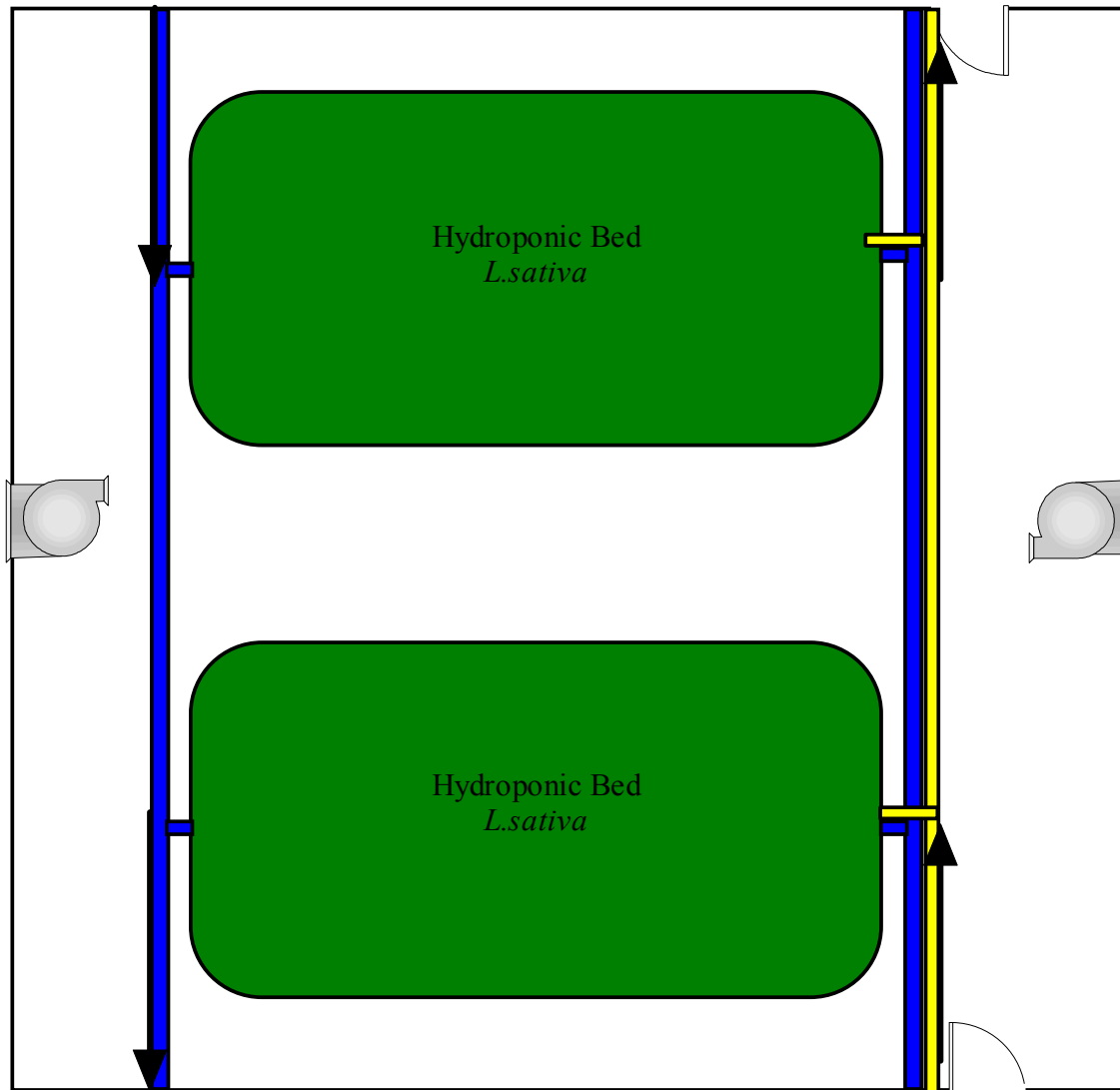


Figure 3. Aquaponics research greenhouse fish section design. Figure legend lists the fish and filtration system components.



Bed = (2.4m x 4.8m x 0.46m) = 5,436 liters

Each bed = 7 Plant Boards 62 plants / board (15 cm off center)

Total Plants / Bed = 434 Total Plants / Bay - 868 Total Plants (bay 2 & 3) = 1,736

Total liters of H₂O / Plant Bay = 10,872 liters

Total liters for Plant Bays 2 & 3 = 21,743 liters

2 swamp coolers per plant bay

Figure 4. Aquaponics research greenhouse plant section design. Figure legend lists the schematics of the hydroponic plant beds. Yellow represents airlines for oxygen delivery, and blue represents water flowing to the hydroponic beds and back to the fish component.

Sweetwater® Centrifugal 1.1 kW pump (Aquatic Eco-Systems Inc. Apoka, FL USA) delivers water back to the fish aquaculture system at a rate of 340 liter/minute. A Sweetwater 0.9 kW regenerative blower was used to provide aeration to the fish tanks, biological filter, water collection tank and hydroponic beds. Aero-Tube™ (Tekni-Plex Inc. Ridgefield, NJ USA) perforated aeration tubing lined the inner perimeter of the growout tanks to provide aeration. Air stones (15 cm x 4 cm) were used to provide aeration to the fingerling tanks. Water from the fish aquaculture system was filtered through a mechanical and a biological filter prior to being distributed to the plants. A Polygeyser™ PG7-PR filter from International Filter Solutions (IFS) (Marion, TX USA) served as the mechanical filter and a biological filter. The PG7 was plumbed inline with an additional 189 L container filled with Bio-Spheres™ (Aquatic Eco-Systems Inc. Apoka, FL USA) and lined with Aero-Tube™ to provide additional biological filtration. The biological filter contained approximately 0.3 m³ of bio-ball filter media for supporting the maximum fish load (150 kg). Trickling biological filters can convert approximately 1-2 grams of nitrogen per square meter per day (Parker 2002). A HiBlow (HiBlow USA Inc. Saline, MI USA) HP-80 linear air pump delivered air to the PG7 filter at a rate of 1.13 m³ per hour for backwashing the beads. The filter was purged daily. Typically five liters of sludge were removed from the system per filter purge.

Each hydroponic bed contained 1,400 l of water. An AquaFlo 0.19 kW pump delivers water to the four hydroponic plant beds at a rate of 340 liters / minute (85 liters per minute per bed). The water was constantly in circulation between the aquaculture and hydroponic sections. There are two plant growing bays, each plant bay contains two hydroponic beds. One of the hydroponic beds was used to produce the plants for the

experiment. The second hydroponic bed was used to maintain proper fish to plant ratios throughout the trials. Two hydroponic treatments which included lettuce plants grown with aquaponics nutrient solution, and the other with a standard hydroponic lettuce nutrient solution were established for each trial. Two replications of the standard lettuce hydroponic nutrient solution were established by submerging two 0.61 meter by 1.22 meter acrylic boxes into the aquaponic beds and filling them with the standard hydroponic lettuce nutrient solution. This procedure maintained similar water temperature and atmospheric conditions (air temperature, relative humidity, and PAR) for the aquaponics and hydroponic plant nutrient treatments.

The experiments were conducted from January through May 2009. The aquaponics system was designed to operate to produce tilapia (*O. niloticus*) for harvest on a monthly basis and for lettuce to be harvested on a weekly basis. Fish tank density ranged from 30 – 80 kg per tank depending on fish growth and/or planting density. Fish are grown to harvest at one kilogram. Previous studies conducted at the University of Arizona Environmental Research Lab determined that 5 kilograms of fish fed 2% biomass per day throughout the growth cycle of lettuce would yield 32 heads of lettuce per square meter (Licamele et al 2009).

The input and output resources of the aquaponic system that are required to produce fish and lettuce are shown in Figure 5. The primary resources needed for fish growth are feed and water. The primary resources needed for plant growth are water, nutrients (from the fish), and sunlight. The three main outputs of the system are fish, lettuce, and the sludge collected from the filtration system. Other inputs into the system include energy for moving water, energy for heating and cooling, oxygen (aeration) for fish respiration, and

air (carbon dioxide) for photosynthesis. Water is lost solely through evaporation from the system and transpiration from the plants. The schematic also outlines the consumption and generation of oxygen and nitrogen in the system. Oxygen is consumed by the biological organisms in the system. Nitrogen is introduced via the fish feed and then processed by the fish and filtration into dissolved nitrate-nitrogen. In an aquaponics system resources are conserved over time. The water is recirculated in the system from the fish (nutrient charge) to the plants (nutrient sink).

Hypothesis and Specific Aims

The focus of this dissertation is to present an integrated farming technique which can increase resource use efficiency without reducing production. The goal was to be able to produce food from freshwater that to sustain the world population's food demand. Lettuce can be grown hydroponically using aquaponics fish water. The fish to plant ratio required to grow lettuce with minimal nitrogen retention in the system was evaluated. It was hypothesized that lettuce can be grown hydroponically utilizing aquaponics fish water and additional supplementation to achieve equal growth and quality of lettuce grown within traditional hydroponic fertilizer solutions. The growth and quality (as determined by the chlorophyll concentration in leaves) of lettuce grown with aquaponic fish water and supplementation was compared to the growth and quality of lettuce grown in a hydroponic solution. The environmental conditions were monitored and controlled to achieve optimal plant growth. It was hypothesized that integrating plant production with fish aquaculture effluent can significantly reduce the potential ammonia-nitrogen and nitrate-nitrogen concentrations in aquaculture water. The amount of nitrogen deposited in

the leaves of the lettuce plants was determined and the potential nitrogen remediation was calculated.

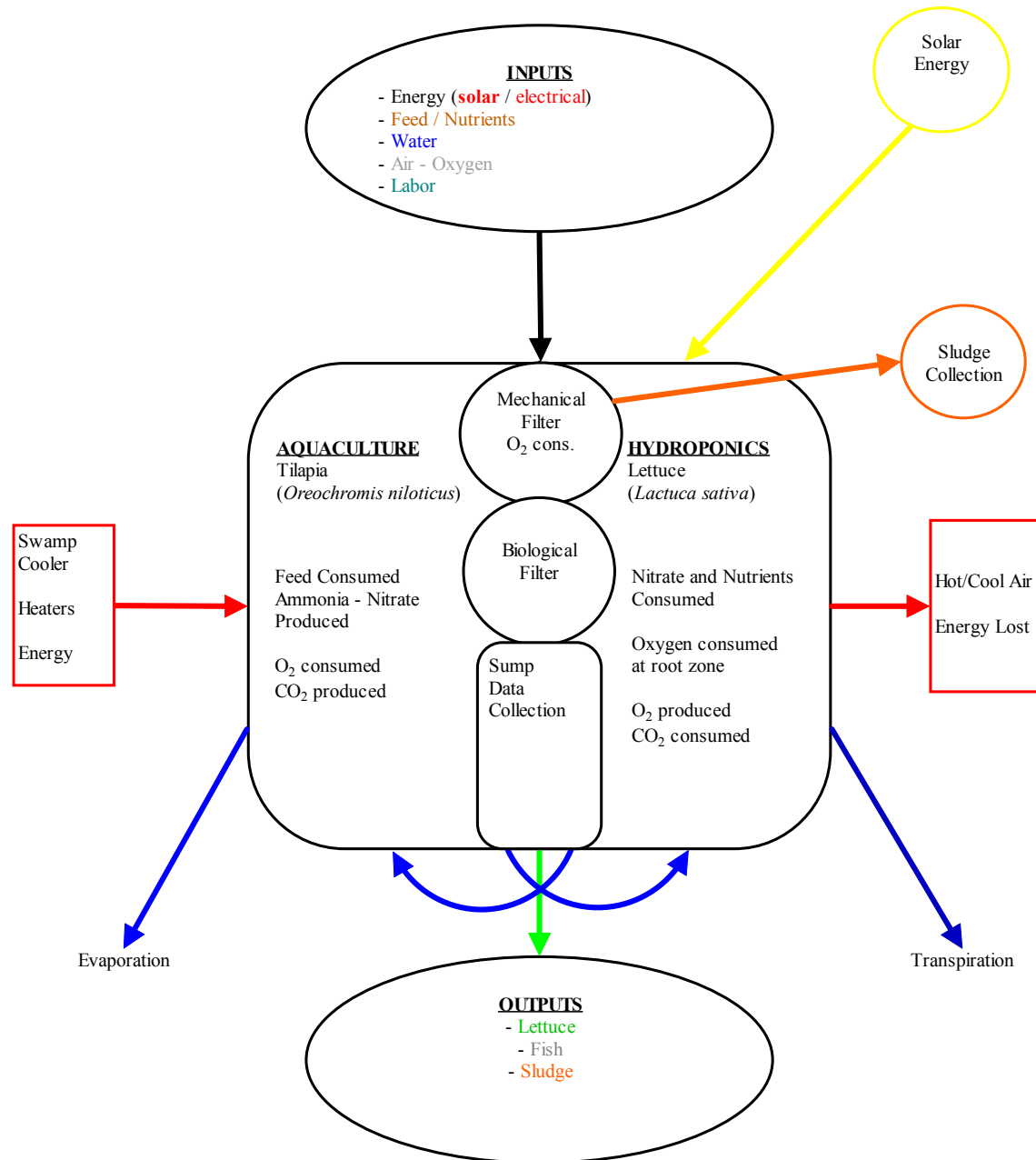


Figure 5. Flow chart of the inputs and outputs of an aquaponics system. The three primary outputs are fish, lettuce, and processed fish sludge. Inputs include water, feed (nutrients), and sunlight. Energy is also utilized for moving water and delivering oxygen to the water.

PRESENT STUDY

OVERALL SUMMARY

The contributions of this dissertation are included in three manuscripts, each in a separate appendix. Each manuscript includes an introduction, methods, results and discussion. The following is a summary of the most important findings of each manuscript.

OPTIMAL FISH (*Oreochromis niloticus*) TO PLANT (*Lactuca Sativa* CV. REX) RATIOS FOR A CONTROLLED ENVIRONMENT AQUAPONICS SYSTEM (APPENDIX A)

The goal of the first study was to determine the optimal stocking density of tilapia to grow a square meter of lettuce crops (32 lettuce heads at 15 cm spacing). Experiments were conducted at the University of Arizona's Environmental Research Lab (ERL) in the fish greenhouse. Preliminary experiments were conducted to test the efficacy of different hydroponic plant cultivars and to let the system mature so the biological organisms in the filter and system could be established. A randomized block experimental design was used to determine if there was a significant difference in growth of lettuce (*L. sativa* cv. Rex) that were fed nutrients from three different stocking densities of tilapia (*O. niloticus*).

Nile tilapia (*O. niloticus*) and Butterhead Lettuce (*L. sativa* cv. REX) were used in the aquaponics experiments. Tilapias were stocked into their respective total tank biomass densities of 2 kg m⁻³, 5 kg m⁻³ and 8 kg m⁻³ of fish per cubic meter. Prior experiments conducted at the ERL revealed that approximately one kilogram of fish feed can yield 20 heads of lettuce (Fitzsimmons 1992). Fish were fed 2% of the total stocking biomass daily throughout the trials. Treatments received 40 grams, 100 grams or 160

grams of feed per day depending on the tank stocking biomass. A total of 1.4 kg of feed was fed to each replicate of the 2 kg m⁻³ fish biomass treatment. A total of 3.5 kg of feed was fed to each replicate of the 5 kg m⁻³ fish biomass treatment. A total of 5.6 kg of feed was fed to each replicate of the 8 kg m⁻³ biomass treatment. Tilapias were fed a Star Milling Company Tilapia Diet. The tilapia diet was comprised of 32% protein, and delivered nutrients to the fish and then the lettuce plants. A nutrient analysis of the fish feed determined the amount of individual nutrients that are delivered to the aquaponics system via the feed.

Fish biomass and FCR was determined for each experimental replicate. The fish were weighed and stocked in their respective tanks three days prior to the beginning of the experiment and were harvested at day 37. The mean initial stocking weight of fish (*O. niloticus*) for the aquaponics experiment was 202.68 ± 30.35 grams per fish. Fish were stocked at mean densities of 10.67 ± 2.89 fish per tank for the 2 kg m⁻³ treatments, 27.33 ± 1.15 fish per tank for the 5 kg m⁻³ treatments and 33.67 ± 0.58 fish per tank for the 8 kg m⁻³ treatments. There was a total of 15 data plants per treatment replicate (n = 15). Border plants were excluded in the experimental analysis. The aquaponics plant trials lasted 35 days which is the time it takes for the lettuce to reach harvest weights of approximately 150 grams.

It was determined that 5 kg m⁻³ of Nile tilapia (*O. niloticus*) fed 2% of their body weight daily will yield on average 4.7 kg of lettuce (*L. sativa* cv. Rex) in 35 days. The lettuce from the 2 kg m⁻³ fish density treatment had similar wet weight as the 5 kg m⁻³ fish density treatment, however the dry weight was significantly (P<0.05) higher for the 5 kg m⁻³ treatment. The coloration of the lettuce leaves from the 2 kg m⁻³ fish treatment

was a pale yellow compared to the 5 kg m⁻³ fish treatment indicating nutrient deficiency and less chlorophyll content. The 5 kg m⁻³ fish treatment had significantly more head dry weight at harvest indicating more vegetative biomass. There was a significant difference in biomass accumulation of dry weight of the lettuce head between the 5 kg m⁻³ fish treatment and the other treatments. The 5 kg m⁻³ fish treatment yielded the most marketable lettuce biomass per square meter of deep bed hydroponics lettuce. The lettuce was greener and more marketable from the 5 kg m⁻³ fish treatment as well. The 2 kg m⁻³ fish treatment had a net yield of 0.65 kg of fish and 4.32 kg of lettuce. The 5 kg m⁻³ fish treatment had a net yield of 1.58 kg of fish and 4.65 kg of lettuce in a 35 day harvest cycle. The total biomass of fish and plant production for the 5 kg m⁻³ fish treatment was 6.23 kg of both fish and plants. For the 5 kg m⁻³ fish treatment the ratio of net fish biomass to harvestable lettuce biomass is 0.34 (1.58 kg fish biomass / 4.65 kg lettuce biomass). It will take approximately 0.34 kg of fish biomass to yield 1 kg of harvestable lettuce biomass. One kilogram of fish will yield 2.94 kilograms of lettuce. These are the production expectations for the species of fish (*O. niloticus*), the fish feed and feeding rate (2%), plant cultivar (*L. sativa* cv. Rex), deep bed hydroponic system, mean daily PAR of 28.64 mols m⁻², and mean air temperature and water temperature of 27.1°C and 24.4°C respectively.

COMPARISON OF LETTUCE (*Lactuca sativa* CV. REX) GROWN WITH TILAPIA (*Oreochromis niloticus*) EFFLUENT AND NUTRIENT SUPPLEMENTATION VERSUS A HYDROPONIC SOLUTION (APPENDIX B)

The goal of this study was to determine if there is a significant difference in growth (head wet weight and head dry weight) and leaf chlorophyll content index (CCI%) between lettuce (*Lactuca sativa* cv. Rex) grown with aquaponics water and nutrient supplements versus a hydroponic solution. A recirculating aquaponics system was engineered and designed at The University of Arizona's Controlled Environment Agriculture Center to grow tilapia and lettuce. Tilapia (*Oreochromis niloticus*) were grown in an intensive recirculating aquaculture system conjoined with deep bed hydroponic lettuce (*L. sativa* cv. Rex and Tom Thumb). In all trials, aquaponics water with the proper nutrient supplementation can yield equal or greater biomass and CCI% as compared to growth in conventional hydroponic solutions. Mean wet weight for aquaponics lettuce (176.75 ± 31.04 grams) was equal to or greater than lettuce grown with hydroponic solution (148.55 ± 21.71 grams). The CCI% for lettuce grown from aquaponic water plus supplements (9.89 ± 0.89) was not significantly different ($p \leq 0.05$), or significantly greater ($p \leq 0.05$), than lettuce grown in hydroponics solution. The efficacy of utilizing aquaponics water as a base nutrient source for hydroponic vegetable production was demonstrated. Integrating fish and plant production will lead to utilizing resources more efficiently thus delivering more sustainable food production with less environmental impacts. Aquaponic system with proper supplementation can yield lettuce of equal biomass and quality to conventional agriculture and hydroponic applications.

The University of Arizona aquaponics research greenhouse was designed to produce tilapia (*O. niloticus*) for harvest on a monthly basis and lettuce to be harvested on a weekly basis. Fish tank density ranges from 30 – 80 kg per tank depending on fish growth and/or planting density. Fish are grown out to 1 kg for harvest. It was determined from previous studies conducted at the University of Arizona Environmental Research Lab that 5 kilograms of fish fed 2% biomass per day will yield 32 heads of lettuce (Licamele et. al. 2009).

The aquaponics system was online for 7 months prior to the onset of the experimental trials. During the experimental trials there was an initial stocking density of a total of 105 kg of fish supplying 21 m² of plant growing area (128 lettuce heads a week). One growout tank had 30 kg of fish, a second had 30 kg of fish, a third was stocked with 35 kg of fish, and a fourth was stocked with 15 kg of fish. Mean fish weight at stocking was 310 grams per fish. Fish feeding was the same throughout the trials. Fish were fed 2% of the standing biomass daily (2.1 kilograms). Fish were harvested throughout the trials and the mean Feed Conversion Ratio (FCR) and mean survival was recorded for the aquaponics system. Fish were harvested, or sorted and put back into the respective tanks for further growout and maintaining system biomass load for plant growth.

Nutrient supplements were added to the aquaponics system throughout the trials to maintain targeted water chemistry parameters. One kilogram of Dolomite 65 Ag Lime was added to the sump every two weeks to maintain a pH of 6.8. Dolomite is comprised of 22.7 % calcium and 11.8% magnesium. Organic Materials Review Institute (OMRI) certified Biomin® amino acid chelated minerals were provided by JH Biotech™. During

the first week of the first trial 275 ml of Biomin® iron (5%) and 50 ml of Biomin® Zinc (7%) were added to the aquaponics system. The second week of the first trial 275 ml of Biomin® iron (5%), 50 ml of Biomin® Zinc (7%), and 50 ml of Biomin® Manganese (5%) were added to the aquaponics system. In the second trial during the second week, and the first week of the third trial, 800 ml of Biomin® iron (5%), 50 ml of Biomin® Zinc (7%), and 50 ml of Biomin® Manganese (5%) were added to the aquaponics system. In the third week of the third trial 100 ml of Biomin® iron (5%) and 50 ml of Biomin® Manganese (5%) were added to the system. Levels equal to or greater than 0.01 mg/l of all nutrients were maintained in the aquaponics water throughout the entire duration of the third trial. The supplements were added to the sump and allowed to dissolve into the system for 24 hours before taking a water sample. A hydroponic solution was formulated for lettuce and used in the hydroponic treatments. A 500 ml water sample was collected every week from the aquaponics system and sent to the lab for analysis. Water samples were tested by the Soil and Plant Laboratory Incorporated (Santa Clara, California).

Lettuce seeds were transplanted one week after germination into the hydroponic boards. Three trials were conducted for this experiment. The wet weight (grams) was measured at harvest (28 days after transplant) and samples were dried in a drying oven at 50°C for 72 hours before dry weight (grams) was measured. The Chlorophyll Concentration Index (CCI %) was measured on three different leaves with an Apogee CCM-200 prior to harvesting for each data plant. Lettuce was harvested 35 days after transplant for the first trial. The transplant time for the following two trials was changed to 28 days because head market weights were achieved more quickly. There were six data

plants per treatment with two replicates for each aquaponic and hydroponic treatment per trial. This totaled 12 data plants for the aquaponic treatment and 12 data plants for the hydroponic treatment per trial for trials one and two. Trial three there were 24 data plants per treatment for *L. sativa* cv. Rex. At the end of the trials the fish growout tanks were harvested, weighed (kg) and restocked with fish accordingly. The mean final weight (kg) and feed conversion ratio (FCR) was calculated for the tilapia fish aquaculture component. The data was analyzed using a paired t-test.

The system nutrients reached a steady state after 6 months of producing lettuce and fish prior to the onset of the experimental trials. A protocol to maintain pH and proper water chemistry parameters was developed. Iron, zinc, and manganese had reached minimal levels (<0.01) and were targeted for nutrient additions. The water chemistry analysis for the first trial revealed that the nitrate-nitrogen ($\text{NH}_3\text{-N}$) concentration ranged from 18 mg/l to 65 mg/l throughout the duration of the trials. The nitrate-nitrogen concentration for the second trial ranged from 18 mg/l to 62 mg/l and the third trial ranged from 48 mg/l to 89 mg/l. Ammonia was not present in the first trial and reached a level of 1 mg/l near the end of the second and third trials. The iron concentration in the aquaponics water ranged from 0.00 mg/l to 0.13 mg/l. The concentration of manganese ranged from 0.00 mg/l to 0.17 mg/l. The concentration of zinc in the aquaponics water trial ranged from 0.00 mg/l to 0.05 mg/l. Throughout the duration of the third trial iron, manganese and zinc maintained detectable levels (>0.01 mg/l) in the aquaponics system water.

The first trial lettuce (*L. sativa* cv. Rex) was analyzed to determine if there was a significant difference in head wet weight (grams), head dry weight (grams), and CCI%.

