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Analysis of Plant Biomass Production Comparing Decoupled Aquaponics Against Equivalent Single-Loop Aquaponic and Hydroponic Systems Growing Lactuca Sativa

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ANALYSIS OF PLANT BIOMASS PRODUCTION COMPARING DECOUPLED AQUAPONICS AGAINST EQUIVALENT SINGLE-LOOP AQUAPONIC AND HYDROPONIC SYSTEMS GROWING *LACTUCA SATIVA*

by

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ABSTRACT

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Aquaponics is an emerging method of agriculture in which fish and plants are grown in an enclosed and recirculating environment. The method mimics a relationship found in nature where fish waste provides nutrients for plants and plants cleanse the water for the benefit of the fish. This symbiotic relationship has proven to be a sustainable method of agriculture in which there is less water use, no need for pesticides or herbicides, recycling of nutrient waste and a smaller spatial footprint. However, the production of both plants and fish in a recirculating aquaponics system has produced less yield and profit when compared to its competitive counterparts, hydroponics and RAS (recirculating aquaculture system) methods. To address this issue, the traditional single recirculation aquaponics system (SRAPS) has been reengineered to a *decoupled* recirculation aquaponics system (DRAPS). The main differences of the DRAPS system is 1) solid waste from fish is mineralized and sent to the hydroponic plant grow system; 2) water from hydroponic system does not return to the fish; and 3) separate aquatic environments for fish and plants allow for the caretaker to create ideal growing conditions based on the specific needs of the culture organism. The objective of this study was to compare the yield of lettuce,

Lactuca sativa, against three systems, hydroponics, SRAPS and DRAPS. The results of this study suggest that aquaponic environments provide a more habitable environment for plants to thrive over comparable hydroponic environments. Further research must be done to refine DRAPS and create a sustainable agriculture system to feed a growing world with less resources.

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CHAPTER 1: INTRODUCTION

1.1 Merits of Aquaponics

Climate change, water and fossil fuel scarcity as well as food shortage is linked to an increasing demand of water, food and energy due to a growing world population (Godfray et al., 2010). Furthermore, agriculture uses 70% of the global freshwater resources (Pimentel et al., 2004) and thus freshwater availability is one of the most important issues for maintaining food security. In order to achieve a high food quantity without adverse impacts on the environment, innovative and sustainable food production systems are urgently needed (Godfray et al., 2010). Aquaponics, which is the combined production of aquaculture and plants in hydroponics, is a sustainable option to reduce fertilizer and water use (Maucieri et al., 2018). The main advantage of aquaponics is the double use of resources; water and nutrients, used firstly for fish and secondly for plant production (Rakocy 2012; Tyson, Treadwel et al., 2011; Goddek et al., 2015).

Aquaponic systems have been proposed as a sustainable development of common recirculating aquaculture systems (RAS) (Blidariu & Grozea, 2011). Aquaponics combines the benefits of RAS by integrating plants to be grown with hydroponic plant production. This type of agriculture mimics the relationship of fish and plants in nature. Fish provide nutrients for the plants and plants purify the water, a mutually beneficial relationship for both organisms. However, the execution of altering an ecosystem and using it for commercial production has its obstacles. Currently, aquaponics has lower productivity of both fish and plants in comparison to separate recirculating systems (RAS and hydroponics) (Engle, 2015; Monsees et al., 2017; Quagrainie et al., 2018). When combining fish and plant production there are often compromises with abiotic factors such as nutrient availability for the plants. It is plausible that

reengineering the traditional model of aquaponics could improve nutrients availability for the plants and reduce waste from the aquaculture unit .

