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Small scale raft aquaponics: Evaluation of hybrid striped bass growth and plant uptake potential

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SMALL SCALE RAFT AQUAPONICS: EVALUATION OF HYBRID STRIPED BASS GROWTH AND PLANT UPTAKE POTENTIAL

BY

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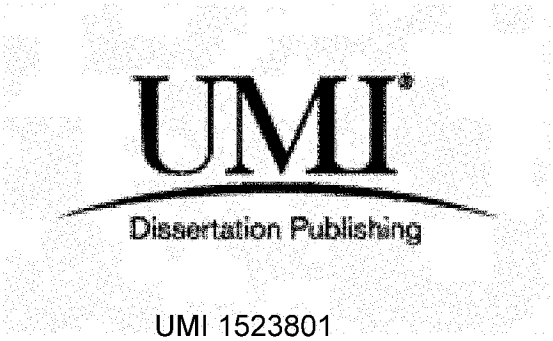

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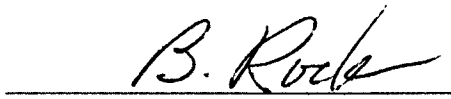

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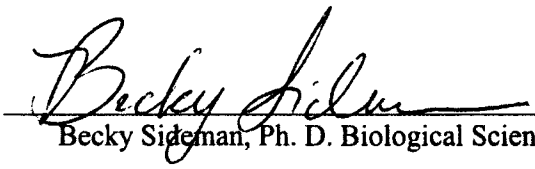

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ABSTRACT

SMALL SCALE RAFT AQUAPONICS: EVALUATION OF HYBRID STRIPED BASS GROWTH AND PLANT UPTAKE POTENTIAL

BY

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University of New Hampshire, May, 2013

Hybrid striped bass (♂*Morone saxatilis x* ♀*Morone chrysopos*) were successfully cultivated in two separate 48 day trials (t_1 and t_2) with seedlings of lettuce (*Lactuca sativa* cv. Rex) and pac choi (*Brassica rapa* cv. Win-Win) within a shared recirculating aquaponic system. It was determined that fish stocking density had no significant effect on the mean yield of lettuce and pac choi biomass in t_1. Stocking density did have a significant effect on the elemental nutrients generated within the aquaponic systems. A nutrient deficiency was exhibited by the leaf tissue of pac choi grown with fish stocked at low a density (0.82 kg/m^3). The deficiency could not be differentiated between Mg or Mn. A higher fish stocking density (1.64 kg/m^3) effectively provided the essential nutrients required by plants for normal healthy growth. A decrease in day length and lack of supplemental lighting contributed to a significant decrease in the mean yield of lettuce and pac choi biomass in t_2.

CHAPTER I

INTRODUCTION

Problem Statement

The theory behind aquaponics is that fish wastes (nitrogen, phosphorous) provide the nutrients necessary for plant growth in a shared recirculating system. Typically, however, essential nutrients such as potassium (K^+), iron (Fe^{2+}), calcium (Ca^{2+}), manganese (Mn^{2+}), and various other crucial elements required for plant growth are low in concentration throughout aquaponic waters (Seawright et al., 1998; Rakocy et al, 2006). Moreover, Seawright et al (1998) suggested that standard commercial fish diets lack the required range and ratio of elements for prolonged continuous plant growth. To correct and limit the influence of these deficiencies on plant development within aquaponic waters, synthetic fertilizers (KOH, FeEDTA, CaC_2O_4) are often supplemented to optimize plant growth (Rakocy et al, 2006) with the intent to ultimately enhance overall revenue. As a result, the practice of aquaponics and a substantial portion of previous research have been constrained to fish species tolerant of water enriched with these synthetic chemicals, most notably tilapia.

Recent development in aquaponics has proven beneficial in supplying much needed fresh vegetables and protein to lower income, urban areas. Tilapia, perch, arctic char, blue gill, largemouth bass, pacu, channel catfish, rainbow trout, Asian sea bass (barramundi), murray cod, jade perch, koi, bester sturgeon, white shrimp, and goldfish have all been successfully cultured in aquaponics (Diver, 2006; Rakocy et al., 2006; Lennard and Leonard., 2006; Nelson, 2008; Dediu et al., 2012; Mariscal-Lagarda et al., 2012). Despite a range of successful cultures, there is a need

to investigate the use of other fish species, particularly those that have undergone genetic improvement, to match specific environmental (e.g. temperature) conditions.

Little research has been conducted using hybrid striped bass (HSB) in aquaponic systems, although the fish are widely grown in ponds throughout the United States (Ludwig, 2004). The focus of this research was to investigate the culture of hybrid striped bass (♂*Morone saxatilis x* ♀*Morone chrysopos*) with vegetable species that have lower requirements for nutrients such as leafy greens (Rakocy et al., 2006), lettuce (*Lactuca sativa*), and pac choi (*Brassica rapa*).

The experiment had three objectives: (1) determine if stocking density of the hybrid striped bass had any effect, positive or negative, on the available nutrients for plant growth, (2) compare the mean plant biomass derived from aquaponic water with traditional hydroponics (enriched water) under equal environmental conditions, and (3) determine if any potential nutrient deficiencies are expressed by the lettuce and/or pac choi in aquaponic systems.

Hydroponics

Hydroponic is recognized as growing plants in a inert soilless media, typically in water rich in nutrients (Jones, 2005). Early experimentation with hydroponics revealed that plants require a total of 16 elements (C, H, O, S, N, P, K, Ca, Mg, Fe, B, Cl, Cu, Mn, Mo, Zn) for continuous normal healthy growth (Jones, 2005). The role and function of these elements are well understood today, and are described in Table 1.

Table 1. Description of the 16 essential elements and their role in vascular plants. (Taken from Goetz et al., 1983)

Element	Form in which absorbed	Some functions
Macronutrients		
Carbon	CO_2	Carbohydrates, both structural (cellulose of the cell wall) and functional (energy sources such as starch and glucose), proteins, fats, etc.
Hydrogen	HOH	Also in carbohydrates, proteins, fats, etc.; functional in energy transfer mechanisms; osmotic and ionic balance
Oxygen	O_2	Also in carbohydrates, proteins, fats; required in free form for aerobic metabolic processes
Nitrogen	NO_3^-m or NH_4^+	Amino acids, proteins, nucleotides, nucleic acids, chlorophyll, and coenzymes
Potassium	K^+	Enzymes, amino acids, and protein synthesis; activator of many enzymes; opening and closing of stomata
Calcium	Ca^{+2}	Calcium of cell walls; cell membrane permeability; enzyme cofactor
Phosphorus	H_2PO_4 or HPO_4^{-2}	Formation of high-energy phosphate compounds; nucleic acids; phosphorylation of sugars; several essential coenzymes; phospholipids
Magnesium	Mg^{+2}	Part of the chlorophyll molecule; activator of many enzymes
Sulfur	SO_4^{-2}	Some amino acids and proteins; coenzyme A
Micronutrients		
Iron	Fe^{+2}	Chlorophyll synthesis, cytochromes, and ferredoxin
Chlorine	Cl^-	Osmosis and ionic balance; probably essential in photosynthesis in the reactions in which oxygen is produced
Copper	Cu^{+2}	Activator of some enzymes
Manganese	Mn^{+2}	Activator of some enzymes
Zinc	Zn^{+2}	Activator of many enzymes
Molybdenum	MoO_4^{-2}	Nitrogen metabolism
Boron	BO^{-3} or $B_4O_7^{-2}$ (borate or tetraborate)	Influences Ca^{+2} utilization; functions unknown

It's crucial to maintain a balance in the number and ratio of elements within a hydroponic solution (Jones, 2005). If one or more elements are low in concentration (mg/L) within the growing solution, a deficiency will be expressed (Table 2). Additionally, if the ratio of specific

elements, especially bivalent cations (Ca^{+2}, Mg^{+2}, Cu^{+2}, Mn^{+2}, Fe^{+2}) are skewed within the growing solution, a deficiency may occur as a result of competition among these major ions at the root zone, which is commonly known as competitive inhibition (Goetz et al., 1983; Jones, 2005). Illustrated in Table 3, the common range of important elements within numerous nutrient solution used in traditional hydroponic growth are given. The justification for the wide range and discrepancy between and among the concentrations in Table 3, are attributed to the method of hydroponic growing and the specific crop being cultivated (Jones, 2005).

Table 2. Description of various deficiency symptoms expressed in leaf tissue (Taken from Goetz et al., 1983)

Symptoms	Deficient element
Older leaves affected first	
Effects mostly generalized over whole plant; lower leaves dry up and die	
Plants light green; lower leaves yellow, drying to brown; stalks become short and slender	Nitrogen
Plants dark green; often red or purple colors appear; lower leaves yellow, drying to dark green; stalks become short and slender	Phosphorus
Effects mostly localized; mottling or chlorosis; lower leaves do not dry up but become mottled or chlorotic; leaf margins cupped or tucked	
Leaves mottled or chlorotic, sometimes reddened; necrotic spots; stalks slender	Magnesium
Mottled or chlorotic leaves; necrotic spots small and between veins or near leaf tips and margins; stalks slender	Potassium
Necrotic spots large and general, eventually involving veins; leaves thick; stalks short; rosetting of leaves	Zinc
Young leaves affected first	
Terminal buds die; distortion and necrosis of young leaves (terminal die-back)	
Young leaves hooked, then die back at tips and margins	Calcium
Young leaves light green at bases; die back from base; leaves twisted	Boron
Terminal buds remain alive but chlorotic or wilted, without necrotic spots	
Young leaves wilted; without chlorosis; stem tip weak	Copper
Young leaves not wilted; chlorosis occurs	
Small necrotic spots; veins remain green	Manganese
No necrotic spots	
Veins remain green	Iron
Veins become chlorotic	Sulfur

Table 3. Common range of important elements within a nutrient solution used in traditional hydroponic growth studies. (Taken from Jones, 2005; pg 94).

	Range in Concentration, ppm				
Element	*Barry (1996)*[a]	*Jones (1997)*[b]	*Yuste/ Costincar (1999)*[c]	*10*[a] *(5)*[d]	*11*[a] *(5)*[e]
Nitrogen (N)	70 to 250	100 to 200	47 to 284 (NO_3-N) 14 to 33 (NH_4-N)	140 to 300	100 to 200
Phosphorus (P)	15 to 80	30 to 50	4 to 448	31 to 80	15 to 90
Potassium (K)	150 to 400	100 to 200	65 to 993	160 to 300	80 to 350
Calcium (Ca)	70 to 200	100 to 200	50 to 500	100 to 400	122 to 220
Magnesium (Mg)	15 to 80	30 to 70	22 to 484	24 to 75	26 to 96
Sulfur (S)	20 to 200		32 to 640	32 to 400	
Boron (B)	0.1 to 0.6	0.2 to 0.4	0.1 to 1.0	0.06 to 1.0	0.4 to 1.5
Copper (Cu)	0.05 to 0.3	0.01 to 0.1	0.005 to 0.15	0.02 to 0.75	0.07 to 0.1
Iron (Fe)	0.8 to 6.0	2 to 12	Trace to 20	0.75 to 5.0	4 to 10
Manganese (Mn)	0.5 to 2.0	0.5 to 2.0	0.1 to 1.67	0.1 to 2.0	0.5 to 1.0
Molybdenum (Mo)	0.05 to 0.15	0.05 to 0.20	0.001 to 2.5	0.001 to 0.04	0.05 to 0.06
Zinc (Zn)	0.1 to 0.5	0.05 to 0.10	0.05 to 0.59	0.04 to 0.7	0.5 to 2.5

[a] Barry, Carl, 1996, Nutrients: *The Handbook of Hydroponic Nutrient Solutions*, Casper Publications Pty Ltd., Narrabeen, NSW, Australia.

[b] Jones J. Benton, Jr. 1997, *Hydroponics: A Practical Guide for the Soilless Grower*, S Lucie Press, Boca Raton, FL.

[c] Yuste M.P. and Costincar J. (Eds.), 1999, Handbook of Agriculture, Marcel Dekker, New York, NY.

[d] *Growing Edge*, 1999, 10(5):13

[e] *Growing Edge*, 2000, 11(5):25

Sources: Barry, Carl., 1996, Nutrients: *The Handbook of Hydroponic Nutrient Solutions*, Casper Publications Pty Ltd., Narrabeen, NSW, Australia; Jones, J. Benton, Jr., 1997, *Hydroponics: A Practical Guide for the Soilless Grower*, St. Lucie Press, Boca Raton, FL; Yuste and Costincar (Eds.), 1999, Handbook of Agriculture, Marcel Dekker, New York, NY.

Aquaponics

Aquaponics is the integration of two farming methods, recirculating aquaculture (RA) and hydroponics. Recirculating aquaculture is a farming practice where aquatic organisms are grown within a closed system, whereas hydroponics is the cultivation of plants in a soilless matrix. Early experimentation with hydroponics revealed that fish waste has a nutrient potential to sustain plant growth (Naegel, 1977).

Fish excrete ammonia (NH_4) directly into the water from the gills, which is transformed by a series of aerobic bacteria (*Nitrosomonas* sp. and *Nitrobacter* sp.) into nitrates (NO_3) by a biological process called nitrification (Losordo et al., 1998; Masser et al., 1999; Tyson et al., 2004). Nitrogen and other waste products produced by the fish are absorbed by the roots of plants in an aquaponic system. These compounds are then sequestered by the vegetation and utilized to construct root, leaf, and stem tissue (Connolly and Trebic, 2010). The process of plants assimilating these compounds from the water, essentially provides an additional biological filter, and the vegetation could possibly provide additional revenue if marketed correctly.

It is estimated that 2.2 pounds of ammonia is excreted from fish for every 100 pounds of food metabolized (Masser et al., 1999). Ammonia exists in two forms: un-ionized ammonia (NH_3) and ionized ammonium (NH_4^+). Ammonia is toxic to fish, and needs to be cleansed from the systems before reaching lethal levels. Un-ionized ammonium (NH_3) can cause tissue damage and slow growth at concentrations as low as 0.02 mg/L (Masser et al., 1999). Plants require both NH_3 and NO_3 to grow (Jones, 2005), which are provided sufficiently by the combination of fish and bacteria (Seawright et al., 1998).

Fish excrete other dissolved metabolic wastes such as CO_2 which ultimately reacts with H_2O, forming carbonic acid (H_2CO_3), eventually decreasing the overall pH within a recirculating system (Masser et al., 1999). Nitrifying bacteria are significantly faster at converting NH_3 into NO_3 at a high pH and as a result recirculating aquaculture systems tend to maintain a pH of 8.5 (Tyson et al., 2004). A decrease in pH results in a direct reduction in the rate of nitrification, and will eventually discontinue at a pH of 5.5 (Tyson et al., 2004). The major implications of a low pH is the collection of toxic NH_3 to lethal levels (> 0.02 mg/L), which results in stress and potentially death of both fish and plants (Masser et al., 1999; Jones, 2005). Specifically in recirculating aquaculture, where plants are absent, chemical buffers such as sodium bicarbonate ($NaHCO_3$) are added to stabilize the pH for both fish and bacteria (Losordo et al., 1998; Masser et al., 1999). Aquaponics systems where plants are present, $NaHCO_3$ should never be supplemented

because a high sodium (Na^+) level in the presence of chloride (Cl^-) is toxic to plants (Rakocy et al., 2006). Bower et al. (1981) explored the pH buffering capacity of various filtrants (crushed oyster shell, limestone, and dolomitic limestone) within a recirculating systems, and discovered that these filtrants, particularly crushed oyster shell, successfully reduce the rapid acidification of culture water.

Hybrid Striped Bass

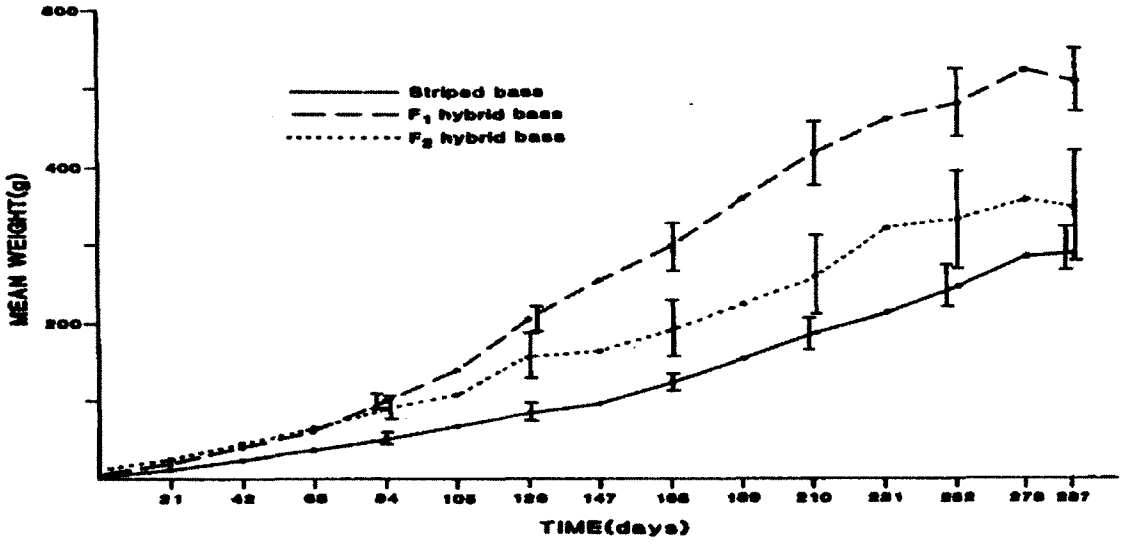
Hybrid striped bass (HSB) are a cross between the anadromous striped bass (*Morone saxatilis*) native to the Atlantic waters of the east coast of North America and the white bass (*M. chrysops*), a fresh water resident to the Mississippi River Basin (Hodson, 1989; Kohler, 2004; Ohs et al., 2008). Initially the first hybrids were produced in South Carolina by the combination of eggs reared from female *M. saxatilis* and sperm from male *M. chrysops* (Hodson, 1989). This "original" cross, referred as the Palmetto Bass, is currently less common than the "reciprocal" cross which is classified as the Sunshine Bass (Ohs et al., 2008). This is due to the constraints of sexual maturity, as female striped bass reach maturity in 5-7 years, while male striped bass reach maturity in 2-3 years (Kohler, 2004). Delaying production for 5-7 years to reach fertility and sustaining the health of large brood stock (sexually mature fish used for spawning), make the palmetto bass an unfavorable cross for production (Kohler, 2004).

During the 1960's, HSB were stocked in southern reservoirs, where the species grew in popularity as a sportfish (Kohler, 2004). In 1980, the commercial aquaculture industry began to raise HSB as a food fish in response to a drastic decline in wild striped bass populations (Hodson, 1989). The U.S. HBS market is estimated to generate 11 million pounds annually from a total of 61 facilities (Kohler, 2004).

Experimentation with striped bass and its various hybrids have shown that the reciprocal cross are superior in both growth (Figure 1) and survival when cultured in recirculating

aquaculture systems (Smith et al., 1985). Furthermore, reciprocal cross hybrids have shown to exhibit less growth variation in comparison with striped bass (Figure 1; Smith et al., 1985), which is important when working with piscivorous species because small fish may be eaten by larger fish. Despite consistent weight and length sampling typically found in traditionally aquaculture growth studies, the reciprocal cross hybrids have been reported to have exceptionally high survival rates (Smith et al., 1985).