The mean chlorophyll concentration index of *L. sativa* cv. Rex was $10.1\% \pm 0.90\%$ for the aquaponics treatment and $11.4\% \pm 0.95\%$ for the hydroponic treatment. There was no significant difference ($p \leq 0.05$) in mean head wet weight (grams) and mean head dry weight (grams) for the Rex cultivar grown with aquaponics and hydroponic solution. There was a significant difference ($p \leq 0.05$) in the mean chlorophyll concentration index (%) between the *L. sativa* CV. Rex grown with aquaponics water and hydroponic solution.

The second trial consisted of a comparison of the Rex cultivar grown in aquaponics water plus supplements and hydroponic solution. The mean head wet weight and mean head dry weight were significantly different ($p \leq 0.05$) between lettuce grown with aquaponics solution plus supplements and hydroponic solution. The aquaponic lettuce had a greater mean head wet weight (182.79 ± 26.90 grams) and mean head dry weight (6.29 ± 0.74 grams at harvest). There was no significant difference ($p \leq 0.05$) in CCI% between *L. sativa* cv. Rex grown with aquaponics water plus supplements and hydroponic solution. The mean CCI% for the aquaponics lettuce was $8.5\% \pm 0.63\%$ and the mean CCI% for the lettuce grown with the hydroponic solution was $8.8\% \pm 0.65\%$.

The third trial was another comparison of the Rex lettuce cultivar. The mean head wet weight was significantly different ($p \leq 0.05$) between lettuce grown with aquaponics solution plus supplements and hydroponic solution. The aquaponic lettuce had a greater mean head wet weight (176.75 ± 31.04 grams) than the hydroponic lettuce at harvest. The mean head dry weight was not significantly different ($p \leq 0.05$) between lettuce grown with aquaponics (4.36 ± 0.78 grams) solution plus supplements and hydroponic (4.60 ± 0.60 grams) solution. There was a significant difference ($p \leq 0.05$) in CCI% between *L.*

sativa cv. Rex grown with aquaponics water plus supplements and hydroponic solution. The mean CCI% for the aquaponics lettuce was $9.89 \pm 0.89\%$ and for the hydroponics lettuce was $8.71 \pm 0.45\%$.

Fish tanks were harvested at the end of the trials. The mean FCR for the fish was 2.56 ± 0.64 and mean fish survival was $82.94\% \pm 0.07$. Fish stocking densities of 110 and 114 fish per growout tank yielded a lower FCR (3.03 and 2.82 respectively) than tanks stocked with 80 fish (1.83). There were no mortalities from the supplement additions or other water chemistry parameters.

NITROGEN REMEDIATION AND NUTRIENT DYNAMICS IN A CONTROLLED ENVIRONMENT AQUAPONICS SYSTEM (APPENDIX C)

The goal of this study was to determine if there is a significant difference in nutrient composition of lettuce (*L. sativa* cv. Rex) grown with aquaponics water plus nutrient supplementation and hydroponic solution. The nutrient dynamics of the aquaponic system was examined through water chemistry analysis. The nutrient flows were monitored and tailored to achieve nutrient concentrations for optimal plant growth. The amount of nitrogen removed from the aquaponics system through lettuce biomass accumulation was determined. In conclusion, aquaponics water plus supplementation can grow *L. sativa* cv. Rex with equal biomass accumulation and chlorophyll concentration index. This indicates that aquaponics lettuce can be produced with similar yield and quality compared with conventional hydroponic solutions when proper supplementation is added.

One head of *L. sativa* cv. Rex (176.75 ± 31.03) will deposit approximately 5.96 grams of nitrogen (3.38% per dry gram lettuce). One kilogram of fish will yield 6.4 lettuce heads (1,128 grams) and fixate 38.13 grams of nitrogen. There was a significant increase ($p \leq 0.05$) in percent composition of the micronutrients zinc, manganese, and boron in the leaves of lettuce (*L. sativa* cv. Rex) grown with aquaponics water compared to lettuce grown in hydroponic solution. The mean head wet weight was significantly different ($p \leq 0.05$) between lettuce grown with aquaponics solution plus supplements and hydroponic solution. The aquaponic lettuce had a greater mean head wet weight (176.75 ± 31.04 grams) than the hydroponic lettuce at harvest. The mean head dry weight was not significantly different ($p \leq 0.05$) between lettuce grown with aquaponics (4.36 ± 0.78 grams) solution and hydroponic (4.60 ± 0.60 grams) solution. There was a significant difference ($p \leq 0.05$) in CCI% between *L. sativa* cv. Rex grown with aquaponics water plus supplements and hydroponic solution. The mean CCI% for the aquaponics lettuce was $9.89 \pm 0.89\%$ and for the hydroponics lettuce was $8.71 \pm 0.45\%$. The mean FCR for the fish was 2.56 ± 0.64 and mean fish survival was $82.94\% \pm 0.07$. Fish stocking densities of 110 and 114 fish per growout tank yielded a lower FCR (3.03 and 2.82 respectively) than tanks stocked with 80 fish (1.83). This research also determines that lettuce (*L. sativa* cv. Rex) can be grow with aquaponics water plus nutrient supplementation and yield adequate nutritional value compared to lettuce grown in a hydroponic solution.

A nitrogen model was constructed for the aquaponics system. For every kilogram of feed put into the system, 59.7 grams of nitrogen was added to the system. A total of 2.1 kg of fish feed was added to the system daily. Approximately 125.37 grams of

nitrogen were added to the aquaponics system daily via the feed (5.97% nitrogen in feed). There was a total of 4.39 kg of nitrogen introduced to the aquaponics system over the 35 day experiment. The fish digest 90% of the proteins in the feed (Timmons et al. 2002) which was comprised of 5.97% nitrogen. Aquaponics lettuce was comprised of 5.22% nitrogen. There was approximately 5 liters of sludge per day collected from the mechanical filter. The sludge was aqueous and consisted of approximately 2% dry sludge (Timmons et al. 2002). Nitrogen loss that is unaccounted for consists of nitrogen gas, algae, bacteria and micro fauna within the aquaponics system. The nitrogen model for this aquaponics system outlines the flow of nitrogen within this system. Nitrogen was utilized for growth in both fish and lettuce. Nitrogen was also lost in the aquaponics system via removal of the processed fish sludge. Other nitrogen sinks in the system consists of bacteria, nitrogen gas, and micro-fauna that reside in the aquaponics system. This nitrogen loss via these mechanisms was not quantified. The aquaponics system utilized approximately 93% of the daily nitrogen input with the rest either remaining in solution as nitrate nitrogen or lost to other mechanisms. The aquaponics system had residual nitrate nitrogen in the water. Algae did not proliferate in the system because of the competition of nutrients with lettuce and the constant grazing of algae by the fish in the fish component. In an aquaponics system nitrogen is either assimilated by organisms in the system or remains in dilute in the water. Nitrogen is not discharged from the system in a form that leads to eutrophication.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The objective of this study was to determine if there is a difference in biomass accumulation and chlorophyll concentration index (CCI%) in lettuce (*Lactuca sativa* cv. Rex) grown with aquaponics water plus nutrient supplementation and hydroponic solution. The second goal of the study was to determine if there is a significant difference in nutrient composition of lettuce (*L. sativa* cv. Rex) grown with aquaponics water plus nutrient supplementation and hydroponic solution. The green coloration in *Lactuca sativa* cv. Rex varied between lettuce grown with aquaponics water, aquaponics water with supplementation, and a hydroponic solution (Figure 3). Tilapia and lettuce were harvested from the system on a regular basis (Figure 4).

The environmental parameters will affect plant physiology, thus plant growth (Resh 2001) which will in turn affect the nutrient uptake. Hydroponic nutrient solutions are formulated for specific plants. Characterizing the water chemistry of an aquaponics system will enable growers to tailor their aquaponics water with individual supplements in order to achieve maximum productivity and crop quality. Determining the optimum water temperature for an aquaponics system is a tradeoff between maximizing fish growth and plant growth. Water temperatures ranging from 24°C to 28°C will yield good lettuce production in relation to biomass generation and quality. Nile tilapia will grow at a better rate at higher temperatures (Chervinski 1982), however in order to obtain optimal lettuce production temperatures were kept cooler and below optimum (30°C). Decreased water temperatures (<28°C) can lead to a lower FCR for the tilapia (Timmons et al. 2002). Future studies should be conducted in order to determine the optimal water

A



B



C



Figure 6. Picture of lettuce (*L. sativa* cv. Rex) grown with aquaponics water (A), aquaponics water plus supplementation (B), and hydroponic solution (C).

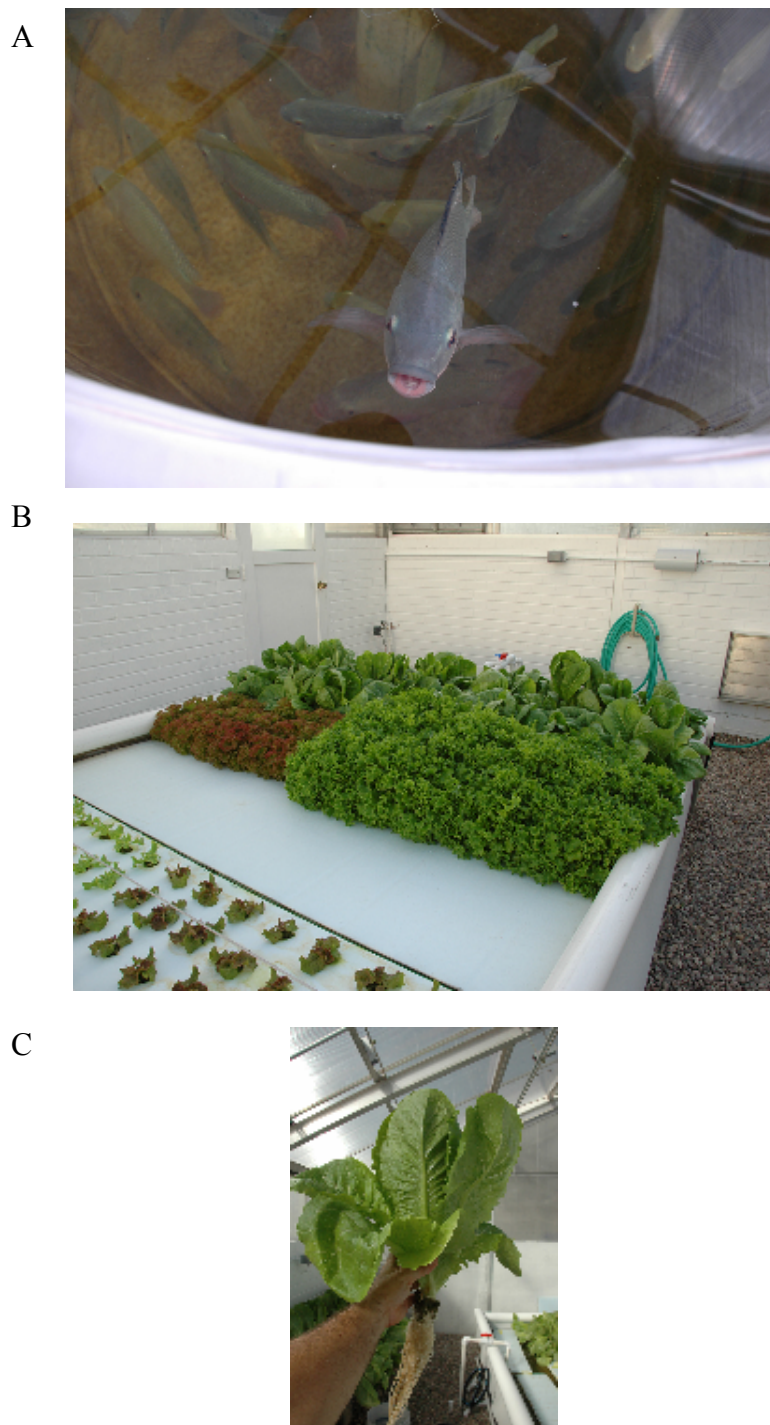


Figure 7. Fish and lettuce grown in the University of Arizona Controlled Environment Aquaponics Greenhouse. Tilapia (*O. niloticus* spp.) (A), and varieties of lettuce were grown in the aquaponics system including romaine, red and green oak, and butterhead (B). An organic romaine cultivar of lettuce (*L. sativa* cv. Rom) (C).

temperature for an aquaponics system. The cost to heat and cool water to achieve optimal temperatures for the fish and plants needs to be evaluated. Elevated water temperature set points for an aquaponics system provide warmer root zone temperatures for the plants. Further development of aquaponics system will take precise control and monitoring of the fish, plants, environment, and water chemistry which can be achieved with greenhouse and filtration technologies.

Fish production was similar and the plant treatments did not seem to have an effect on growth. In a system of larger magnitude the plant uptake of nutrients can compete with algae and bacteria thus potentially clearing up water quality for the fish. It is recommended that 100 grams of fish feed per day will yield 45.1 grams of fish growth and 132.86 grams of lettuce biomass per day under the specified environmental parameters. These formulations can be scaled up to fit commercial designs. Changing the feed can yield different results if the nutrient analysis of the feed is different. Increasing protein content in the feed for feeding trout or other species would change the ratio of fish needed to grow one kilogram of lettuce. The amount of feed needed to produce a kilogram of harvestable head lettuce would be less in terms of nitrogen. However, the other essential nutrients for proper fish growth might not be present at the same levels in feeds made of different sources. The nutrient accumulation of other essential nutrients for growth can also change from the type of fish feed used in an aquaponics system. Nutrient depletion over an extended period of time in aquaponics systems is common (Rakocy et al 1993, Seawright et al. 1998). Developing a commercial aquaponics greenhouse prototype will involve the need for nutrient supplementation not limited to but including iron (Fitzsimmons 1992, Rakocy et al. 1993 and Seawright et al. 1998).

The filtration and biological processing of the fish water for plant nutrient uptake can also influence the system. Future studies should include the integration of filtration technologies to remove solids and dissolve solids within the system. Controlled aquaponic systems can increase productivity, feed use efficiency, and water use efficiency over conventional farming practices. The ability to produce crops intensively with minimal to no impact to the environment will be essential in order for human civilization to be able to meet future food demands without negatively impacting the environment. Continuous operation of an aquaponics system can lead to the production of beneficial organisms in the water medium. Bacteria, phytoplankton, and zooplankton can become established in a system given the correct environmental surroundings and can increase growth and health of the plants (Sabidov 2004). If beneficial organisms can thrive in the aquaponics system then it is possible for non beneficial organisms to also reside in the system given a particular set of environmental surroundings. The balance of nutrients into the system with nutrient uptake by the target organisms (fish and plants) can be crucial in establishing beneficial organisms and reducing the establishment of harmful organisms. These harmful organisms thrive off similar nutrients that the fish and plants require for growth. Harmful bacteria and algal blooms indicate poor water quality. Water quality is affected by the nutrients put into the systems, the density of organisms within the systems, and the biological processing of the water within the system.

Aquaponics water plus supplementation can grow *L. sativa* cv. Rex with equal or greater biomass accumulation and chlorophyll concentration index. All of the macronutrients analyzed remained mostly constant throughout the duration of the experiment with a slight increase in the end. The micronutrients also remained in steady a

steady state again with a very slight increase near the end of the experiment. Sulfate accumulated throughout the duration of the experiment due to the content in the fish feed being at concentrations higher than that needed for fish and plant fixation. The *L. sativa* cv. Rex lettuce needs nutrient supplementation including iron, manganese and zinc to be added to the aquaponics water to yield marketable lettuce. The level of the supplementation in this experiment was sufficient and can possibly be reduced with further study. A total of 900 ml of Biomin® iron (5%), 100 ml of Biomin® Zinc (7%), and 100 ml of Biomin® Manganese (5%) were added to the aquaponics system throughout the growout cycle of the lettuce.

The need for supplementation will depend on the system water chemistry parameters and the plants nutrient demand. Nutrient requirements can be different for each plant cultivar and must be evaluated to achieve optimal production and plant health. Lettuce can be grown consistently in an aquaponics system thus using water, nutrients and space more efficiently than conventional agriculture leading to a more sustainable food production system. The aquaponics system has produced lettuce with equal or greater chlorophyll concentration indices. It was determined that aquaponics water provides a good source of nutrients for plant growth. The nitrogen, phosphorous and potassium in the aquaponics system reached a near steady state with the lettuce production indicating that fish effluent at the densities established can provide a stable source of macronutrients for plant growth. The micronutrients in the aquaponics system must be monitored and adjusted to the crop being grown. It is concluded that lettuce grown with aquaponics water plus nutrient supplementation grows at similar rates to lettuce grown with hydroponic solutions. Supplementation rates will vary depending on

the fish to plant ratio, system design and environmental parameters. Nutrients do not accumulate at the same rate in relation to each other so certain supplements would be needed to match the nutrient demand of the target crop. It has been supported that iron, zinc and manganese accumulate at different rates in aquaponic system (Seawright 1998) and therefore must be supplemented accordingly. Nutrients can also be recovered from the solid fish wastes. The sludge from the aquaponics system has a nutrient composition that can be utilized if applied to field crops, soil amendments, or dissolved back into solution. By utilizing the fish sludge complete recovery of nutrients from the system can be achieved.

Lettuce (*L. sativa* cv. Rex) can be grow with aquaponics water plus nutrient supplementation and yield adequate nutritional value compared to lettuce grown with hydroponic solution. Values reported for the nutrient composition in the lettuce are similar to lettuce grown in conventional agriculture and hydroponics (Resh 2001). The composition of macronutrients and micronutrients in the growing solution can determine the nutrient content, growth, and health of lettuce (Resh 2001). Lettuce has a low efficiency in using nitrogen but needs nitrogen constantly to promote growth (Welch et al. 1983). Aquaponic systems deliver the amount of nitrogen needed for lettuce growth allowing for the assimilation of nitrogen through photosynthesis without developing a build up or sink of nitrogen within the system.

Lettuce and other plant crops prove a valuable remediation tool for fish farming and aquaculture in general. Greenhouse or conventional agriculture can integrate lettuce production to existing aquaculture farms to remediate nitrogen and nutrient discharge. This will in turn generate a marketable product in the lettuce, but reduce the costs for

fertilizers and reduce environmental degradation commonly associated with large scale agriculture. If one kilogram of fish will yield 6.4 lettuce heads (1,128 grams) and deposit 38.13 grams of nitrogen, then a farm can calculate the amount of greenhouse space needed to remediate the current discharge of fish effluent. The impacts from this can be tremendous to the environment and costs to farmers. Further research regarding the production of different crops that require different nutrient demands can lead to different nutrient supplementation protocols. The OMRI listed nutrient supplement Biomins™ from JH Biotech allowed for specific nutrients to be targeted and added to the system. This enables the aquaponics system to stay within organic standards by utilizing OMRI listed nutrient products along with processed fish effluent to produce sustainable organic vegetables. Future research at the aquaponics greenhouse will focus on integrating varieties of vegetable crops and tailoring the water chemistry using Biomin™ nutrient supplementations to produce crops of equal or greater yields to agriculture and hydroponic systems.

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**APPENDIX A: OPTIMAL FISH (*OREOCHROMIS
NILOTICUS*) TO PLANT (*LACTUCA SATIVA*)
RATIOS FOR A CONTROLLED ENVIRONMENT
AQUAPONICS SYSTEM**

Journal of the World Aquaculture Society

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ABSTRACT

The goal of this study was to determine the optimal stocking density of tilapia to grow 32 lettuce heads (15 cm spacing). A randomized block experimental design was used to determine if there was a significant difference in growth of lettuce (*L. sativa* cv. Rex) that were fed nutrients from three different stocking densities of tilapia (*O. niloticus*). Each experimental treatment was a recirculating aquaponics system totaling 1600 liters. The fish tank was 1000 liters which flowed to a biological filter, and then a one square meter hydroponic bed. Three fish densities were stocked with a total biomass of 2 kg m⁻³, 5 kg m⁻³, and 8 kg m⁻³ of fish and fed 2% of their respective biomass daily. The tilapia diet was comprised of 32% protein, and delivered nutrients to the fish and then the lettuce plants. The hydroponic lettuce plot consisted of a square meter area with 32 lettuce seedlings planted at 15 cm spacing. Environmental parameters (temperature, relative humidity and PAR) were monitored throughout the experiment. Biomass data of the lettuce (head wet weight and head dry weight) determined if there was a significant difference in lettuce yield. The Feed Conversion Ratio (FCR) was calculated for the different fish stocking densities. Water chemistry was analyzed throughout the experiments to determine the levels of ammonia, nitrite, and nitrate. The pH, dissolved oxygen and electrical conductivity were also monitored. Plant biomass data was compiled to test for a significant difference between treatments via an ANOVA. The data demonstrated that 5 kg m⁻³ fish density treatment of tilapia had significantly ($P < 0.05$) greater lettuce growth (Head dry weight) compared to the 2 kg m⁻³ treatment for a one square meter plot of lettuce (32 plants). The water chemistry results demonstrated that nitrate and phosphate accumulation in the system reached a near steady state in the 5 kg

m^{-3} fish density treatment whereas the ammonia and nitrate continued to accumulate in the 8 kg treatment. It was concluded that 5 kg m^{-3} of tilapia will grow 32 marketable lettuce heads (one square meter plot). The nutrient input (nitrogen) into the system reached a steady state thus concluding that the amount of nitrogen into the system reached equilibrium with the nitrogen requirements of the lettuce biomass. It was concluded that one kilogram of fish fed 2% of its body weight per day will yield 2.94 kilograms of lettuce in a 35 day period under the experiments environmental conditions.

INTRODUCTION

Aquaponics has received much interest in recent years. The ability to increase production through integration of fish aquaculture and plant production has been demonstrated (Fitzsimmons 1991; Fitzsimmons 1992; Rakocy et al. 1993; McMurtry et al. 1997; Chaves 2000; McIntosh and Fitzsimmons 2003; Sabidov 2004; Castro et al. 2006; Diver 2006). Coupling fish aquaculture with hydroponic plant culture has been proven to be feasible and more sustainable than conventional agriculture systems (McMurtry et al. 1990; Fitzsimmons 1992; Rakocy et al. 1992; Rakocy and Nair 1987; Rakocy and Hargreaves 1993). The economic feasibility of aquaponic systems for producing food fish and crops has been evaluated and proven to be potentially profitable (Rakocy et al. 1997, Adler et al. 2000, Rakocy et al. 2004, Neori et al. 2004). Popular food crops such as lettuce, basil, tomatoes and strawberries have been successfully cultivated with the use of fish effluent as the primary fertilizer (McMurtry et al. 1997, Takeda et al. 1997, Rakocy et al. 2004, Hanson et al. 2008). The success of commercial aquaponics to consistently produce high quality yields is dependent on the engineering and design of the biological processes occurring within a given biological system. The goal of this study was to determine the optimal ratio of fish biomass to lettuce biomass in a controlled environment aquaponics system. The nutrient cycling in the system occurs via bio-processing of fish effluent and retention in fish, plants, bacteria, and various microorganisms. A greenhouse will allow for consistent monitoring and control of environmental parameters, thus allowing for optimal crop production.

The primary resources used in animal or crop production are water, nutrients, light, and land. Intensive recirculating aquaculture systems (RAS) can produce more fish

per liter of water than other types of aquaculture systems (Timmons et al. 2002). And greenhouse hydroponics (GHH) production can produce from five to ten times the output as compared to conventional agriculture (Resh 2001; Hannan 1998). Water from RAS systems can be used in GHH to intensify production by utilizing resources as efficiently as possible. Integration of lettuce culture with fish aquaculture systems can reduce water usage by 20-27% (Chavez et al. 2000). Combining aquaculture with agriculture or greenhouse crop production can increase food production efficiency. World food demand is increasing as populations continue to rise and the resources available to produce food are finite. In order to provide enough food in the future to feed humanity, the management of those resources must be optimized to produce the most amount of food per unit of resource.

The integration of a RAS with hydroponic plant production is referred to as aquaponics. Aquaponic systems incorporate specific design parameters in which the fish water flows through a mechanical filter, into a biological filter, and then to the hydroponic plants (Rakocy et al. 2006). Mechanical and biological filtration processes are important components for RAA (Losordo et al. 1999). The fish component provides the water with nutrients via feeding and excretion. A mechanical filter is used to remove large particulate matter from the fish wastes prior to being processed via the biological filter. Removal of large particulate matter prior to the biological filtration unit will reduce heterotrophic bacteria populations (Losordo 1999), thus reducing competition with nitrifying bacteria. The biological filtration promotes the growth of nitrifying bacteria which converts ammonia to nitrate (Timmons et al. 2002). Ammonia can be toxic to plants at solution concentrations greater than $0.5 \mu\text{mol} / \text{liter}$ (Britto and Kronzucker

2002). Ammonia and nitrite are toxic to fish at high concentrations, however, nitrate is not (Timmons 2002). After the water is processed it is then delivered to the hydroponic beds where nutrients are taken up by the plants. Dissolved nutrients from fish are similar to nutrients used in hydroponics systems (Rakocy et al. 2006). The water then flows to the sump where it is pumped back to the fish system. Accumulated nutrients in fish aquaculture systems can lead to algae and bacterial growth that can be harmful to fish and lead to off flavors (Fitzsimmons 2006).