1.2 Limitations of Traditional Aquaponics

Classic aquaponic systems are commonly referred to as coupled, single-loop, or single recirculation aquaponic systems, “SRAPS,” as to be referred to as from here on. SRAPS were described already more than 30 years ago (Sneed et al., 1975; Naegel 1977). Here, the aquaculture unit and hydroponic (HP) unit are arranged in a single loop where water is directed from the aquaculture unit to a solids removal system, to a biofilter, then to a hydroponic unit and back to the aquaculture unit (See Figure 1B.). Inevitably, such systems provide the same water quality for both, fish and plants, which inevitably represent a compromise in the rearing conditions for each product (Azad et al., 2016). The SRAPS design became common practice for aquaponic users as a result of research from The University of Virgin Islands in the 1980’s (Rakocy et al., 2011; Rakocy, 1989; Rakocy et al., 1997). The University of Virgin island design made use of several main components including: rearing tanks, sump, clarifier, filtration unit, degassing tank, hydroponic tanks and a range of equipment with the system designed to produce tilapia and leafy green produce. The sludge collected in the clarifier is drained periodically and is either discarded or used as fertilizer for soil-grown crops. The waste of sludge represents an inefficiency in the system and drives the efforts to reengineer aquaponic systems.

The complimented method of aquaponics is referred to as decoupled recirculation aquaponic systems (DRAPS). DRAPS may be a realistic solution to increase control of water quality parameters in the system and to prevent any adverse interactions between plant and fish units (Kloas et al., 2015). DRAPS is similar to SRAPS by that it still has biofiltration and solid waste

capture. DRAPS is different because it retains solid waste from fish, a normally discarded byproduct, and mineralizes the waste through an aerobic process. Second, water does not return from the hydroponic unit to the aquaculture unit; this allows for a greater concentration of nutrients within the hydroponic unit of the system. Separation of aquaculture and hydroponic units also allows for the independent manipulation of water quality parameters like temperature, pH, and nutrients. Research lead by Boris Delaide and Simon Goddek has made strides in validating the effectiveness of DRAPS. Their research demonstrated that aquaponic systems could surpass the growth rates found in conventional hydroponic systems and (Delaide et al., 2016a; Goddek et al., 2016).

In regards to pH optimization, for optimal nutrient uptake, plants prefer a pH between 5.8 and 6.2 in hydroponic environments, whereas cold-water fish (*Perca flavescens*) and the aerobic bacteria used for nitrification (Nitrobacter and Nitrosomonas) have a pH optimum of ~7 to 9 (Rakocy 2012; Masser et al., 1999). To accommodate both species, it is suggested to maintain a level of 7.0 to 7.6 in SRAPS (Delaide et al., 2016). This type of aquaponics may be ideal for classroom demonstrations or backyard farmers, but there is a lack of control that hinders the success of industrial aquaponics (Love et al., 2015). With DRAPS, there is the opportunity to manually adjust hydroponic water with additives, such as, white vinegar or citric acid to decrease the pH with no impact to the fish (Tyson et al., 2008).

Temperature is known to be the most sensitive and variable environmental factor across different species of fish (Kucharczyk, 2015). The ability to manipulate temperature in DRAPS gives the freedom to farmers to accommodate various fish species or temperature cycle the fish based on spawning season without impact to the plants (Sandström et al., 1997). *Lactuca sativa*

prefer consistency in temperature ranging from to 20° to 25°C (Wheeler et al., 1993; Sakamoto and Suzuki 2015). Whereas, temperatures for yellow perch can range from 5° to 20°C throughout the annual spawning cycle (Kolkovski & Dabrowski, 1998; Linkenheld, 2019).

Traditional aquaponic systems (SRAPS) typically maintain the same flow velocity through the whole system (Bailey & Ferrarezi, 2017). Most aquaponic farmers put an emphasis on the production and profitability of the plants and fish serve merely as a supply of nutrients (Engle, 2015). A consequence of this is fish are grown in limited stocking densities because the plants require a relatively low exchange rate of water (Endut et al., 2009). To achieve maximum output from aquaponics, fish must be fully stocked and flow velocity fast enough to allow maximum stocking (Quagrainie et al., 2018).