Figure 1. Growth results (mean ± 1 S.E.) of striped bass, reciprocal cross F_1 hybrids (white bass / striped bass hybrid), and F_2 hybrid bass (F_1 original x F_1 original) reared in a recirculating brackish water system. (Taken from Smith et al., 1985)



Plants and Nutrient Supplementation

Aquaponics utilizes a natural process of plant nutrient uptake to biologically remediate wastes generated during traditional aquatic farming. Much like the fish in a closed recirculating aquaculture system, the plants in an aquaponic system require a number of specific parameters for healthy growth. Various nutrients (NO_3^-, PO_4^{-2}, and SO_4^{-2}) required by plants for growth and development are found to be supplemented in sufficient quantities through the direct input of the fish feed and water source (Rakocy et al., 2006). Nutrients such as: potassium (K^+), magnesium

(Mg^{+2}), calcium (Ca^{+2}), iron (Fe^{+2}), manganese (Mn^{+2}), copper (Cu^{+2}), boron (B^{+3}), and molybdenum (MO^{+6}) have been reported insufficient through the direct input of the fish feed, and thus are limiting nutrients in aquaponic systems (Rakocy et al, 2006). Moreover, Rakocy et al., (2006) indicated that aquaponic systems require chemical supplementation to maintain healthy plant growth: iron (Fe as iron chelate containing 10 percent iron by weight), potassium (K as potassium hydroxide) and calcium (Ca as calcium oxide or calcium hydroxide). Seawright et al., (1998) also reported P, K, and Fe deficiencies in aquaponic water receiving standard aquaculture diets. Rakocy et al., (2006) and Seawright et al., (1998) both cultured tilapia.

Flowering crops such as cucumber and tomato require slightly higher levels of potassium for fruiting (Rakocy et al., 2006). Leafy greens such as lettuce (*Lactuca sativa*) and herbs such as basil require much less potassium then fruiting species, and thus are more common in aquaponics (Rakocy et al., 2006).

Spectral Properties of Plants

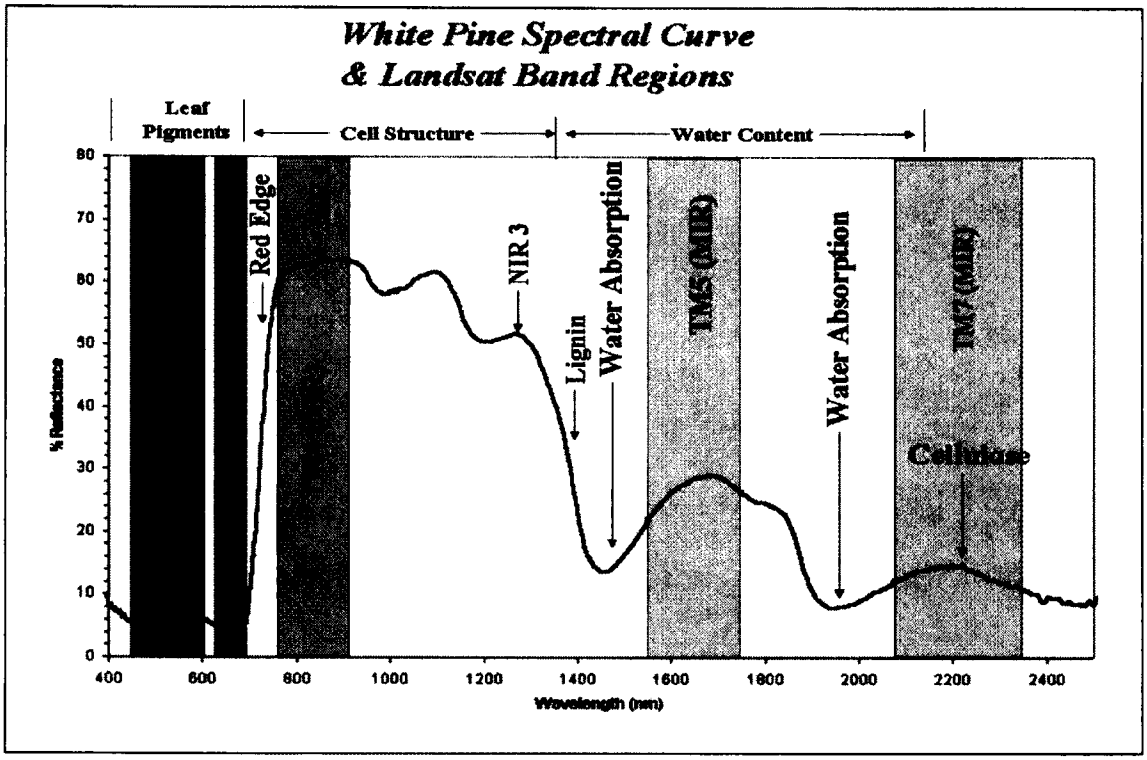
There is a increasing need to quantify and monitor the health of agricultural crops, in particular those grown in aquaponics where the health of the plants are directly tied to the health of the integrated system. If the productivity of plants could be examined instantaneously by non-destructive means, the potential of aquaponics would increase greatly as mineral deficiencies, which are characteristic of aquaponics (Rakocy et al., 2006), could be potentially identified. The ability to distinguish and categorize a particular nutrient deficiency early, especially within plant tissues grown in a closed system shared with fish, may prove to significantly increase crop yield and reduce unnecessary additions of synthetic fertilizers typically used in aquaponics (Rakocy et al., 2006).

Spectral reflectance properties, in particular those of plants, have been successfully used to identify anatomical changes in the leaf as a result of various stressors (Rock et al., 1986). The Visible Infrared Intelligent Spectrometer (VIRIS) used in this study is a GER 2600 reflectance

spectrometer that measures percent reflectance in the wavelength range of 350-2500nm. The instrument contains two detectors: a silicon (Si) detector measuring wavelengths in 350-1050 nm and a lead sulfide (PbS) detector measuring reflectance in 1050-2500nm.The VIRIS is used to detect anatomical and physiological variations in vegetation (Rock et al., 1986). The VIRIS obtains spectral coverage at approximately 2.0 nanometer spectral resolution from 0.40 to 1.10 micrometers and 4.0 nanometers resolution from 1.10 to 2.50 micrometers. Figure 2 presents a typical reflectance curve for healthy vegetation. Absorbance in the visible portion of the electromagnetic spectrum, centered around 0.48 and 0.68 μm, corresponds to pigment absorption (chlorophylls a & b, and carotenoids). The strong reflectance from 0.75 - 1.3μm represents the near-infrared (NIR) plateau and is indicative of the cellular conditions of foliage and/or canopies. The sudden rise in the reflectance curve between 0.68 μm and the NIR plateau is referred to as the red edge. Reflectance in the mid-infrared between 1.45 and 1.8 μm records foliar and canopy moisture (Rock et al., 1986). These three regions described above (mid-IR, NIR, and visible) have been used to monitor and detect healthy and stressed vegetation (Rock et al., 1986).

In this study, we have explored the spectral reflectance properties of plants grown within various waters, some grown in traditional hydroponic solutions, while others were grown in a system shared with fish. A supplementary objective was to associate tissue nutrient levels with VIRIS data.

Figure 2. Typical reflectance spectra of healthy vegetation. (Taken from Rock and Carlson, 2013)



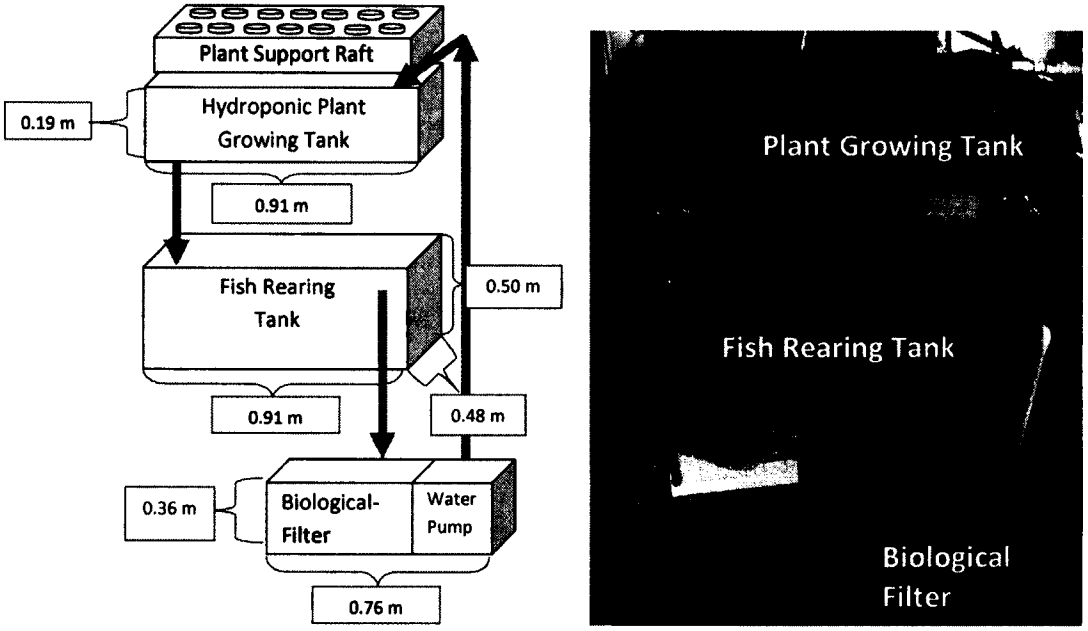
CHAPTER II

MATERIAL AND METHODS

Experimental Raft Aquaponic System

Twelve floating raft aquaponic systems were constructed and stationed within the Macfarlane Greenhouse located on the campus of the University of New Hampshire (UNH). Each individual aquaponic system held a total volume of 390 liters of fresh water (< 0.5 ppt), each consisting of: 80 L hydroponic plant rearing tank, 208 L fish rearing tank, and 102 L biological filter (Figure 3).

Figure 3. Outline (left) of one individual experimental raft aquaponic system (not to scale). Arrows illustrate the direction of water circulation (not to scale). Picture (right) of one functional system stationed in the Macfarlane Greenhouse (Durham, New Hampshire). Fish rearing unit and hydroponic growing tank were wrapped in black plastic to reduce algae growth. Fish netting placed above fish rearing tank prevented fish escape.



The experimental systems were identical in design, each outfitted with a individual water pump which recirculated water at a continuous rate. Water within the fish rearing tank would exit (effluent) and enter the biological filter by way of gravity through a PVC pipe (Figure 3). After the water had passed completely through the filter, a water pump circulated the filtered water directly to the uppermost point of the system, which was the plant growing tank. A PVC ball-valve attached to the effluent end of the circulation pump allowed for control over the speed (*ad libitum*) of recirculation. The plant growing tank was flooded, which created a pool 18 cm in depth. Each aquaponic unit was outfitted with a plant support raft, which was constructed from a 2.54 cm thick sheet of polystyrene measuring 60 cm in width and 90 cm in length. The polystyrene was buoyant and had twelve evenly spaced predrilled 5cm holes, which served to hold individual pots of plants. Water would exit the plant growing tank through a pipe, and flowed back into the fish rearing tank.

The experiment had 4 treatments running in triplicate. The treatments were as follows: (1) low fish density, (2) high fish density, (3) negative control (no fish) and, (4) positive control (no fish + traditional hydroponic synthetic fertilizer) (Figure 4).

Figure 4. Organization of the twelve independent experimental aquaponic systems stationed within the Macfarlane Greenhouse. Each rectangle is an independent recirculating aquaponic system (see Figure 3) and is labeled with a corresponding treatment. The positive control was stationed on a separate bench top to the left side of greenhouse to prevent contamination from the synthetic salts within the positive control.

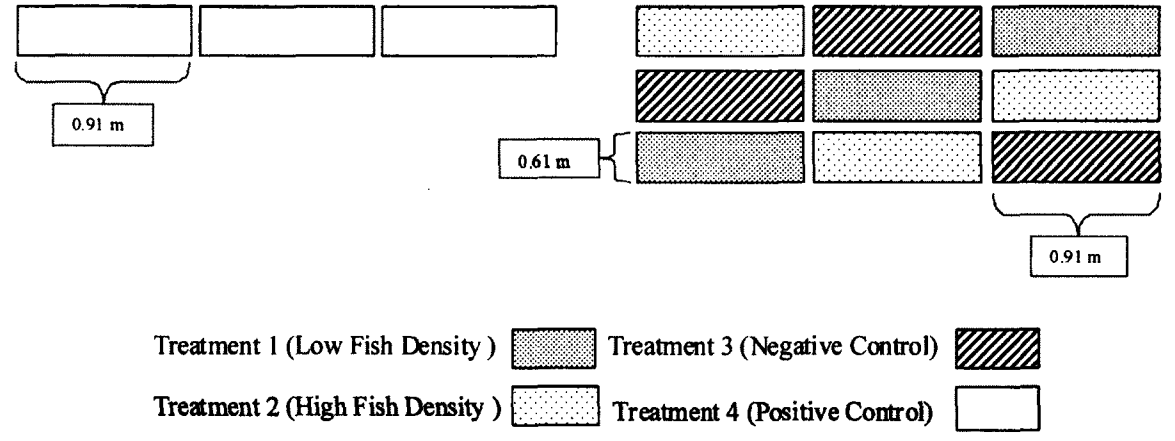
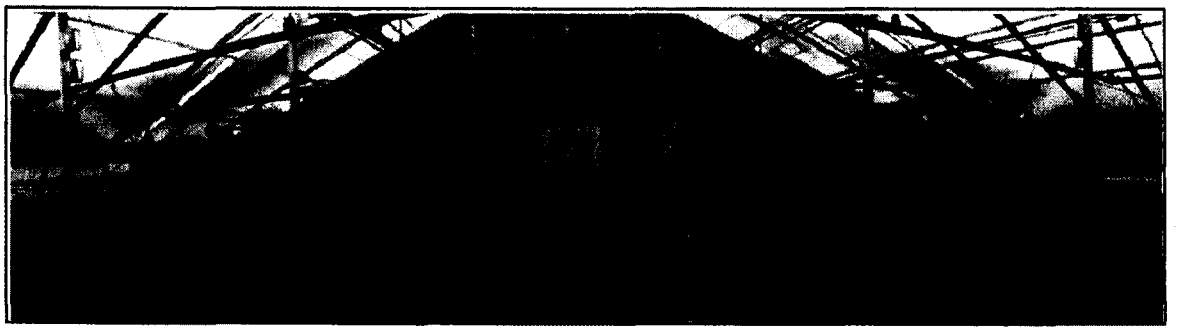


Figure 5. Photograph of the 12 experimental systems in the Macfarlane Greenhouse, UNH (Durham, NH).



The investigation had two experimental trials (t_1 and t_2) each consisting of 48 days (t_1; Sept 9th, 2012 - Oct 27th 2012 and, t_2; November 3rd, 2012 - December 21nd, 2012). Six of the 12 experimental systems were stocked with hybrid striped bass, while the remaining 6 experimental systems were not stocked with fish and would serve to represent the two control groups (negative control and positive control). Each individual high and low fish density experimental unit in t_1 had a total initial fish biomass (total fish mass / volume of fish rearing unit) of 1.64 kg/m^3 (50 fish) and 0.82 kg/m^3 (25 fish), respectively. Each individual high and low fish density experimental unit in t_2 had an total initial fish biomass of 2.43 kg/m^3 (20 fish) and 1.22 kg/m^3 (10 fish), respectively. Equal numbers of *L. sativa* and *B. rapa* were stocked throughout the twelve plant rearing tanks at a fixed density of 24 plants/m^2 (number of plants / dimension of the hydroponic raft growing area).

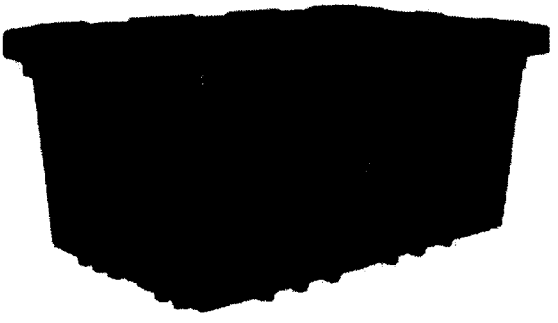
Bacterial Culture and Biological Filter

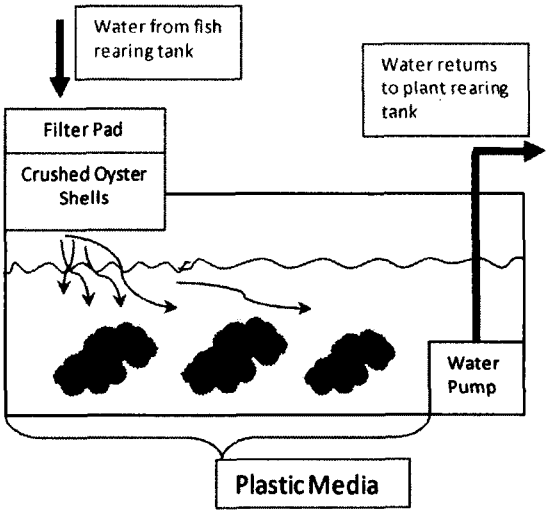
To guarantee successful biological filtration (nitrification) throughout the course of experimentation, a bacterial culture was established prior to the arrival of the fish. A 190L rain barrel was filled with fresh water (< 0.5 ppt), stocked with 40L of disinfected Kaldnes® biofilm media[1], and outfitted with an active air stone. The rain barrel was initially enriched with 2.4g of

ammonium chloride (4.00 mg/L) and inoculated with 0.59 L of Proline® Bacteria[1]. Ammonium chloride was combined with water in a 2 L Nalgene bottle and slowly dosed (1L/12h) to the bacteria culture by placing a clamp on a hose connecting the bottom of the Nalgene bottle to the culture barrel. The bacteria culture was monitored daily for ammonia, nitrites, and nitrates with an API freshwater master kit[1]. Ammonia levels were maintained initially at 5.0 mg/L for the first week, and decreased to a minimum of 1.0 mg/L throughout the remained of culturing period of four weeks.

On September 8th, one day prior to introducing the hybrid striped bass to the aquaponic systems, six biological filters (Figure 6) that would serve to support fish were inculcated with 20 L of media from the initial bacteria culture.

Figure 6. The image below (left) is a side view of a 31"L X 21"W X 14"H, 102.20 liter rectangular plastic tote, which served as both a biological filter and a sump. The diagram below (right) is a descriptive outline of the tote and the various components which construct a complete mechanical and biological filter with the additional aid of a pH buffering mechanism (crushed oyster shell).





Fish waste water entered the biological filter, passing through a filter pad (collects solid waste) and then a small tray of crushed oyster shells that maintained the pH. The first stage (filter pad and oyster shell) of the filter acted as a clarifier, collecting suspended solids released by fish,

[1] Reference to a specific brand does not imply endorsement by the University of New Hampshire or National Aeronautic Space Administration (NASA).

while the second stage (retangular tote) was filled with plastic beads which increased the surface area for nitrification by bacterica (*Nitrosomonas* sp. and *Nitrobacter* sp.). The final stage of the filteration process is a water pump which returns the water to the hydroponic plant rearing tank.

Fish

Three hundred reciprocal-cross hybrid striped bass (♂*Morone saxatilis x* ♀*Morone chrysopos*) were purchased and shipped from Keo Fish Farm hatchery in Lonoke, Arkansas to the Aquaculture Research Center (ARC) in Durham, New Hampshire. Importation and scientific permits were acquired from the New Hampshire Fish and Game Department prior to purchase (Appendix A). Institutional Animal Care and Use Committee (IACUC) approval was obtained preceding acquisition of the fish (Appendix B).

On August 7th, 2012 the 1.0 gram hybrids arrived at the Manchester Airport, Manchester New Hampshire and were transported to the ARC. The fish were shipped in a slightly opaque plastic bag housed in a sealed insulated box. Once at the ARC, the bag containing the fish was removed from the insulation box and allowed to float unopened above a single 2,400 L fresh water recirculating aquaculture tank. After temperature within the bag containing the fish was of equal temperature with the aquaculture tank (18.1 ^{O}C), one cup of tank water was introduced to the bag containing fish. This process was repeated every 30 seconds until the fish bag was competently filled with water. This acclimation process was important as the water used in transportation possessed different water parameters than the culturing tank.