Maintaining proper water chemistry parameters requires balancing the fish, nitrifying bacteria, and the plant's physiological requirements in one system. Tilapia can tolerate a pH from acidic to alkaline (pH 5-11) (Chervinski 1982) and a wide range of salinity concentrations (Watanabe et al. 2002). Plants grow best and uptake nutrients at a lower (5.5-6.5) pH (Resh 2001). Nitrifying bacteria is inhibited below a pH of 6.5 with an optimum pH at 7.8 (Antoniou et al. 1990) depending on bacterial species and temperature (Tyson et al. 2007). Electrical conductivity levels range between 1-2 $\mu\text{S cm}^{-1}$ for hydroponic lettuce production (Resh 2001). Lettuce will grow well at a pH range of 5.5-6.5 (Resh 2001, Islam et al. 1980). Optimal water temperature for tilapia (*O. niloticus*) ranges between 28-35°C (Chervinski 1982) while lettuce grows best at water temperatures between 21-25°C (Resh 2001). These water parameters can influence the physiology of the organisms within the aquaponic system and must be monitored and controlled for optimal system performance. Filtration technologies can be incorporated into recirculating aquaculture systems or aquaponics systems in order to maintain optimum targeted water chemistry parameters.

The nutrients in the system can achieve a steady state with given inputs (feed) and outputs (fish, plants, particulate matter). The concept of aquaponic systems is to balance the nutrients within a given system so that there is no excess accumulation of nutrients, nitrogen in particular. Nutrients are delivered to the system via feed which is consumed, assimilated and excreted by the fish. The conversion of feed to usable energy and somatic growth in fish is 25-30% (Rakocy & Hargreaves 1993). Studies by Quillere et al. (1995) revealed a 60% nitrogen recovery (fish 31% and tomato plants 28%) from an aquaponics system. Balancing the amount of nutrients produced from the fish system with the nutrient requirements of the plants can lead to optimized resource utilization and system productivity. Excess nutrients can decrease water clarity thus initiating algal, fungal or bacterial growth (Timmons 2002, Losordo et al. 1999). In aquaponics systems, dissolved waste nutrients from the aquaculture system are retained within the aquaponics system and absorbed by the plants (Rakocy et al. 2006). Therefore, it is necessary to develop an optimal ratio of fish density to plant density. Determining the nutrient inputs, the biological processes and outputs will help elucidate the efficiency and performance of an aquaponics system.

Prior research has mapped out the feasibility of aquaponic lettuce production (Rakocy 1989b, Fitzsimmons 1992, Chaves 2000, Castro et al. 2006, Diver 2006,), ratio of fish to cropping area (Fitzsimmons 1991, Rakocy et al. 1993), crop feasibility (Rakocy 1989a, Sabidov 2004, Sabidov 2007), nutrient composition (Seawright et al. 1998) and polyculture of aquatic organisms (Quillere et al. 1995, Neori et al. 2004). Aquaponic systems are comprised of multiple biological systems (fish, plants, and microorganisms) functioning synergistically with one another. Design of the aquaponic system will also

influence the biological activity of the system. Research that focuses on the individual system components in a controlled environment can yield insight into the maximum productivity of aquaponics. Combining greenhouse environmental control with the recirculating aquaponic system will enable a more controlled analysis of the interactions between nutrients provided by the fish and plant uptake of those nutrients. This research integrates two intensive growing systems, RAS and greenhouses to optimize environmental parameters in order to achieve maximal system efficiency. Lettuce (Lactuca sativa cv. Rex.) and Nile tilapia (Oreochromis niloticus) were grown in an aquaponics system to determine the optimal fish to plant ratios. System productivity was evaluated. Water chemistry analysis allows for determination of the amount of soluble nutrients in the aquaponics system. The amount of nitrate in the system will be dependent on the amount of fish feed introduced, which is determined by the fish biomass. The goal of this study was to determine the optimal fish density that would support a specified lettuce density for an intensive aquaponics system. Water chemistry analysis (ammonia, nitrite, and nitrate), plant biomass analysis, and fish growth were analyzed.

MATERIALS AND METHODS

Experiments were conducted at the University of Arizona's Environmental Research Lab (ERL) in the fish greenhouse. The fish greenhouse glazing consisted of double layer polyethylene plastics. Greenhouse environmental control consisted of evaporative cooling and natural gas heating to maintain temperatures throughout the growing seasons. Environmental parameters (air and water temperature, humidity, and photosynthetic active radiation) were monitored and controlled for optimal plant production. Preliminary experiments were conducted to test the effects of different hydroponic plant cultivars and air temperature set points. The system was allowed to mature for 8 weeks so the biological organisms in the filter and system could be established.

EXPERIMENTAL DESIGN

A randomized block experiment was designed to evaluate the difference in plant growth and nitrate accumulation of aquaponic systems fed nutrients from three different fish stocking density treatments. A total of nine aquaponic test systems were constructed consisting of three treatments with three replicates per treatment. Each aquaponic treatment system consisted of a 1000 liter (1 m^3) fiberglass fish tank plumbed in line to a 95 liter plastic drum which overflowed into a 200 liter plastic drum. This was connected to a one square meter per growing bed constructed of wood with dimensions 1 meter long by 1 meter wide by 15.25 cm deep and lined with a black plastic liner. A 5 cm drain was plumbed at the bottom of the West side of each bed. A 2 cm thick hydroponic styrofoam board was cut to the hydroponic bed size and used to float the lettuce heads to allow for

the roots to be suspended in the water. Deep bed hydroponics was found to be the more efficient hydroponic system compared to sand or gravel ebb and flood systems in delivering nutrients to the roots at a consistent and constant rate from the fish systems (Lennard and Leonard 2004). This drain fed water to another 200 liter drum which housed a water pump that would deliver water back to the fish tank at rate of 1-3 liters per second. The water volume of the each system was 1600 liters of water (Figure 1).

Water was replenished to each aquaponic treatment system to compensate for water loss from evapotranspiration. Water loss was approximately 5% of the system volume per week (0.05% - 1.8% daily). The water in the system cycled from the fish to the plants and back to the fish approximately every 5-10 minutes to assure complete mixing and delivery of fish nutrients to the plants. The biological filter contained 2 kilograms of small polyethylene filter beads topped with 1.8 m² of nylon bird netting material. For each aquaponic system one air diffuser (7.62 cm length) was placed at the bottom of the cylinder to aerate and stir the beads, one air diffuser was placed in the fish tank and one air diffuser was placed in plastic drum that housed the pump. The netting material was manually shaken out inside the filter every week to prevent filters from clogging and overflowing. Particulates would dissolve back into solution or collect in the 200 liter plastic drum.

FISH AND PLANTS

The target fish used in the aquaponics experiments was the Nile tilapia (O. niloticus) and the target plant used was a Butterhead Lettuce (L. sativa cv. REX). Tilapias were stocked into their respective total tank biomass densities of 2 kg m⁻³, 5 kg m⁻³ and 8

kg m⁻³ of fish per cubic meter. Prior experiments conducted at the ERL revealed that approximately one kilogram of fish feed can yield 20 heads of lettuce (Fitzsimmons 1992). Fish were fed 2% of the total stocking biomass daily throughout the trials. Treatments received 40 grams, 100 grams or 160 grams of feed per day depending on the tank stocking biomass. A total of 1.4 kg of feed was fed to each replicate of the 2 kg m⁻³ fish biomass treatment. A total of 3.5 kg of feed was fed to each replicate of the 5 kg m⁻³ fish biomass treatment. A total of 5.6 kg of feed was fed to each replicate of the 8 kg m⁻³ biomass treatment. Tilapias were fed a Star Milling Company Tilapia Diet (Table 1). A nutrient analysis of the fish feed determined the amount of individual nutrients that are delivered to the aquaponics system via the feed (Table 1).

Tilapia starting and final densities were recorded and the Feed Conversion Ratio (FCR) was determined for each of the treatments. The FCR is calculated by dividing the amount of fish feed into the system by the net fish biomass increase for the duration of the growth period. Fish biomass and FCR was determined for each experimental replicate. The fish were weighed and stocked in their respective tanks three days prior to the beginning of the experiment and were harvested at day 37. The mean initial stocking weight of fish (O. niloticus) for the aquaponics experiment was 202.68 ± 30.35 grams per fish. Fish were stocked at mean densities of 10.67 ± 2.89 fish per tank for the 2 kg m⁻³ treatments, 27.33 ± 1.15 fish per tank for the 5 kg m⁻³ treatments and 33.67 ± 0.58 fish per tank for the 8 kg m⁻³ treatments. Each individual fish tank was plumbed in line with a standard hydroponic planting bed consisting of a density of 32 lettuce plants per square meter. A total of 32 plants per square meter yielded 15 data plants per treatment replicate. Border plants were excluded in the experimental analysis.

Lettuce (*L. sativa* cv. Rex) was planted in 2.5 x 5 centimeter rockwool cubes. The seedlings were transplanted to 2 cm thick polystyrene boards at a spacing of 15.25 cm off center. Lettuce was seeded in a misting bed for 14 days prior to transplanting. The aquaponics plant trials lasted 35 days which is the time it takes for the lettuce to reach harvest weights of approximately 150 grams. The lettuce was harvested at 35 days. Lettuce was harvested and plant data was collected at the end of the experiment. Plant height (cm) was collected by measuring from the top of the hydroponic board to the upper most section of the plant crown. Plant diameter (cm) was measured from the two outer most leaves across the plant crown. Lettuce harvest head wet weight (g) was recorded. Plants were placed in a drying oven for 72 hours and head dry weight (g) was recorded.

ENVIRONMENTAL PARAMETERS AND WATER CHEMISTRY

The water temperature (C°), air temperature (C°), relative humidity (%) and the photosynthetic active radiation ($\mu\text{mol}/\text{m}^2\text{s}$) were monitored continuously with a Campbell (Campbell Scientific Inc. Logan, Utah USA) 21X Data logger (averaged values every 15 minutes). An aspirator was designed to house the dry/wet bulb temperature thermocouples (Copper-Constantine) for accurate temperature and relative humidity readings. A Li-Cor (Li-Cor Biosciences Lincoln, NE USA) PAR sensor was mounted on the truss of the greenhouse above the lettuce canopy. The pH and electrical conductivity ($\mu\text{S}/\text{cm}$) was measured on a weekly basis with a pH ep4 Pen and a Dist5EC/TDS Pen made by Hanna Instruments (Woonsocket, RI USA). The dissolved oxygen (mg/l) and temperature (C°) were monitored on a weekly basis using a YSI (Yellow Springs, OH

USA) 550A handheld dissolved oxygen meter. The environmental parameters for the trials are listed in Table 2. Water samples were collected from each treatment at the beginning of the experiment, 12 days after transplant, 24 days after transplant, and 35 days after transplant. Water samples were tested for ammonia, nitrite and nitrate (Kjeldhal digestion colorimetry). Total Phosphate-P was also determined for each treatment using colorimetry. The samples water samples were analyzed by the Water Quality Center at the Environmental Research Lab.

DATA ANALYSIS

Experiments were conducted in the beginning of the fall from September to October. The data was analyzed using JMP analytical software. An ANOVA was applied to determine if there was a significant ($p = 0.05$) difference in plant biomass (head wet weight, head dry weight, plant height and plant diameter) between lettuce grown with effluent from the three respective fish densities (2kg, 5kg, and 8kg). Post-hoc students T-tests were used to determine significant (0.05) differences between treatments. The residual nitrate and phosphate concentration (mg/l) in the water for each treatment was examined to determine if nitrate or phosphate was a limiting factor among the three treatments. The hypothesis to be tested is that there is a significant difference in biomass of hydroponically grown lettuce (*L. sativa* cv. Rex) grown off nutrients from aquaculture effluent at different fish (*O. niloticus*) densities (2 kg m^{-3} , 5 kg m^{-3} and 8 kg m^{-3}).

Aquaponics Research System

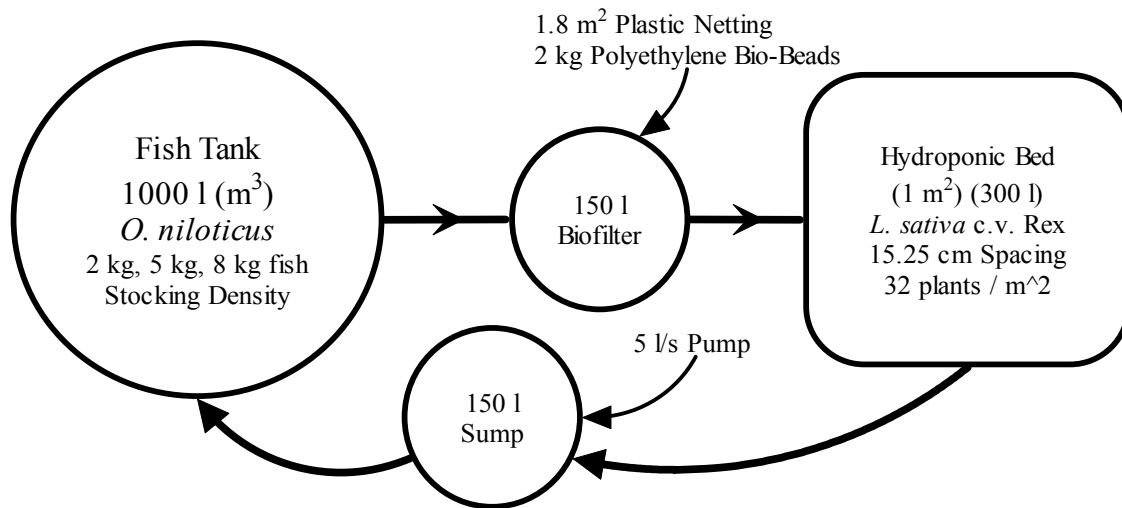


FIGURE 1. This is a schematic of one of the replicates of the aquaponics research system. The biological filter media consisted of 2 kg of bead media with 1.8 m² bird netting on top. Water is pumped from the sump then flows via gravity through the fish tank through the biological filter, then into the hydroponic plant bed finally returning to the sump. The water flow of the system is 5 liters per second. A total of 32 lettuce heads were planted per square meter. The respective fish density was allocated for each replicate.

Star Milling Co. Tilapia Feed Proximate Analysis (% composition)	
Crude Protein	35%
Crude Fat	5%
Crude Fiber	4%
Ash	9%
Nutrients (% dry diet)	
N	5.97%
P	1.53%
K	1.46%
Ca	1.61%
Mg	0.26%
Na	0.24%
S	0.46%

TABLE 1. List of tilapia feed analysis used in the aquaponics research trials. The feed is a manufactured tilapia diet by Star Milling Company. A nutrient analysis of the feed shows the relative concentrations of nitrogen and phosphorous that is delivered to the aquaponics system.

ENVIRONMENTAL PARAMETERS	Daily Mean Values (9/9/07 - 10/13/07)
Air Temperature C°	
T_{\min} C°	24.8 °C
T_{\max} C°	29.1 °C
T_{ave} C°	27.1 °C
Water Temperature °C	
T_{\min} C°	23.2 °C
T_{\max} C°	25.7 °C
T_{ave} C°	24.4 °C
Relative Humidity (%)	
Mean R.H. %	66%
PAR (mol m ⁻²)	
PAR Daily mols m ⁻²	28.64 mols m ⁻²
PAR Total mols m ⁻²	1002.4 mols m ⁻²

TABLE 2. A list of the environmental parameters for the aquaponics trial. The water temperature, air temperature, relative humidity and photosynthetic active radiation (PAR) were monitored and recorded using a 21x Campbell Scientific Data Logger. Readings were recorded every 10 seconds and averaged over 5 minute intervals continuously throughout the experiment.

RESULTS

FISH BIOMASS

The mean increase in average fish biomass was greatest in the 5 kg m⁻³ fish density treatment (1.58 ± 0.12 kg) (Table 3). The 8 kg m⁻³ fish treatment yielded the greatest increase in fish biomass (Table 3). The 8 kg m⁻³ fish treatment had the highest FCR of 3.10 ± 0.27 (Table 3). The 5 kg m⁻³ fish treatment had an FCR of 2.22 ± 0.17 , which was similar to the 2 kg m⁻³ fish treatment that had an FCR of 2.19 ± 0.34 (Table 3). Tilapia aquaculture feed conversion ratios typically range from 1.5 -2.0 (Watanabe et al 2002). All treatments had an increase in fish biomass throughout the experiment. The 2 kg m⁻³ and 5 kg m⁻³ fish density treatments had 100% survival. One replicate of the 8 kg m⁻³ fish density treatment had 88% survival. All feed distributed to the tanks was consumed by the fish or dissolved into the system within a 24 hour period.

PLANT BIOMASS

There was a significant ($P < 0.05$) difference in the mean head wet weight, mean head dry weight, mean plant height and mean plant diameter of *L. sativa* cv. Rex grown from the three fish densities (2 kg m⁻³, 5 kg m⁻³ and 8 kg m⁻³). The mean head wet (145.47 ± 33.94 grams) and head dry weight (7.10 ± 1.53 grams) was highest in the 5 kg m⁻³ fish biomass treatment (Figure 2 and Figure 3). The 8 kg m⁻³ treatment had the lowest head wet (96.63 ± 22.90 grams) and head dry weight (5.53 ± 0.92 grams) (Figure 2 and Figure 3). The 5 kg m⁻³ fish treatment had the greatest mean plant height (13.95 ± 2.07 cm) and plant diameter (19.05 ± 2.21 cm) (Figure 4). The 5 kg m⁻³ fish treatment accumulated the most biomass and size compared to the other treatments.

A post hoc t-test determined there was no significant ($P < 0.05$) difference in mean lettuce head wet weight between the 2 kg m^{-3} fish density treatment and the 5 kg m^{-3} treatment. There was a significant ($P < 0.05$) difference in mean lettuce head wet weight between the 5 kg m^{-3} fish density treatment and the 8 kg m^{-3} treatment. Mean lettuce head dry weight was significantly different for the 5 kg m^{-3} fish density treatment compared to the 2 kg m^{-3} and 8 kg m^{-3} treatments. There was a significant ($P < 0.05$) difference in mean plant height of lettuce grown in the 5 kg m^{-3} fish density treatment compared to the 2 kg m^{-3} and 8 kg m^{-3} treatments. There was no significant ($P < 0.05$) difference for mean lettuce plant diameter grown in the 2 kg m^{-3} and 5 kg m^{-3} fish density treatments. There was a significant ($P < 0.05$) difference in mean lettuce plant diameter between the 5 kg m^{-3} fish density treatment compared to the 8 kg m^{-3} treatment.

The 2 kg m^{-3} fish density treatment received 1,400 grams of feed over a 35 day period, had a mean FCR of 2.19 ± 0.34 , and produced on average 620 ± 100 grams of fish and $4,048 \pm 38.71$ grams of lettuce. The 5 kg m^{-3} fish density received 3,500 grams of feed over a 35 day period, had a mean FCR of 2.22 ± 0.17 , and produced $1,550 \pm 120$ grams of fish and $4,364 \pm 33.94$ grams of lettuce. The lettuce in the 2 kg m^{-3} fish density treatment was not as green as the lettuce in the 5 kg m^{-3} fish density treatment. The 8 kg m^{-3} fish density received 5,600 grams of feed over a 35 day period, had a mean FCR of 4.73 ± 1.61 , and produced $1,270 \pm 360$ grams of fish and $2,899 \pm 30.00$ grams of lettuce.

WATER CHEMISTRY

The mean pH of each fish density treatment all had hydrogen ion concentrations within a pH 7 (Table 4). The pH for all treatment replicates did decrease throughout the

duration of the experiment. The pH decreased in the 2 kg m⁻³, 5 kg m⁻³ and 8 kg m⁻³ treatments by 0.40, 0.70 and 0.80 pH units, respectively. The dissolved oxygen concentration for the 2 kg m⁻³ (7.91 ± 0.36 mg/l) and 5 kg m⁻³ (6.76 ± 0.41 mg/l) fish density treatments was maintained above the optimum of 5 mg/l for fish growth (Timmons et al. 2002). The mean dissolved oxygen levels for the 8 kg m⁻³ fish density treatment was 4.82 ± 2.07 mg/l (Table 4). The electrical conductivity increased in all treatments as the experiment progressed and was significantly different among all treatments ($P < 0.05$). At the end of the trial the electrical conductivity was highest in the treatment with the highest fish density of 8 kg m⁻³, and the 5 kg m⁻³ fish density treatment was greater (920 ± 0.08 μ S/cm) than the 2 kg m⁻³ treatment (830 ± 0.04 μ S/cm) (Table 4).

Ammonia and nitrite were minimal to none detectable during the experiments for all treatments. Ammonia nitrogen (NH₃-N) levels ranged from 0.04 mg/l to 0.69 mg/l and were undetectable after two weeks into the experiment. Nitrite was undetectable throughout the entire experiment for all treatments. Nitrifying bacteria populations in the biological filters were established prior to the experiment to reduce elevated ammonia and nitrite concentrations. Nitrate and phosphate concentrations increased as the experiment progressed and more feed was added (Figure 5 and Figure 6). Nitrate concentration was highest in the 8 kg m⁻³ treatment and was continuing to rise throughout the duration of the experiment (Figure 5). Nitrate concentrations at the end of the experiment in the 5 kg m⁻³ fish density treatment increased 1.2 mg/l between sampling. . Nitrate concentration in the 5 kg m⁻³ treatment reached a near steady state at the end of the experiment (Figure 4). The 2 kg m⁻³ and 8 kg m⁻³ fish density treatments had larger nitrate concentration increases of 12.49 mg/l and 24.81 mg/l compared to the 5 kg m⁻³

treatment. Phosphate continued to rise in all three treatments to the end of the experiment (Figure 5). The increase in phosphate concentration for the 2 kg m⁻³ and 5 kg m⁻³ fish density treatments were 3.61 and 3.25 mg/l, respectively. The increase in phosphate concentration in the 8 kg m⁻³ fish density treatment was much higher (6.94 mg/l) than the other treatments. This data indicates that nitrates and phosphates in the 8 kg m⁻³ fish density treatments compared to the 2 kg m⁻³ and 5 kg m⁻³ treatments accumulate at a faster rate than the lettuce plants can uptake nutrients.

Fish Data	Treatment 1	Treatment 2	Treatment 3
Initial Fish Biomass (kg)	2 kg m ⁻³	5 kg m ⁻³	8kg m ⁻³
Mean Initial Fish Weight (g)	196 ± 0.04	183 ± 0.01	238 ± 0.01
Mean Number Fish per tank	10.67 ± 2.89	27.33 ± 1.15	33.67 ± 0.58
Mean Harvest Fish Biomass (kg)	2.65 ± 0.10	6.58 ± 0.12	9.82 ± 0.15
Mean Net Biomass Increase (kg)	0.65 ± 0.10	1.58 ± 0.12	1.82 ± 0.15
Mean Survival %	100%	100%	88%
Total Feed (35 day trial)	1.4 kg	3.5 kg	5.6 kg
Mean Feed Conversion Ratio (FCR)	2.19 ± 0.34	2.22 ± 0.17	3.10 ± 0.27
Plant Data	Treatment 1	Treatment 2	Treatment 3
Lettuce Biomass Head Wet Weight (kg)	4.32 ± 0.04	4.65 ± 0.03	3.09 ± 0.02
Lettuce Biomass Head Dry Weight (g)	201 ± 1.56	227 ± 1.53	1.77 ± 0.92

Table 3. Fish and plant data from an aquaponics experiment comparing the growth of a one square meter hydroponic plot of lettuce (*L. sativa* c.v Rex) irrigated with fish effluent comprised of three different fish (*O. niloticus*) stocking densities (2 kg m⁻³, 5 kg m⁻³, and 8 kg m⁻³). There were 32 heads of lettuce per square meter hydroponic plot.