Recent energy analysis and industry surveys of aquaponic systems concluded that profitability is greater when a plant-centric production approach is adopted (Chaves et al., 1999; Laidlaw & Magee, 2014; Love et al., 2015). However, to improve plant growth in plant-centric SRAPS, micronutrient supplementation (e.g., iron, calcium, and potassium) is often required (Bittsánszky et al., 2016; Roosta, 2011). Furthermore, early aquaponic research concluded that industrial applications of aquaponics require artificial nutrient supplementation, such as chelated iron (Rakocy et al., 1992; Seawright et al., 1998), these studies make these assumptions without even considering the utilization of the solid waste effluent. Essential nutrients which are otherwise supplemented, are found in the fish sludge (i.e., feces and uneaten feed). The mobilization of nutrients from fish sludge plays a key role in optimizing the resource utilization and thus in improving the sustainability of aquaponic systems (Goddek et al., 2016; Monsees et

al., 2017; Zhang et al., 2020). DRAPS aims to close the gap in nutrient deficiency by utilizing the solid waste product from the fish and mineralizing it into a soluble form.

1.3 Nutrient Mineralization

The mineralization chamber is a key component of the DRAPS design and sets it apart from the traditional SRAPS designs. When fish are cultured, only a small proportion of the feed is converted (25–30%) to useable energy (Rakocy et al., 2006). The balance of nutrients is excreted in solid and dissolved fractions. Then these byproducts are separated by either gravity or physical filtration. Unfortunately, the concentrated sludge produced after the solid filtration stage, comprising organic matter and valuable nutrients, is most often discarded (Naylor et al., 1999). Notably, up to 50% in dry matter of the feed ingested is excreted as solids by fish (Chen, Coffin, & Malone, 1997), and most of the nutrients that enter aquaponic systems via fish feed accumulate in these solids (Neto & Ostrensky, 2015). Hence, effective solid filtration removes, for example, more than 80% of the valuable phosphorus (Monsees, et al., 2017) that could otherwise be used for plant production.

During this mineralization process, the macronutrients (i.e. N, P, K, Ca, Mg and S) and micronutrients (i.e. Fe, Mn, Zn, Cu, B and Mo) that were bound to the organic molecules in the sludge are released into the water in their ionic forms (Delaide et al., 2019). The addition of a mineralization chamber in an aquaponics system creates an aerobic environment to break down organic matter (Khiari et al., 2019). This process aims to reduce waste and reduce dependency on external fertilizer (Delaide et al., 2019). By applying these additional treatment steps in aquaponic units, the water and nutrient recycling efficiency is improved and the dependency on

external fertilizer can be reduced, thereby enhancing the sustainability of the system in terms of resource utilization.

Aquaponics usually operates at considerably low nutrient concentrations (not more than 75-100 mg/l of NO_3-N) (Rakocy et al., 2004; Lund 2014; Endut et al., 2016). However, it has been shown in experimental studies that complemented aquaponic water (i.e., supplementation of otherwise lacking nutrients) promotes plant growth compared to hydroponics (Delaide et al., 2016; Saha et al., 2016; Ru et al., 2017). It is understood that most of the nutrients that enter aquaponic systems via fish feed, accumulate in the solid part of the RAS wastewater (Schneider et al., 2005; Neto & Ostrensky, 2013). Therefore, there is a high potential to recycle these nutrients (Jung & Lovitt, 2011; Monsees et al., 2017). Reintroducing them into the aquaponic water via natural mineralization of fish sludge seems to be a promising way to improve the aquaponic system production performance. Hence, sludge mineralization could be a contributing factor to close the loop to a higher degree, to save water and lower the environmental impact of food production (Delaide et al., 2015).

1.4 Research Objectives and Hypotheses

In agriculture research, metrics of wet mass, dry mass and root length are standard ways of identifying the overall health of the plant and growth performance (Anderson et al., 2017; Buzby & Lin, 2014; Cometti et al. , 2013; Licamele, 2009; Sapkota et al., 2019). Several studies have evaluated the use of DRAPS for industrial systems but fail to compare it against hydroponic systems of similar scales (Goddek et al., 2016; Khiari et al., 2019; Monsees et al., 2017; Zhang et al., 2020). This study addresses this knowledge gap by comparing hydroponics, SRAPS and DRAPS side-by-side. The objective of this study was to compare yields of lettuce (*Lactuca sativa*) grown

in conventional hydroponic solutions to those grown in complemented and traditional aquaponic solutions.