Water quality in the aquaculture tanks was maintained as follows: ammonia (< 0.05 mg/l), nitrites (< 0.5 mg/l), nitrates (< 210 mg/l), dissolved oxygen (> 6.5), temperature (24 C $^{+}$/- 2 C), salinity (2 ppt) (Hodson, 1989; Losordo et al., 1998; Masser et al., 1999; Rakocy et al., 2006). The fish remained at the ARC for a period of approximately one month to ensure the health of the fish. The hybrids were initially fed a commercial diet (Salmon fry #2 [52 % protein,

16 % fat]; Nelson and Sons, Murray, Utah) at a rate of 8.1 g per tank, six times daily with an automatic feeder. The fish were slowly weaned onto a 1.5 mm steelhead trout excreted diet [45% protein, 16% fat]; Nelson and Sons, Murray, Utah) by reducing the percent of the initial feed over the course of 2 weeks, where by the end of the weaning period all fish were fed the 1.5 mm trout diet.

On September 9th, 2012 the hybrids were transported from the ARC to the Macfarlane Greenhouse where they were stocked to a specified density throughout six aquaponic systems. Three aquaponic systems were stocked to a density of 1.64 kg/m^3 and 0.82 kg/m^3, which corresponds to 50 and 25 fish in the high and low density systems, respectively. There was no pre-established stocking density for hybrid striped bass in raft aquaponics. To establish a baseline, the amount of feed required in supporting the square footage of the plants within the growing chamber, and the amount of fish required to be reared to sustain plant growth had to be considered. To understand the dynamics of potential fish waste, (Seawright et al., 1998:Kemeh and Brown, 2001; Rackocy et al., 2006) stocking densities were reviewed and assisted in establishing a stocking density for hybrid striped bass in aquaponics.

The remaining 47 hybrids were transported back to the ARC, where weight and length were measured to establish a starting average for t_1. The surplus hybrids were stocked back within the original 2,400 L recirculating tank were they remained in the event restocking was necessary due to unlikely mortalities in the aquaponic systems.

The fish were allowed to acclimate to the water conditions within the greenhouse for a period of seven days prior to germinating plant seed. This acclimation period was instituted to ensure that biological filtration (nitrification) was successfully established, and to ensure the health of the fish before investing plant seed. Furthermore, this delay reduced the likelihood that the plants were exposed to lethal nitrite levels (> 5.0 mg/L) that are common in the initial cycling period found in closed recirculating systems.

Plants

Following the acclimation period, 96 pac choi (*Brassica rapa* cv. Win-Win) and 96 butter head lettuce (*Lactuca sativa* cv. Rex) seeds were sowed into individual 5cm Rockwool® cubes[1]. The seed used in both trials were purchased from Johnny's Selected Seeds located in Winslow, Maine. The seeds were placed in a separate germination section of the greenhouse (24.5 - 26.7 °C, relative humidity 45%) for a duration of one week to optimized early development of seedlings. Seeds of both lettuce and pac choi germinated within 5 days post sowing. Following the 1 week germination period, the seedlings were transferred and placed into 5cm net pots. Six pac choi and six lettuce were randomly selected, and placed at random through each of the twelve aquaponic units. All plants were harvested on day 42, post germination.

Operation and Protocols

Water Quality

The water used to the fill each individual system was conditioned through an ion exchange column to remove any heavy metals, a carbon filter to remove any organics, and finally a water softener to help increase the buffering compactly of the water (pH). Surplus water was stored in three 190 L rain barrels which were used to top off any water lost to evaporation or transpiration in the aquaponic systems.

Water quality (temperature and dissolved oxygen) was sampled daily from only one of the three replicates for each treatment, which allowed for all tanks to be sampled within three days. Dissolved oxygen (DO) and temperature (^{O}C) were measured daily (0800 - 0930 hr) with an YSI DO 200A meter[1], and maintained at levels > 5.5 mg/L and 20 ^{O}C $^{+}$/- 2 C, respectively. Ambient temperature within the greenhouse was maintained with supplemental heat and/or with automated window vents, at 18.0 – 21.0 ^{O}C during the day (0600 – 1900 hr) and 15.0 - 18.0 ^{O}C during the night (1900 – 0600 hr). A HM Digital COM-100 EC/TDS/Temp meter[1] was used to

measure electrical conductivity (EC) in microsiemens (mS). All fish rearing tanks that contained fish were siphoned daily to remove uneaten food and waste. The waste water was siphoned directly onto the first stage of the bio-filter (filter pads). The filter pads were washed daily with municipal tap water and rinsed with deionized water to remove any trace fluoride or chlorine particles. Once a week, an API freshwater master test kit[1] was used to measure the levels (mg/L) of ammonia, nitrites, and nitrates. Additionally, pH was measured weekly with a portable HANNA HI9813 EC/pH meter[1].

Trial one (t_1) had three specific collection dates (9/24, 10/15, 10/26) in which water was sampled from each of the twelve systems. Water samples were only collected in trial one (t_1), and obtained specifically from the effluent of the hydroponic unit, and stored in 175 mL Nalgene containers. Samples were processed on an Agilent-Varian Vista AXCCD inductively coupled plasma atomic emission spectrometer (IPC-OES)[1] to obtain concentration (mg/L) of all cations present. A Metrohm-Peak[1] 761 IC was used to obtain concentration (mg/L) of anions. Organic carbon and total nitrogen measurements were attained with a Shimadzu TOC-VCHP Carbon Analyzer[1] coupled with a TNM-1 Nitrogen Analyzer[1]. Ammonia (NH3) and nitrates (NO3) were measured using a Lachat Quickchem QC8500 Automated Ion Analyzer[1].

Feeding

To establish a feeding regime, fish in each aquaponic system were fed twice daily to apparent satiation for the initial two weeks with a commercial diet (1.5 mm excreted steelhead trout [45% protein, 16% fat]; Nelson and Sons, Murray, Utah). The amount eaten in each tank was determined by weighing the amount of feed before and after feeding. The average amount fed was calculated individually for each tank at the end of the two week calibration period. This procedure was performed again halfway through the experimental period, and was only of one week in duration.

[1] Reference to a specific brand does not imply endorsement by the University of New Hampshire or National Aeronautic Space Administration.

Biological-Control

To control and reduce the number of destructive insects (aphids, white fly, and thrips), three biological controls were used: *Aphidius colemani* and *Harmonia axyridis* (aphid control), *Eretmocerous eremicus* (sweet potato whitefly control). The greenhouse staff (Jonathan Ebba) conducted the release of these bio-controls.

Nutrient Supplementation

Three of the twelve systems were enriched with traditional inorganic salts and served as the positive control (hydroponic) for the entirety of the experiment. Enrichment scheduled rate differed according to the crop maturity (seedling, early growth, late growth), following the guidelines set forth by the manufacture, General Hydroponics ™. A three part solution (Flora Series) was mixed to a specific concentration and introduced into each of the three positive controls (Table 14. Appendix C).

Sampling Protocol

Plant Above-ground and Below-ground Biomass

Plants were removed from their respective treatment, cut at the base, and placed on a paper towel to remove any excess water. After excess water was removed from the plant tissue, the plant was placed on a scale and weight was recorded. Weighed plants were placed in a paper bag, labeled with plant identification-code and then dried in an air-flow oven for 72 hours at 70 ^{0}C. Plants were weighed after drying to obtain wet weight / dry weight derived water content. The same protocol was used to sample root tissue.

Fish Length and Weight

A sample (t_1: n=30 high density, n=25 low density; t_2: n=20 high density, n=10 low density) of the hybrid striped bass were collected individually from each of the six systems with a

fish net and placed in a large disinfected plastic cooler. Each fish was weighed (g) on an electronic scale to establish weight gain (ending weight – initial weight). Fish length was measured by use of a metric tape (cm) to establish physical growth (ending length – initial length).

Production characteristics of hybrids striped bass were calculated with the following formulas:

Feed conversion ratio (FCR) : (total dry feed weight / [final fish biomass – initial fish biomass])

Survival rate (%) : (N_t / N_o x 100 (n_t = total number of fish at harvest from initial stocking; N_o = initial number of fish stocked)

Specific growth rate (SGR) : 100 x (In W_t - In W_o) / t (W_o = initial wet weight; w_t = final wet weight; t = number of days)

Feed Intake (FI) : 100 x (amount of feed consumed) x (average tank biomass)$^{-1}$ x (number of days)$^{-1}$

Fish Restocking

Following conclusion of trial one (t_1), fish were placed in a separate plastic cooler containing an active air stone and transferred to the ARC, were they remained for one week. The fish were fed once a day to satiation while the aquaponic systems in the greenhouse were prepared for the second trial (t_2). During the one week transformation period, all aquaponic systems were drained and refilled with new ARC water.

To ensure the health of the nitrifying bacteria which typically colonize exposed surfaces, the experimental systems were not disinfected between trials and were filled with new water within 24hrs post transfer of the fish. After the tanks were refilled and the temperature stabilized, the low and high treatment were restocked with hybrids from the same cohort as t_1. The number of HSB stocked in the low and high density treatments in t_2 were reduced to 10 and 20 fish, respectively. The reduction in the number of fish was to ensure that t_1 and t_2 had a similar total weight of fish within a given system (kg/cm^3).

Plant Tissue Sampling

Tissue analysis was performed on twenty-four plants, representing one pac choi and one lettuce from each treatment. The plants were initially rinsed in tap water, disinfected with 0.2N HCl solution and rinsed in DI water. The leaves selected for tissue analysis were the newest mature leaves from each plant. The plants were placed in a paper bag, labeled with a specific plant identification code, and dried in an air flow oven at 70 ^{O}C for 72 hours. Dried tissue was placed in a plant tissue grinder and ground into a fine power. The ground material was placed in a labeled plastic container. The samples were sent to JR PETERS Inc., in Allentown, PA for tissue (macronutrient and micronutrient) analyses. Plant tissue analysis was performed on the tissues from t_1 only.

Spectral Analysis

Four plants (two lettuce and two pac choi) were removed from each of the 12 hydroponic units and placed individually in separate labeled Ziploc[1] plastic bags. Each plant was processed independently during the spectral scans. Plant leaves were detached and placed in a 10 cm black sampling dish with the surface of the leaf facing upwards. The leaf tissue was scanned by the VIRIS with the new emergent tissue arranged on top with ascending older tissue underneath the newer tissue. Each sample was scanned under the GER 2600 at three different angles (0°, 90°, 180°) to account for geometric influence. After the initial three scans, the arrangements of leaves were switched so the older leaves were on top of the new emergent tissue.

Data were processed in VIRIS-Spec, in which the Red Edge Inflection Point (REIP) was calculated by using the first derivative of the portion of the spectral curve found in the spectral wavelength range of 680-750 nm (Rock et al., 1988). The TM 5/4 (TM54) ratio was calculated with the average reflectance in band 5, divided by the average reflectance in band 4 (Rock et al., 1986). The TM 4/3 (TM43) was calculated in a similar approach, however the average reflectance of band 4 was divided by band 3. The NIR 3/1 (NIR31) was calculated with the average

reflectance of the NIR-3 peak divided by the average reflectance of NIR-1 peak, which were both found along the NIR-plateau (Rock and Carlson., 2013). The reflectance values from 400-2000 nm was the subject of evaluation as this is the only region of spectral importance for vegetation. The spectrometer was mounted on a tripod facing a hemispherical light source angled at 45°. A white Spectrolon panel was scanned by the GER 2600 to establish a reference source.

Statistical methods

All treatment replicates for t_1 and t_2 were analyzed with a one-way ANOVA, and processed in JMP$^{(TM)}$ 9[1] (SAS Institute, Cary, NC). Unless stated otherwise, the statistical difference between treatments was set to $P \leq 0.05$. Tukey's honestly significant differences (HSD) and Students-t test were used to compare means between specific treatments.

CHAPTER III

RESULTS

Summary

The higher fish stocking density had a direct positive influence on the concentration (mg/L) of various nutrients that collected in the aquaponic systems (Table 7). Stocking density of hybrid striped bass had no significant influence on the mean yield of lettuce and pac choi in t_1 and t_2 (Table 5), although significant differences were found in t_2 with respect to the mean yield of pac choi. A reduction in day length (Figure 11) and the lack of supplemental lighting within the greenhouse was likely the main contributor to the reduction in the mean yield expressed in t_2 compared to t_1.

Hybrid striped bass performed well in t_1 with no reported moralities, while eleven moralities were reported in t_2, specifically within the high density treatments. The pac choi tissue in the low density fish treatments expressed interveinal chlorosis[2] throughout the duration of t_1 (Figure 12), while the pac choi in the high density treatment expressed interveinal chlorosis (old tissue) after the seventh week of t_1. With regards to the second trial (t_2), neither pac choi nor lettuce expressed visible signs of interveinal chlorosis.

Water Quality

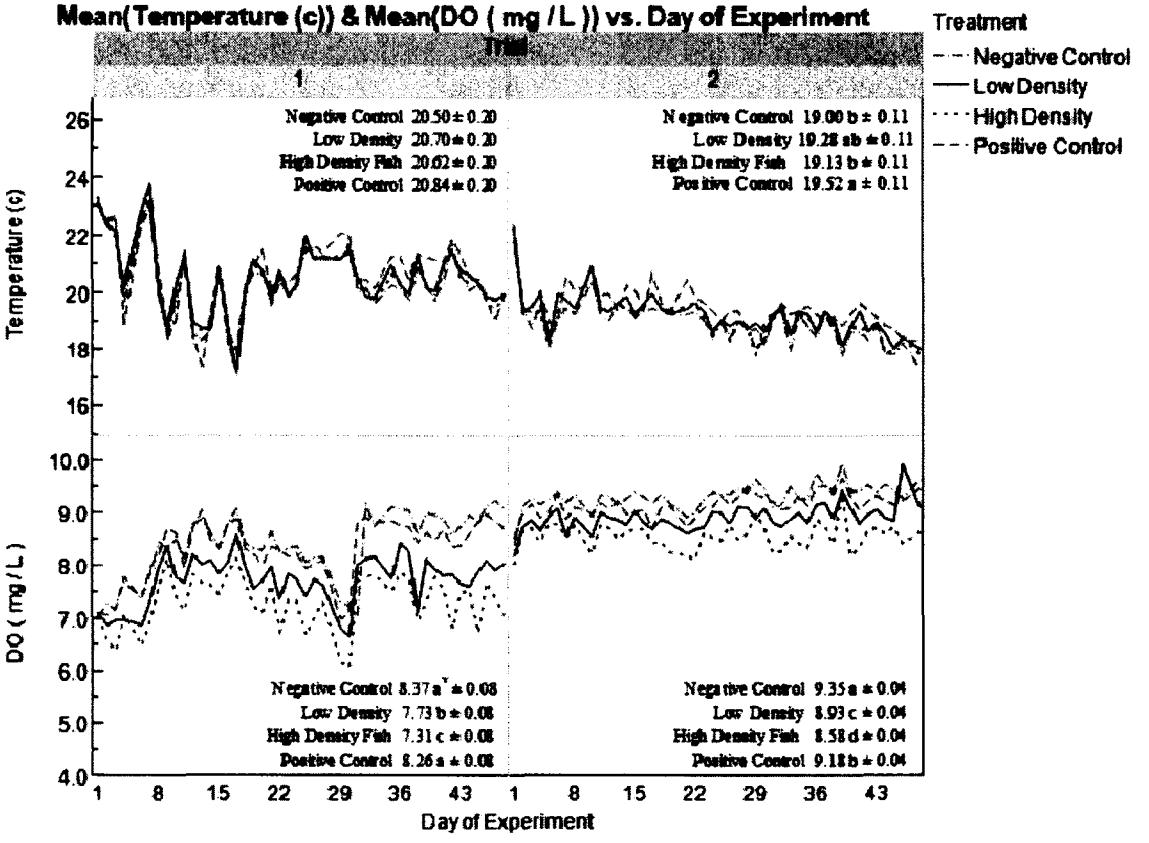
Temperature and Dissolved Oxygen

Dissolved oxygen (DO) and temperature maintained aquaculture production standards (> 6.5mg/L and 18 ^{o}C - 30 ^{o}C, Smith et al., 1985) within all experimental systems throughout the

[2] Interveinal chlorosis refers to the yellowing of leaf tissue, isolated specifically between the venial margins that scatter throughout the leaf tissue, and is a result of decreased chlorophyll concentrations.

duration of t_1 and t_2. There were no significant differences in the mean water temperature between the low and high density fish treatments for the duration of t_1 or t_2; however, there were significant differences in the mean dissolved oxygen (DO) between the low and high fish density treatments (Figure 7).

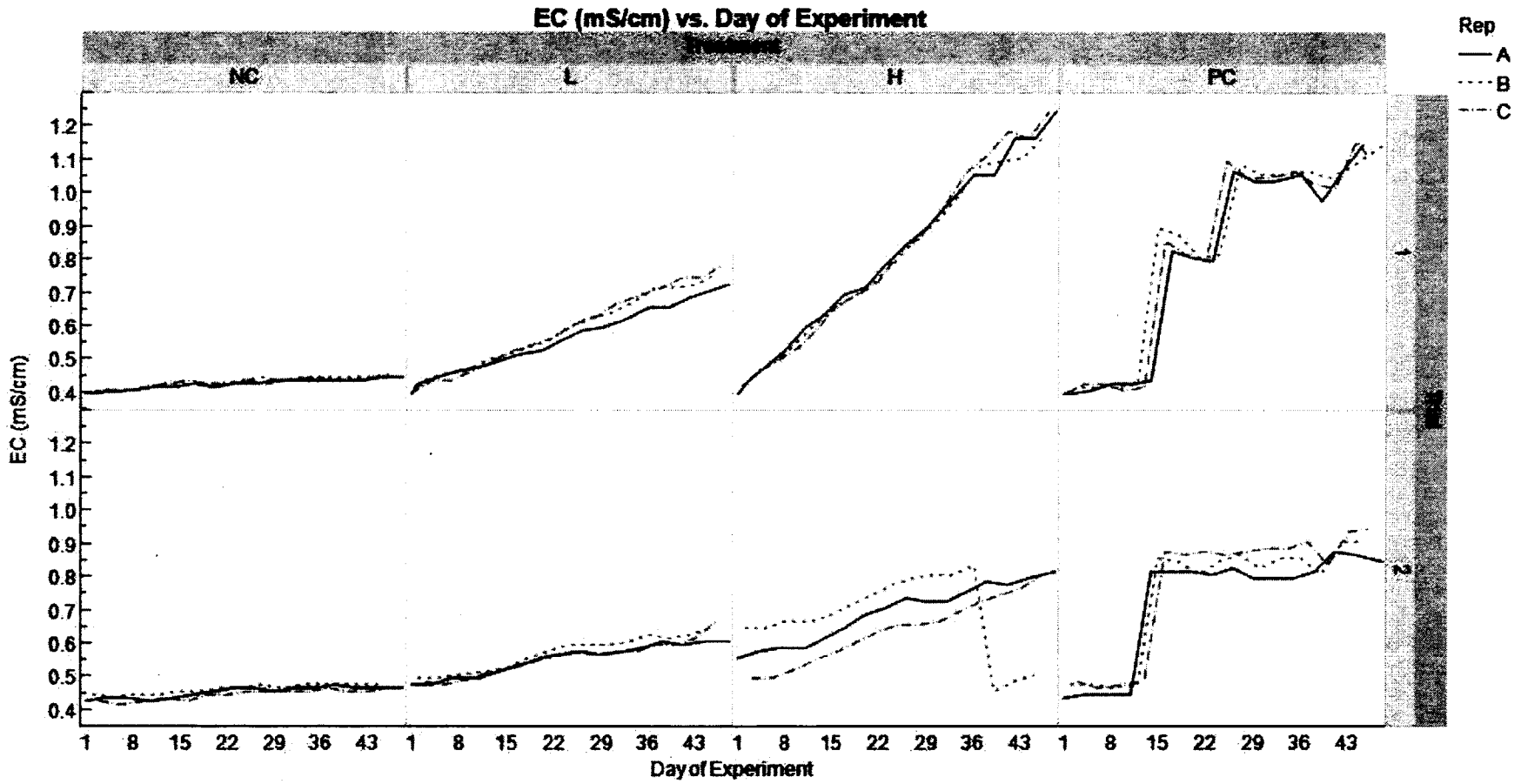
Figure 7. Mean temperature and dissolved oxygen by treatment for the duration of t_1 and t_2. [y]Means followed by different lowercase letters indicate significance based off a Tukey's test ($P \leq 0.05$, $n = 3$). The absence of letters indicates that there are no significant differences among treatments.