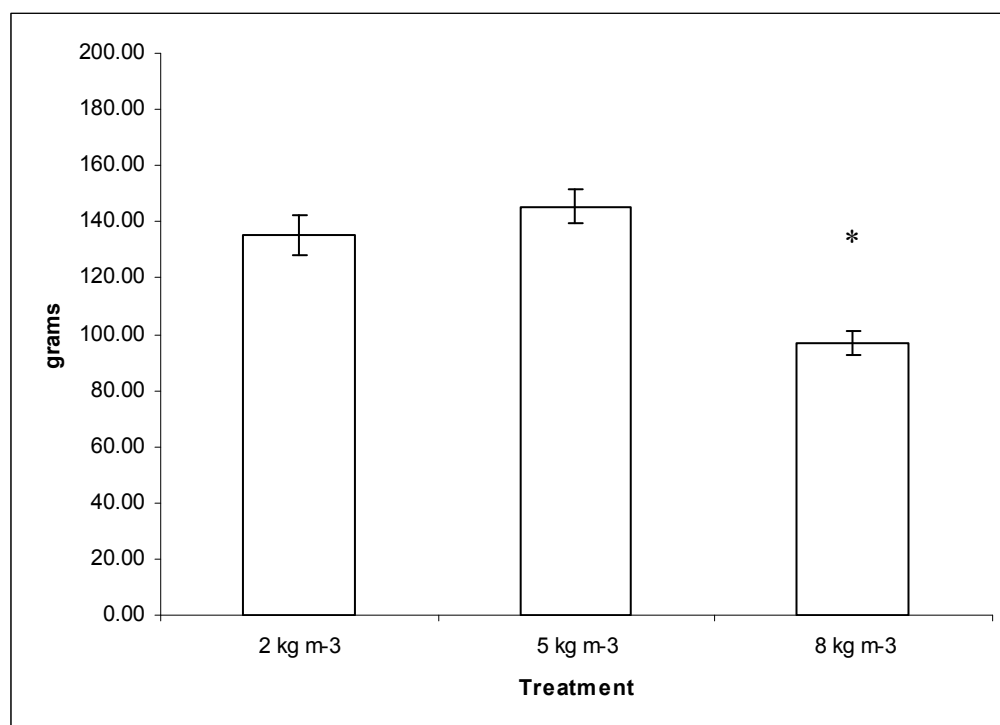


Figure 2. Mean head wet weight of Lettuce (*L. sativa* cv. Rex) in an aquaponics system fed nutrients from three different fish (*O. niloticus*) densities (2 kg m⁻³, 5 kg m⁻³ and 8 kg m⁻³). Fish were fed %2 of the stocking biomass daily. Lettuce was harvested 35 days after transplant. Error bars represent SEM, * represents $p \leq 0.05$ as determined by ANOVA.

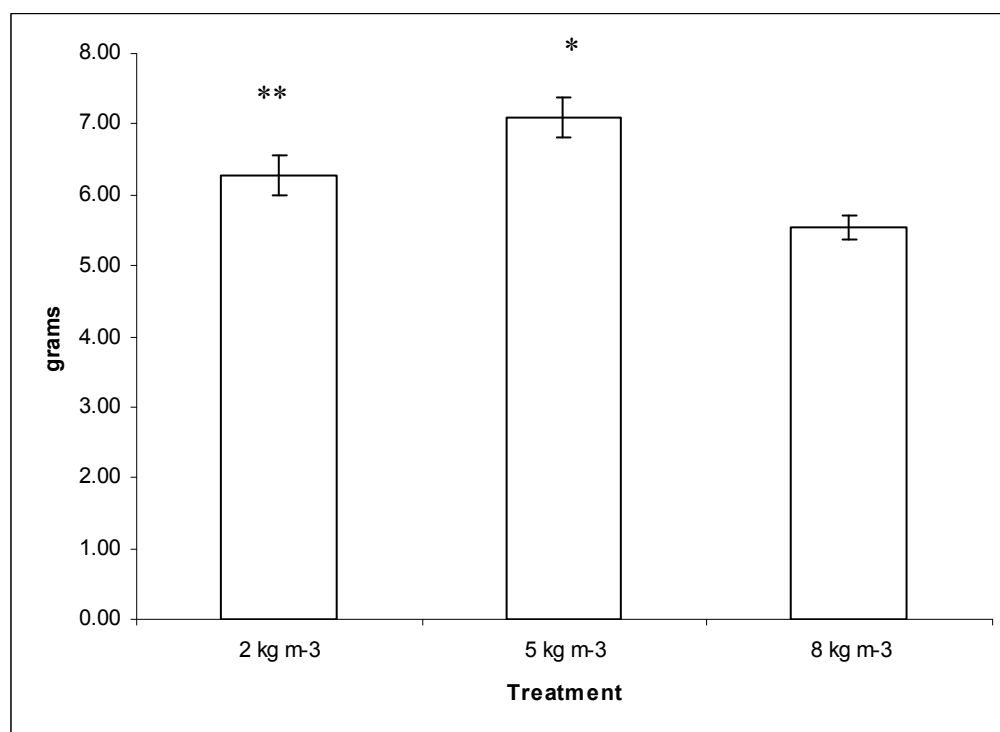


Figure 3. Mean head dry weight of Lettuce (*L. sativa* cv. Rex) in an aquaponics system fed nutrients from three different fish (*O. niloticus*) densities (2 kg m⁻³, 5 kg m⁻³ and 8 kg m⁻³). Fish were fed %2 of the stocking biomass daily. Lettuce was harvested 35 days after transplant. Error bars represent SEM, *, and ** represents $p \leq 0.05$ as determined by ANOVA.

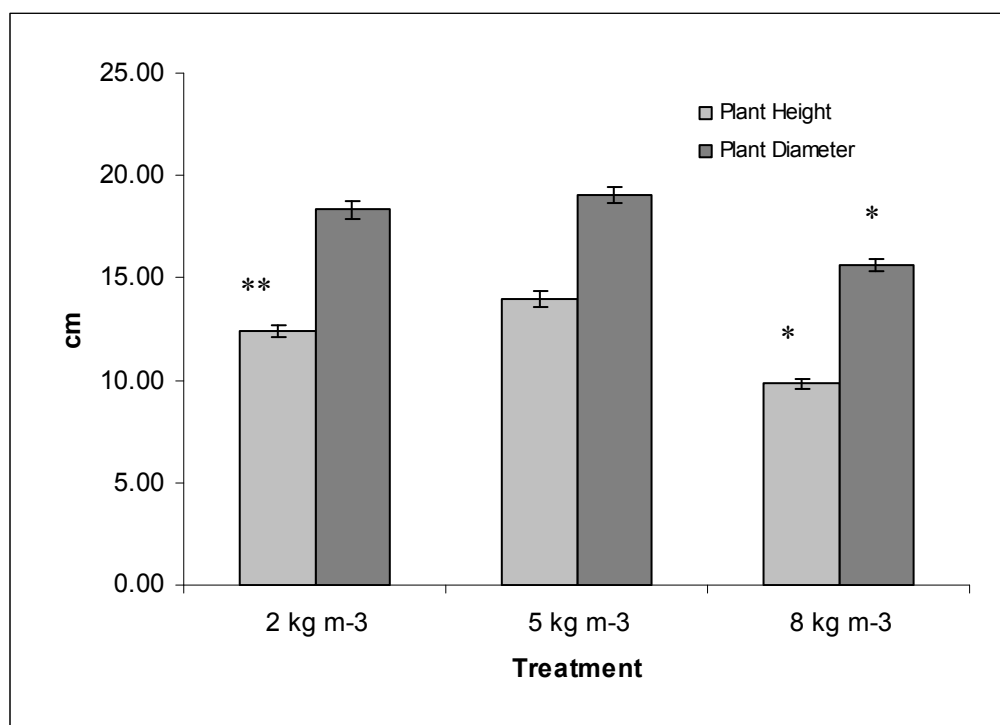


Figure 4. Mean height and diameter of Lettuce (*L. sativa* cv. Rex) in an aquaponics system fed nutrients from three different fish (*O. niloticus*) densities (2 kg m⁻³, 5 kg m⁻³ and 8 kg m⁻³). Fish were fed %2 of the stocking biomass daily. Lettuce was harvested 35 days after transplant. Error bars represent SEM, *, and ** represents $p \leq 0.05$ as determined by ANOVA.

Treatment	Initial pH	Final pH	Initial EC ($\mu\text{S/cm}$)	Final EC ($\mu\text{S/cm}$)	Initial D.O. (mg/L)	Final D.O. (mg/L)
2 kg m ⁻³	8.1 \pm 0.19	7.7 \pm 0.38	0.80 \pm 0.04	830 \pm 0.04	8.1 \pm 0.26	7.91 \pm 0.35
5 kg m ⁻³		7.4 \pm 0.41		920 \pm 0.08		6.76 \pm 1.08
8 kg m ⁻³		7.3 \pm 0.32		1000 \pm 0.13		4.82 \pm 2.07

Table 4. Mean water chemistry parameters for the three fish density (*O. niloticus*) treatments (2 kg m⁻³, 5 kg m⁻³, and 8 kg m⁻³). Readings were recorded at noon on a weekly basis throughout the trials.

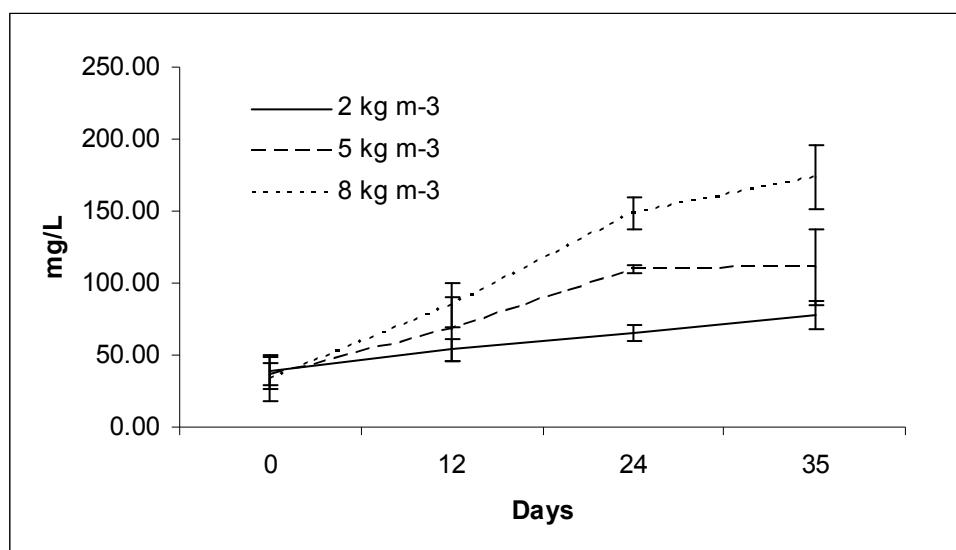


Figure 5. Mean nitrate concentration in an aquaponics system fed nutrients from three different fish (*O. niloticus*) densities (2 kg m⁻³, 5 kg m⁻³ and 8 kg m⁻³). Fish were fed %2 of the stocking biomass daily. Lettuce was harvested 35 days after transplant.

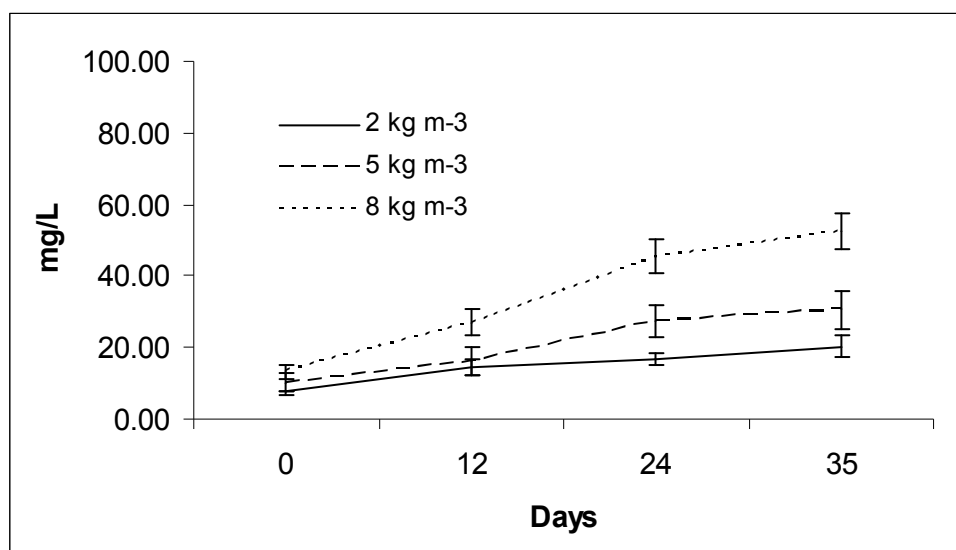


Figure 6. Mean phosphate concentration in an aquaponics system fed nutrients from three different fish (*O. niloticus*) densities (2 kg m⁻³, 5 kg m⁻³ and 8 kg m⁻³). Fish were fed %2 of the stocking biomass daily. Lettuce was harvested 35 days after transplant. Error bars represent SEM.

DISCUSSION

It was concluded that 5 kg m⁻³ of Nile tilapia (*O. niloticus*) will yield on average 4.7 kg of lettuce (*L. sativa* cv. Rex) in 35 days under the specified environmental conditions. The mean lettuce head wet weight was approximately 145.87 ± 42.66 grams. An increase in PAR would have an effect on the growth of the lettuce, possibly increasing the biomass accumulation and nitrogen demand. A marketable lettuce head of 150 grams can be achieved with hydroponic solution in 21-35 days with a minimum PAR of 17 mols per day (Both et al 1994). The lettuce from the 2 kg m⁻³ fish density treatment had similar wet weight as the 5 kg m⁻³ fish density treatment, however the dry weight was significantly ($P < 0.05$) higher for the 5 kg m⁻³ treatment. The coloration of the lettuce leaves from the 2 kg m⁻³ fish treatment was a pale yellow compared to the 5 kg m⁻³ fish treatment indicating nutrient deficiency and less chlorophyll content. The 5 kg m⁻³ fish treatment had significantly more head dry weight at harvest indicating more vegetative biomass. There was a significant difference in biomass accumulation of dry weight of the lettuce head between the 5 kg m⁻³ fish treatment and the other treatments. The 5 kg m⁻³ fish treatment yielded the most marketable lettuce biomass per square meter of deep bed hydroponics lettuce. The lettuce was greener and more marketable from the 5 kg m⁻³ fish treatment as well. The 2 kg m⁻³ fish treatment had a net yield of 0.65 kg of fish and 4.32 kg of lettuce. The 5 kg m⁻³ fish treatment had a net yield of 1.58 kg of fish and 4.65 kg of lettuce in a 35 day harvest cycle. The total biomass of fish and plant production for the 5 kg m⁻³ fish treatment was 6.23 kg of both fish and plants.

For the 5 kg m⁻³ fish treatment the ratio of net fish biomass to harvestable lettuce biomass is 0.34 (1.58 kg fish biomass / 4.65 kg lettuce biomass). It will take

approximately 0.34 kg of fish biomass to yield 1 kg of harvestable lettuce biomass. One kilogram of fish will yield 2.94 kilograms of lettuce. This is the production expectations under the assumptions of the species of fish (O. niloticus), the fish feed and feeding rate (2%), and plant cultivars (L. sativa cv. Rex) used in this experiment under the specified environmental parameters (Table 2). Optimizing environmental parameters such as PAR air temperature, and water temperature could yield increased lettuce biomass accumulation and fish growth. Changing the feed can yield different results if the nutrient analysis of the feed is different. Increasing protein content in the feed for feeding trout or other species would change the ratio of fish needed to grow one kilogram of lettuce. The amount of feed needed to produce a kilogram of harvestable head lettuce would be less in terms of nitrogen. However, the other essential nutrients for proper fish growth might not be present at the same levels in feeds made of different sources. The nutrient accumulation of other essential nutrients for growth can also change from the type of fish feed used in an aquaponics system.

The FCR of the 8 kg m⁻³ fish treatment was higher than expected because of some mortality in one of the replicate tanks. The FCR for tilapia in the aquaponics system (FCR 2.2) was close to the expected tilapia aquaculture FCR of 1.5-2.0 (Watanabe et al 2002). Increasing water temperature could increase the FCR of for the fish system however this could have negative effects on lettuce growth. The plant treatments did not seem to have an effect on growth. In a system of larger magnitude the plant uptake of nutrients can compete with algae and bacteria thus potentially clearing up water quality for the fish. The dissolved oxygen was low in the 8 kg m⁻³ fish treatment compared to the other two treatments. Dissolved oxygen in all treatments never reached levels (7 mg/l)

equal to the other treatments. The respiration from the fish, plant root zone, and microorganisms in the system was greater than the capacity to deliver oxygen in this experiment. In order to sustain the biological oxygen demand of larger densities of fish and plants more airstones, increased air delivery, or injection of pure oxygen would be required. A decrease in oxygen is not beneficial for fish or plant growth (Timmons et al. 2002; Resh 2001).

Electrical conductivity, nitrate and phosphate increased in all the treatments as more fish feed was added on a daily basis. The nitrate and phosphate concentration in the 5 kg m^{-3} fish treatment reached a near steady state at the end of the experiment. Nitrate concentration in the 5 kg m^{-3} fish treatment reached a steady state at approximately 110 mg/l by the end of the experiment with no negative effect on growth. Phosphate concentration in all treatments was greater than 20 mg/l. This indicates that the amount of nitrogen and phosphate into the system was equivalent to the amount being taken up by the plants. Nitrogen that is not accounted for in the aquaponic system can be lost via fixation by bacteria, algae, and micro fauna that reside in the system, or in the form of nitrogen gas (N_2). An increase in more nutrients for the 8 kg m^{-3} fish treatment did not yield an increase in plant biomass compared to the other treatments.

It was determined that 100 grams of fish feed per day will yield 45.1 grams of fish growth and 132.86 grams of lettuce biomass per day. These result are comparable to production from hydroponic and aquaculture systems (FCR 2.2; 150 gram head). These formulations can be scaled up to fit commercial designs. Nutrient depletion over an extended period of time in aquaponics systems is common (Rakocy et al 1993, Seawright et al. 1998). Developing a commercial aquaponics greenhouse prototype will involve the

need for nutrient supplementation not limited to but including iron (Fitzsimmons 1992, Rakocy et al. 1993 and Seawright et al. 1998).

The filtration and biological processing of the fish water for plant nutrient uptake can also influence the system. Future studies should include the integration of filtration technologies to remove solids and dissolve solids within the system. Controlled aquaponic systems can increase productivity and feed use efficiency. The ability to produce crops intensively with minimal to no impact to the environment will be essential in order for human civilization to be able to meet future food demands without negatively impacting the environment. Continuous operation of an aquaponics system can lead to the production of beneficial organisms in the water medium. Bacteria, phytoplankton, and zooplankton can become established in a system given the correct environmental surroundings and can increase growth and health of the plants (Sabidov 2004). If beneficial organisms can thrive in the aquaponics system then it is possible for non beneficial organisms to also reside in the system given a particular set of environmental surroundings. The balance of nutrients into the system with nutrient uptake by the target organisms (fish and plants) can be crucial in establishing beneficial organisms and reducing the establishment of harmful organisms. Particularly because these harmful organisms thrive off of the similar constituents that the fish and plants thrive off of (water and nutrients). Harmful bacteria and algal blooms indicate poor water quality. Water quality is affected by the nutrients put into the systems, the density of organisms within the systems, and the biological processing of the water within the system.

Future studies should include chlorophyll analysis of the plant leaves. Visual observations of plant nutrient deficiency symptoms can also be used to determine plant

health. The environmental parameters will affect plant physiology, thus plant growth (Resh 2001) which will in turn affect the nutrient uptake. Hydroponic nutrient solutions are formulated for specific plants. Characterizing the water chemistry of an aquaponics system will enable growers to tailor their aquaponics water with individual supplements in order to achieve maximum productivity and crop quality.

Future studies will look into developing a commercial prototype scaled up from the fish to plant ratios. Control of environmental parameters will allow for testing the effect of air temperature, humidity, and PAR on plant physiology in the aquaponics system and thus the nutrient dynamics of the system. Determining the optimum water temperature for an aquaponics system is a tradeoff between maximizing fish growth and plant growth. Water temperatures ranging from 24°C to 28°C will yield good lettuce production in relation to biomass generation and quality. Nile tilapia will grow at a faster rate at higher temperatures (Watanabe et al 2002), however in order to obtain optimal lettuce production temperatures were kept cooler and below optimum (30°C). Decreased water temperatures (<28°C) can lead to a lower FCR for the tilapia (Timmons et al. 2002). Increasing water temperature could yield a lower FCR 1.5-2.0, however the growth of the lettuce would also be affected. Future studies should be conducted in order to determine the optimal water temperature for an aquaponics system. The price to heat and cool water to achieve optimal temperatures for the fish and plants needs to be evaluated. Elevated water temperature set points for an aquaponics system provide warmer root zone temperatures for the plants. Warmer root zone temperatures coupled with providing cooler air temperatures can provide optimal growing conditions for the lettuce. Further development of aquaponics system will take precise control and

monitoring of the fish, plants, environment, and water chemistry which can be achieved with greenhouse and filtration technologies.

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**APPENDIX B: COMPARISON OF LETTUCE
(*LACTUCA SATIVA* CV. REX) GROWN WITH
TILAPIA (*OREOCHROMIS NILOTICUS*) EFFLUENT
AND NUTRIENT SUPPLEMENTATION VERSUS A
HYDROPONIC SOLUTION**

Journal of Plant Nutrition

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ABSTRACT

The goal of this study was to determine if there is a significant difference in growth (head fresh weight and head dry weight) and leaf chlorophyll content index (CCI%) between lettuce (*Lactuca sativa* cv. Rex) grown with aquaponics water and nutrient supplements a hydroponic solution. A recirculating aquaponics system was designed to grow tilapia and lettuce. Tilapia (*Oreochromis niloticus*) were grown in an intensive recirculating aquaculture system conjoined with deep bed hydroponic lettuce (*L. sativa* cv. Rex and Tom Thumb). Aquaponics water with the proper nutrient supplementation can yield equal or greater biomass and CCI% as compared to growth in conventional hydroponic solutions. Mean wet weight for aquaponics lettuce (176.75 ± 31.04 grams) was equal to or greater than lettuce grown with hydroponic solution (148.55 ± 21.71 grams). The CCI% for lettuce grown from aquaponic water plus supplements (9.89 ± 0.89) was not significantly different ($p \leq 0.05$) than lettuce grown in a hydroponics solution. This research demonstrates the efficacy of utilizing aquaponics water as a base nutrient source for hydroponic vegetable production. Integrating fish and plant production can produce plants of equal biomass and color compared to a hydroponic solution. Integrated fish and plant systems can lead to more sustainable food production with less environmental impacts. Aquaponic systems with proper supplementation can yield lettuce and other potential vegetable crops of equal biomass and quality (coloration) compared to agriculture and hydroponic production.

INTRODUCTION

The production of fish and vegetables through the integration of fish aquaculture and plant production has been demonstrated (Fitzsimmons 1991; Fitzsimmons 1992; Rakocy et al. 1993; McMurtry et al. 1997; Chaves 2000; McIntosh and Fitzsimmons 2003; Sabidov 2004; Castro et al. 2006; Diver 2006). Coupling fish aquaculture with hydroponic plant culture is more sustainable than conventional agriculture systems by reducing the environmental impact from eutrophication (McMurtry et al. 1990; Fitzsimmons 1992; Rakocy et al. 1992; Rakocy and Nair 1987; Rakocy and Hargreaves 1993). Popular food crops, such as lettuce, basil, tomatoes, and strawberries, have been successfully cultivated with the use of fish effluent as the primary fertilizer (McMurtry et al. 1997, Takeda et al. 1997, Rakocy et al. 2004, Hanson et al. 2008). Intensive recirculating aquaculture systems (RAS) can produce more fish per liter of water than other types of aquaculture systems (Timmons et al. 2002), therefore reducing water used. Greenhouse hydroponics production can produce from five to ten times more output compared to conventional agriculture (Resh 2001; Hannan 1998). Given these increased yields, aquaponic systems can yield similar crop production compared to hydroponic systems (Sabidov 2004). Water from aquaculture systems can be integrated with plant production utilizing resources more efficiently, potentially reducing water usage by 20-27% (Chaves et al. 2000).

The fish component of an aquaponics system provides the water with nutrients via feeding and excretion. A mechanical filter is used to remove large particulate matter from the fish wastes prior to being processed by the biological filter. Removal of large particulate matter prior to biological filtration will reduce heterotrophic bacteria

populations (Losordo 1999), thus reducing competition with nitrifying bacteria and promoting conversion of ammonia to nitrate (Timmons et al. 2002). This is important in preventing ammonia toxicity in fish (>2 mg/l) (Timmons et al 2002; Watanabe et al 2002). Depending on plant species sensitivity symptoms of ammonia toxicity appear with external ammonia concentrations above $0.1 - 0.5 \mu\text{mol/L}$ (Britto and Kronzucker 2002). Lettuce will grow in ammonia concentrations of 2 mg/l (Resh 2001). Ammonia and nitrite are toxic to fish at high concentrations, however, nitrate is not (Timmons 2002). After the water is processed by the mechanical and biological filters, it is then delivered to the hydroponic beds where nutrients are taken up by the plants. Dissolved nutrients from fish are similar in composition to the nutrients required for hydroponic growth of plants (Rakocy et al. 2006). Certain accumulated nutrients (nitrogen, phosphorous, potassium) in fish aquaculture systems can lead to algae and bacterial growth that can be harmful to fish and lead to off flavors (Fitzsimmons 2006). In aquaponics systems, the plants use dissolved nutrients from the fish aquaculture component thus reducing their accumulation in the RAS. Plants compete for these same nutrients as algae thus leading to reduced algae concentrations in aquaponics system compared to RAS.

Maintaining proper water chemistry parameters requires balancing the fish's, nitrifying bacteria's, and the plant's physiological requirements in one system. Tilapia can tolerate a pH from acidic to alkaline (pH 5-11) (Chervinski 1982) and a wide range of salinity concentrations (Watanabe et al. 2002). Plants grow best and uptake nutrients at a lower pH (5.5-6.5) (Resh 2001). Specifically, lettuce will grow well in a pH range of 5.5-6.5 (Resh 2001, Islam et al. 1980). Nitrifying bacteria is inhibited below a pH of 6.5, with an optimum pH of 7.8 depending on bacterial species and temperature (Antoniou et al.