The following experiment analyzes aquaponics in a novel approach by comparing equivalent systems of hydroponics, SRAPS and DRAPS side-by-side. Many studies have evaluated plant performance by comparing equivalent hydroponic and aquaponic systems (Anderson et al., 2017) or SRAPS and DRAPS (Monsees et al., 2017). However, there has yet to be a study comparing the performance of all three grow systems. The DRAPS system will utilize a mineralization chamber to aerobically breakdown solid fish waste and also create an optimal environment for plant growth promoting microorganisms (PGPM). The study will measure *Lactuca sativa* success based on wet bio mass (at time of harvest), root length and dry mass.

Several hypotheses were addressed in this trial. The null hypothesis are stated as: H_o 1. Grow system treatment will have no significant difference on biomass production of *Lactuca sativa*. H_o 2. Grow system treatment will have no significant difference on pH of water in the root zone of *Lactuca sativa*. H_o 3. Grow system treatment will have no significant difference on root length of *Lactuca sativa*. The alternative hypotheses are stated as: H_a 1. There is a significant difference between grow system treatments based on biomass production of *Lactuca sativa*. H_a 2. There is a significant difference between grow system treatments based on pH of water in the root zone of *Lactuca sativa*. H_a 3. There is a significant difference between grow system treatments based on root length of *Lactuca sativa*.

CHAPTER 2: MATERIALS AND METHODS

2.1 Experimental Design

Two identical trials were conducted between November 2018 and March 2019 in the Aquaculture Teaching and Research Facility of the University of Wisconsin-Milwaukee, School of Freshwater Sciences (Milwaukee, Wisconsin, USA). Trial 1 began on December 6, 2018 and ended on January 10, 2019. Trial 2 began on February 4, 2019 and ended on March 17, 2019. The air temperature and relative humidity in the laboratory were recorded daily in order to control the similar climate conditions between Trial 1 and Trial 2.

The experimental setup consisted of three treatment systems, hydroponic (HP), single recirculating aquaponics system (SRAPS) and decoupled recirculating aquaponics system (DRAPS). A schematic flow diagram of the three system designs is shown in Figure 1. The figure illustrates the direction of flow within the system and identifies the main components for each treatment. There were three replicates of each treatment system. Each system used the nutrient film technique (NFT) to grow *Lactuca sativa* in a flow-through channel. Each were designed with equivalent parameters regarding environmental conditions (light, humidity, temperature), water volume, water exchange rate, and growth time.

Lactuca sativa (Territorial Seed Company, Cottage Grove, Oregon) was the selected lettuce strain used in the experiment. Seeds were organic and clay coated for optimum germination. This species was chosen due to the abundance of hydroponic and aquaponic research using this species (Anderson et al., 2017; Delaide et al., 2016a; Delaide et al., 2016b; Wheeler et al., 1993) and access to detailed records regarding nutrient requirements and diagnosis of nutrient deficiencies (Broadley et al., 2000; Kasozi et al., 2019). *Lactuca sativa* was

germinated in Rockwool cubes at 32°C. Initially, 400 seeds were germinated, after 12 days, the 270 largest and most robust plants were transferred into 5 cm diameter net pots and randomly distributed across the nine replicate grow systems. *Lactuca sativa* was grown in the NFT system for 40 days. After which, *Lactuca sativa* was harvested and measured for wet biomass (g), root length (mm) and dry biomass (g).

For aquaponic systems, yellow perch (*Perca flavescens*) were used as the aquaculture product and waste producing organism. Each 190 L aquaculture tank contained 20 yellow perch with a total starting biomass of 5.1±0.4 kg. Fish were fed a balanced pellet diet (Ziegler Finfish Perch, Zeigler Bros., Inc., Gardners, Pennsylvania) composed of 45% protein, 12% fat at a rate of 0.2% biomass per day. The low feed rate was established to balance the low consumption rate of the fish and reduce food waste in the system.