Electrical Conductivity (EC)

The EC of the conditioned water used to initially fill all experimental systems at commencement of t_1 and t_2, was 0.41 mS/cm. Illustrated in Figure 8, the four spikes in the EC found within the positive control during t_1, represents the four enrichment periods, where liquid fertilizer was supplemented to each respective system. The positive control only had two enrichment periods in t_2, and is illustrated by two isolated spikes in the EC (Figure 8).

Figure 8. Electrical conductivity (mS/cm) by replicate (A, B, C) for the duration of t_1 and t_2.



The discrepancy between the initial EC in the low and high fish treatments in t_2, is a result of not extracting the biological active media from the biological filter between the conclusion of t_1, and the commencement of t_2. This small difference in the initial EC between t_1 and t_2, suggests that the basic ionic composition of nutrients moving within each independent system, was different at the initiation of the second experiment.

Fish Biomass

The final mean individual fish weight and length did not significantly differ between the low and high density treatment groups in either t_1 or t_2 (Table 4). Aggressive feeding behavior was exhibited by the HSB throughout the entire duration of t_1. This aggressive behavior was not observed in t_2, in fact the feeding behavior was much reduced, and decreased over the duration of the experiment.

Table 4. Characteristics of stocking density of hybrid striped bass in experimental raft aquaponics for the duration of two (t_1 and t_2) 48 day trials.

	Trial 1		Trial 2	
	Low Density n = 3	High Density n = 3	Low Density n = 3	High Density n = 3
# Fish / System	25	50	10	20
Initial fish weight (g)	8.8 ± 0.23		26.6 ± 0.48	
Initial biomass / tank (g)	179.0 ± 0.46	358.1 ± 0.46	266 ± 6.15	532.1 ± 6.15
Final fish weight (g)	26.5 ± 0.72	26.7 ± 0.65	37.7 ± 1.84	36.7 ± 1.35
Final biomass / tank (g)	662.03 ± 6.40	1334.8 ± 5.85	377.5 ± 11.19	733.4 ± 9.36
FI (% ABW/d)	1.76 ± 0.07	1.73 ± 0.05	1.45 ± 0.07	1.21 ± 0.08
SGR (% day^{-1})	2.30	2.31	0.73	0.67
Feed conversion ratio (FCR)	1.13 ± 0.03	1.11 ± 0.01	2.37 ± 0.18	2.17 ± 0.33
Sex (M/F)	Mixed		Mixed	
Survival rate (%)	100	100	100	81.7

Int fish wt, final wt, FCR, input costs are LSmeans ± standard error

Total int, FI, and total final biomass are LSmean ± standard deviation

Eleven mortalities were reported in t_2, isolated exclusively from two of the high density replicates (A and B). High density replicate B contributed eight of the eleven deaths. In response to unidentifiable reasons for mortality, the remaining hybrids within high density replicate B were

temporarily removed from their respective aquaponic system, and 100 percent of the water was replaced with new fresh water. The effect of the water exchange is well illustrated by a sudden drop in the EC of replicate B (Figure 8). The overall feeding behavior increased within several days after water exchange. An overall decline in fish feed intake was noted t_2 in comparison with t_1, which is an indicator that the fish were under stress in t_2 (Table. 4). The low density fish tanks did have a greater feed intake in comparison to the high density fish tanks in t_2. The limitation of space in the higher density tanks as a result of the larger size fish (cm) in t_2 may have perhaps negatively influenced feed intake.

Plant Biomass

A high degree of variance in the weight of the foliage and roots harvested from negative control, coupled with a insufficient amount of tissue generated within the treatment resulted in the elimination of the negative control from statistical analysis within this study. Furthermore, the tissue grown in the negative control expressed sever nutritional deficiencies.

Table 5. Final mean wet and dry biomass by treatment and plant type for t_1 and t_2.

			Wet Biomass (g / plant)		Dry Biomass (g / plant)	
Trial	Treatment	Plant Type PC = Pac Choi L= Lettuce	Above-ground [LSmean ± SE]	Below-ground [LSmean ± SE]	Above-ground [LSmean ± SE]	Below-ground [LSmean ± SE]
1	Low Density Fish	L	49.67 ± 3.01	6.29 ab[y] ± 0.71	2.43 ± 0.20	0.18 a ± 0.04
	High Density Fish	L	57.95 ± 3.11	7.12 a ± 0.71	2.67 ± 0.19	0.11 ab ± 0.04
	Positive Control	L	47.95 ± 3.01	4.54 b ± 0.71	2.20 ± 0.19	0.01 b ± 0.04
	Low Density Fish	PC	181.65 b ± 14.21	12.68 a ± 0.92	8.69 b ± 0.70	0.70 a ± 0.07
	High Density Fish	PC	204.15 b ± 14.21	15.02 ab ± 0.92	9.71 b ± 0.70	0.69 a ± 0.07
	Positive Control	PC	341.94 a ± 14.68	16.33 a ± 0.95	13.42 a ± 0.73	0.38 b ± 0.07
2	Low Density Fish	L	7.93 a ± 0.31	1.21 b ± 0.13	0.32 ± 0.03	0.04 b ± 0.01
	High Density Fish	L	8.41 a ± 0.31	1.73 a ± 0.13	0.32 ± 0.03	0.07 a ± 0.01
	Positive Control	L	6.77 b ± 0.31	1.33 ab ± 0.13	0.21 ± 0.04	0.04 b ± 0.01
	Low Density Fish	PC	16.89 b ± 1.12	1.76 b ± 0.24	0.72 b ± 0.07	0.10 b ± 0.01
	High Density Fish	PC	25.06 a ± 1.12	2.67 a ± 0.24	1.10 a ± 0.07	0.16 a ± 0.01
	Positive Control	PC	27.03 a ± 1.12	2.48 ab ± 0.24	1.16 a ± 0.07	0.09 b ± 0.01

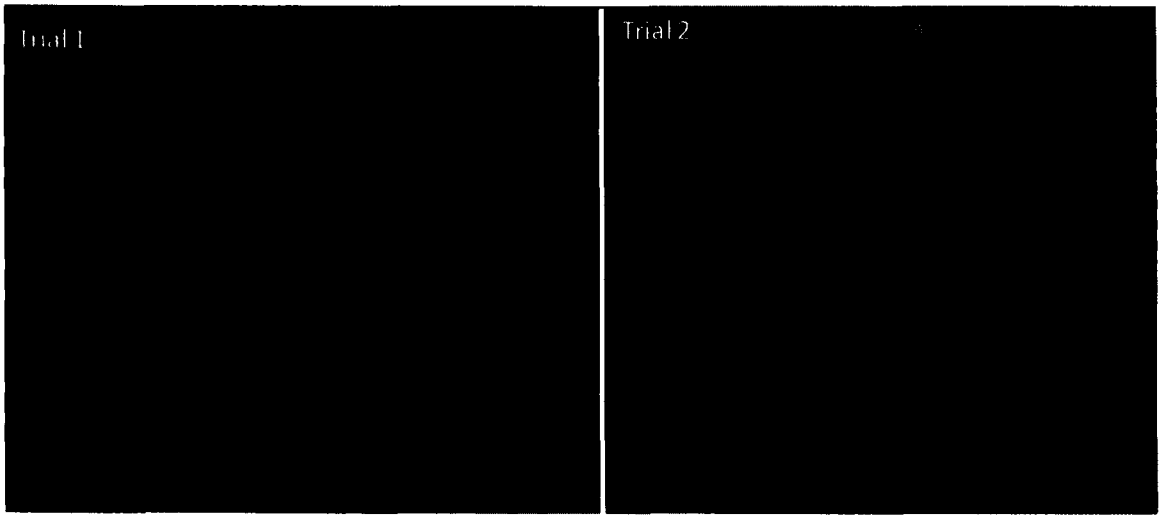
[y]Means followed by different lowercase letters indicate significance based off Tukey's test ($P \leq 0.05$, $n = 3$) and apply within individual trials only and not across trials. The absence of letters indicates that there are no significant differences among factors.

Lettuce: (*Lactuca sativa* cv. Rex)

Comparison of the low and high fish density treatments for t_1 and t_2 revealed that there were no significant differences in the mean above-ground wet and dry lettuce biomass (Table 5). A significantly greater amount of below-ground biomass (wet and dry) was generated in the high density fish treatments in comparison to the low density fish treatment in t_2, while no significant differences were determined in t_1. The mean above-ground and below-ground (wet and dry) lettuce biomass in t_1 and t_2 were greater in the high and low fish density treatments in comparison to positive control, except for wet below-ground biomass from the low density treatment in t_2 (Table 5).

The low density fish treatment and the positive control were not determined to be significantly different with respect to mean above-ground and below-ground wet lettuce biomass in t_1. Dried below-ground biomass reveled a significant difference between the two treatments in t_1. In t_2, no significant differences were determined between the biomass yield from the low density fish treatment and the positive control, except for wet above-ground biomass (Table 5). No significant differences were found between the positive control and the high density fish treatment in either t_1 and t_2, except for the wet above-ground biomass and dry below-ground biomass in t_2. The physical appearance and size of the lettuce plants in t_2 were of a lighter shade of green and were smaller in comparison with lettuce in t_1 (Figure 9).

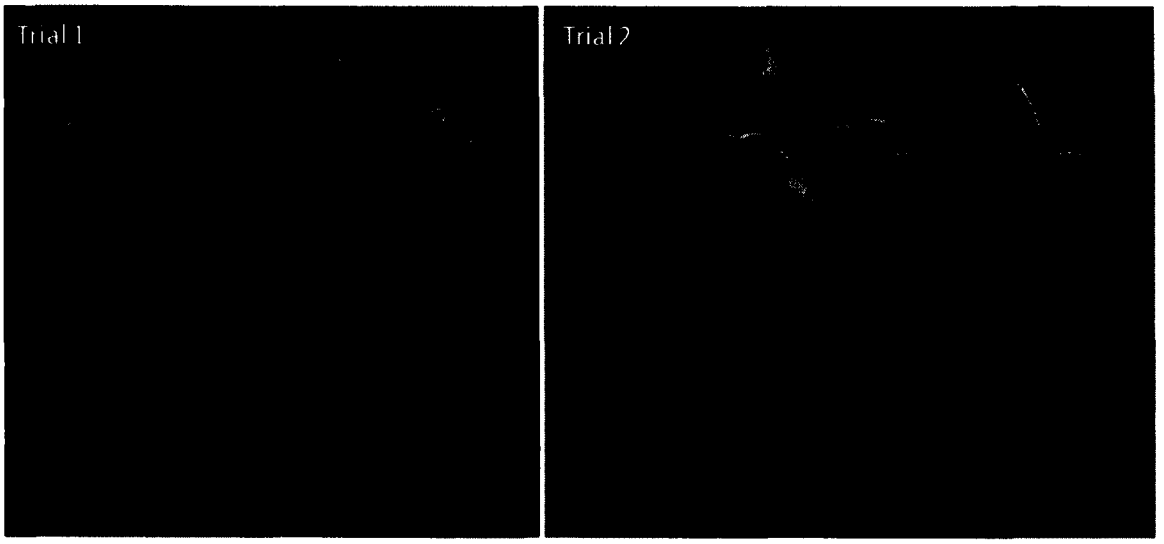
Figure 9. (Left) lettuce yield t_1 and (right) lettuce yield t_2 (not to scale).



Pac choi (*Brassica rapa* cv. Win-Win)

The mean above-ground and below-ground wet and dry pac choi biomass were not significantly different between the low and high density fish treatments in t_1, but were significantly different in t_2 (Table 5). The mean above-ground wet and dry pac choi biomass from the positive control was significantly greater than the two fish treatment (low and high) in t_1. No significance differences were determined between the high density fish treatment and the positive control in t_2, except with respect to dried below-ground biomass. The dried below-ground biomass was significantly less in the positive control in comparison to the two fish treatments in t_1, while the high density fish treatment in t_2 was significantly greater than the low density fish treatment and positive control. The positive control and the high density fish treatments were determined to be not significantly different from one another in t_1, but were determined to be significantly different from the low density fish treatment. The physical appearance and size of the pac choi plants in t_2 were of a lighter shade of green and were smaller in comparison with pac choi in t_1 (Figure 10).

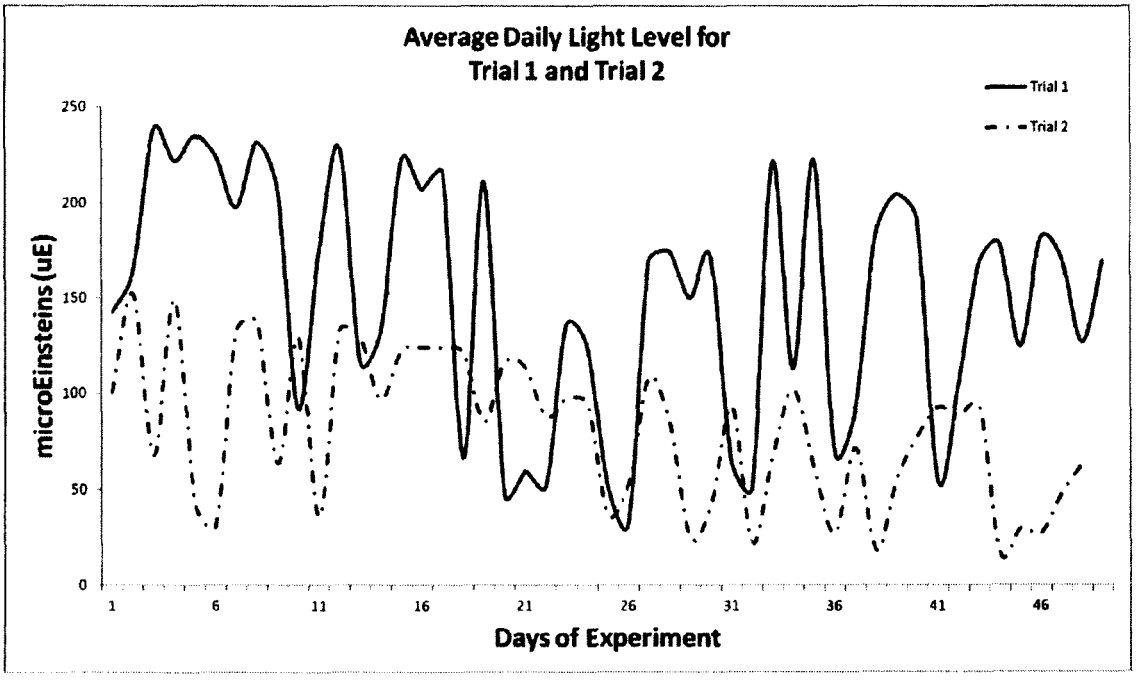
Figure 10. (Left) pac choi yield t_1 and (right) pac choi yield t_2 (not to scale).



The mean yield of both lettuce and pac choi differed significantly between trials; with t_1 yielding significantly more tissue than t_2 (Figures 9 and 10). The differences correspond to a decrease in the length of solar day, and the absence of supplemental lighting within the greenhouse, which resulted in a decrease in photosynthetically active radiation (PAR), with a mean of 151 and 81 μE/day for t_1 and t_2, respectively (Figure 11). The sudden dips found throughout Figure 11 illustrates the difference in light between a cloudy day and a sunny day.

Plants within the greenhouse are typically stationed on a bench-top, or placed on the ground. The design of the experimental systems used in this investigation resulted in the elevation of plants to a height not usually found in the greenhouses. The elevated plant bed resulted in a dangerous proximity to the high density discharge lighting (high pressure sodium) found used in the greenhouse. The heat released from the high pressure sodium bulbs at this closer proximity could dry out and damage the foliage, and in response, supplemental light was not used.

Figure 11. Average daily light for the duration of t_1 and t_2. Measurement (microEinsteins) was taken with a light sensor in adjacent greenhouse every 15 minutes and averaged over a 24 hour period to obtain daily average.



Tissue Analysis

The elemental concentration of N, P, Fe, B, Mo (Table 6) within the tissues of pac choi and lettuce in the fish treatments did not significantly differ from the positive control, and were above the minimal range of healthy tissue (Jones, 2005). The concentration of potassium (K), and manganese (Mn) in the positive control surpassed the concentration of the tissues harvested from the low and high density fish treatments. Elevated levels of aluminum (Al), zinc (Zn), calcium (Ca), and copper (Cu) were found in tissues harvested from the fish treatments.

Table 6. Elemental composition of lettuce and pac choi dry tissue for t_1 only.

		Macronutrients (%)				
Treatment	Plant Type	N	P	K	Ca	Mg
Low Density Fish	Lettuce	5.12 ± 0.40	0.73 ± 0.03	2.31 b[y] ± 0.15	2.57 a ± 0.17	0.22 b ± 0.01
High Density Fish	Lettuce	5.27 ± 0.40	0.72 ± 0.03	3.25 a ± 0.15	1.89 a ± 0.17	0.21 b ± 0.01
Positive Control	Lettuce	4.92 ± 0.40	0.87 ± 0.04	3.82 a ± 0.15	1.09 b ± 0.17	0.29 a ± 0.01
Low Density Fish	Pac Choi	4.82 ± 0.31	0.56 ± 0.05	1.42 b ± 0.21	2.26 b ± 0.30	0.24 ± 0.03
High Density Fish	Pac Choi	5.17 ± 0.31	0.51 ± 0.05	2.22 b ± 0.21	2.95 ab ± 0.30	0.26 ± 0.03
Positive Control	Pac Choi	5.64 ± 0.31	0.50 ± 0.05	3.66 a ± 0.21	1.91 b ± 0.30	0.27 ± 0.03

		Micronutrients (mg / kg)						
Treatment	Plant Type	B	Fe	Mn	Cu	Zn	Mo	Al
Low Density Fish	Lettuce	25.53 ± 2.01	48.67 ± 6.81	23.67 b ± 15.56	15.90 ± 2.04	122.00 a ± 7.16	0.28 ± 0.12	14.00 a ± 1.12
High Density Fish	Lettuce	25.57 ± 2.01	68.73 ± 6.81	69.63 ab ± 15.56	17.70 ± 2.04	105.77 a ± 7.16	0.18 ± 0.12	12.07 ab ± 1.12
Positive Control	Lettuce	29.33 ± 2.01	53.63 ± 6.81	108.63 a ± 15.56	11.60 ± 2.04	16.30 b ± 7.16	0.23 ± 0.12	7.63 b ± 1.12
Low Density Fish	Pac Choi	31.87 ± 4.52	56.40 ± 4.52	41.73 ± 23.89	19.07 a ± 2.01	158.00 ab ± 42.47	1.86 ± 0.35	15.65 ± 3.15
High Density Fish	Pac Choi	35.97 ± 4.52	56.67 ± 4.52	99.30 ± 23.89	22.33 a ± 2.01	254.00 a ± 42.47	2.40 ± 0.35	12.19 ± 3.15
Positive Control	Pac Choi	47.77 ± 4.52	55.40 ± 4.52	119.40 ± 23.89	10.33 b ± 2.01	17.07 b ± 42.47	1.88 ± 0.35	5.31 ± 3.15

[y]Means followed by different lowercase letters indicate significance based off Tukey's test ($P \leq 0.05$, $n = 3$). The absence of letters indicates that there are no significant differences among factors.
Values are LSmean ± SE

Interveinal chlorosis was expressed by the pac choi grown in low density treatment in t_1 (Figure 12), and was explicit in the older vegetation, not the younger most recent vegetative growth (Figure 13). The pac choi harvested from the high density treatments did not express interveinal chlorosis to the severity seen in the low density treatment. The lettuce harvested from all treatments (excluding the negative control) appeared healthy and had no distinguishing differences were observed with respect to appearance.