1990; Tyson et al. 2007). Electrical conductivity levels range between 1-2 mS cm⁻¹ for hydroponic lettuce production (Resh 2001), well below the levels (2000 μ S cm⁻¹) that can be toxic to Nile tilapia (Timmons 2002). Optimal water temperature for Nile tilapia (*Oreochromis niloticus*) ranges between 28-35° C (Chervinski 1982) while lettuce grows at water temperatures between 21-25°C (Resh 2001). These water parameters can influence the physiology of the organisms within the aquaponic system and must be monitored and controlled for optimal system performance. Lettuce grows best at air temperatures between 16 – 25° C and can bolt in air temperature above 28 – 30° C (Resh 2001). Day temperatures of 24° C and night temperatures of 19°C coupled with photosynthetic active radiation (PAR) levels of 17 mol/m² day were found to produce marketable lettuce heads in 24 days after transplant (Both et al. 1994). A marketable lettuce head weighs a minimum of 150 grams and can be achieved in 3-4 weeks after transplant under optimal growing conditions (Both et al. 1994; Resh 2001).

Macronutrients and micronutrients are essential for proper plant growth (Marschner 1995). It has been documented that over time aquaponic systems require some nutrient supplementation not delivered from the fish feed (Rakocy et al. 2006; Seawright 1998). The nutrients are affected by the amount of feed put into the system, the fish to plant ratios, and environmental parameters (Rakocy et al 2006; Seawright 1998; Fitzsimmons 1991). Prior studies at the University of Arizona Environmental Research Lab determined that nitrogen and phosphate were not depleted when a stocking density of 5kg of tilapia were fed 100 grams of feed daily throughout the duration of the growing cycle of 32 heads of lettuce (Licamele unpublished data 2009). Iron, zinc, and copper have been documented to be plant micronutrients that can be depleted in aquaponic

systems (Rakocy et al. 2006; Seawright 1998; Fitzsimmons 1992) and are important for plant health (Resh 2001). Prior observations on aquaponic systems revealed that iron, manganese, and zinc became depleted over time and must be supplemented to achieve optimal plant growth (Licamele unpublished data 2009). Iron and manganese play an important role for photosynthesis in plants. Iron plays a role in chlorophyll synthesis in plants (Marschner 1995). Manganese is primarily associated with photosystem II reactions (Marschner 1995). Zinc plays a role in the structure of plants and is associated with plant hormones responsible for cell elongation in plants (Marschner 1995). These nutrients were targeted for supplementation in the aquaponics system. Plants with equal CCI% will be photosynthesizing and fixing nutrients at similar rates and thus should have similar morphology.

Combining greenhouse environmental control with the recirculating aquaponic system will enable a more controlled analysis of the interactions between nutrients provided by the fish and plant uptake of those nutrients. Prior studies have shown that lettuce in an aquaponics system can be produced with similar growth as hydroponics solution (Licamele et al. 2009). The quality of lettuce grown with aquaponics water must also be analyzed to determine the efficacy of aquaponic vegetable production. Lettuce (*Lactuca sativa* cv. Rex and *Lactuca sativa* cv. Tom Thumb) and Nile tilapia (*O. niloticus*) were grown in a controlled environment aquaponics greenhouse system to compare the differences in growth and quality of lettuce to a standard hydroponic solution. Plant quality was determined by comparing the chlorophyll concentration index of the same plant cultivar grown in both an aquaponics and hydroponic system. This method was used to quantify green coloration in the lettuce. The goal of this study was to

determine if there is a significant difference in growth and quality of lettuce grown in aquaponics water plus supplements or a hydroponic solution.

MATERIALS AND METHODS

Aquaponics System Design and Protocol

The University of Arizona Aquaponics Greenhouse (UAAG) structure developed and operated by the UA-CEAC is a free standing steel A-frame greenhouse with a double wall polycarbonate glazing. The greenhouse is oriented in a north-south configuration. The height to the gutter is 2 meters and height to the ridge is 4 meters. The greenhouse is divided into three separate 7.6 meter by 7.4 meter compartments, each with individual environmental controls. Double wall polycarbonate panels are used to divide the greenhouse. The north section houses the fish aquaculture and water filtration systems and the two south bays house the hydroponic beds.

The aquaculture component of the UAAG is comprised of four 1,300 liter growout aquaculture tanks, four 190 liter fry and fingerling tanks, and a 3,200 liter gallon water collection tank. The aquaculture component consists of a total of 9,160 liters. The tanks were designed and fabricated by GSE Incorporated (Houston, TX USA). The water from the fish aquaculture and plant hydroponic systems drains into the collection tank. A Sweetwater® Centrifugal 1.5 horsepower pump (Aquatic Eco-Systems Inc. Apopka, FL USA) delivers water back to the fish aquaculture system at a rate of 340 liter/minute. A Sweetwater 1 ¼ horsepower regenerative blower was used to provide aeration to the fish tanks, biological filter, water collection tank and hydroponic beds. Aero-Tube™ (Tekni-Plex Inc. Ridgefield, NJ USA) lined the inner perimeter of the growout tanks to provide aeration. Air stones (15 cm x 4 cm) were used to provide aeration to the fingerling tanks. Water from the fish aquaculture system is filtered through a mechanical and a biological filter prior to being distributed to the plants. A Polygeyser™ PG7-PR filter from

International Filter Solutions (IFS) (Marion, TX USA) served as the mechanical filter and a biological filter. The PG7 was plumbed inline with an additional 50 gallon drum filled with Bio-Spheres™ (Aquatic Eco-Systems Inc. Apoka, FL USA) and lined with Aero-Tube™ to provide additional biological filtration. The biological filter contains approximately 0.3 m³ of bio-ball filter media for supporting the maximum fish load (150 kg). Trickling biological filters can convert approximately 1-2 grams of nitrogen per square meter per day (Parker 2002). A HiBlow (HiBlow USA Inc. Saline, MI USA) HP-80 linear air pump delivered air to the PG7 filter at a rate of 1.13 standard cubic feet per hour for backwashing the beads. The filter was purged daily removing five liters of sludge from the system.

Each hydroponic bed holds 1,400 liters of water. An AquaFlo ¼ horsepower pump delivers water to the four hydroponic plant beds at a rate of 340 liters / minute (85 liters per minute per bed). The water is constantly in circulation between the aquaculture and hydroponic sections. Aero-Tube™ was used to line the perimeter of the beds to aerate and circulate the water within the bed. There are two plant growing bays, each plant bay houses two beds. One of the hydroponic beds housed the experimental trials. The second hydroponic bed was used to maintain proper fish to plant ratios throughout the trials. Two hydroponic treatments were set up per trial each consisting of a 0.61 meter by 1.22 meter acrylic box submerged into the aquaponic beds and filled with a hydroponic lettuce solution. This was done to maintain similar water temperature and atmospheric conditions (air temperature, relative humidity, and PAR) for the aquaponics and hydroponic plant treatments. The experiments were conducted from March through May 2009.

The UAAG was designed to produce tilapia (*O. niloticus*) for harvest on a monthly basis and lettuce to be harvested on a weekly basis. Fish tank density ranges from 30 – 80 kg per tank depending on fish growth and/or planting density. Fish are grown out to 1 kg for harvest. It was determined from previous studies conducted at the University of Arizona Environmental Research Lab that 5 kilograms of fish fed 2% biomass per day throughout the growth cycle of lettuce will yield 32 heads of lettuce (Licamele et al 2009). The aquaponics system had been in operation for seven months prior to the onset of the experimental trials and the water in the system reached a steady state.

The seeds were germinated for one week prior to transplant. During the experimental trial there was an initial stocking density of a total of 105 kg of fish supplying 21 m² of plant growing area (a total of 672 lettuce heads). This density was based on the design ratio from previous studies (Licamele et al. 2009). One growout tank had 30 kg of fish, a second had 30 kg of fish, a third was stocked with 35 kg of fish, and a fourth was stocked with 15 kg of fish. Mean fish weight at stocking was 310 grams per fish. Fish were fed 2% of the standing initial biomass daily (2.1 kilograms) throughout the growout cycle of lettuce. The feed used in the experimental trials was manufactured by Star Milling Company (Perris, CA USA) (Table 1). Fish were harvested and the mean Feed Conversion Ratio (FCR) and mean survival was determined for the aquaponics system. Fish were harvested, or sorted and put back into the respective tanks for further growout and maintaining system biomass load for plant growth.

Nutrient Supplementation:

Nutrient supplements were added to the aquaponics system throughout the trials to maintain targeted water chemistry parameters (Table 2). One kilogram of Dolomite 65 Ag Lime was added to the sump every two weeks to maintain a pH of 6.8. Dolomite is comprised of 46.0% calcium carbonate (CaCO_3), 38.5% magnesium carbonate (MgCO_2), 22.7 % calcium (Ca), and 11.8% magnesium (Mg). Organic Materials Review Institute (OMRI) certified Biomin® amino acid chelated minerals were provided by JH Biotech™ (Ventura, CA USA). The first week 800 ml of Biomin® iron (5%), 50 ml of Biomin® Zinc (7%), and 50 ml of Biomin® Manganese (5%) were added to the aquaponics system. On the third week of the trial 100 ml of Biomin® iron (5%) and 50 ml of Biomin® Manganese (5%) were added to the system. Levels equal to or greater than 0.01 mg/l of all nutrients were maintained in the aquaponics water throughout the entire duration of the experimental trial. All supplements were mixed into a 20 liter bucket and diluted with water prior to being added to the system. The supplements were added to the sump and allowed to dissolve into the system for 24 hours before taking a water sample. A hydroponic solution based off a half strength Hoagland's solution was formulated for lettuce and used in the hydroponic treatments (Table 2). The aquaponics targeted water chemistry parameters are listed in Table 2. Water chemistry analysis allows for determination of the amount of soluble nutrients in the aquaponics system. A 500 ml water sample was collected every week from the aquaponics system and sent to a lab for analysis. Water samples were tested by the Soil and Plant Laboratory Incorporated (Santa Clara, CA USA).

Monitoring of Environmental Parameters:

Environmental parameters were monitored and controlled via a network of sensors connected to a Campbell Scientific™ 21X Data logger. The air temperature (°C) was monitored with a Campbell Scientific™ 107-L temperature probe. The relative humidity (%) was monitored with a Campbell Scientific HMP50-L Vaisala™ sensor housed in an aspirator. A LI-COR™ 190 Quantum sensor was used to capture photosynthetic active radiation ($\mu\text{mol m}^{-2}\text{s}^{-1}$) available to the plants. Water parameters were monitored in real time using the Campbell Scientific™ 21X Data Logger and sensors placed in the water collection tank. A Copper-Constantine thermocouple was constructed to measure water temperature (C°), a Hanna HI 3001 electrical conductivity ($\mu\text{S/cm}$) sensor, a Hanna HI 1001 pH sensor, and an Oxyguard Campbell Scientific CS512 dissolved oxygen (mg/l) sensor were used to monitor water parameters in real time throughout the duration of the experiment. The data was processed and stored on a computer in the control room on the North side of the greenhouse. The mean water and environmental parameters are listed in Table 3.

Biomass and Chlorophyll Concentration Index:

Lettuce seeds were transplanted one week after germination into the hydroponic boards. A total of three trials were conducted for this experiment. The Chlorophyll Concentration Index (CCI %) was measured on three different leaves with an Apogee CCM-200 prior to harvesting for each data plant. Lettuce was harvested 35 days after transplant for the first trial. The transplant time for the following two trials was changed to 28 days because head market weights were achieved quicker. The wet weight (grams)

was determined at harvest (28 days after transplant) and samples were dried in a drying oven at 50°C for 72 hours before dry weight (grams) was measured. There were six sample plants per treatment with two replicates for each aquaponic and hydroponic treatment per trial. This totaled 12 data samples (n=12) for the aquaponic treatment and 12 data samples (n=12) for the hydroponic treatment per trial for trials one and two. There were 24 data plants per treatment for *L. sativa* cv. Rex. At the end of the trials the fish growout tanks were harvested, weighed (kg) and restocked with fish accordingly. The mean final weight (kg) and feed conversion ratio (FCR) was calculated for the tilapia fish aquaculture component. The data was analyzed using a paired t-test.

Star Milling Co. Tilapia Feed	
Crude Protein	35%
Crude Fat	5%
Crude Fiber	4%
Ash	9%
Nutrient Analysis	
N	5.97%
P	1.53%
K	1.46%
Ca	1.61%
Mg	0.26%
Na	0.24%
S	0.46%
Cu	15 mg/Kg
Zn	143 mg/ Kg
Mn	93 mg/ Kg
Fe	161 mg/ Kg
B	18 mg/ Kg

TABLE 1. Tilapia fish diet manufactured by Star Milling Company. The manufacturer's label is listed in the table. A nutrient analysis of the feed shows the relative concentrations of nutrients per gram of feed.

Solution Concentrations	Lettuce	
	Solution	Aquaponics
pH	6.50	6.80
Electrical Conductivity (dS/m)	1.80	1.00
Ammonia NH ₃ -N (mg/L)	0.00	0.00
Nitrite NO ₂ (mg/L)	8.00	0.00
Nitrate NO ₃ -N (mg/L)	180.00	50.00
Boron (B) (mg/L)	0.25	0.20
Calcium (Ca) (mg/L)	150.00	60.00
Copper (Cu) (mg/L)	0.10	0.05
Iron (Fe) (mg/L)	1.50	0.05
Magnesium (Mg) (mg/L)	30.00	30.00
Manganese (Mn) (mg/L)	0.25	0.05
Molybdenum (Mo) (mg/L)	0.05	0.05
PO ₄ -P (mg/L)	25.00	50.00
Potassium (K) (mg/L)	125.00	150.00
Sulfate (SO ₄)-S (mg/L)	100.00	50.00
Zinc (Zn) (mg/L)	0.20	0.03

Table 2. Water chemistry target parameters for the aquaponics research trials and the hydroponic lettuce solution.

RESULTS

Water Chemistry Analysis and Environmental Parameters:

The mean water temperature for the duration of the trials was $28.8 \pm 1.7^{\circ}\text{C}$. The mean dissolved oxygen was 5.4 ± 0.5 mg/l, the mean pH was 6.8 ± 0.2 , and the mean electrical conductivity was 0.9 ± 0.2 mS/cm for the aquaponics system water (Table 3). The water and air environmental parameters for the trials are listed in Table 3. The mean air temperature for the first trial was $19.7 \pm 2.3^{\circ}\text{C}$, the second trial was $21.7 \pm 3.5^{\circ}\text{C}$, and the third trial was $21.4 \pm 3.0^{\circ}\text{C}$ (Table 3). The mean relative humidity was greater for the second trial ($47.23 \pm 6.85\%$) and third trial ($46.71 \pm 6.71\%$) than the first trial ($39.79 \pm 7.44\%$) (Table 3). The mean daily integrated photosynthetic active radiation for the first trial was 16.52 ± 5.13 mol $\text{m}^{-2} \text{d}^{-1}$ and was greater than the second trial (13.37 ± 4.06 mol $\text{m}^{-2} \text{day}^{-1}$) but not the third trial (18.70 ± 2.42 mol $\text{m}^{-2} \text{day}^{-1}$) (Table 3).

The system was in operation for six months prior to the onset of the experimental trials. A protocol to maintain pH and proper water chemistry parameters was developed. Iron, zinc, and manganese had reached minimal levels (<0.01) and were targeted for nutrient additions. The water chemistry analysis for the first trial revealed that the nitrate-nitrogen ($\text{NH}_3\text{-N}$) concentration ranged from 18 mg/l to 65 mg/l throughout the duration of the trials (Figure 1). The nitrate-nitrogen concentration for the second trial ranged from 18 mg/l to 62 mg/l and the third ranged from 48 mg/l to 89 mg/l (Figure 1). Ammonia was not measurable in the first trial and reached a level of 1 mg/l near the end of the second and third trials (Figure 1). The iron concentration in the aquaponics water for the first trial ranged from 0.01 mg/l to 0.08 mg/l (Figure 2). The iron concentration in the aquaponics water for the second trial ranged from non detectable to 0.13 mg/l and the third trial ranged from 0.01 mg/l to 0.13mg/l (Figure 2). The concentration of manganese

in the first trial ranged from non detectable mg/l to 0.17 mg/l (Figure 2). The concentration of manganese in the second trial ranged from 0.02 mg/l to 0.04 mg/l and the third trial ranged from 0.02 mg/l to 0.05 mg/l (Figure 2). The concentration of zinc in the aquaponics water for the first trial ranged from non detectable to 0.04 mg/l (Figure 2). The concentration of zinc in the aquaponics water for the second trial ranged from 0.03 mg/l to 0.04 mg/l and the third trial ranged from 0.03 mg/l to 0.05 mg/l (Figure 2). Throughout the duration of the third trial iron, manganese and zinc maintained detectable levels (>0.01 mg/l) in the aquaponics system water.

Biomass Analysis and Chlorophyll Concentration Index:

In the first trial, lettuce (*L. sativa* cv. Rex) was analyzed to determine if there was a significant difference in head wet weight (grams), head dry weight (grams), and CCI% between aquaponics and nutrient supplements versus hydroponics. The mean CCI% of *L. sativa* cv. Rex was $10.1\% \pm 0.90\%$ for the aquaponics treatment and $11.4\% \pm 0.95\%$ for the hydroponic treatment. There was no significant difference ($p \leq 0.05$) in mean head wet weight (grams) and mean head dry weight (grams) for the Rex cultivar grown with aquaponics and hydroponic solution (Figure 5 and Figure 6). There was a significant difference ($p \leq 0.05$) in the mean chlorophyll concentration index (%) between the *L. sativa* CV. Rex grown with aquaponics water and hydroponic solution (Figure 4).

The second trial consisted of a comparison of the Rex cultivar grown in aquaponics water plus supplements and hydroponic solution. There was a significant difference ($p \leq 0.05$) in mean head wet weight between lettuce grown with aquaponics solution plus supplements and hydroponic solution (Figure 5). The aquaponic lettuce had

a greater mean head wet weight (182.79 ± 26.90 grams) at harvest compared to the hydroponic solution. There was no significant difference ($p \leq 0.05$) in CCI% between *L. sativa* cv. Rex grown with aquaponics water plus supplements and hydroponic solution (Figure 6). The mean CCI% for the aquaponics lettuce was $8.5\% \pm 0.63\%$ and the mean CCI% for the lettuce grown with the hydroponic solution was $8.8\% \pm 0.65\%$.

The third trial was another comparison of the Rex Lettuce cultivar. The mean head wet weight was significantly different ($p \leq 0.05$) between lettuce grown with aquaponics solution plus supplements and hydroponic solution (Figure 7). The aquaponic lettuce had a greater mean head wet weight (176.75 ± 31.04 grams) than the hydroponic lettuce at harvest (Figure 7). The mean head dry weight was not significantly different ($p \leq 0.05$) between lettuce grown with aquaponics (4.36 ± 0.78 grams) solution plus supplements and hydroponic (4.60 ± 0.60 grams) solution (Figure 7). There was a significant difference ($p \leq 0.05$) in CCI% between *L. sativa* cv. Rex grown with aquaponics water plus supplements and hydroponic solution (Figure 8). The mean CCI% for the aquaponics lettuce was $9.89 \pm 0.89\%$ and for the hydroponics lettuce was $8.71 \pm 0.45\%$ (Figure 8).

Fish tanks were harvested at the end of the trials. The mean FCR for the fish was 2.56 ± 0.64 and mean fish survival was $82.94\% \pm 0.07$. Fish stocking densities of 110 and 114 fish per growout tank yielded a lower FCR (3.03 and 2.82 respectively) than tanks stocked with 80 fish (1.83). Any fish mortality that occurred was from fish jumping out of the tank. There were no mortalities from the supplement additions or other water chemistry parameters. Fish were fed the same amount of feed throughout the trials to deliver a consistent amount of nutrients to the aquaponics system.

		<u>Trial 1</u>	<u>Trial 2</u>	<u>Trial 3</u>
H₂O	T_{H₂O} C°	29.0 ± 1.8	28.4 ± 1.4	29.0 ± 1.8
	D.O. (mg/l)	5.2 ± 0.9	5.5 ± 0.3	5.6 ± 0.3
	pH	6.8 ± 0.3	6.8 ± 0.1	6.9 ± 0.1
	EC (mS/cm)	1.0 ± 0.3	0.9 ± 0.1	0.9 ± 0.2
Air	T_{air} C°	19.7 ± 2.2	21.7 ± 3.5	21.4 ± 3.0
	RH%	39.8 ± 7.4	47.2 ± 6.9	46.7 ± 6.7
	PAR (mol/m² day)	16.5 ± 5.1	13.4 ± 4.1	18.7 ± 2.4

Table 3. Mean water chemistry and environmental parameters for the aquaponic research trials.

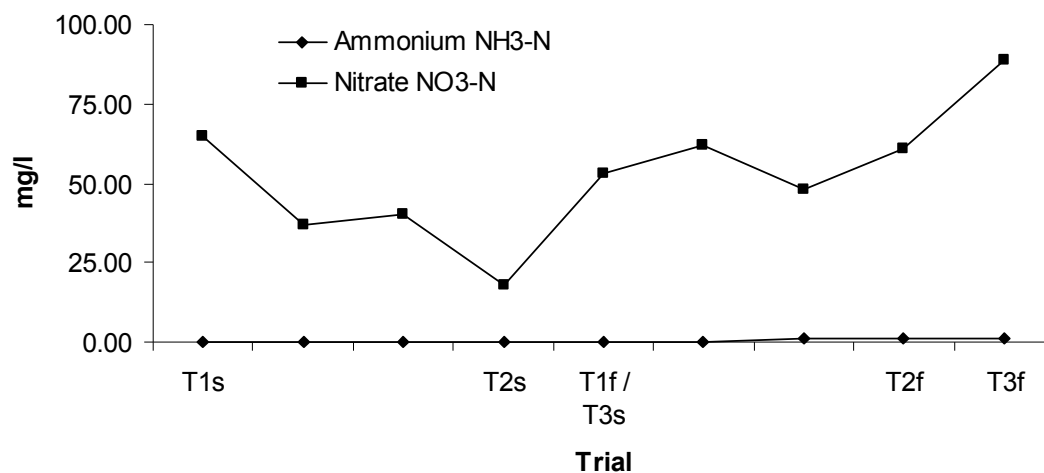


Figure 1. Water chemistry analysis for the aquaponics experiments. Ts is when trials start, Tf is when trials finish. This graph represents the concentration of ammonia-nitrogen and nitrate-nitrogen in the aquaponics water. Nitrate-nitrogen concentration ranged from 18 mg/l to 89 mg/l. The hydroponic solution concentration of nitrate nitrogen was of 180 mg/l.

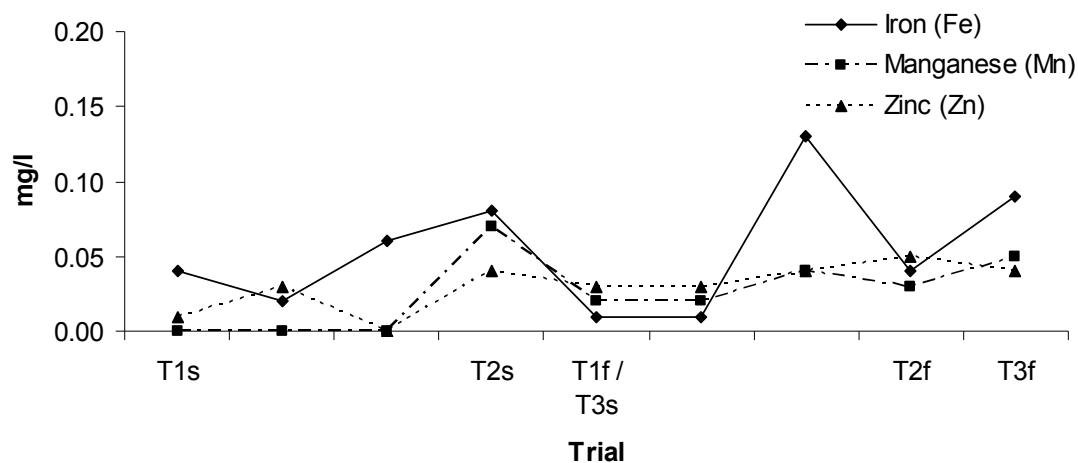


Figure 2. Water chemistry analysis for the aquaponics research trials. Ts is when trials start, Tf is when trials finish. This graph represents the concentration of iron, manganese, and zinc in the aquaponics water throughout the duration of the trials. The concentration of iron, manganese, and zinc in the hydroponic solution was 1.50, 0.25, and 0.20 mg/l respectively.