Each replicate system had two gutters, 2.4 meters in length, serving as the structure for NFT (Nutrient Film Technique) plant growth. The depth of the gutter provided 2 cm of water depth for submerged plant roots to uptake nutrients. Each gutter grows 15 Lactuca sativa plants, totaling 30 plants per replicate system. NFT systems were housed in 96x48x78" mylar reflective grow tents (CoolGrows.com, Fujian, China), three tents total, three replicates per tent, one tent for each system type (See Figure 2). Each grow tent had three 250 watt LED grow lights providing full spectrum light with red, blue, white, IR and UV light spectrums (Shenzhen Phlizon Technology Co., Ltd. Guangdong, China). Lights automatically turned on and off to reflect a 16-hour day and 8-hour night cycle. Humidity and temperature were measured daily and vents were adjusted to maintain consistent environmental conditions between each grow tent. Small fans mounted in each grow tent promoted air circulation.

2.2 Engineering the Grow Systems

Photos of the experimental arrangement are available in Figure 2. The hydroponic (HP) systems consisted of a sump made from 60 L tote bins each filled with 45 L of water. Each HP system held 48 liters of water and nutrient dosage was based on this volume. Nutrients were sourced from Nature's Source Professional Plant Food 10-4-3 (Ball DPF, LLC., Sherman, Texas). Nature's Source is an all-purpose plant food providing essential nutrients at concentrations of 10% nitrogen in both water soluble & insoluble forms (NH_3, NO_3, $CO(NH_2)_2$), 4% phosphoric acid (P_2O_5), 3% soluble potash (K_2O), calcium, magnesium, plus micronutrients, sulfur, boron, copper, iron, manganese, molybdenum and zinc. The hydroponic system was dosed with 250 mg/L Nitrate (NO_3) concentration, as it is the recommended dosage for *Lactuca sativa* in hydroponic culture (Sapkota et al., 2019). There were 3.5 ounces of hydroponic nutrient added at the beginning of each trial to achieve the target N concentration of 250 mg/L. Throughout the trial water was added to compensate for evaporation and replacement water was added at a rate of 0.4 ounces of nutrient solution per liter. Nutrient composition was not consistent amongst treatments, rather the hydroponic nutrient was added to create ideal growth conditions and serve as the control treatment for the experiment. While aquaponic treatments may have had more dilute nutrients, they serve as the experimental treatments and were not given supplemental nutrients.

Each aquaponic system consisted of an aquaculture tank, solids removal chamber and moving bed biofilm reactor. The solids removal chambers were built from 19 L square bottom buckets and used a 10 cm diameter pipe to slow the flow of incoming water which allowed solid particles to settle to the bottom. A moving bed biofilm reactor was made from 60 L tote bins and

approximately 5 L of K1 biofiltration media (Evolution Aqua Ltd., Wigan, England). Aeration was sourced from air pumps (Hydrofarm Active Aqua Air Pump, 4 Outlets, 6W) and one air stone measuring 2.5 cm length per bio reactor.

Dolomite chips were added to the aquaculture sump tank of each aquaponics system for the purpose of increasing buffering capacity. The submerged dolomite chips are made of magnesium carbonate ($MgCO_3$) which will react with carbonic acid yielding magnesium bicarbonate $Mg(HCO_3^-)_2$ [$H_2CO_3 + MgCO_3 \rightarrow Mg(HCO_3^-)_2$] (Boyd et al., 2016). The produced bicarbonates react with free hydrogen, thus maintaining pH through the aquaculture system.

To move water through the systems, submersible water pumps were used. Damper valves on each water pump were manipulated to have equal flow rates for all systems (15 L/min) (Endut et al., 2009). HP systems each had one water pump per system to move water from sump tank to NFT trough. Single-loop aquaponics had two pumps per system, both in a shared sump tank. One pump moving water to the aquaculture tank and the other moving water to the NFT troughs (See Figure 2).