The pac choi harvested from the positive control was brittle, and required special handing to remain intact. The pac choi harvested from the low and high density fish treatments did not exhibited a brittle characteristic, in fact, the pac choi was quite elastic.

The greenhouse was outfitted with automated vents which would open and close to regulate the temperature within the greenhouse. The temperature during the duration of t_1 would reach day time temperatures above 26 ^{o}C. The pac choi from the fish treatment would wilt during these warm, sunny (cloudless) days, while the pac choi grown in the positive control never expressed wilting.

Figure 12. Photograph taken of an individual pac choi leaf from the low density treatment before harvest, t_1. The leaf is expressing interveinal chlorosis.

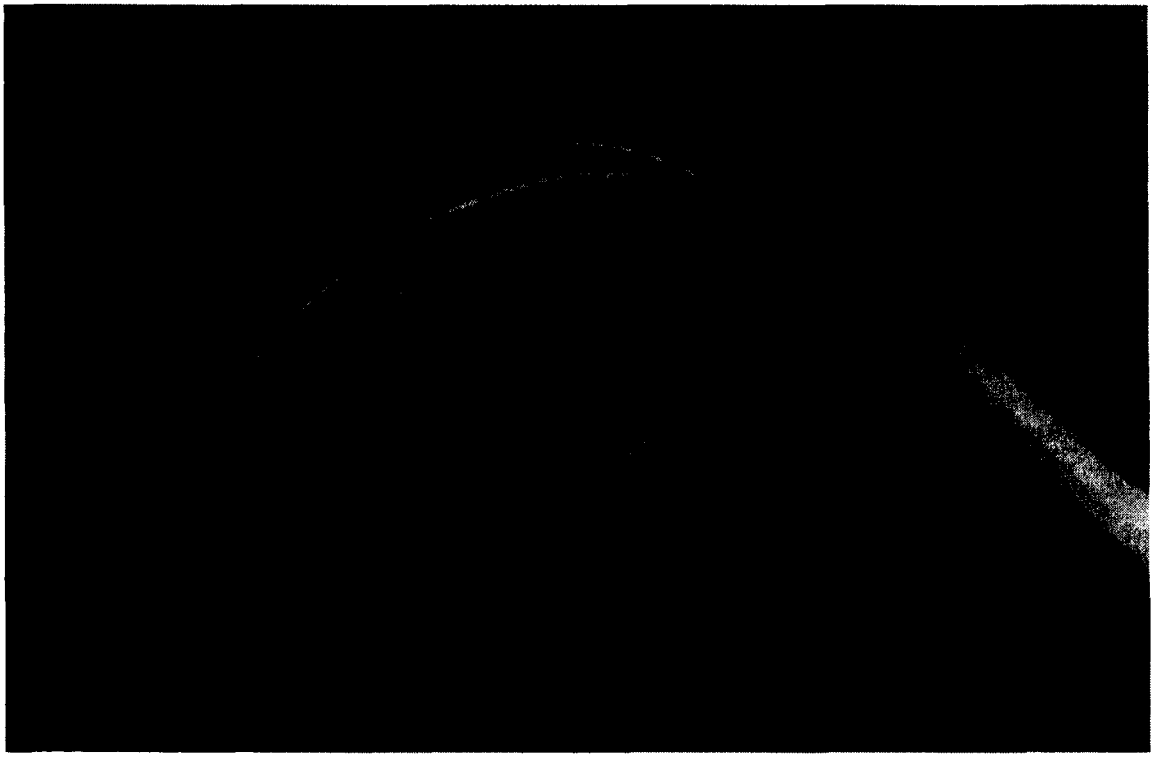


Figure 13. Photograph (A) of a plant growing tank connected to the low density fish treatment (replicate C) on day of harvest, t_1. Note the interveinal chlorosis only in the older leaves of the pac choi. Photograph (B) has been enlarged to illustrate no interveinal chlorosis in the young, most recent vegetation.

Nutrient Concentration

The mean concentration of all elements found in the water are presented in Table 7 and were greater in the high density treatment, in comparison to the low density treatment. The difference between the two fish treatments was typically half the concentration of the high density treatment and can be seen reflected in the EC (Figure 8), where the final EC of the low density treatments was half the value reported in the high density treatment.

The final mean concentration of calcium (Ca^{+2}) in the waters of the high density treatment exceeded that of the positive control (Table 7). The final mean concentration of K, P, Mg, S, Fe, Mn, Zn, and Cu in the water from the low and high density treatments were significantly less than the levels present in the water of the positive control.

The final mean concentration of both NH_4 and NO_3 were significantly different between the water of the low and high density treatments, while composition of elemental nitrogen in the tissue (Table 6) harvested from both treatments, were above acceptable (N% > 4.20) levels, and did not significantly differ (Jones, 2005). Nitrate levels in the high density fish treatment (Table 7) were well above levels found in a traditional hydroponic solutions (see Table 3).

Table 7 Final elemental concentration (mg/L) of the water derived from each treatment at the end of t_f only.

		Macronutrients (mg / L)					
Treatment	TDN (mg / L)	NH4 N / L	NO3 (mg / L)	K (mg / L)	Ca (mg / L)	P (mg / L)	Mg (mg / L)
Intial water supply	0.27	0.04	1.45	1.94	5.75	0.13	0.50
Negative Control	0.13 d ± 2.06	0.00 c ± 0.01	0.18 d ± 7.97	1.30 b ± 0.84	9.36 c ± 2.68	0.09 b ± 0.95	0.52 d ± 0.18
Low Density Fish	52.31 c ± 2.06	0.22 b ± 0.01	219.26 b ± 7.97	0.20 b ± 0.84	52.70 b ± 2.68	1.21 b ± 0.95	1.89 c ± 0.18
High Density Fish	110.66 a ± 2.06	0.29 a ± 0.01	477.18 a ± 7.97	0.59 b ± 0.84	127.56 a ± 2.68	3.33 b ± 0.95	3.91 b ± 0.18
Positive Control	70.28 b ± 2.06	0.01 c ± 0.01	67.20 c ± 7.97	49.07 a ± 0.84	59.36 b ± 2.68	12.64 a ± 0.95	16.18 a ± 0.18

	Micronutrients (mg / L)					
	S (mg / L)	Fe (mg / L)	Mn (mg / L)	Zn (mg / L)	Cu (mg / L)	Na (mg / L)
Intial water supply	7.09	0.00	0.00	0.00	0.00	62.01
Negative Control	7.83 d ± 0.29	0.00 b ± 0.02	0.00 b ± 0.01	0.00 c ± 0.00	0.00 c ± 0.00	70.40 c ± 0.85
Low Density Fish	11.29 c ± 0.29	0.02 b ± 0.02	0.00 b ± 0.01	0.01 bc ± 0.00	0.01 bc ± 0.00	74.41 b ± 0.85
High Density Fish	15.87 b ± 0.29	0.02 b ± 0.02	0.01 b ± 0.01	0.01 b ± 0.00	0.02 b ± 0.00	84.08 a ± 0.85
Positive Control	33.09 a ± 0.29	0.98 a ± 0.02	0.06 a ± 0.01	0.20 a ± 0.00	0.12 a ± 0.00	78.18 b ± 0.85

Means followed by different lowercase letters indicate significance based off a Tukey's test ($P \leq 0.05$, n=3). The absence of letters indicates that there are no significant differences among factors.

Values are LSmeans ± SE

Spectral Analysis

The spectral indices presented in Table 5 revealed significant differences between treatments in TM54 and NIR31 for lettuce, while pac choi expressed significance differences only in REIP (Table 8). The remaining indices for either pac choi and lettuce were not determined to be significantly different among trials. Lettuce always had a low REIP, NDVI, and TM43 (a standard vegetation index), compared with pac choi. The low REIP, NDVI, and TM43 exhibited by the lettuce, in comparison with pac choi, suggests that lettuce was lower in both chlorophyll and biomass. TM54 and NIR31 expressed the opposite relationship, where lettuce exhibited a greater value (Table 8). The lower TM54 expressed by the pac choi in comparison to the lettuce within trials is an indication that pac choi held a greater degree of leaf moisture (Table 8). The higher NIR31 expressed by the lettuce is an indication that lettuce was more phenologically advanced than pac choi.

The REIP exhibited by the pac choi in t_2 was much lower than the REIP in t_1 (Table 8), which suggests low chlorophyll levels within the pac choi tissue in t_2. The strong decline in the REIP expressed by the pac choi between trials was not reported with respect to the lettuce (Table 8), which suggests that chlorophyll levels within the lettuce tissue were no different between trials. The TM54 in t_2 was greater in both the lettuce and pac choi tissue in comparison to t_1, which suggests that both crops contained less leaf moisture in second trial.

Table 8. Spectral indices of pac choi and lettuce for t_1 and t_2.

Trial	Treatment	Plant Type PC = Pac Choi L= Lettuce	REIP [LSmean ± SE]	NDVI [LSmean ± SE]	TM43 [LSmean ± SE]	TM54 [LSmean ± SE]	NIR31 [LSmean ± SE]
1	Low Density Fish	L	703.57 ± 0.71	0.76 ab ± 0.01	7.35 ± 0.31	0.44 b ± 0.01	0.71 b ± 0.01
	High Density Fish	L	702.41 ± 0.71	0.77 a ± 0.01	7.89 ± 0.31	0.47 a ± 0.01	0.75 a ± 0.01
	Positive Control	L	702.28 ± 0.71	0.75 b ± 0.01	7.05 ± 0.31	0.42 b ± 0.01	0.71 b ± 0.01
	Low Density Fish	PC	719.15 b ± 1.30	0.82 ± 0.01	10.12 ± 0.30	0.38 ± 0.01	0.68 ± 0.01
	High Density Fish	PC	723.13 a ± 1.30	0.83 ± 0.01	10.61 ± 0.30	0.39 ± 0.01	0.68 ± 0.01
	Positive Control	PC	723.63 a ± 1.30	0.83 ± 0.01	10.74 ± 0.30	0.37 ± 0.01	0.66 ± 0.01
2	Low Density Fish	L	702.27 ± 0.61	0.74 ± 0.01	6.60 ± 0.15	0.56 a ± 0.01	0.84 a ± 0.01
	High Density Fish	L	702.80 ± 0.61	0.74 ± 0.01	6.72 ± 0.15	0.54 ab ± 0.01	0.82 b ± 0.01
	Positive Control	L	703.55 ± 0.61	0.74 ± 0.01	6.62 ± 0.15	0.52 b ± 0.01	0.82 b ± 0.01
	Low Density Fish	PC	705.10 b ± 2.24	0.79 b ± 0.01	8.61 b ± 0.26	0.47 a ± 0.01	0.76 a ± 0.01
	High Density Fish	PC	708.22 ab ± 2.24	0.81 a ± 0.01	9.48 a ± 0.26	0.44 ab ± 0.01	0.76 ab ± 0.01
	Positive Control	PC	712.72 a ± 2.24	0.81 a ± 0.01	9.52 a ± 0.26	0.43 b ± 0.01	0.73 b ± 0.01

[Y]Means followed by different lowercase letters indicate significance based off Student's t test ($P \leq 0.05$, $n = 3$). The absence of letters indicates that there are no significant differences among factors.

Values are LSmeans ± SE

Spectral reflectance curves of both crops (lettuce and pac choi) harvested from t_1 and t_2 exhibit spectral differences, and are illustrated in Figures 14 - 17. The NIR plateau (750 - 1300 nm) exhibited by the lettuce in t_1 (Figure 14) were similar among all treatment groups except the negative control. The reduction in reflectance of lettuce demonstrated by the negative control in t_1 and t_2 (Figures 14 and 15), is likely attributed to a lack of leaf material in the field of view of the spectrometer. The reduction in the slope of the NIR-plateau, which is present in the spectral curve of the negative control in comparison to the other treatments (Figure 14), contributes directly to a significant change in the indices, but is not presented in Table 8 because of the lack of adequate tissue for a representative sample.

A downward shift in the reflectance of the NIR-plateau was exhibited by the lettuce in t_2 (Figure 15) in comparison to t_1 (Figure 14). The downward shift (reduction in slope) exhibited by the lettuce in t_2 resulted in the direct alteration in the indices presented in Table 8. An example of the effect of a reduction in the slope of the NIR-plateau is illustrated by the reduction in band 4 (Figure 14 and 15), which decreased the ratio of TM band 5/4, thus resulting in an overall change in the indices in t_2 (Table 8). NIR31 was another index that was directly impacted as a result of the reduction of the slope along the NIR-plateau.

Figure 14. Reflectance curves for lettuce by treatment group for t_1 only.

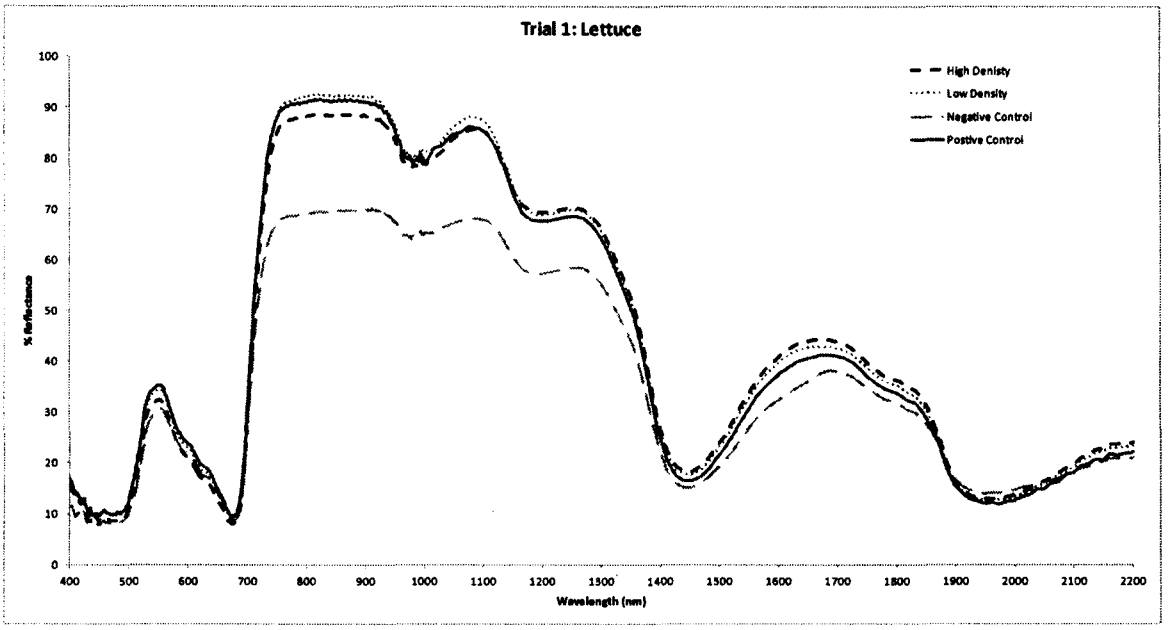
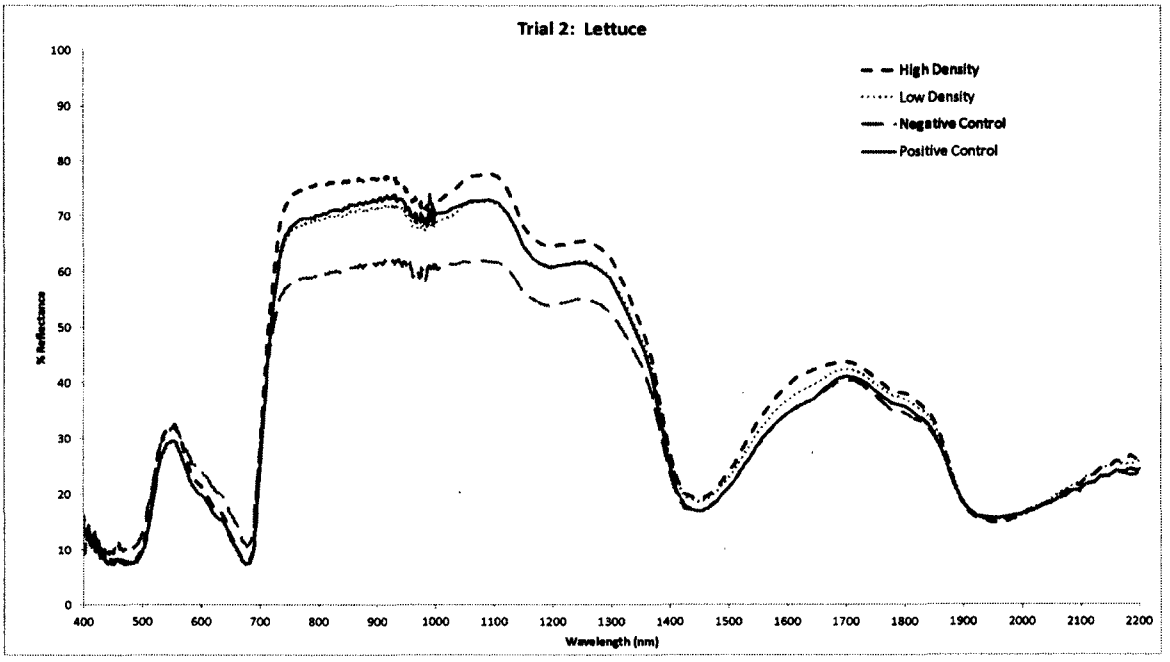


Figure 15. Reflectance curves for lettuce by treatment group for t_2 only.



Lettuce (Figure 14 and 15), in comparison to pac choi (Figure 16 and 17), had a significantly greater reflectance in the chlorophyll absorbance (480 - 680 nm) band. To the eye the lettuce was a lighter shade of green in comparison to pac choi, which contributed to the difference in the reflectance in the visible portion of the reflectance curve between the two crops.

Furthermore, lettuce had an obvious narrow and shallow chlorophyll well (660 - 680 nm) in comparison with pac choi, which suggests less chlorophyll in the lettuce tissue. The narrow chlorophyll well exhibited by the lettuce had a directly influenced on the slope of the REIP, which is why the REIP is highly correlated with chlorophyll levels.

Pac choi harvested from the positive control and the high density fish treatment in t_1 (Figure 16), expressed a deep broad chlorophyll well (480 - 680 nm) in comparison to the narrow and shallow chlorophyll well exhibited by the lettuce (Figure 14 - 15). However, the pac choi from the low density fish treatment in t_1, which exhibited interveinal chlorosis (Figure 12), expressed an increase in reflectance found in both the visible and NIR portions of the reflectance curves (Figure 16). The direct result of the increase in reflectance had little impact on the NIR31 and TM54, which suggests that the slope of the NIR-plateau were similar among treatment groups. The REIP with respect the low density fish treatment in t_1 (Table 8) was significantly affected, possibly by the narrowing of the chlorophyll well, which resulted in a blue shift of the red edge (Rock et al., 1988). The negative control had a narrow and shallow well in both t_1 and t_2.