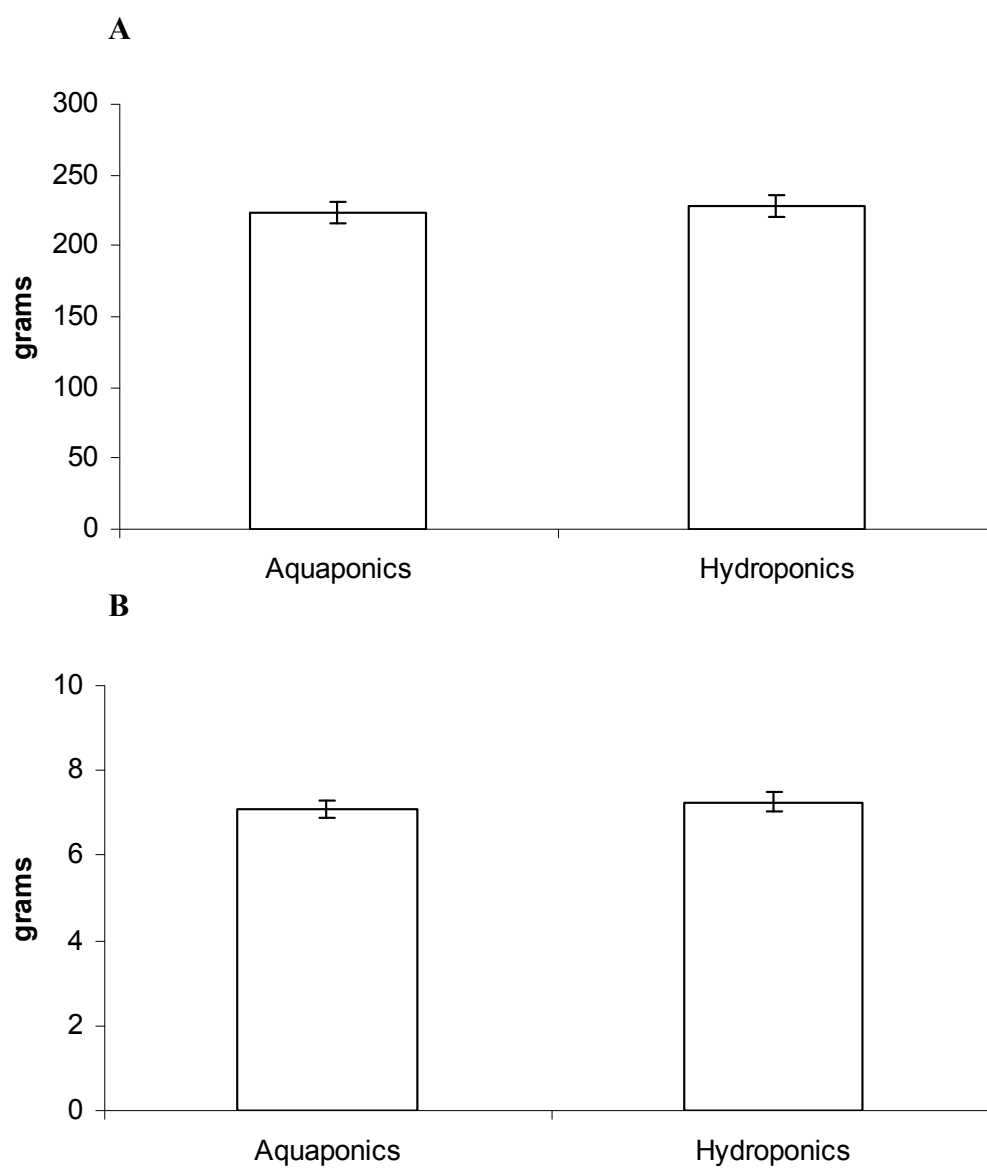


Figure 3. Mean head wet weight (grams) of *L. sativa* cv. Rex grown with aquaponics water and hydroponic solution for the first trial (A). Mean head dry weight (grams) of *L. sativa* cv. Rex grown with aquaponics water and hydroponic solution for the first trial (B). Error bars represent SEM, *represents $p \leq 0.05$ as determined by ANOVA.

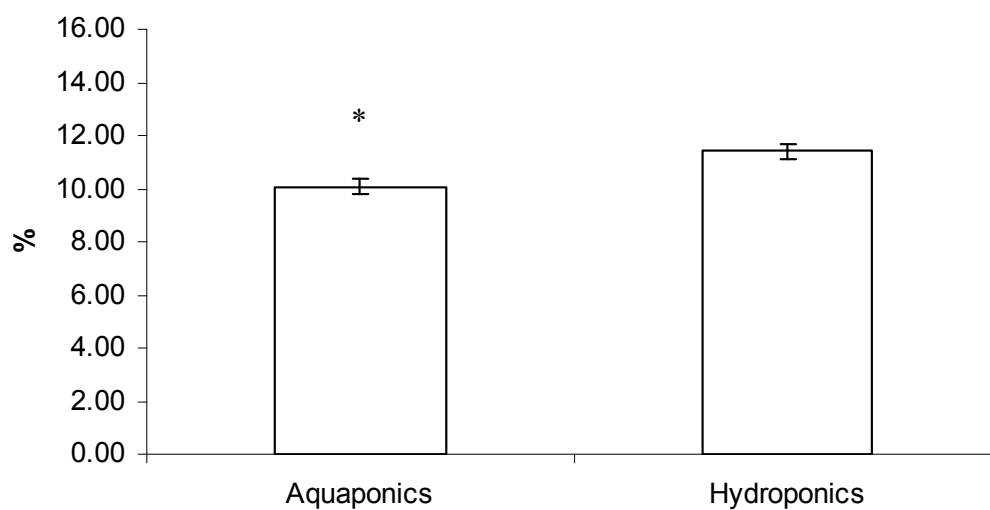


Figure 4. Mean chlorophyll concentration index (%) of *L. sativa* cv. Rex grown with of aquaponics and hydroponic solution for the first trial. There is a significant difference ($p \leq 0.05$) in CCI% of lettuce (*L. sativa* cv. Rex) grown with aquaponics water and hydroponic solution. Error bars represent SEM, * represents $p \leq 0.05$ as determined by ANOVA.

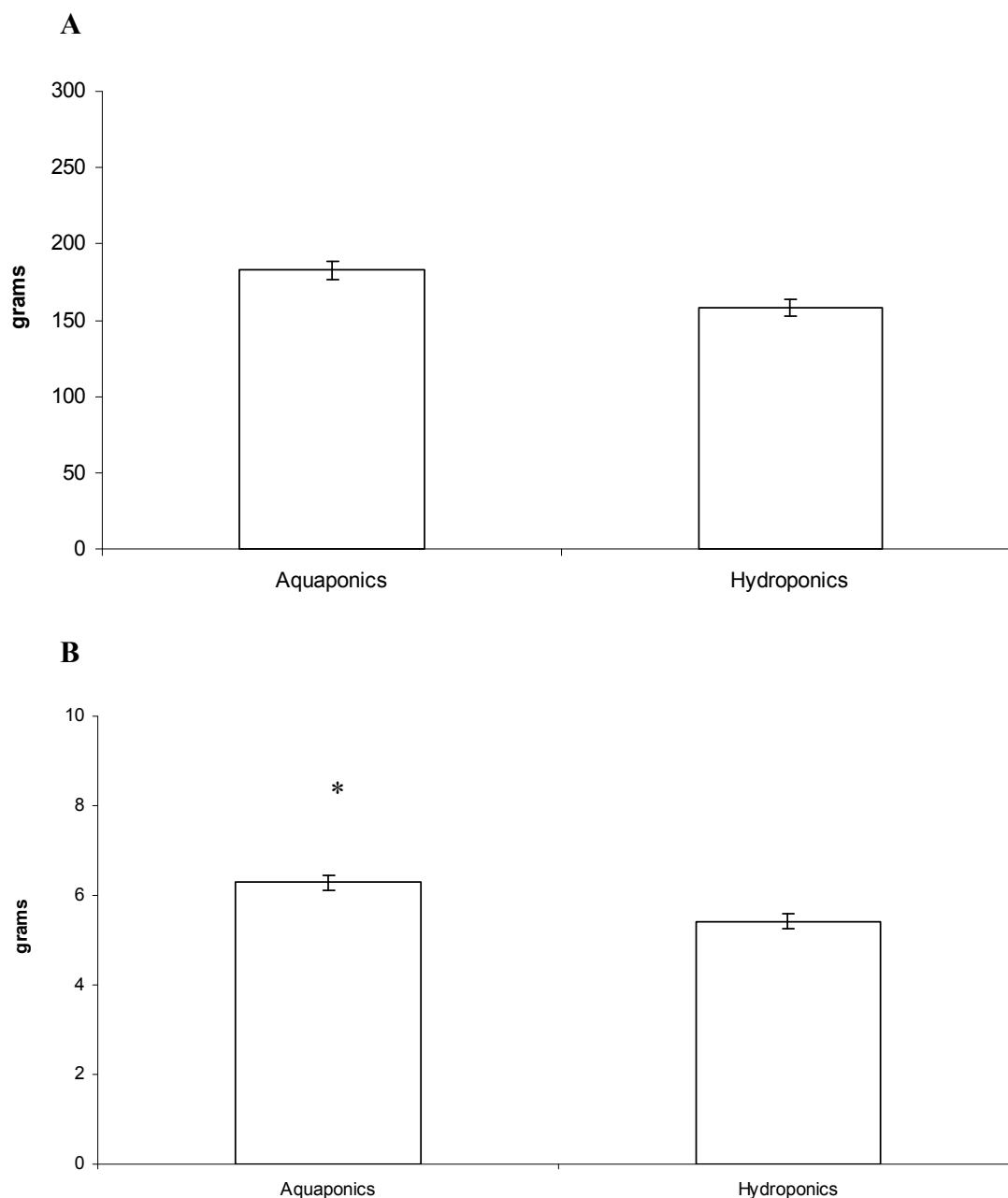


Figure 5. Mean head wet weight (grams) of *L. sativa* cv. Rex grown with aquaponics water and hydroponic solution for the second trial (A). There is a significant difference ($p \leq 0.05$) in mean head wet weight of lettuce (*L. sativa* cv. Rex) grown with aquaponics water and hydroponic solution. Mean head dry weight (grams) of *L. sativa* cv. Rex grown with aquaponics water and hydroponic solution for the second trial (B). There is a significant difference ($p \leq 0.05$) in mean head dry weight of lettuce (*L. sativa* cv. Rex) grown with aquaponics water and hydroponic solution. Error bars represent SEM, * represents $p \leq 0.05$ as determined by ANOVA.

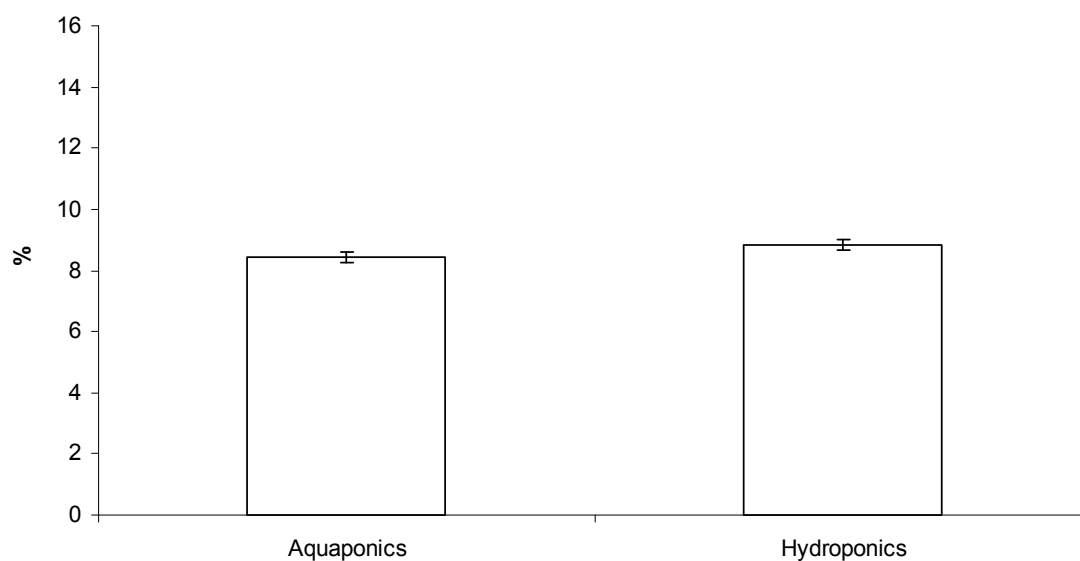


Figure 6. Mean chlorophyll concentration index (%) of *L. sativa* cv. Rex grown with aquaponics water and hydroponic solution for the second trial. There is no significant difference ($p \leq 0.05$) in mean CCI% of lettuce (*L. sativa* cv. Rex) grown with aquaponics water and hydroponic solution. Error bars represent SEM, * represents $p \leq 0.05$ as determined by ANOVA.

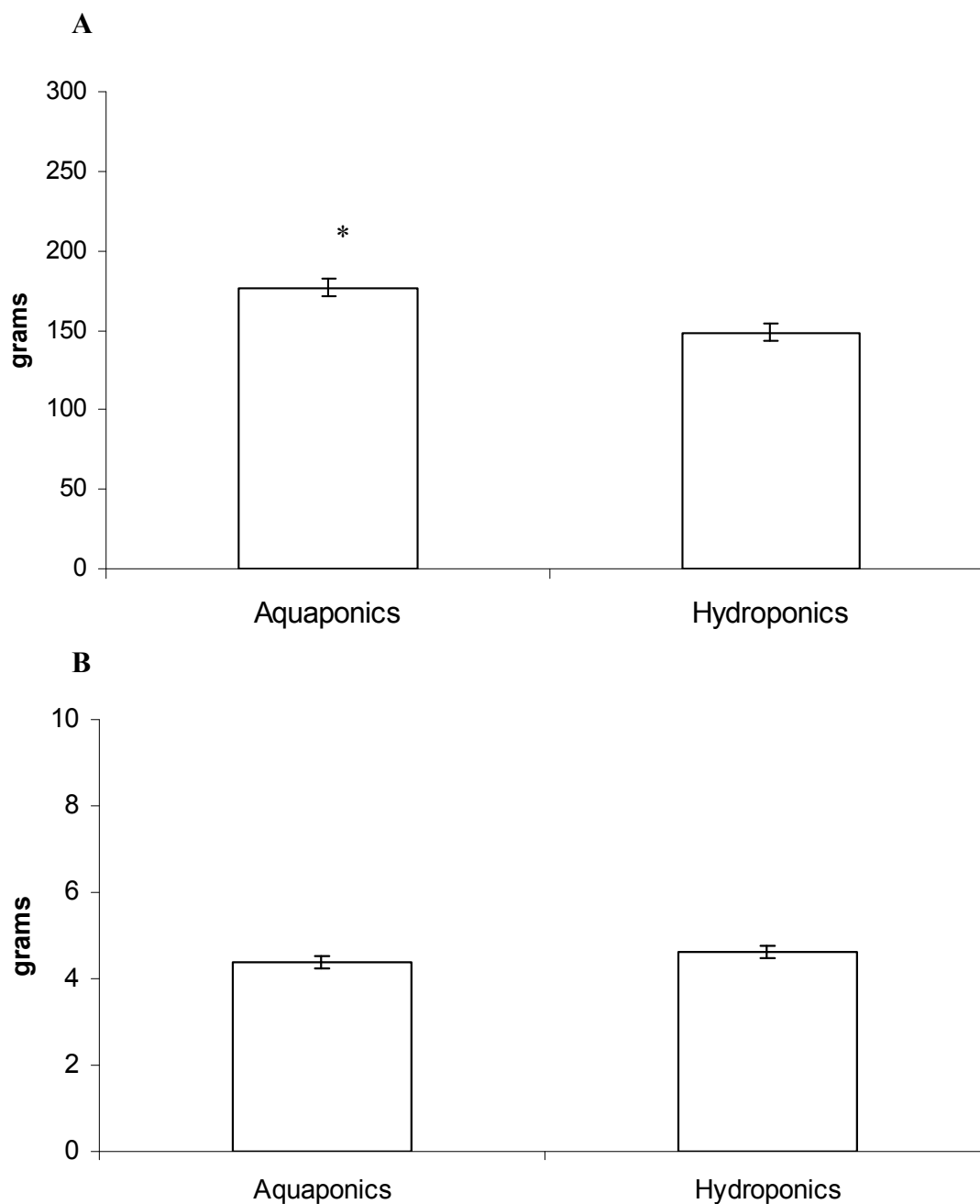


Figure 7. Mean head wet weight (grams) of *L. sativa* cv. Rex grown with aquaponics water and hydroponic solution for the third trial (A). There is a significant difference ($p \leq 0.05$) in mean head wet weight of lettuce (*L. sativa* cv. Rex) grown with aquaponics water and hydroponic solution. Mean head dry weight (grams) of *L. sativa* cv. Rex grown with aquaponics water and hydroponic solution for the third trial (B). There is no significant difference ($p \leq 0.05$) in mean head dry weight of lettuce (*L. sativa* cv. Rex) grown with aquaponics water and hydroponic solution. Error bars represent SEM, * represents $p \leq 0.05$ as determined by ANOVA.

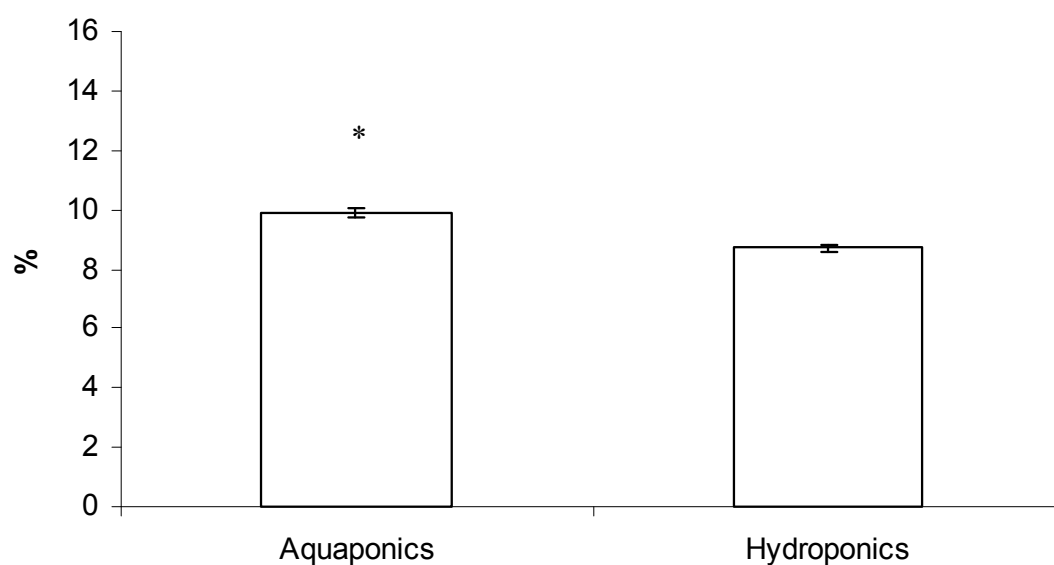


Figure 6. Mean chlorophyll concentration index (%) of *L. sativa* cv. Rex grown with aquaponics water and hydroponic solution for the third trial. There is a significant difference ($p \leq 0.05$) in mean CCI% of lettuce (*L. sativa* cv. Rex) grown with aquaponics water and hydroponic solution. Error bars represent SEM, * represents $p \leq 0.05$ as determined by ANOVA.

DISCUSSION

It has been demonstrated that aquaponics water plus supplementation can grow *L. sativa* cv. Rex and *L. sativa* cv. Tom Thumb with equal or greater biomass accumulation and comparative chlorophyll indices. This indicates that aquaponics lettuce can be produced with similar yield and quality compared with conventional hydroponic solutions when proper nutrient supplementation is added. The micronutrients Iron, manganese and zinc were added to the aquaponics water along with the addition of dolomite to increase the buffering capacity of the water. These additions to the aquaponics water yielded *L. sativa* cv. Rex of equal fresh head weight and quality (coloration). The need for supplementation will depend on the system water chemistry parameters and the plants nutrient demand. The lettuce in the first trial grew larger than lettuce in the second and third trial because lettuce was allowed to grow for 35 days after transplant compared to 28 days after transplant. In the third trial lettuce showed no significant difference in dry weight but had a significant difference in wet weight in favor of the aquaponics lettuce. The CCI% was also significantly greater in the aquaponics lettuce thus increasing the photosynthetic capability of the lettuce and possibly driving more water accumulation in the lettuce.

Lettuce can be grown an aquaponics system utilizing nutrients from the processed fish waste. The integration of fish and plant systems can yield more efficient production and less environmental impact compared to conventional agriculture leading to a more sustainable food production system. The aquaponics system has produced lettuce with equal or greater growth and chlorophyll concentration indices. It was determined that aquaponics water provides a good source of nutrients for plant growth. The nitrogen,

phosphorous and potassium in the aquaponics system reached a near steady state with the lettuce production indicating that fish effluent at the densities established can provide a stable source of macronutrients for plant growth. From this study we conclude that lettuce grown with aquaponics water plus nutrient supplementation grows at similar rates to lettuce grown with hydroponic solutions. Supplementation rates will vary depending on the fish to plant ratio, system design and environmental parameters. Nutrients do not accumulate at the same rate in relation to each other so certain supplements would be needed to match the nutrient demand of the target crop. It has been supported that iron, zinc and manganese accumulate at different rates in aquaponic system (Seawright 1998) and therefore, must be supplemented accordingly. Nutrients can also be recovered from the solid fish wastes.

Further research regarding the production of different crops that require different nutrient demands can lead to different nutrient supplementation protocols. The OMRI listed nutrient supplement BiominTM from JH Biotech allowed for specific nutrients to be targeted and added to the system. This enables the aquaponics system to stay within organic standards by utilizing OMRI listed nutrient products along with processed fish effluent to produce sustainable organic vegetables. Future research should focus on integrating varieties of vegetable crops and tailoring the water chemistry using BiominTM nutrient supplementations to produce crops of equal or greater yields to agriculture and hydroponic systems.

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APPENDIX C: NITROGEN REMEDIATION AND NUTRIENT DYNAMICS IN A CONTROLLED ENVIRONMENT AQUAPONICS SYSTEM

Agriculture, Ecosystems and the Environment

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ABSTRACT

The goal of this study was to determine if there is a significant difference in nutrient composition of lettuce (*L. sativa* c.v. Rex) grown with aquaponics water plus nutrient supplementation and hydroponic solution. The nutrient dynamics of the aquaponic system was examined through water chemistry analysis. The nutrient flows were monitored and tailored to achieve nutrient concentrations for optimal plant growth. The amount of nitrogen removed from the aquaponics system through lettuce biomass accumulation was determined. In conclusion, aquaponics water plus supplementation can grow *L. sativa* cv. Rex with equal biomass accumulation and chlorophyll concentration index equivalent to traditional hydroponics. This indicates that aquaponics lettuce can be produced with similar yield and quality compared with conventional hydroponic solutions when proper supplementation is added. One head of *L. sativa* cv. Rex (176.75 ± 31.03) will deposit approximately 5.96 grams of nitrogen (3.38% per dry gram lettuce). One kilogram of fish will yield 6.4 lettuce heads (1,128 grams) and fixate 38.13 grams of nitrogen. There was a significant increase ($p \leq 0.05$) in percent composition of the micronutrients zinc, manganese, and boron in the leaves of lettuce (*L. sativa* cv. Rex) grown with aquaponics water compared to lettuce grow in hydroponic solution. The mean head wet weight was significantly different ($p \leq 0.05$) between lettuce grown with aquaponics solution plus supplements and hydroponic solution. The aquaponic lettuce had a greater mean head wet weight (176.75 ± 31.04 grams) than the hydroponic lettuce at harvest. The mean head dry weight was not significantly different ($p \leq 0.05$) between lettuce grown with aquaponics (4.36 ± 0.78 grams) solution plus supplements and hydroponic (4.60 ± 0.60 grams) solution. There was a significant difference ($p \leq 0.05$) in

CCI% between *L. sativa* cv. Rex grown with aquaponics water plus supplements and hydroponic solution. The mean CCI% for the aquaponics lettuce was $9.89 \pm 0.89\%$ and for the hydroponics lettuce was $8.71 \pm 0.45\%$. The mean FCR for the fish was 2.56 ± 0.64 and mean fish survival was $82.94\% \pm 0.07$. Fish stocking densities of 110 and 114 fish per growout tank yielded a lower FCR (3.03 and 2.82 respectively) than tanks stocked with 80 fish (1.83). Lettuce (*L. sativa* cv. Rex) can be grown with aquaponics water plus nutrient supplementation and yield adequate nutritional value compared to lettuce grown with hydroponic solution.

INTRODUCTION

Water and land resources are decreasing and as demand for their utilization to produce food increases their value will increase. Resources used in farming of food consist primarily of water, land, and feed. All three of these resources are limited. Agriculture accounts for approximately 70% of the global water use (Rakocy et al. 2006). Farm irrigation is one of the major sinks for freshwater in the world. Non-point source pollution from agriculture comprises nitrogen and causes environmental degradation (Fitzsimmons 1992). It is speculated that a 10% drop in irrigation water can save more water than water used by consumers (Rogers 2008). Improving water use by agriculture and food production is critical in order to supply the demand for food in the future (FAO 2003). Establishing proper water management practices will lead to an increase in water use efficiency and a reduction in environmental pollution. Phosphorous and nitrogen pollution of surface waterways is attributed to agricultural runoff (Fitzsimmons and Posadas 1997). Elevated nitrogen and phosphorous levels have been documented to have negative impacts on aquatic ecosystems (Fitzsimmons and Posadas 1997). Reducing the amount of nitrogen in agriculture and aquaculture discharge water can reduce the environmental impact from non point source pollution. This can be done through proper fertilizer application rates, reducing water discharge by recirculating water, and using biological organisms to remediate the water. This information will lead to more conservative agricultural practices that maximum production.