The deep and broad chlorophyll well (480 - 680 nm) exhibited by the pac choi in t_1 (figure 16), became more shallow and narrow in the second trial (Figure 17), which corresponds to the reduction in the REIP in t_2 (Table 8). Furthermore, the overall reflectance of the visible portion of the spectral curves of pac choi across all treatment groups was greater in t_2 (Figure 17), except the negative control which decreased. There was a noticeable reduction in the NIR-plateau expressed by pac choi in the low density fish treatment in t_2 (Figure 17), in comparison to t_1.

Figure 16. Reflectance curves for pac choi by treatment group for t_1 only

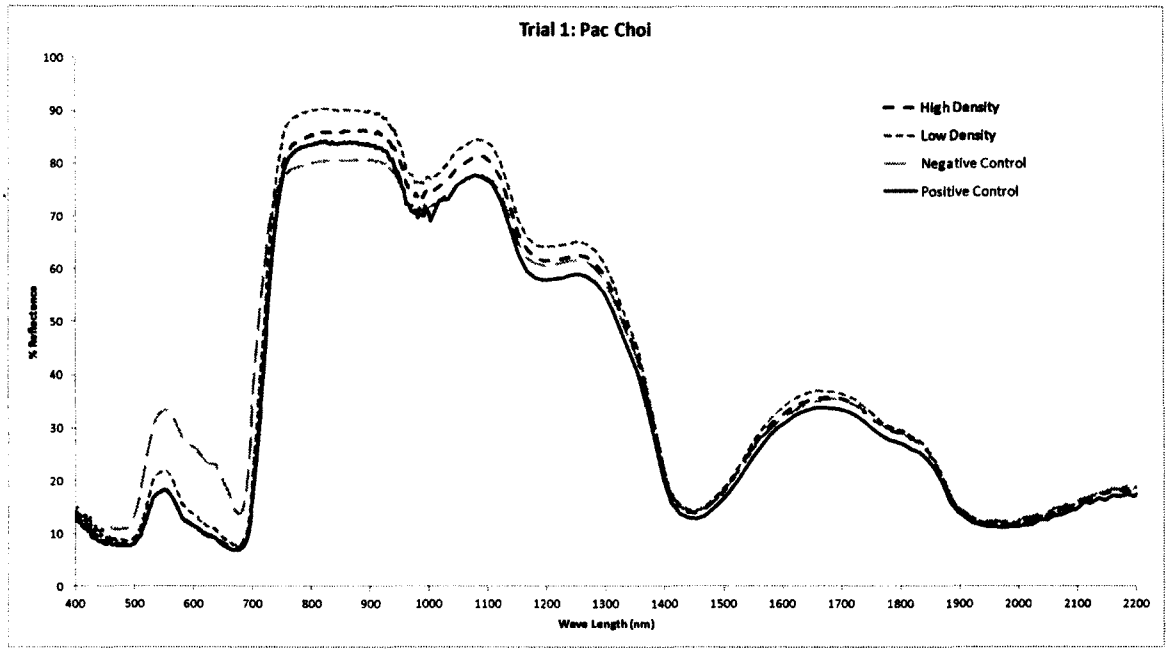
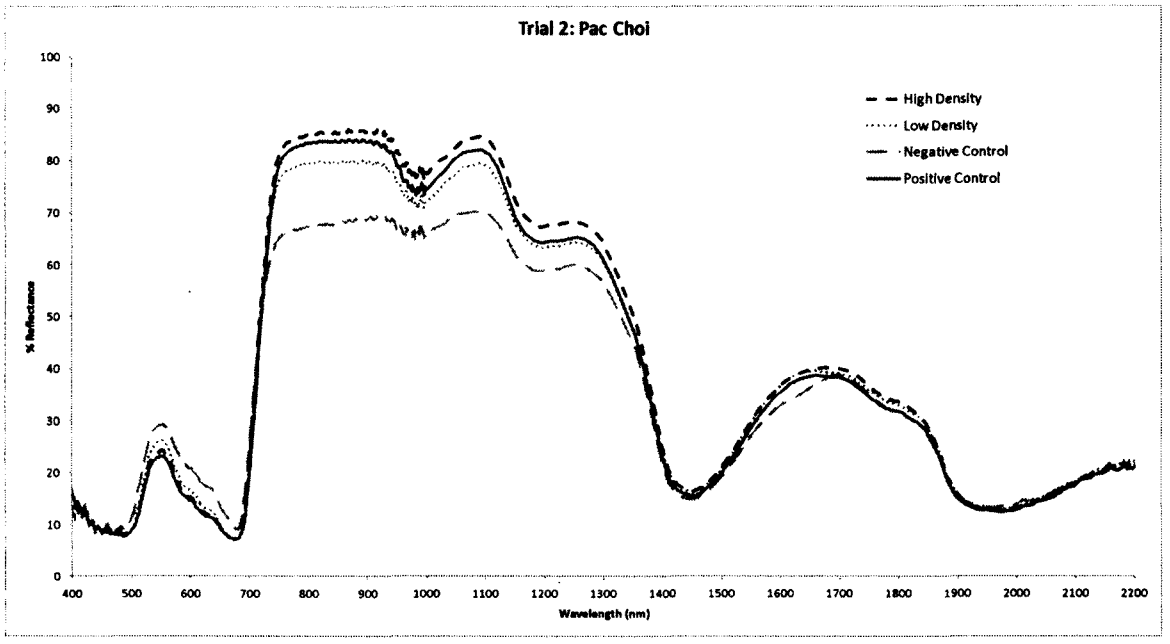


Figure 17. Reflectance curves for pac choi by treatment group for t_2 only.



CHAPTER IV

DISCUSSION

Summary

This study confirms that hybrid striped bass (♂*Morone saxatilis* x ♀*Morone chrysopos*) can be successfully integrated with pac choi (*Brassica rapa* cv. Win-Win) and lettuce (*Lactuca sativa* cv. Rex) in a closed recirculating systems. The results of this study suggest that the standard fish diet used in this study did provide the nutrients necessary for vegetative growth. Elements within the water such as K, P, Mg, S, Fe, Mn, Zn, and Cu were reported to be well below that of conventional hydroponics standards for optimum plant growth, which is similar to other studies (Seawright et al., 1998; Rakocy et al., 2006). Additional research should investigate alternative methodology in providing these nutrients directly to the system with the intention of avoiding enrichment with synthetic fertilizers.

Analytical investigation of the plant tissues revealed that plants grown with fish at the densities studied are relatively low in K, and Mn in relation to the positive control, while elements such as N, P, Fe, B, Mg, and Mo were similar to the positive control. Furthermore, elements such as Ca, Al, Zn, and Cu were elevated in the plants harvested from the low and high density fish tanks in relation to the positive control.

Spectral investigation of the plant tissues obtained from the experimental systems have proven to be useful and accurate in diagnosing signs of elemental deficiencies. The results of this research suggest that a change in reflectance both amplitude and slope along the NIR-plateau and specifically the REIP appear to be directly associated with the health of the plant tissue, which supports the finding by other studies (Rock et al., 1986; Rock et al., 1988). TM band 5/4 indicated

that the crops in t_2 were becoming stressed, which suggests that this index may be a useful indicator of overall stress.

EC and Nitrogen Cycling

The rate at which the EC accumulates within a given system, is an indication of the rate at which nutrients are released and/or collected within that system. The greater fish biomass found in the high density treatments clearly had a positive effect on the rate at which the EC increased within the experimental systems (Figure 8), which is illustrated by a difference in the overall slope of EC between the low and high density treatments. The higher EC, in turn reduced the nutrient deficiency expressed by the pac choi in the low density treatment.

The final concentration of nitrates in the water from the high density treatment (Table 7) exceeds twofold the recommended concentration (200 mg/L) for traditional hydroponic growth (Table 3). Naegel (1977) reported 1200 mg/L of nitrates within the first seven weeks of experimentation, to later be reduced and maintained at 200 mg/L. The reduction was possible because the researcher integrated a microbial denitrification bed, which converted dissolved nitrates (NO_3) into various forms of nitrogen gas. Rakocy et al. (2006) introduced orchard netting in a denitrification bed, and reported a release of methane and hydrogen sulfide as byproduct. Denitrification has proven to reduce and control the accumulation of nitrates in aquaponic systems, while the byproducts of the cycle may serve as a potential energy source (CH_4). The accumulation of nitrates (Table 7) in this study suggests that denitrification is required.

Another alternative to reduce the acumination of nitrates in long term aquaponic cultivation would be to dispense some of the water in a system to crops not connected to the integrated system. The water in an aquaponic systems matures (Table 7) and becomes biologically active (microbial rich) with time, which could certainly be used as a liquid fertilizer.

Dissolved Oxygen and Temperature

The difference in the mean temperature between t_1 and t_2 is likely as a result of a reduction in the day length, which reduced the overall heat generated from solar radiation (Figure 11). The sudden drop in DO experienced in t_1 (day 29), was a result of a campus wide power outage, which discontinued the operation of the air compressor in the greenhouse (figure 7). The lack of supplemented air, especially in the tanks supporting fish, resulted in a drastic decline in the DO.

The discrepancy (0.41 mg/L) between the DO within the low and high density fish systems is a result of a combination of physical and biological factors. Physically, the experimental aquaponic systems most likely reached an oxygen saturation point, as the oxygen supplied to each system was from compressed air, not pure oxygen. Biologically, the greater overall fish biomass (Table 4) in the high density treatments require overall more oxygen to sustain the greater number of fish. Furthermore, the higher fish biomass resulted in an increased rate of ammonia production (Table 7), and in response, more nitrifying bacteria were established throughout the system. The process of nitrification in the high density treatments was elevated by the greater overall biomass, and as a result, the demand for oxygen was augmented, which are observed as a decrease in the overall DO (Figure 7).

Fish

To generate revenue after the initial costs of a large capital investment associated with recirculating systems, and absolute necessity of skilled and technical management required to maintain a functioning commercial system, a niche market may be essential (Rakocy et al., 2006). Hybrid striped bass (HSB) would indeed be a niche market if grown specifically within New England, and could be furthermore promising if the operator could integrate plants, which this study has proven possible. It is important to note the fact that HSB have been reported to perform

poorly in aquaponic systems, as the fish is intolerant of the potassium (K) which is often supplemented in aquaponics (Rakocy et al., 2006). This study suggest that K may need to be supplemented for longer term growth regimes.

Considering the reports of the hybrids' potential intolerance in aquaponics made the eleven mortalities experienced during the duration of t_2 concerning. The dead fish did not appear to have any lesions or any indications of damage to the external surfaces. Ammonia and nitrite levels were monitored in each fish tank weekly with an API Freshwater Test kit, and the results of these tests never revealed toxic levels of either form of nitrogen. Mortalities were most likely attributed to the stress placed on the fish during transfer from the greenhouse to the ARC (holding facility), and again from the ARC to the greenhouse between the conclusion of t_1 and commencement of t_2.

The majority of commercially cultured hybrid striped bass are typically grown in brackish (> 5.0 ppt) water (Smith et al. 1985). The fish in this experiment were grown in freshwater (< 0.5 ppt), which may have induced stress, as one of its parents (*Morone saxatilis*) is a salt water resident and the hybirds could be prone to requiring saline conditions. Furthermore, the sudden change in the ionic composition of the water found in the holding facility (ARC) and the aquaponic systems at the start of t_2, may explain the stress, and the reduced feeding behavior exhibited during t_2. It is unfortunate that elemental concentration of the water within the experimental systems were not conducted in t_2, as it may have provided insight on the ratio of elements present within the water. The decision to move the fish to the ARC after concluding t_1, was to ensure the health of the fish as Hurricane Sandy approached, and the greenhouse was not outfitted with electrical backup.

The reported mean weight gain of the HSB grown in the aquaponic systems, were substantially less than reported by other studies where hybrids were cultured in intensive tank systems (Smith et al., 1985; Kemeh and Brown, 2001). The difference in HSB weight gain between this study and others cited above could be attributed to the higher mean water

temperature in the cited studies (22 - 28 ^{O}C) in comparison to this study (19 - 20 ^{O}C), and the longer duration (287 days) of experimentation found in the cited studies. Furthermore, fish in the cited studies above were cultured in brackish waters, which may help the fish osmoregulate. It has been reported that HSB stocked at densities greater than 5 kg/m^3 exhibit a schooling behavior, which has been shown to positively influence and stimulate feeding behavior among other individuals within the school (Kemeh and Brown. 2001). The low number of fish in t_2 (Table 4), specifically those in the low density treatment (10 fish) may have been negatively influenced by the low stocking density which could potentially explain the reduced feeding behavior .

Plants

It is suggested (Rakocy et al, 2006) that when the EC is greater than 3.5mmoh/cm, a water change is required to reduce potential phytotoxic[3] levels. The high density treatments had a mean EC of 1.25 mS/cm at the conclusion of t_1. Giving this relatively high rate of increasing EC (Figure 8) in such a short period (48 days), accompanied with toxic levels of NO_3 (> 477.18 ± 7.97; Table 7) in the high density treatment, suggests that the high density systems would require frequent water changes to reduce the accumulation of nutrients to phytotoxic[3] levels. On the other hand, increasing in the capacity of vegetation within the plant growing tank could potentially reduce the rate of accumulation of certain nutrients (i.e. nitrates). It should be noted that a nutrient deficiency (likely Mn) was reported in the pac choi from a low density treatment, which suggests that the high density treatment is advantageous for the growth of pac choi.

The composition of the plant tissues (Table 6) raised in waters shared with hybrid striped bass, especially those from the low density treatment, are low in K, and Mn. The lettuce tissue harvested from this study in comparison to lettuce harvested in Seawright et al. (2006) investigation, were markedly lower in K, Fe, Mn, which are crucial elements for plant development. The nutritional value of the vegetation is also vitally important, especially for those

[3] A substance or mixture of substances that have a toxic (poisonous) effect on plant, usually leading to the death of the plant (i.e. excessive fertilization)

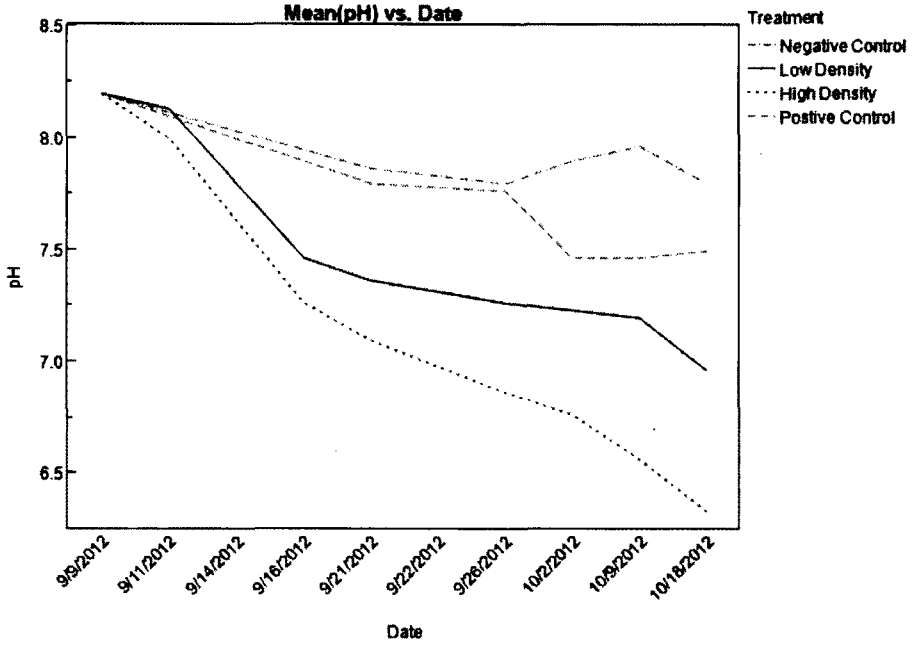
(vegetarians) who depend on vegetables for the majority of the nutritional needs. If the tissues harvested from aquaponics were in fact relatively low in vital nutrients (Table 6) in comparison to traditional farming practices, the added value of an integrated system may not hold as great of a benefit if there is a loss of nutritional value with regards to the crop being cultivated.

Four weeks into t_1, interveinal chlorosis was expressed by pac choi in the low density treatments (Figure 12), specifically in the older tissue (Figure 13), which suggests a Mg deficiency. The pac choi harvested from the high density treatment never expressed the severity of interveinal chlorosis experienced by those in the low density treatment. The difference between the low and high density treatments with regards to the physical appearance of the pac choi suggests that a elemental deficiency is directly associated with a lower fish density.

Interveinal chlorosis is an indication of either a magnesium (Mg) or manganese (Mn) deficiency. Mg is the central element within a chlorophyll molecule and its function is enzymatic activation for the transfer of energy (Jones, 2005) . A Mg deficiency is expressed in the older tissue and can be triggered by a low concentration (< 50 mg/L Mg) in the growing medium, or as a results of competitive inhibition between cations such as Ca^{2+}, K^{+}, Mn^{2+}, and Fe^{2+} (Goetz et al., 1983; Jones, 2005). It is critical to maintain a balance among these major cations, which is not present in our study (Table 7).

Mn is required for electron transport during photosynthesis, and its deficiency is expressed in the newly emergent foliage (Jones, 2005) as opposed to the older foliage of the pac choi. Despite the low (Tables 6 and 7) concentration of Mn within both the tissue and water shared with the HSB, the deficiency was characteristic of Mg based on the symptomology cited in Table 2.

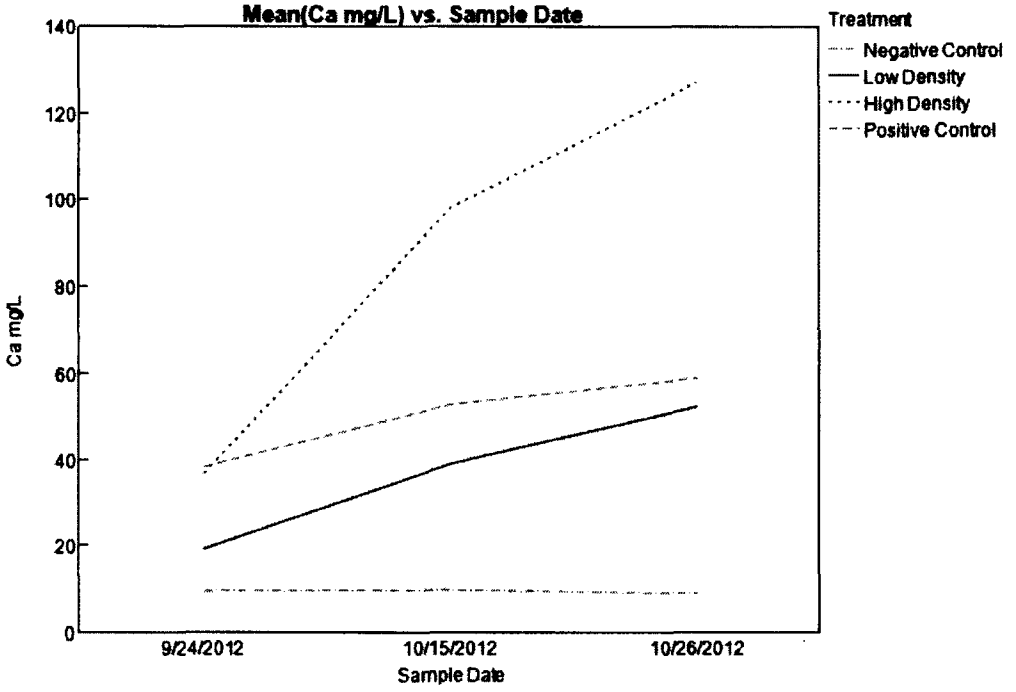
Figure 18. Mean pH by treatment group over the duration of t_1 only.



The pH of all experimental systems decreased over the duration of t_1 (Figure 18). The high fish density treatment had the greatest rate of pH decline, followed by the low density, positive control, and finally the negative control. The large discrepancy between the control groups (positive and negative) and the fish treatments is likely attributed to the release of CO_2 by the fish and bacteria, which reacted with water to form carbonic acid .

The rate of pH decline within the treatments containing fish is rather alarming. A low pH can have a significant effect on the rate of nitrification, and it's suggested that nitrification will be inhibited at a pH of 5.5 (Tyson et al., 2004). The given rate of pH decline presented in Figure 18 suggests that the pH may become a serious issue in a prolonged culture if left uncorrected.

Figure 19. Mean concentration (mg/L) of Calcium within each treatment over the duration of t_1 only.



Rakocy et al. (2006) reported insufficient levels of Ca^{+2} within aquaponic systems, and supplemented $Ca(OH)_2$ to increase Ca levels to a health range (100-200 mg/l) (Jones 2005). The implementation of gravel and / or sand in aquaponic production has proven to enhance mean calcium concentrations (Rakocy et al., 2006; Sikawa et al., 2010). The concentrations of Ca in the water (Table 7) and in the plant tissue (Table 6) were adequate in this study despite any addition of synthetic $Ca(OH)_2$, which is usually supplemented to improve plant growth. The origin of Ca in this experiment is likely a combination sourced from the direct input of the fish feed (Rafiee and Saad, 2005) and the release of calcium carbonate ($CaCO_3$) as a result of the acidification of the oyster shell.

The deficiency expressed in the pac choi is perhaps a result of a combination of factors. Ca levels in the waters of the fish treatments were elevated in comparison K and Mg (Table 7). The Ca levels, especially those in the high density fish treatment (Figure 19), may have caused competitive inhibition between Ca and Mg. It is important to understand that Mg becomes less

available to a plant at a lower pH (Figure 20), which was present at the conclusion of t_1 (Figure 18). In contrast, the complete absence of Mn in the waters of the experimental systems (Table 7) suggests that Mn was a limiting element, which may have resulted in the expressed interveinal chlorosis. Furthermore, it is important to understand that Mn is less available to plants at a high pH (Figure 20), which was present at the start of the experiment (Figure 18).

Figure 20. Effect of pH on the availability of essential nutrients for plant uptake. (Taken for Goetz et al., 1983)

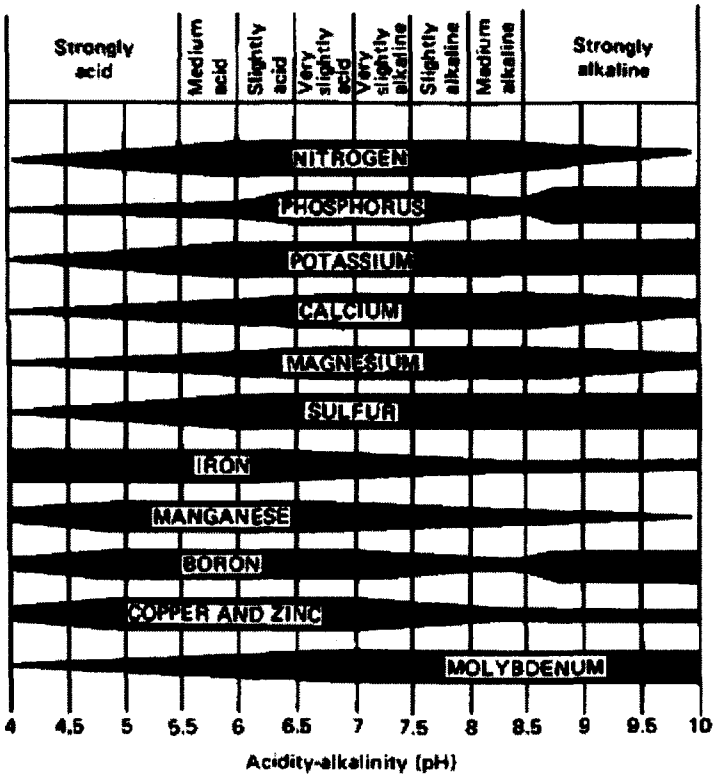


FIG. 9. The effect of soil pH on availability of nutrients to plants. The thickness of the horizontal band represents the relative solubility and, thus, the availability of the nutrient. Taken from Bidwell (1979).

The plant stalk of the pac choi grown in the positive control (hydroponic solution) were reported to be brittle, which made handling of the crop quite difficult. Many stalks were broken and damaged when sampled at the end of each trial. The fragile and delicate nature of the pac

choi harvested from the positive control could prove to be a negative characteristic if the crop require transportation to reach a consumer. The pac choi harvested from the low and high density fish treatments did not express this brittle characteristic, in fact the stacks of these pac choi were elastic. An explanation for the reported characteristically differences between the pac choi harvested from the positive control with that of the fish treatments, is most likely attributed to either over fertilization within the waters of the positive control, or the lack of a particular element(s).

Spectral Properties of Plants

The spectral determination of chlorophyll levels (REIP's, Table 8) in this study has been successfully used to indicate the health and productively of each experimental system. Despite that the stocking density of HSB was determined to not significantly influence mean plant yield (Table 5), the VIRIS was able to detect the subtle differences which correlated with yield (Figures 14 - 17) . The significant difference in the pac choi REIP's (Table 8) in t_1 suggests that the pac choi harvested from the low density treatment was under stress due to low chlorophyll concentrations. Interveinal chlorosis was visual and apparent throughout the pac choi leaf tissues in t_1 (Figure 12), which may have contributed to the greater reflectance in both the visual and NIR-plateau.

The differences in the visible portion of the reflectance curves (480-680nm) between lettuce and pac choi is due in part to the intensity of leaf color expressed by the crops. The difference is most likely attributed to lettuce exhibiting a lighter shade of green due to low levels of chlorophyll (low REIPs) in comparison to pac choi. The pac choi harvested from the low density treatment expressed interveinal chlorosis (Figure 12), which may have ultimately effected the spectral properties of the plant (Figure 16). The increase in reflectance in both the visible and the NIR plateau (750 - 1300 nm), along with the reduction in the REIP's clearly indicate stress in

the pac choi grown in the low density fish treatments (Rock et al., 1988). The visible portion of the spectral curves exhibited by the pac choi were elevated in t_2 in comparison to t_1, which may have contributed to an overall reduction in the REIP's expressed by pac choi among all treatments in t_2.

The spectral curves exhibited by lettuce were discernibly different between t_1 and t_2 (Figure 14 and 16). There was a considerable shift downwards (reduced reflectance) in the near-infrared (NIR) plateau, which suggests lettuce was under stress in t_2 (Rock et al., 1988), likely attributed to the effect of the low light levels on the cellular structure of the leaf. Sun leaves which are located in the top of a forest canopy are typically thicker than shade leaves which occupy the lower portion of a forest canopy. The thickness and the ordination of the cells within a plant can drastically influence reflectance properties, which may have cause the difference in REIP between t_1 and t_2. Leaf thin sectioning was not explored in this experiment and should be considering in further research. The minor shift in wavelength position of the chlorophyll absorption feature in comparison to the distinctive shift in the REIP has been reported in other studies (Rock et al., 1988), and further suggests distinguishing differences between trials.

Conclusion

Rakocy et al. (2006) suggested that the concentration of nutrients dissolved throughout an aquaponic system is influenced by a fish to plant ratio. The more fish you culture in a system, the greater the potential of nutrients found available in the system for plant uptake, which essentially provides the opportunity for additional plants. Seawright et al. (1998) suggested that the relative proportions of soluble nutrients made available to the plants by fish excretion do not mirror the proportions of nutrients assimilated by normal growing plants. Therefore, the rate of change in the concentration for individual nutrients differ, resulting in the inability of aquaponic systems

functioning for a prolonged period of time as nutritional deficiencies occur from an elemental imbalance.

It has been suggested that to accommodate the skewed concentration of nutrients that accumulate in result of prolonged cultures, the producer could potentially introduce these elements through supplementing the fish feed or foliar application (Roosta and Hamidpour, 2011). Integration of multiple crops could also prolong cultivation time if the farmer is knowledgeable of basic water chemistry, and introduces specific crops to remediate specific elemental nutrients (i.e nitrates). Introducing these elements through means of a specific formulated diet could be an alternative to supplementation, however the diet would have to generated at commercially acceptable rates (Seawright et al., 1998). Furthermore the complexity of a balanced fish diet must not mirror that of just one species of plant, but many. The diet could be in the form of larvae, more specifically fly pupae (e.g. *Hermetia illucens*), which have proven to recycle omega-3 fatty acids (St-Hilaire et al., 2007). Soldier fly cultivation could be an avenue for nutrient supplementation if the larvae could incorporate specific elements (e.g Mg, Mn, K) during development, ultimately releasing these elements into the aquaponic waters for plant uptake.

To maximize growth of the fish and plants, and thus revenue, the system must be operational at all times. The 48 day experimental period provides a short-term understanding of the behavior of small scale raft aquaponics. Further research exploring the effects of long term experimental duration, much like those provided by Dr. Rakocy, may provided some insight into the behavior of farming by means of small scale raft aquaponics.

The value of adding a spectral investigation of the plant tissues grown in the experimental systems has proven to be beneficial in diagnosing early signs of stress. Further research investigating the effect of select elemental deficiencies on the reflectance curves could possibly provide insight into non-destructive methods of diagnosing early signs of elemental stress. If this were possible, the effects of monitoring the health of crops grown by means of traditional

agriculture, and especially those grown within aquaponics, would serve to be of enormous benefit to the overall production of crops, worldwide.

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APPENDICES

Appendix A

New Hampshire
Fish and Game Department

HEADQUARTERS: 11 Hazen Drive, Concord, NH 03301-6500
(603) 271-3421
FAX (603) 271-1438

www.WildNH.com
e-mail: info@wildlife.nh.gov
TDD Access: Relay NH 1-800-735-2964

Permit No. MFD 1228
June 21, 2012

TO WHOM IT MAY CONCERN:

Under the authority contained in RSA 214:29, permission is hereby granted to **Calvin Diessner and David Bertinsky, UNH, Morse Hall, 8 College Rd. Durham, N.H. 03824,** to possess for educational purposes, hybrid striped bass (*Morone saxatilis*).

This permit is subject to the following conditions:

1. The permitted species may be acquired from Keo Fish Farm, Keo, Arkansas. An importation permit must be obtained from New Hampshire Fish & Game Law Division prior to importation. Up to 300 fish are permitted for study in 2012
2. The imported specimens shall be maintained in a closed-system aquarium at the Macfarlane Greenhouse on the UNH Campus.
3. There shall be no release of the study fish used in this project. At the completion of experimentation, all test subjects must be euthanized.
4. No specimens collected as authorized by this permit may be sold.

The permittee shall furnish the Executive Directory, by January 31, 2012, a written report containing the approximate number and species taken and possessed and the disposition of any possessed species.

This permit shall expire December 31, 2012, unless sooner revoked or rescinded. No collections may be made after December 31, 2012, without renewal of this permit.

Glenn Normandeau
Executive Director

GN/BWS/vjb

cc: Sandy Falicon, Rules Coordinator
Marine Fisheries Division
Law Enforcement
Lt. Jeffrey A. Marston

REGION 1
629B Main Street
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REGION 2
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REGION 3
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REGION 4
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New Hampshire
Fish and Game Department

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(603) 271-3421
FAX (603) 271-1438

www.WildNH.com
e-mail: info@wildlife.nh.gov
TDD Access: Relay NH 1-800-735-2964

Permit No. 2012-12

PERMIT TO IMPORT WILDLIFE OR FISH

UNDER THE AUTHORITY CONTAINED UNDER RSA 207:14, PERMISSION IS HEREBY GRANTED TO:

Name	Street Address	Town/City, State, Zip Code
Calvin Diesner	**8 College Road**	**Durham, NH 03824**

To Import **Striped Bass** Number: **300**

Species to be imported under this permit are from: **Keo Fish Farm**
6444 Highway 164N
Keo, AR 72083

Conditions of the Permit

1. Any species imported into the state shall be imported in compliance with all applicable State and Federal laws or rules, such as the NH Dept. of Agriculture, US Dept. Of Agriculture, or the US Fish and Wildlife Service.
2. Persons importing wildlife, including fish, pursuant to the requirements of Fis 803, shall notify the department prior to the importation into the state.
3. This permit issued in conjunction with permit No. MFD 1228. Said importation must abide by the conditions set forth in permit No. MFD 1228.
4. **This permit expires December 31, 2012**

Permittee Signature

Glenn Normandeau
Executive Director

July 12, 2012
Date of Issue

cc: Lt. Marston CO Benvenuti Marine Fisheries Division File

REGION 1
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Appendix B

University *of* New Hampshire

Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

02-Aug-2012

Berlinsky, David L
Biological Sciences, Rudman Hall
Durham, NH 03824

IACUC #: 120702
Project: Influence of Hybrid Striped Bass Stocking Density on Plant Growth in an Aquaponic System
Category: C
Approval Date: 25-Jul-2012

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category C on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics or other assessments.* The IACUC made the following comment(s) on this protocol:

The ARO needs to approve the Macfarlane Greenhouse location for vertebrate animal housing and use before any animals may be purchased for that location.

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jill A. McGaughy, Ph.D.
Chair

cc: File

Appendix C

Table 9. Mean Nutrient Concentration (mg/L) over three sampling periods for the duration of t_1 only.

Sampling Event (days into experiment)	Treatment	TDN (mg/L)	Macronutrients (mg/L) NH4 N/L	NO3 (mg/L)	K (mg/L)	Ca (mg/L)	P (mg/L)	Mg (mg/L)	Micronutrients (mg/L) S (mg/L)	Fe (mg/L)	Mn (mg/L)	Zn (mg/L)	Cu (mg/L)	Na (mg/L)
Day 0	Intial water supply	0.27	0.04	1.45	1.94	5.75	0.13	0.50	7.09	0.00	0.00	0.00	0.00	62.01
Day 15	Negative Control	0.39 c ± 0.53	0.01 c ± 0.04	1.04 c ± 2.09	1.96 c ± 0.57	9.96 c ± 1.04	0.18 c ± 0.16	0.57 c ± 0.17	7.46 d ± 0.20	0.00 c ± 0.00	0.00 b ± 0.00	0.00 d ± 0.00	0.00 c ± 0.00	64.14 c ± 0.66
	Low Density Fish	17.41 b ± 0.53	0.19 b ± 0.04	68.75 b ± 2.09	4.33 c ± 0.57	19.62 b ± 1.04	0.80 bc ± 0.16	1.10 bc ± 0.17	9.53 c ± 0.20	0.01 bc ± 0.00	0.00 b ± 0.00	0.00 c ± 0.00	0.02 b ± 0.00	67.18 b ± 0.66
	High Density Fish	37.19 a ± 0.53	0.27 b ± 0.04	154.49 a ± 2.09	6.94 b ± 0.57	37.02 a ± 1.04	1.40 b ± 0.16	1.85 b ± 0.17	12.29 b ± 0.20	0.02 b ± 0.00	0.00 b ± 0.00	0.01 b ± 0.00	0.02 b ± 0.00	70.61 a ± 0.66
	Postice Control	36.90 a ± 0.53	5.27 a ± 0.04	147.04 a ± 2.09	39.59 a ± 0.57	38.61 a ± 1.04	14.37 a ± 0.16	9.89 a ± 0.17	26.01 a ± 0.20	0.38 a ± 0.00	0.21 a ± 0.00	0.09 at 0.00	0.06 a ± 0.00	68.15 ab ± 0.66
Day 36	Negative Control	0.35 d ± 1.28	0.01 c ± 0.02	0.77 d ± 6.96	1.69 c ± 0.69	10.16 d ± 2.17	0.13 d ± 0.36	0.55 d ± 0.18	7.58 d ± 0.31	0.00 b ± 0.01	0.00 b ± 0.01	0.00 b ± 0.00	0.00 c ± 0.00	66.46 c ± 0.88
	Low Density Fish	43.10 c ± 1.28	0.21 b ± 0.02	169.32 c ± 6.96	4.35 c ± 0.69	39.32 c ± 2.17	1.82 c ± 0.36	1.78 c ± 0.18	11.31 c ± 0.31	0.02 b ± 0.01	0.00 b ± 0.01	0.01 b ± 0.00	0.01 b ± 0.00	71.67 b ± 0.88
	High Density Fish	90.65 a ± 1.28	0.44 a ± 0.02	389.01 a ± 6.96	9.51 b ± 0.69	98.57 a ± 2.17	3.57 b ± 0.36	3.61 b ± 0.18	15.41 b ± 0.31	0.02 b ± 0.01	0.00 b ± 0.01	0.01 b ± 0.00	0.02 b ± 0.00	80.64 a ± 0.88
	Postice Control	64.66 b ± 1.28	0.01 c ± 0.02	265.66 b ± 6.96	51.11 a ± 0.69	53.19 b ± 2.17	14.06 a ± 0.36	12.77 a ± 0.18	27.40 a ± 0.31	0.71 a ± 0.01	0.07 a ± 0.01	0.16 a ± 0.00	0.10 a ± 0.00	72.54 b ± 0.88
Day 47	Negative Control	0.13 d ± 2.06	0.00 c ± 0.01	0.18 d ± 7.97	1.30 b ± 0.84	9.36 c ± 2.68	0.09 b ± 0.95	0.52 d ± 0.18	7.83 d ± 0.29	0.00 b ± 0.02	0.00 b ± 0.01	0.00 c ± 0.00	0.00 c ± 0.00	70.40 c ± 0.85
	Low Density Fish	52.31 c ± 2.06	0.22 b ± 0.01	219.26 b ± 7.97	0.20 b ± 0.84	52.70 b ± 2.68	1.21 b ± 0.95	1.89 c ± 0.18	11.29 c ± 0.29	0.02 b ± 0.02	0.00 b ± 0.01	0.01 bc ± 0.00	0.01 bc ± 0.00	74.41 b ± 0.85
	High Density Fish	110.66 a ± 2.06	0.29 a ± 0.01	477.18 a ± 7.97	0.59 b ± 0.84	127.56 a ± 2.68	3.33 b ± 0.95	3.91 b ± 0.18	15.87 b ± 0.29	0.02 b ± 0.02	0.01 b ± 0.01	0.01 b ± 0.00	0.02 b ± 0.00	84.08 a ± 0.85
	Postice Control	70.28 b ± 2.06	0.01 c ± 0.01	67.20 c ± 7.97	49.07 a ± 0.84	59.36 b ± 2.68	12.64 a ± 0.95	16.18 a ± 0.18	33.09 a ± 0.29	0.98 a ± 0.02	0.06 a ± 0.01	0.20 a ± 0.00	0.12 a ± 0.00	78.18 b ± 0.85

[1]Means followed by different lowercase letters indicate significance based off a Tukey's test ($P \leq 0.05$, n=3). The absence of letters indicates that there are no significant differences among factors.
Values are means ± SE

Table 10. Nutrient concentration (mg/L)of the water by treatment group, t_1 only.