Aquaponics is the integration of recirculating fish production systems with hydroponic plant production to utilize the fertilizers efficiently. Coupling fish aquaculture with hydroponic plant culture is more sustainable than conventional agriculture systems

(McMurtry et al. 1990; Fitzsimmons 1992; Rakocy et al. 1992; Rakocy and Nair 1987; Rakocy and Hargreaves 1993). It takes approximately 500 liters of water to produce \$100 of product (fish and lettuce), whereas producing cattle takes more than 100 times as much water to produce \$100 of product (Rakocy et al. 2004). Dissolved nutrients from fish are similar to the nutrients required for hydroponic growth of plants (Rakocy et al. 2006). The production of fish and vegetables through the integration of fish aquaculture and plant production has been demonstrated (Fitzsimmons 1991; Fitzsimmons 1992; Rakocy et al. 1992; McMurtry et al. 1997; Chaves 2000; McIntosh and Fitzsimmons 2003; Sabidov 2004; Castro et al. 2006; Diver 2006). Popular food crops, such as lettuce, basil, tomatoes and strawberries, have been successfully cultivated with the use of fish effluent as the primary fertilizer (McMurtry et al. 1997, Takeda et al. 1997, Rakocy et al. 2004, Hanson et al. 2008). Intensive RAS can produce more fish per liter of water than other types of aquaculture systems (Timmons et al. 2002) therefore reducing water used. Greenhouse hydroponics production can produce from five to ten times more output compared to conventional agriculture (Resh 2001; Hannan 1998). Aquaponic systems can yield similar crop production compared to hydroponic systems (Sabidov 2004). Water from RAS systems can be used in greenhouse hydroponics to intensify production by utilizing resources more efficiently, potentially reducing water usage by 20-27% (Chavez et al. 2000).

The concept of aquaponics is to balance the nutrients within a given system. Nutrients are delivered to the system through an input source, in this case fish feed. Protein content in the feed dictates the amount of nitrogen that is available to the plants after the fish assimilate and process the nutrients (Timmons 1996). The density of fish,

protein content in the feed, and the feeding rate determine the nutrient loading of the system. Aquatic animals convert feed to biomass at better rates than other terrestrial food animals (Parker 2002). Fish require less energy for body support and are cold blooded therefore expending less metabolic energy for body temperature regulation. This will yield low feed conversion ratios (FCR), thus producing more product per feed unit. Wastewater from aquaculture farms primarily discharge water composed of nutrients and organic matter, which is suitable for plant growth. Water from intensive recirculating fish culture systems can have total ammonia levels up to 19.2 mg/l, nitrate levels over 500 mg/L, phosphate levels up to 53 mg/L, and potassium levels up to 150 mg/L (Fitzsimmons 1992). These nutrient levels are suitable for plant growth and can be manipulated by increasing fish biomass, feed rate, or by increasing the protein levels in the feed.

Nitrogen is used for protein and amino acid synthesis. Proteins make up structural tissues, transport oxygen or hemoglobin, regulate reactions as hormones, and catalyze biochemical reactions as enzymes (Parker 2002; Santamaria 2002). Protein is the primary nutrient for fish growth and can account for more than 60 percent of fish feed cost (Hatch and Kinnucan 1993). The Nitrogen cycle is critical for sustaining life by conversion of harmful waste buildup (ammonia) from animal excretion to a less toxic form (nitrates) for plant uptake. Ammonia ($\text{NH}_3\text{--NH}_4^+$) is oxidized by *Nitrosomonas spp.* to nitrite (NO_2^-) (Timmons et al. 2002). Nitrite is then oxidized by *Nitrobacter spp.* to nitrate (NO_3^-) which is taken up by the lettuce plants for growth (Broadly et al. 2003). The nitrification process is an acid forming process which releases H^+ in turn increases the pH of the water (Timmons et al. 2002). Calcium hydroxide and potassium hydroxide, are used for

pH stabilization of aquaponic systems (Rakocy et al. 2006). The nitrogen is removed from the system when the plants are harvested.

Plants use ammonia and nitrates for growth (Marschner 1995). Nitrate is taken up by the plant at better rates than ammonia nitrite can be toxic to plants (Britto and Konzucker 2002). Depending on plant species sensitivity symptoms of ammonia toxicity appear with external ammonia concentrations above 0.1 - 0.5 $\mu\text{mol/L}$ (Britto and Kronsucker 2002). Ammonia concentrations at elevated levels can inhibit nutrient uptake in plants by altering the ionic capacity of the water medium. The fish component of an aquaponics system provides the water with nutrients via feeding and excretion. Plants can uptake nitrate build up in fish systems serving as a natural filter that can generate profits simultaneously with the fish.

Lettuce (*Lactuca sativa* cv.) is commonly cultured in hydroponic and aquaponic systems. It is a hardy plant that has a fast growth rate (Resh 2001). A marketable lettuce head weighs a minimum of 150 grams and can be achieved in 3-4 weeks after transplant under optimal growing conditions (Both et al. 1994; Resh 2001). Lettuce can deposit a large amount of nitrogen to its leaves and the nitrogen deposition can be manipulated by plant density and nitrogen availability (Seawright 1998). Fish and plants require water chemistry that can overlap in range. Tilapia can tolerate a pH from acidic to alkaline (pH 5-11) (Chervinski 1982) and a wide range of salinity concentrations (Watanabe et al. 2002). Lettuce will grow best in a pH range of 5.5-6.5 (Resh 2001, Islam et al. 1980). Nitrifying bacteria is inhibited below a pH of 6.5, with an optimum pH of 7.8 depending on bacterial species and temperature (Antoniou et al. 1990; Tyson et al. 2007). Electrical conductivity levels range between 1-2 mS cm^{-1} for hydroponic lettuce production (Resh

2001), well below the levels ($2000 \mu\text{S cm}^{-1}$) that can be toxic to Nile tilapia (Timmons 2002). Optimal water temperature for Nile tilapia (*Oreochromis niloticus*) ranges between $28\text{--}35^\circ\text{C}$ (Chervinski 1982) while lettuce grows best at water temperatures between $21\text{--}25^\circ\text{C}$ (Resh 2001). Lettuce grows best at air temperatures between $16\text{--}25^\circ\text{C}$ and will bolt in air temperature above $25\text{--}28^\circ\text{C}$ (Resh 2001). Day temperatures of 24°C and night temperatures of 19°C coupled with photosynthetic active radiation (PAR) levels of $17 \text{ mol/m}^2 \text{ day}$ were found to produce marketable lettuce heads in 24 days after transplant (Both et al. 1994). These water parameters can influence the physiology of the organisms within the aquaponic system and must be monitored and controlled for optimal system performance.

The goal of this study was to develop a nitrogen budget of an aquaponics system. The amount of nitrogen removed from the aquaponics system through lettuce biomass accumulation was determined. A second objective was to determine if there was a significant difference in nutrient composition of lettuce (*L. sativa* cv. Rex) grown with aquaponics water plus nutrient supplementation and hydroponic solution. The nutrient dynamics of the aquaponic system was examined through water chemistry analysis. The nutrient flows were monitored and tailored to achieve recommended nutrient concentrations for expected plant growth.

MATERIALS AND METHODS

Aquaponics System Design and Protocol

The University of Arizona Aquaponics Greenhouse (UAAG) structure is a free standing steel A-frame greenhouse with a double wall polycarbonate glazing. The greenhouse is oriented in a north-south configuration. The height to the gutter is 2 meters and height to the ridge is 4 meters. The greenhouse is divided into three separate 7.6 meter by 7.4 meter compartments, each with individual environmental controls. Double wall polycarbonate panels are used to divide the greenhouse. The north section houses the fish aquaculture and water filtration systems and the two south bays house the hydroponic beds.

The aquaculture component of the UAAG is comprised of four 1,300 liter growout aquaculture tanks, four 190 liter fry and fingerling tanks, and a 3,200 liter gallon water collection tank. The aquaculture component consists of a total of 9,160 liters. The tanks were designed and fabricated by GSE Incorporated (Houston, TX USA). The water from the fish aquaculture and plant hydroponic systems drains into the collection tank. A Sweetwater® (Aquatic Eco_Systems Inc. Apoka, FL USA) Centrifugal 1.5 horsepower pump delivers water back to the fish aquaculture system at a rate of 340 liter/minute. A Sweetwater 1 ¼ horsepower regenerative blower was used to provide aeration to the fish tanks, biological filter, water collection tank and hydroponic beds. Aero-Tube™ (Tekni-Plex Inc. Ridgefield, NJ USA) lined the inner perimeter of the growout tanks to provide aeration. Air stones (15 cm x 4 cm) were used to provide aeration to the fingerling tanks. Water from the fish aquaculture system is filtered through a mechanical and a biological filter prior to being distributed to the plants. A Polygeyser™ PG7-PR filter from

International Filter Solutions (IFS) (Marion, TX USA) served as the mechanical filter and a biological filter. The PG7 was plumbed inline with an additional 50 gallon drum filled with Bio-Spheres™ (Aquatic Eco-Systems Apoka, FL USA) and lined with Aero-Tube™ to provide additional biological filtration. The biological filter contains approximately 0.3 m³ of bio-ball filter media for supporting the maximum fish load (150 kg). Trickling biological filters can convert approximately 1-2 grams of nitrogen per square meter per day (Parker 2002). A Hiblow (HiBlow USA Inc. Saline, MI USA) HP-80 linear air pump delivered air to the PG7 filter at a rate of 1.13 standard cubic feet per hour for backwashing the beads. The filter was purged daily removing five liters of sludge from the system.

Each hydroponic bed holds 1,400 liters of water. An AquaFlo ¼ horsepower pump delivers water to the four hydroponic plant beds at a rate of 340 liters / minute (85 liters per minute per bed). The water is constantly in circulation between the aquaculture and hydroponic sections. Aero-Tube™ was used to line the perimeter of the beds to aerate and circulate the water within the bed. There are two plant growing bays, each plant bay houses two beds. One of the hydroponic beds housed the experimental trials. The second hydroponic bed was used to maintain proper fish to plant ratios throughout the trials. Two hydroponic treatments were set up per trial each consisting of a 0.61 meter by 1.22 meter acrylic box submerged into the aquaponic beds and filled with a hydroponic lettuce solution. This was done to maintain similar water temperature and atmospheric conditions (air temperature, relative humidity, and PAR) for the aquaponics and hydroponic plant treatments.

The UAAG was designed to produce tilapia (*O. niloticus*) for harvest on a monthly basis and lettuce to be harvested on a weekly basis. Fish tank density ranges from 30 – 80 kg per tank depending on fish growth and/or planting density. Fish are grown out to 1 kg for harvest. It was determined from previous studies conducted at the University of Arizona Environmental Research Lab that 5 kilograms of fish fed 2% biomass per day throughout the growth cycle of lettuce will yield 32 heads of lettuce (Licamele et. al. unpublished data 2009). The aquaponics system was online for seven months prior to the onset of the experimental trials and the water in the system reached a steady state.

The seeds were germinated for one week prior to transplant. During the experimental trial there was an initial stocking density of a total of 105 kg of fish supplying 21 m² of plant growing area (128 lettuce heads a week). One growout tank had 30 kg of fish, a second had 30 kg of fish, a third was stocked with 35 kg of fish, and a fourth was stocked with 15 kg of fish. Mean fish weight at stocking was 310 grams per fish. Fish were fed 2% of the standing initial biomass daily (2.1 kilograms) throughout the growout cycle of lettuce. The feed used in the experimental trials was manufactured by Star Milling Company (Table 1). Fish were harvested and the mean Feed Conversion Ratio (FCR) and mean survival was determined for the aquaponics system. Fish were harvested, or sorted and put back into the respective tanks for further growout and maintaining system biomass load for plant growth.

Nutrient Supplementation:

Nutrient supplements were added to the aquaponics system throughout the trials to maintain targeted water chemistry parameters (Table 2). One kilogram of Dolomite 65 Ag Lime was added to the sump every two weeks to maintain a pH of 6.8. Dolomite is comprised of 46.0% calcium carbonate (CaCO_3), 38.5% magnesium carbonate (MgCO_2), 22.7 % calcium (Ca), and 11.8% magnesium (Mg). Organic Materials Review Institute (OMRI) certified Biomin® amino acid chelated minerals were provided by JH Biotech™ (Ventura, CA USA) The first week 800 ml of Biomin® iron (5%), 50 ml of Biomin® Zinc (7%), and 50 ml of Biomin® Manganese (5%) were added to the aquaponics system. On the third week of the trial 100 ml of Biomin® iron (5%) and 50 ml of Biomin® Manganese (5%) were added to the system. Levels equal to or greater than 0.01 mg/l of all nutrients were maintained in the aquaponics water throughout the entire duration of the experimental trial. All supplements were mixed into a 20 liter bucket and diluted with water prior to being added to the system. The supplements were added to the sump and allowed to dissolve into the system for 24 hours before taking a water sample. A hydroponic solution was formulated for lettuce and used in the hydroponic treatments (Table 2). The aquaponics targeted water chemistry parameters are listed in Table 2. Water chemistry analysis allows for determination of the amount of soluble nutrients in the aquaponics system. A 500 ml water sample was collected every week from the aquaponics system and sent to a lab for analysis. Water samples were tested by the Soil and Plant Laboratory Incorporated (Santa Clara, California).

Monitoring of Environmental Parameters:

Environmental parameters were monitored and controlled via a network of sensors connected to a Campbell Scientific™ (Campbell Scientific Inc. Logan, Utah USA) 21X Data logger. The air temperature (C°) was monitored with a Campbell Scientific™ 107-L temperature probe. The relative humidity (%) was monitored with a Campbell Scientific HMP50-L Vaisala™ sensor housed in an aspirator. A Li-Cor™ (Li-Cor Biosciences Lincoln, NE USA) 190 Quantum sensor was used to capture photosynthetic active radiation ($\mu\text{mol m}^{-2}\text{s}^{-1}$) available to the plants. Water parameters were monitored in real time using the Campbell Scientific™ 21X Data Logger and sensors placed in the water collection tank. A Copper-Constantine thermocouple was constructed to measure water temperature (C°), a Hanna™ (Hanna Instruments Woonsocket, RI USA) HI 3001 electrical conductivity ($\mu\text{S/cm}$) sensor, a Hanna HI 1001 pH sensor, and an Oxyguard™ CS512 dissolved oxygen (mg/l) from Campbell Scientific sensor were used to monitor water parameters in real time throughout the duration of the experiment. The data was processed and stored on a computer in the control room on the North side of the greenhouse. The mean water and environmental parameters are listed in Table 3.

Data Collection and Statistical Analysis:

Lettuce seeds were transplanted one week after germination into the hydroponic boards at a density of 32 heads per m^2 . There were 24 data plants per aquaponic and hydroponic treatment for *L. sativa* cv. Rex. The wet weight (grams) was taken at harvest (28 days after transplant) and samples were dried in a drying oven at 50°C for 72 hours

before dry weight (grams) was measured. The data was analyzed to test for a significant difference ($p \leq 0.05$) in wet weight, dry weight, and CCI% in lettuce grown with aquaponics water plus supplements and a hydroponic solution. Six data plants per aquaponic and hydroponic treatment were sent to the Soil and Plant Laboratory Inc. (Santa Clara, California) for dry mass analysis. The nutrient content of the lettuce was tested for a significant difference ($p \leq 0.05$) between lettuce grown with aquaponic water plus supplements and hydroponic solution. The amount of nitrogen removed from the aquaponic system via the lettuce was calculated. The nutrient composition of the fish feed and the aquaponics sludge was analyzed by the Soil and Plant Laboratory Incorporated. A student's T-test was applied to test for significant differences ($p \leq 0.05$).

Star Milling Co. Tilapia Feed	
Crude Protein	35%
Crude Fat	5%
Crude Fiber	4%
Ash	9%
Nutrient Content	
N	5.97%
P	1.53%
K	1.46%
Ca	1.61%
Mg	0.26%
Na	0.24%
S	0.46%
Cu	15 mg/Kg
Zn	143 mg/Kg
Mn	93 mg/Kg
Fe	161 mg/Kg
B	18 mg/Kg

TABLE 1. Tilapia fish diet manufactured by Star Milling Company. The manufacturer's label is listed in the table. A nutrient analysis of the feed shows the relative concentrations of nutrients per gram of feed.

Solution Concentrations	Lettuce	
	Solution	Aquaponics
pH	6.50	6.80
Electrical Conductivity (dS/m)	1.80	1.00
Ammonia NH ₃ -N (mg/L)	0.00	0.00
Nitrite NO ₂ (mg/L)	8.00	0.00
Nitrate NO ₃ -N (mg/L)	180.00	50.00
Boron (B) (mg/L)	0.25	0.20
Calcium (Ca) (mg/L)	150.00	60.00
Copper (Cu) (mg/L)	0.10	0.05
Iron (Fe) (mg/L)	1.50	0.05
Magnesium (Mg) (mg/L)	30.00	30.00
Manganese (Mn) (mg/L)	0.25	0.05
Molybdenum (Mo) (mg/L)	0.05	0.05
PO ₄ -P (mg/L)	25.00	50.00
Potassium (K) (mg/L)	125.00	150.00
Sulfate (SO ₄)-S (mg/L)	100.00	50.00

Table 2. Water chemistry target parameters for the aquaponics research trials and the hydroponic solution.

H₂O	T_{H2O} C⁰	28.9 ± 1.8
	D.O. (mg/l)	5.6 ± 0.3
	pH	6.8 ± 0.1
	EC (μS/cm)	0.9 ± 0.2
Air	T_{air} C⁰	21.4 ± 3.0
	RH%	46.7 ± 6.7
	PAR (mol/m² day)	18.7 ± 2.4

Table 3. Mean water chemistry and environmental parameters for the aquaponic research trial.

RESULTS

Environmental Parameters:

The water and air environmental parameters for the trial are listed in Table 3. The mean water temperature for the duration of the trial was 28°C. The mean dissolved oxygen was 5 mg/l, the pH was 6.8, and the mean electrical conductivity was 0.9 ± 0.2 mS/cm for the aquaponics system water (Table 3). The mean air temperature for the third trial was 21.40 ± 3.00 °C (Table 3). The mean relative humidity was $(46.71 \pm 6.71 \%)$ (Table 3). The mean daily integrated photosynthetic active radiation was $(18.70 \pm 2.42 \text{ mol m}^{-2} \text{ day}^{-1})$ (Table 3).

Water Chemistry Analysis:

The nitrate-nitrogen concentration for the trail ranged from 48 mg/l to 89 mg/l (Figure 1A). Ammonia was present reaching a level of 1 mg/l near the end of the experiment (Figure 1A). Macronutrient concentration reached a near steady state throughout the trial, however, sulfate seemed to accumulate at a high rate (Figure 1A). The iron concentration in the aquaponics water ranged from 0.01 mg/l to 0.13mg/l (Figure 1B). The concentration of manganese ranged from 0.02 mg/l to 0.05 mg/l (Figure 1B). The concentration of zinc in the aquaponics water ranged from 0.03 mg/l to 0.05 mg/l (Figure 1B). Throughout the duration of the experiment iron, manganese and zinc maintained detectable levels (>0.01 mg/l) in the aquaponics system water. Concentration of micronutrients such as iron, zinc, manganese, and molybdenum in the aquaponics water did show a reduction in concentration over time (Figure 1B). This indicates the need for micronutrient supplementation at the specified fish to plant ratios of 5 kg of fish to 32 lettuce heads.

Biomass Analysis:

The mean head wet weight was significantly different ($p \leq 0.05$) between lettuce grown with aquaponics solution plus supplements and hydroponic solution (Figure 2). The aquaponic lettuce had a greater mean head wet weight (176.75 ± 31.04 grams) than the hydroponic lettuce at harvest (Figure 2). The mean head dry weight was not significantly different ($p \leq 0.05$) between lettuce grown with aquaponics (4.36 ± 0.78 grams) solution plus supplements and hydroponic (4.60 ± 0.60 grams) solution (Figure 2). There was a significant difference ($p \leq 0.05$) in CCI% between *L. sativa* cv. Rex grown with aquaponics water plus supplements and hydroponic solution (Figure 3). Fish tanks were harvested at the end of the trial. The mean FCR for the fish was 2.56 ± 0.64 and mean fish survival was $82.94\% \pm 0.07$. Fish stocking densities of 110 and 114 fish per growout tank yielded a lower FCR (3.03 and 2.82 respectively) than tanks stocked with 80 fish (1.83). Any fish mortality that occurred was from fish jumping out of the tank. There were no mortalities from the supplement additions or other water chemistry parameters. Fish were fed the same amount of feed throughout the trials to deliver a consistent amount of nutrients to the aquaponics system.

Nutrient Analysis:

The composition of macronutrients and micronutrients in the lettuce grown via aquaponics and hydroponics are shown in Figure 3. There was a significant difference ($p \leq 0.05$) in percent composition per dry gram of lettuce leaf in nitrogen, phosphorous, and magnesium between lettuce grown in the aquaponics water compared with hydroponic solution. There was a greater percent concentration of nitrogen (5.22 ± 0.38 %), phosphorous ($1.14 \pm 0.07\%$), and magnesium (0.74 ± 0.13) in lettuce (*L. sativa* cv. Rex)

grown with aquaponics water as compared to hydroponic solution (Figure 4A). There was no significant difference ($p \leq 0.05$) in calcium composition in the leaves of lettuce (*L. sativa* cv. Rex) grown with aquaponic water (1.94 ± 0.08) and hydroponic solution (1.82 ± 0.14). The concentration of potassium and sulfate in the leaves of lettuce (*L. sativa*. Cv. Rex) was not significantly ($p \leq 0.05$) different in the lettuce grown in aquaponics water and hydroponic solution (Figure 3A). There was a significant increase ($p \leq 0.05$) in percent composition of the micronutrients zinc, manganese, and boron in the leaves of lettuce (*L. sativa* cv. Rex) grown with aquaponics water compared to lettuce grow in hydroponic solution (Figure 3B). There was no significant difference ($p \leq 0.05$) in percent composition in iron and copper in lettuce (*L. sativa* cv. Rex) grown with aquaponics water and hydroponic solution (Figure 3B). The percent nutrient composition in the aquaponics lettuce (*L. sativa* cv. Rex) was greater in all micronutrients except copper (Figure 3B).

Nitrogen Dynamics:

The percent nutrient composition of macronutrients (N-P-K) in the aquaponics lettuce (*L. sativa* cv. Rex) was greater than the hydroponic lettuce (Figure 3A). In the aquaponics lettuce there was $5.22 \pm 0.15\%$ nitrogen per dry gram lettuce (Figure 3A). In the hydroponic lettuce there was $4.62 \pm 0.26\%$ nitrogen per dry gram of lettuce (Figure 3A). The nutrient composition of the fish sludge is listed in Figure 4. The percent nutrient composition of nitrogen (3.38%) and calcium (1.36%) were present in high levels in the sludge in relation to the other macronutrients (Figure 4A). The nutrient composition of the fish sludge consisted of elevated levels of iron (759 mg / l) and zinc (233 mg / l)

(Figure 4B). One head of *L. sativa* cv. Rex (176.75 ± 31.03) will assimilate approximately 9.23 ± 0.05 grams of nitrogen ($5.22 \pm 0.15\%$ per dry gram lettuce). One kilogram of fish will yield 6.4 lettuce heads (1,128 grams) and assimilate approximately 58.88 ± 1.69 grams of nitrogen.

A nitrogen model was constructed for the aquaponics system (Figure 5). For every kilogram of feed put into the system, 59.7 grams of nitrogen is added to the system. A total of 2.1 kg of fish feed was added to the system daily. Approximately 125.37 grams of nitrogen were added to the aquaponics system daily via the feed (5.97% nitrogen in feed). There was a total of 4.39 kg of nitrogen introduced to the aquaponics system over the 35 day experiment. The fish digest 90% of the proteins in the feed (Timmons et al. 2002) which is comprised of 5.97% nitrogen (Table 1). Aquaponics lettuce was comprised of 5.22% nitrogen. There was approximately 5 liters of sludge per day collected from the mechanical filter. The sludge is discharged in aqueous form and is approximately 2% dry sludge (Timmons et al. 2002). Nitrogen loss that is unaccounted for consists of nitrogen gas, algae, and micro fauna within the aquaponics system.