ID	SampleName	SampleDate	DOC mg/L	TDN mg/L	F mg/L	Cl mg/L	Br mg/L	NO3 mg/L	SO4 mg/L	NH4 mg N/L	PO4 mg P/L	Total monomeric Al mg/L	Organic monomeric Al	Al mg/L	Ca mg/L	Fe mg/L	K mg/L	Mg mg/L	Mn mg/L	Na mg/L	P mg/L	Si mg/L	S mg/L	Zn mg/L	Ag mg/L	As mg/L	Ba mg/L	Be mg/L	Cd mg/L	Co mg/L	Cr mg/L	Cu mg/L	Ni mg/L	Pb mg/L	Sb mg/L	Se mg/L	Sr mg/L	Ti mg/L
30900	ARC	9/9/2012	0.68	0.27	0.29	48.46	0.19	1.45	16.88	0.04	0.13	0.06	0.01	-0.01	5.75	0.00	1.94	0.50	0.00	62.01	0.13	6.87	7.08	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00	-0.01	0.00	0.00	0.04	0.00
30901	PCA	9/24/2012	3.84	35.32	0.40	55.04	0.12	140.27	58.08	5.17	14.27	0.03	0.02	-0.01	38.08	0.37	*37.3615	9.27	0.20	68.72	13.74	7.31	25.39	0.09	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.07	0.00	0.02	0.00	0.01	0.28	0.00
30902	PCB	9/24/2012	3.93	38.46	0.41	57.53	0.14	153.53	62.84	5.38	15.70	0.03	0.01	0.00	38.48	0.39	*41.0341	10.18	0.21	69.01	14.83	7.17	26.68	0.09	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.06	0.00	0.01	-0.01	0.00	0.28	0.00
30903	PCC	9/24/2012	3.81	36.91	0.44	52.45	0.17	147.31	61.35	5.26	15.01	0.03	0.01	0.00	38.52	0.37	*40.3633	10.23	0.21	66.73	14.52	7.09	25.96	0.09	0.00	0.03	0.01	0.00	0.00	0.00	0.00	0.06	0.00	0.01	0.01	0.00	0.28	0.00
30904	NCA	9/24/2012	0.70	0.34	0.30	54.15	0.09	0.82	18.07	0.00	0.15	0.02	0.00	0.00	8.45	0.00	1.88	0.49	0.00	64.14	0.15	7.14	7.44	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.06	0.00
30905	NCB	9/24/2012	0.98	0.44	0.36	54.40	0.19	1.10	18.17	0.02	0.18	0.01	0.00	0.00	9.98	0.00	2.00	0.56	0.00	64.66	0.18	7.56	7.52	0.00	0.00	0.04	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.08	0.00
30906	NCC	9/24/2012	0.81	0.41	0.40	52.92	0.27	1.21	17.75	0.01	0.20	0.01	0.00	0.00	11.44	0.00	2.00	0.66	0.00	63.64	0.20	7.36	7.41	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.10	0.00
30907	LA	9/24/2012	3.91	16.52	0.31	59.64	0.14	65.56	21.75	0.18	0.71	0.01	0.00	0.00	17.02	0.01	4.23	0.95	0.00	68.53	0.75	6.77	9.53	0.01	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.00	0.10	0.00
30908	LB	9/24/2012	4.24	17.81	0.33	59.92	0.08	70.50	21.91	0.21	0.88	0.01	0.00	0.00	18.78	0.01	4.19	1.09	0.00	67.64	0.89	6.57	9.51	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.11	0.00
30909	LC	9/24/2012	4.33	17.89	0.30	55.80	0.20	70.19	17.51	0.20	0.73	0.01	0.00	0.00	23.05	0.01	4.58	1.27	0.00	65.38	0.76	6.71	9.54	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.01	0.00	0.14	0.00
30910	HA	9/24/2012	7.19	36.58	0.32	67.24	0.13	153.62	27.32	0.32	1.28	0.02	0.01	0.00	37.97	0.02	7.03	2.01	0.00	71.53	1.31	6.55	12.56	0.01	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.01	0.00	0.19	0.00
30911	HB	9/24/2012	8.24	37.17	0.32	63.38	0.21	154.75	26.29	0.16	1.42	0.02	0.01	0.00	36.78	0.02	6.97	1.90	0.00	69.72	1.44	6.44	12.08	0.01	0.00	0.03	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.00	0.18	0.00
30912	HC	9/24/2012	8.48	37.80	0.37	59.62	0.01	155.11	26.70	0.33	1.45	0.02	0.01	0.00	36.31	0.02	6.81	1.64	0.00	70.59	1.47	6.43	12.22	0.01	0.00	0.03	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.01	0.00	0.18	0.00
31404	PCA	10/15/2012	4.57	62.46	0.51	52.78	0.10	248.21	75.16	0.01	15.72	0.10	0.03	0.01	52.35	0.66	*48.5203	12.15	0.12	72.28	14.43	7.15	26.29	0.15	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.10	0.00	0.01	0.00	0.00	0.36	0.00
31405	PCB	10/15/2012	5.04	65.19	0.31	54.87	0.10	269.10	76.30	0.01	15.86	0.06	0.02	0.01	54.93	0.75	*51.8069	13.02	0.04	73.91	14.97	7.43	27.81	0.16	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.10	0.00	0.01	0.00	0.02	0.38	0.00
31406	PCC	10/15/2012	4.95	66.32	0.28	48.65	0.10	279.68	72.80	0.02	13.40	0.05	0.02	0.01	52.28	0.73	*53.0172	13.15	0.05	71.42	12.77	8.17	28.12	0.16	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.09	0.00	0.01	0.00	0.01	0.37	0.00
31407	NCA	10/15/2012	0.80	0.31	0.35	54.69	0.01	0.35	19.12	0.01	0.14	0.04	0.01	0.01	9.05	0.01	1.72	0.48	0.00	66.51	0.12	6.81	7.53	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00
31408	NCB	10/15/2012	0.93	0.53	0.37	55.53	0.01	1.76	19.36	0.00	0.17	0.03	0.00	0.01	10.67	0.01	1.78	0.57	0.00	67.61	0.15	7.36	7.76	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00
31409	NCC	10/15/2012	1.14	0.21	0.43	52.87	0.26	0.20	18.84	0.01	0.15	0.05	0.01	0.02	10.76	0.00	1.55	0.60	0.00	65.25	0.13	7.10	7.44	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00
31410	LA	10/15/2012	5.65	39.83	0.35	60.70	0.03	156.22	26.24	0.19	1.92	0.02	0.01	0.01	32.72	0.02	3.90	1.51	0.00	72.28	1.77	7.19	11.04	0.00	0.00	0.03	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.16	0.00
31411	LB	10/15/2012	6.31	45.44	0.33	60.91	0.03	176.25	27.51	0.22	2.20	0.03	0.02	0.01	41.44	0.02	4.66	1.86	0.00	73.77	2.08	7.51	11.75	0.02	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.20	0.00
31412	LC	10/15/2012	6.38	44.03	0.37	58.06	0.03	175.49	27.34	0.20	1.84	0.02	0.01	0.01	43.80	0.02	4.50	1.97	0.00	68.94	1.60	6.67	11.15	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.00	0.01	0.21	0.00
31413	HA	10/15/2012	10.61	88.13	0.37	70.57	0.10	375.27	36.68	0.49	3.15	0.03	0.02	0.01	94.36	0.02	9.47	3.65	0.00	81.24	3.19	6.43	15.46	0.01	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.36	0.00
31414	HB	10/15/2012	12.82	90.31	0.15	68.40	0.10	388.53	36.56	0.37	3.30	0.03	0.01	0.01	98.22	0.02	9.77	3.68	0.00	80.36	3.45	6.68	15.28	0.01	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.38	0.00
31415	HC	10/15/2012	12.52	93.50	0.34	64.73	0.10	403.24	37.13	0.45	3.93	0.03	0.01	0.01	103.12	0.03	9.27	3.50	0.01	80.30	4.07	7.41	15.49	0.01	0.00	0.02	0.04	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.38	0.00
31425	PCA	10/26/2012	5.87	65.92	0.29	56.72	0.01	79.14	1583.38	0.01	15.39	0.10	0.03	0.01	59.26	0.93	*45.9325	15.59	0.10	78.90	15.03	8.20	32.18	0.20	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.13	0.00	0.01	0.00	0.00	0.42	0.00
31426	PCB	10/26/2012	6.03	71.95	0.11	58.98	0.01	63.18	1882.01	0.00	14.22	0.06	0.03	0.01	61.31	1.04	*49.6722	16.67	0.04	79.93	13.95	8.31	34.00	0.20	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.12	0.00	0.01	0.00	0.01	0.44	0.00
31427	PCC	10/26/2012	5.98	72.96	0.03	53.79	0.01	59.30	1687.38	0.02	9.11	0.04	0.02	0.01	57.53	0.97	*51.8009	16.27	0.05	75.69	8.94	8.07	33.07	0.21	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.11	0.00	0.02	0.00	0.00	0.41	0.00
31428	NCA	10/26/2012	1.13	0.16	0.40	58.01	0.07	0.15	332.53	0.00	0.10	0.02	0.01	0.00	8.65	0.00	1.64	0.48	0.00	70.07	0.09	6.40	7.81	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.07	0.00
31429	NCB	10/26/2012	0.91	0.14	0.43	59.67	0.23	0.24	325.69	0.00	0.12	0.03	0.01	0.01	9.29	0.00	0.86	0.50	0.00	71.74	0.10	7.73	7.88	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.08	0.00
31430	NCC	10/26/2012	1.00	0.09	0.47	56.51	0.27	0.14	325.10	0.00	0.11	0.03	0.01	0.00	10.13	0.00	1.41	0.58	0.00	68.40	0.09	7.29	7.79	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00
31431	LA	10/26/2012	7.71	47.59	0.41	66.33	0.07	201.98	349.40	0.22	1.14	0.03	0.01	0.01	43.99	0.02	0.20	1.56	0.00	75.30	1.15	7.48	11.04	0.00	0.00	0.03	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.18	0.00
31432	LB	10/26/2012	8.41	54.87	0.37	65.57	0.07	227.25	364.33	0.24	1.40	0.02	0.01	0.01	53.91	0.02	0.19	1.91	0.00	75.01	1.41	7.19	11.40	0.01	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.22	0.00
31433	LC	10/26/2012	8.37	54.47	0.38	63.25	0.07	228.55	389.31	0.19	1.07	0.02	0.01	0.01	60.21	0.02	0.21	2.18	0.00	72.92	1.07	7.26	11.44	0.01	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.00	0.00	0.26	0.00
31434	HA	10/26/2012	13.66	110.29	1.01	76.16	0.36	468.85	459.48	0.32	2.93	0.03	0.02	0.01	128.07	0.02	0.73	3.99	0.01	84.99	3.07	6.36	16.24	0.01	0.00	0.03	0.04	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.44	0.00
31435	HB	10/26/2012	13.08	106.41	0.87	73.81	0.36	462.00	459.08	0.24	2.78	0.03	0.02	0.01	123.42	0.02	0.41	3.89	0.01	83.67	2.91	7.02	15.55	0.01	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.44	0.00
31436	HC	10/26/2012	13.73	115.28	0.98	71.27	0.36	500.68	474.48	0.30	3.86	0.03	0.02	0.01	131.18	0.02	0.62	3.85	0.01	83.60	4.02	7.17	15.81	0.01	0.00	0.03	0.06	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.44	0.00

*Indicates that the sample is above the detectable range of the equipment

Table 11. Elemental composition of lettuce and pac choi (dry weight) for t_1 only.

			Macronutrients (%)					Micronutrients (mg/kg)					
Plant Type	Treatment	Replicate	N	P	K	Ca	Mg	B	Fe	Mn	Cu	Zn	Na
Lettuce	High	a	5.02	0.689	3.55	2.22	0.222	21.6	48	77.9	24.2	112	5865
Lettuce	High	b	5.67	0.77	3.36	1.54	0.195	26.3	81.4	30	14.4	91.3	3813
Lettuce	High	c	5.12	0.699	2.83	1.91	0.22	28.8	76.8	101	14.5	114	4377
Lettuce	Low	a	5.58	0.795	2.42	2.58	0.232	28.3	55.9	21.4	15.5	133	8817
Lettuce	Low	b	5.05	0.697	2.11	2.18	0.212	25.4	49.8	19.6	13.8	102	7818
Lettuce	Low	c	4.72	0.699	2.4	2.94	0.221	22.9	40.3	30	18.4	131	8017
Lettuce	Negative	a	1.48	0.105	2.27	0.352	0.106	28	195*	21.6	16.8	44.7	7695
Lettuce	Negative	b	2.37	0.152	4.65	0.855	0.162	34	29.3	15.2	12.6	106	8681
Lettuce	Negative	c	1.24	0.095	2.17	0.145	0.098	26	18.8	14.1	16.6	50.3	7389
Lettuce	Positive	a	3.73	0.922	4.05	1.19	0.272	26.7	58.8	142	11.6	17.8	4321
Lettuce	Positive	b	5.84	4148*	3.7	1.12	0.3	21.4	53.8	93.2	11	16.6	4148
Lettuce	Positive	c	5.18	0.817	3.7	0.97	0.308	18.9	48.3	90.7	12.2	14.5	3582
Pac Choi	High	a	4.56	0.454	2.15	2.22	0.216	29	59.2	73	18.8	176	7849
Pac Choi	High	b	5.92	0.421	1.76	2.7	0.22	31.6	50.3	62.9	19.1	189	7439
Pac Choi	High	c	5.04	0.66	2.75	3.94	0.347	47.3	60.5	162	29.1	397	8983
Pac Choi	Low	a	4.89	0.572	1.12	2.26	0.251	29.5	66	22	17.8	128	12710
Pac Choi	Low	b	5.34	0.631	1.33	2.35	0.277	39.2	54.9	24.9	20.4	161	11040
Pac Choi	Low	c	4.22	0.484	1.81	2.17	0.19	26.9	48.3	78.3	19	185	7255
Pac Choi	Negative	a	1.71	0.305	2.31	3.86	0.59	54.8	70.8	27.1	84	168	5762
Pac Choi	Negative	b	2.05	0.256	2.64	4.05	0.912	77.5	23.3	26	73.5	744	10670
Pac Choi	Negative	c	1.65	0.318	2.31	3.7	0.666	70.4	19.1	20	76.8	369	4224
Pac Choi	Positive	a	5.93	0.525	3.54	1.79	0.281	54.8	63.8	155	10.8	23.8	2053
Pac Choi	Positive	b	5.63	0.48	3.8	2.14	0.274	46.8	46.8	116	10	12.3	2862
Pac Choi	Positive	c	5.35	0.496	3.63	1.8	0.249	41.7	55.6	87.2	10.2	15.1	2481

*Outliers, Dropped from the data set

Table 12. Elemental composition (mg/kg) of lettuce and pac choi tissue induced with iron and calcium deficient Hoagland solution's.

		Macronutrients (%)				
Treatment	Plant Type	N	P	K	Ca	Mg
0.25 Calcium (Ca)	Lettuce	5.01 ± 0.20	0.76 a[y] ± 0.04	3.60 a ± 0.14	0.37 c ± 0.07	0.41 ± 0.02
0.00 Iron (Fe)	Lettuce	4.89 ± 0.20	0.63 ab ± 0.04	3.50 ab ± 0.14	1.39 a ± 0.07	0.40 ± 0.02
0.25 Iron (Fe)	Lettuce	5.08 ± 0.20	0.53 bc ± 0.04	3.37 ab ± 0.14	1.05 b ± 0.07	0.46 ± 0.02
Control	Lettuce	4.75 ± 0.20	0.47 c ± 0.04	2.98 b ± 0.14	0.89 b ± 0.07	0.45 ± 0.02
0.25 Calcium (Ca)	Pac Choi	4.92 b ± 0.11	0.45 ab ± 0.02	2.52 ± 0.16	1.56 b ± 0.13	1.17 a ± 0.03
0.00 Iron (Fe)	Pac Choi	5.43 a ± 0.11	0.47 a ± 0.02	2.23 ± 0.16	3.13 a ± 0.13	0.95 b ± 0.03
0.25 Iron (Fe)	Pac Choi	5.46 a ± 0.11	0.38 bc ± 0.02	2.40 ± 0.16	2.80 a ± 0.13	0.60 c ± 0.03
Control	Pac Choi	5.51 a ± 0.11	0.37 c ± 0.02	2.13 ± 0.16	3.30 a ± 0.13	0.64 c ± 0.03

		Micronutrients (mg / kg)						
Treatment	Plant Type	B	Fe	Mn	Cu	Zn	Mo	Al
0.25 Calcium (Ca)	Lettuce	36.92 c ± 1.26	56.45 a ± 2.71	51.53 c ± 5.65	12.27 ± 0.50	46.20 ± 2.95	0.28 a ± 0.02	21.80 ± 1.15
0.00 Iron (Fe)	Lettuce	39.47 bc ± 1.26	37.03 b ± 2.71	107.30 a ± 5.65	12.29 ± 0.50	40.90 ± 2.95	0.00 b ± 0.02	14.17 ± 1.15
0.25 Iron (Fe)	Lettuce	45.14 a ± 1.38	59.92 a ± 2.71	76.50 b ± 5.65	12.15 ± 0.50	36.10 ± 2.95	0.00 b ± 0.02	14.77 ± 1.15
Control	Lettuce	43.50 ab ± 1.26	61.05 a ± 2.71	53.78 c ± 5.65	10.98 ± 0.50	41.42 ± 2.95	0.00 b ± 0.02	14.02 ± 1.15
0.25 Calcium (Ca)	Pac Choi	104.97 ± 6.77	70.80 a ± 3.73	103.52 ± 5.70	11.16 c ± 0.63	32.73 ± 2.26	0.47 b ± 0.06	17.07 a ± 1.19
0.00 Iron (Fe)	Pac Choi	124.17 ± 6.77	54.87 b ± 3.73	110.38 ± 5.70	22.62 a ± 0.63	27.83 ± 2.26	1.05 a ± 0.06	12.20 b ± 1.19
0.25 Iron (Fe)	Pac Choi	121.33 ± 6.77	79.80 a ± 3.73	91.62 ± 5.70	14.70 b ± 0.63	31.40 ± 2.26	0.34 b ± 0.06	11.55 b ± 1.19
Control	Pac Choi	103.93 ± 6.77	79.05 a ± 3.73	89.20 ± 5.70	14.15 b ± 0.63	30.33 ± 2.26	0.37 b ± 0.06	9.58 b ± 1.19

[y]Means followed by different lowercase letters indicate significance based off Tukey's test ($P \leq 0.05$, $n = 3$). The absence of letters indicates that there are no
Values are LSmean ± SE

Table 13. Total final fish weight (g) described within each experimental system at the conclusion of t_1 and t_2.

Total final weight (g) within experimental raft aquaponics systems. High density system (total fish = 150, 90 measured) and Low density (total fish = 75, 75 measured).			
	HA	HB	HC
Sum	824.6	766.8	811.3
STDEV	5.8	6.5	5.2
Total Wt	1374.3	1278.0	1352.2
Total t_1 HD ± STDEV	1334.8	5.9	

	LA	LB	LC
Sum	645.5	658.7	681.9
STDEV	4.6	8.3	6.3
Total Wt	645.5	658.7	681.9
Total t_1 LD ± STDEV	662.0	6.4	

Total Final weight (g) within experimental raft aquaponics systems. High density system (total fish = 56*, 56 measured) and Low density (total fish = 30, 30 measured).			
	HA	HB	HC
Sum	704.3	575.0	705.6
STDEV	8.9	9.7	9.9
Total Wt	741.4	676.5	705.6
Total t_2 HD ± STDEV	707.8	9.5	

	LA	LB	LC
Sum	361.3	412.1	359.0
STDEV	9.5	14.3	9.8
Total Wt	361.3	412.1	359.0
Total t_2 LD ± STDEV	373.5	11.2	

*Low number, in result of a fish lost (n = 11) during this experimental trial (t_2).

Table 14. Enrichment regime of synthetic fertilizer in the positive control for t_1 and t_2

Trial 1. Enrichment amount by date

Date	Flora Grow (mL)	Flora Bloom (mL)	Flora Micro (mL)
22-Sep	115.5	115.5	115.5
2-Oct	120	30	90
19-Oct	30	30	30
21-Oct	30	30	15

Trial 2. Enrichment amount by date

Date	Flora Grow (mL)	Flora Bloom (mL)	Flora Micro (mL)
16-Nov	115.5	115.5	115.5
13-Dec	30	30	30