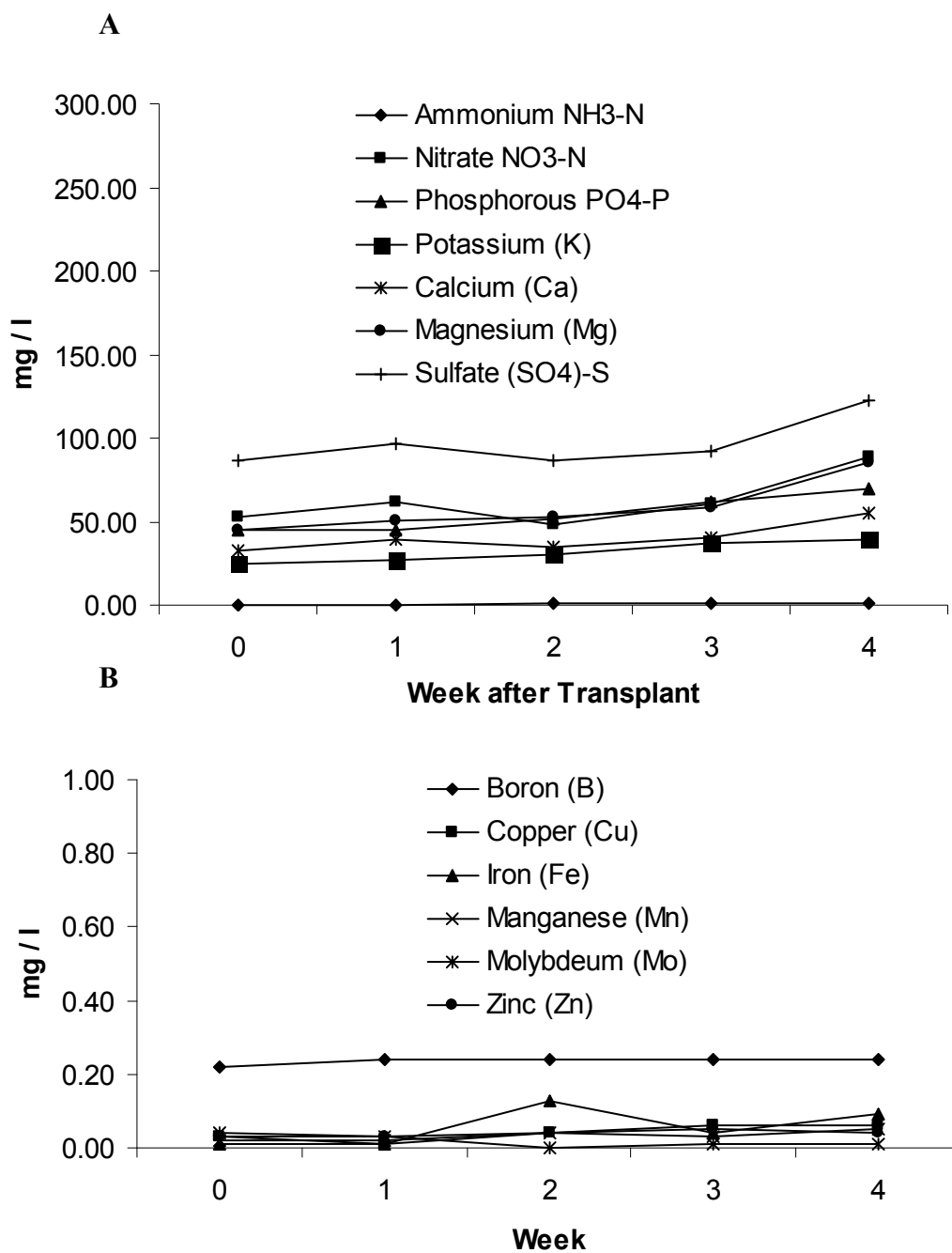


Figure 1. Concentration of macronutrients (A) in the aquaponics water. Concentration of micronutrients in the aquaponics water. Water samples were taken at the onset of the experiment and every week thereafter.

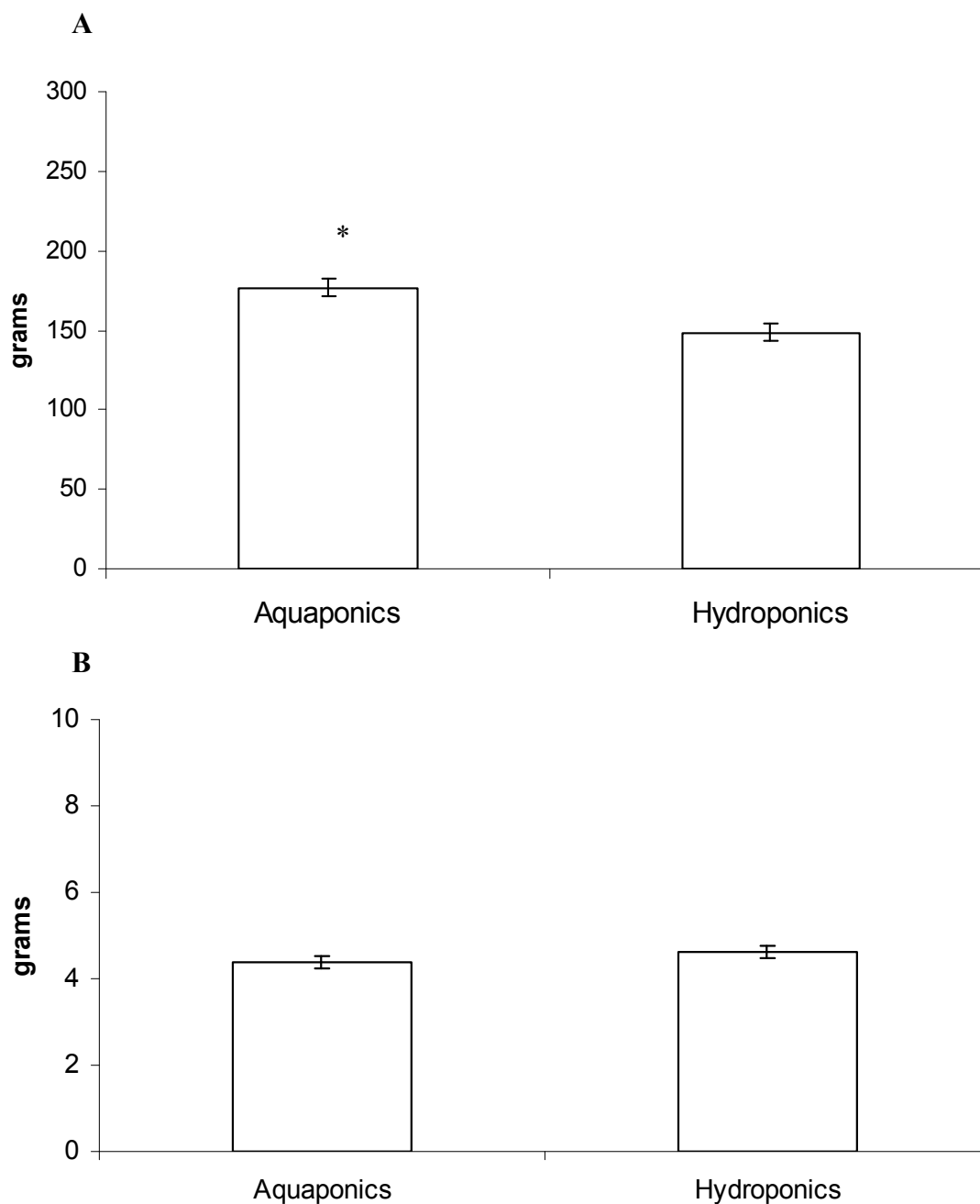


Figure 2. Mean head wet weight (grams) of *L. sativa* cv. Rex grown with aquaponics water and hydroponic solution (A). There is a significant difference ($p \leq 0.05$) in mean head wet weight of lettuce (*L. sativa* cv. Rex) grown with aquaponics water and hydroponic solution. Mean head dry weight (grams) of *L. sativa* cv. Rex grown with aquaponics water and hydroponic solution (B). There is no significant difference ($p \leq 0.05$) in mean head dry weight of lettuce (*L. sativa* cv. Rex) grown with aquaponics water and hydroponic solution. Error bars represent SEM, * represents $p \leq 0.05$ as determined by ANOVA.

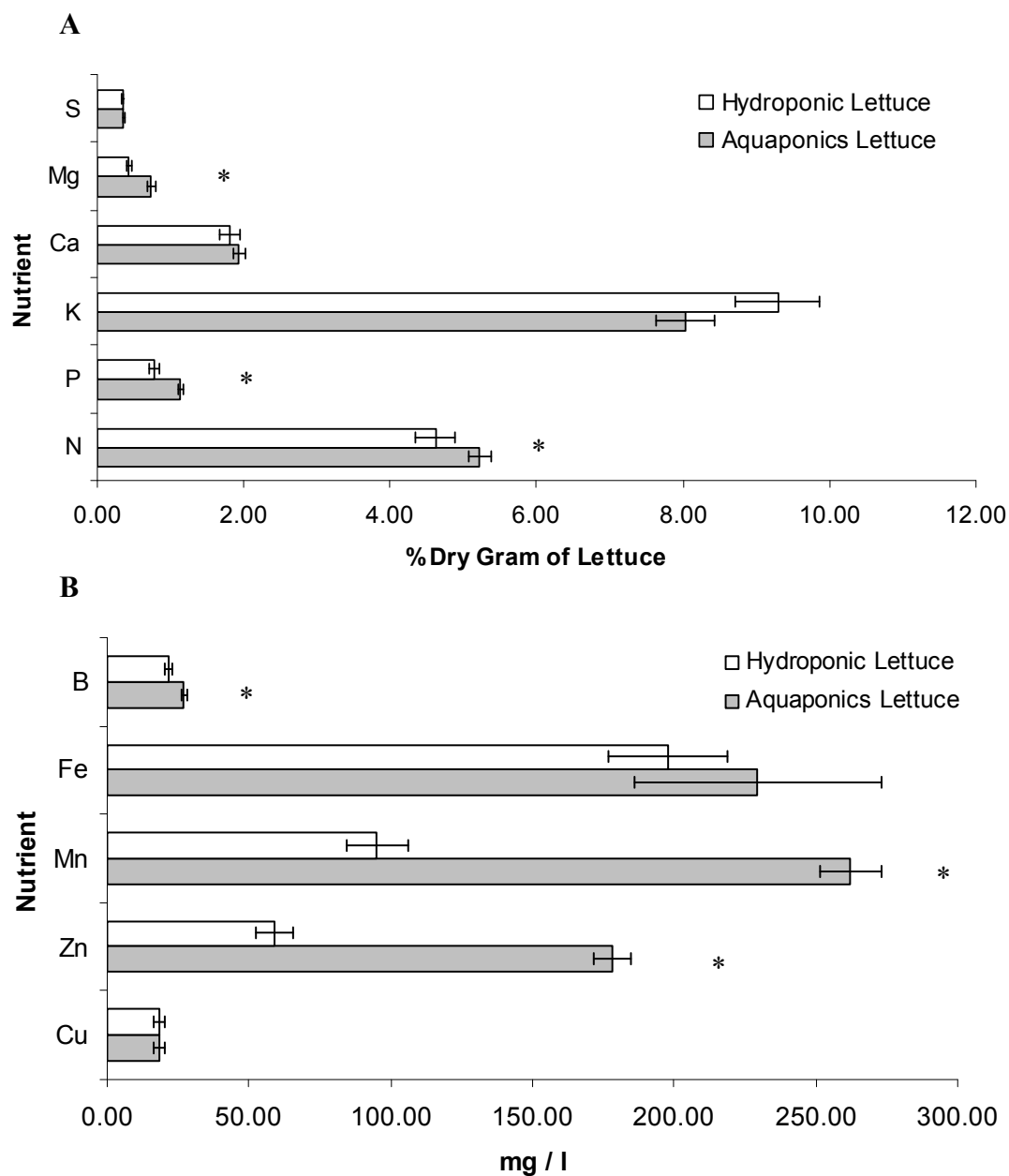


Figure 3. Percent nutrient composition per dry gram of macronutrients (A) and concentration of micronutrients (B) in lettuce (*L. sativa* cv. Rex) grown with aquaponics water plus supplementation and hydroponic solution. Error bars represent SEM, *, and ** represents $p \leq 0.05$ as determined by ANOVA.

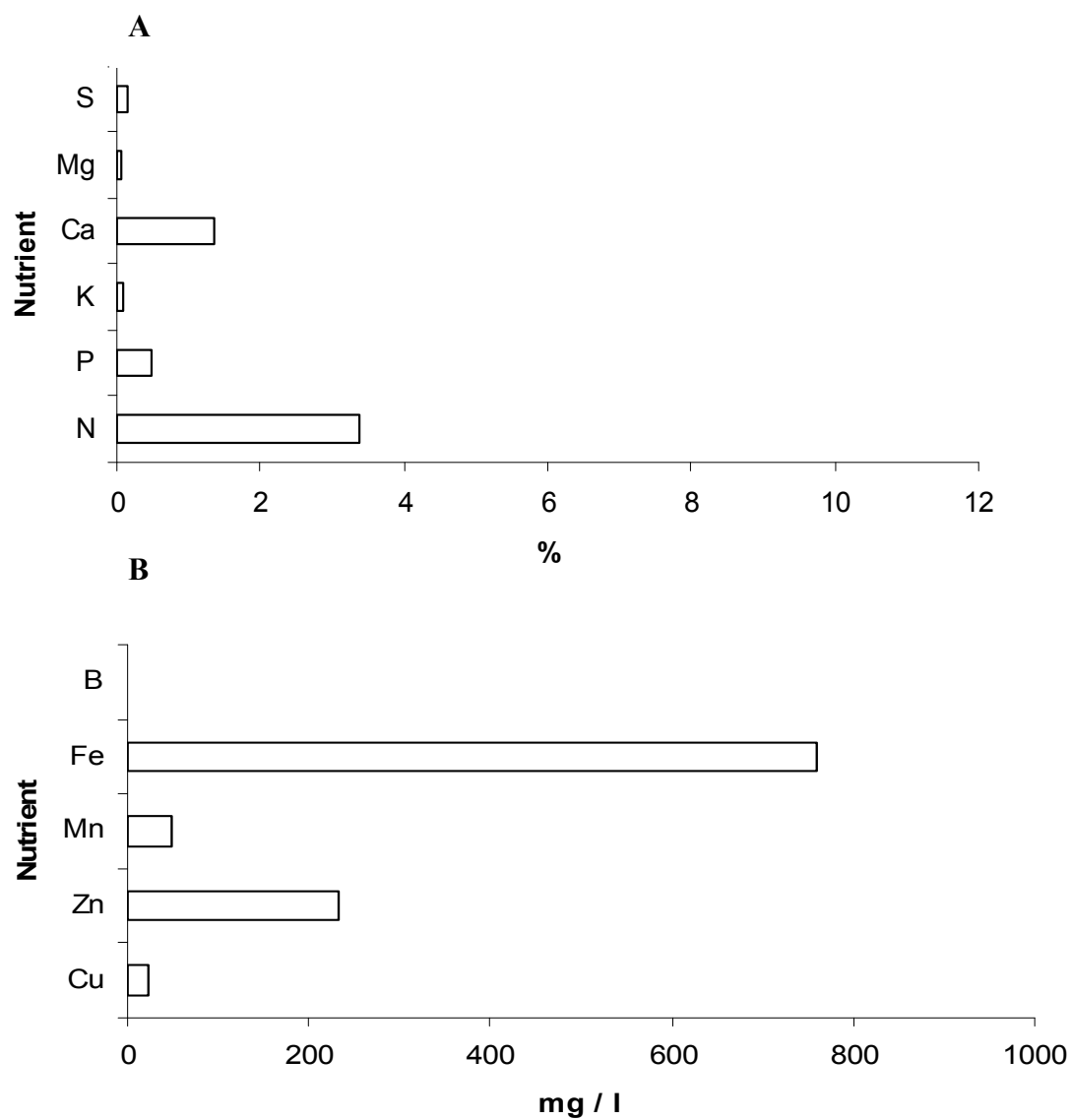


Figure 4. Percent nutrient composition of macronutrients (A) and concentration of micronutrients (B) in the aquaponics fish sludge.

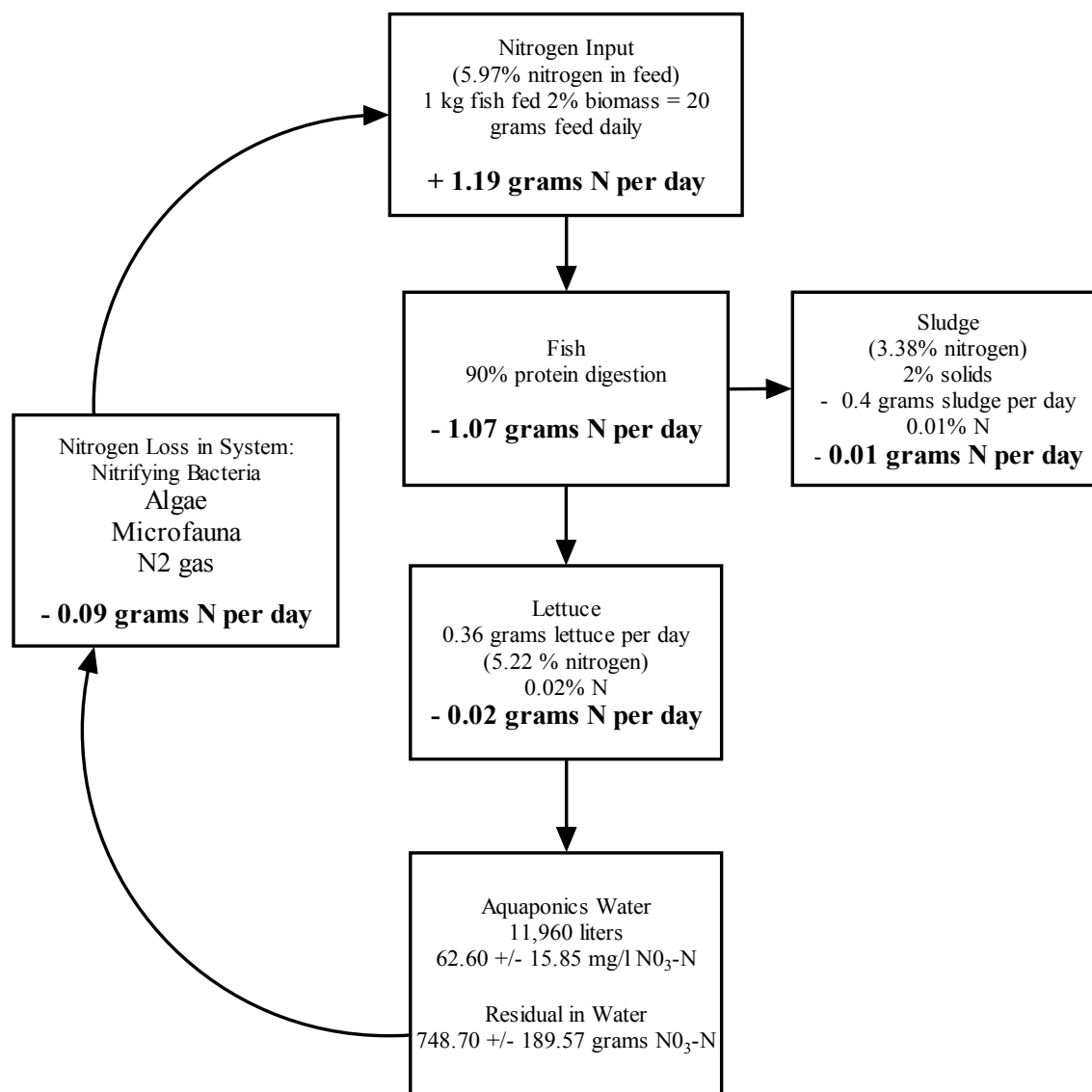


Figure 5. Nitrogen model of an aquaponics system. The nitrogen input in this model is based off of the feed requirement for a kilogram of fish.

DISCUSSION

Aquaponics water plus supplementation can grow *L. sativa* cv. Rex with equal or greater biomass accumulation and chlorophyll concentration index. This study focused on the nitrogen flow in the aquaponic system and the composition of aquaponics lettuce compared to hydroponic lettuce. Aquaponics lettuce can be produced with similar yield and quality compared with conventional hydroponic solutions when proper supplementation is added. All of the macronutrients analyzed remained mostly constant throughout the duration of the experiment with a slight increase in the end. The micronutrients also remained in a steady state with a very slight increase near the end of the experiment. Sulfate accumulated throughout the duration of the experiment due to the content in the fish feed being at concentrations higher than that needed for fish and plant fixation. The *L. sativa* cv. Rex lettuce needs nutrient supplementation including iron, manganese, and zinc to be added to the aquaponics water to yield marketable lettuce. The level of the supplementation in this experiment was sufficient and can possibly be reduced with further study. A total of 900 ml of Biomin® iron (5%), 100 ml of Biomin® Zinc (7%), and 100 ml of Biomin® Manganese (5%) were added to the aquaponics system throughout the growout cycle of the lettuce. The aquaponics lettuce had a higher concentration of nitrogen in the leaves compared to the hydroponic treatment. The concentration of nitrogen in the hydroponic was 180 mg/l compared to the aquaponics water which had nitrogen levels fluctuating around 50 mg/l. However, this is residual nitrogen left in the system water, when feed is introduced more nitrogen is introduced to the system and is assimilated by the plants. Future studies should include real time monitoring of nitrate-nitrogen concentration to determine the diurnal fluctuations in nitrogen in an aquaponics system.

The need for supplementation will depend on the system water chemistry parameters and the plants nutrient demand. Nutrient requirements can be different for each plant cultivar and must be evaluated to achieve optimal production and plant health. Lettuce can be grown consistently in an aquaponics system thus using water, nutrients and space more efficiently than conventional agriculture leading to a more sustainable food production system. The aquaponics system has produced lettuce with equal or greater chlorophyll concentration indices. Aquaponics water provides a good source of nutrients for plant growth. The nitrogen, phosphorous and potassium in the aquaponics system reached a near steady state with the lettuce production indicating that fish effluent at the densities established can provide a stable source of macronutrients for plant growth. The micronutrients in the aquaponics system must be monitored and adjusted to the crop being grown.

Lettuce grown with aquaponics water plus nutrient supplementation grows at similar rates to lettuce grown with hydroponic solutions. Supplementation rates will vary depending on the fish to plant ratio, system design and environmental parameters. Nutrients do not accumulate at the same rate in relation to each other so certain supplements would be needed to match the nutrient demand of the target crop. It has been supported that iron, zinc and manganese accumulate at different rates in aquaponic system (Seawright 1998) and therefore must be supplemented accordingly. Nutrients can also be recovered from the solid fish wastes. The sludge from the aquaponics system has a nutrient composition that can be utilized in soil amendments or dissolved back into solution.

Lettuce (*L. sativa* cv. Rex) can be grown with aquaponics water plus nutrient supplementation and yield a nutritional value compared to lettuce grown with hydroponic solution. Values reported for the nutrient composition in the lettuce are similar to lettuce grown in conventional agriculture and hydroponics (Resh 2001). The composition of macronutrients and micronutrients in the growing solution can determine the nutrient content, growth, and health of lettuce (Resh 2001). Lettuce has a low efficiency in using nitrogen but needs nitrogen constantly to promote growth (Welch et al. 1983).

The nitrogen model for this aquaponics system outlines the flow of nitrogen within the system. Nitrogen was utilized for growth in both fish and lettuce. Nitrogen was also lost in the aquaponics system via removal of the sludge. Nitrogen was also utilized in the system by bacteria and microfauna that reside in the aquaponics system. Nitrogen loss via these mechanisms was not quantified in this model. The aquaponics system had residual nitrate nitrogen in the water. Algae did not proliferate in the system because of the competition of nutrients with lettuce and the constant grazing of algae by the fish in the fish component. In an aquaponics system nitrogen is either assimilated by organisms in the system or remains dilute in the water. Nitrogen is not discharged from the system in a form that causes eutrophication. The aquaponics system utilized approximately 93% of the nitrogen introduced to the system on a daily basis (Figure 5).

Lettuce and other plant crops prove a valuable remediation tool for fish farming and aquaculture. Greenhouse or conventional agriculture can integrate lettuce production to existing aquaculture farms to remediate nitrogen and nutrient discharge. This will in turn generate a marketable product in the lettuce, but reduce the costs for fertilizers and reduce environmental degradation commonly associated with large scale agriculture. In

an aquaponics system resources (nutrients and water) are shared thus potentially increasing net profits compared to the individual systems (aquaculture or hydroponics). If one kilogram of fish will yield 6.4 lettuce heads (1,128 grams) and deposit 38.13 grams of nitrogen, then a farm can calculate the amount of greenhouse space needed to remediate the current discharge of fish effluent. The impacts from this can be tremendous to the environment and costs to farmers. Further research regarding the production of different crops that require different nutrient demands can lead to different nutrient supplementation protocols. The OMRI listed nutrient supplement BiominTM from JH Biotech allowed for specific nutrients to be targeted and added to the system. Aquaponic systems can operate within organic certifying standards by utilizing OMRI listed nutrient products along with processed fish effluent to produce sustainable organic vegetables. Future research at the aquaponics greenhouse will focus on integrating varieties of vegetable crops and tailoring the water chemistry using BiominTM nutrient supplementations to produce crops of equal yields to agriculture and hydroponic system.

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