Arrangement of the DRAPS unit had the same solids removal chamber and moving bed biofilm reactor but differentiates itself from SRAPS with the addition of a mineralization chamber and alternative plumbing not to return water from the NFT plant system to the aquaculture tank. The mineralization chamber was made from a 19 L bucket filled with 3-4 L of 4 cm diameter bioballs and aerated with an air pump and air stone. Solid waste continuously accumulates in the settling chamber and is manually transferred to the mineralization chamber daily. This daily transfer of waste contains a mixture of solid and dissolved fish waste, unconsumed fish feed and water, approximately 5 L is transferred to the mineralization chamber daily. A ‘new’ 5 L batch

displaces some of the 'old' batch into the sump tank of the NFT grow system. This batch method of transferring waste results in a 4-day average retention time in the mineralization chamber. Mineralized waste would then recirculate through the NFT grow system, not to return to the aquaculture tank.

2.3 Data Collection

Lactuca sativa were harvested after the cultivation period (36 days for Trial 1 and 42 days for Trial 2). The fresh weights for the lettuce data were collected immediately in the greenhouse on a scale accurate to 0.01g (Fisher Scientific Education Precision Balance, Thermo Fisher Scientific, Waltham, Massachusetts). Shoots were removed by slicing the hypocotyl at the top of the rockwool plug. Root data were collected from individual plugs measuring from top to the longest root hair, accurate to the nearest millimeter. Root length is a relevant metric for this study because root growth is a characteristic with high plasticity in response to stress factors, such as, nutrient limitation, mineral toxicity, and interactions of plant growth promoting microorganisms (Bartelme et al., 2018; Neumann et al., 2014; Neumann & Römheld, 2007).

At the end of the harvest day, roots and shoots were bagged and combined according to replicate and treatment type. The drying process involved laying lettuce shoots on trays and drying in a drying oven at 70°C for 4-6 days. The dry weights were taken on a scale accurate to 0.01g (Ohaus Adventurer Pro AV4101 Precision Balance, Parsippany, New Jersey). The method of data collection for Trial 1 and Trial 2 differs slightly. Trial 1 harvest measured the total mass of each lettuce plant (shoots and roots together). Trial 2 harvest measured the shoot mass for each lettuce plant and the length of the roots. Both trials measured dry mass as a summation of all plants in each replicate, with three replicates per treatment. Dry mass was not measured for

individual plants. Missing data points occurred due to failed plants. Photographs of lettuce were taken from Trial 2 and will be discussed in the discussion section. The final mass of yellow perch was not measured because the fish served as a source of constant nutrient input into the system and were all fed equal amounts of pellet feed.

2.4 Statistical Analysis

Analyses were conducted using JMP Pro software (version JMP PRO 14.2.0; JMP a Division of SAS, Cary, North Carolina, USA). Take note, there is slight variation in data collection between Trial 1 and Trial 2. Trial 1 data included total (shoot and root) mass, fresh weight (FW) and dry weight (DW). Trial 2 data included root length (RL) and shoot mass in both FW and DW. Root mass was not included in the mass measurements for Trial 2. RL, FW, and DW were treated as response variables. Treatment and trial were treated as fixed effects, and the two NFT grow troughs nested within each treatment was treated as a random effect. Data is presented as mean value ± standard deviation (error bars). DW and FW were analyzed as a ratio (DW/FW) and percentage. Analysis of variance (ANOVA) is used to determine significance. Tukey's honest significant difference is used to determine where these differences occur, utilizing a 95% confidence interval (α=0.05). If the p-value is less than or equal to the alpha ($p < 0.05$), then we reject the null hypothesis, and we say the result is statistically significant. The assumptions of the ANOVA include; each group sample is drawn from a normally distributed population, all populations have a common variance and samples are drawn independently from each other. Multiple range test is conducted to determine where there are differences between treatments. Multiple analysis of variance (MANOVA) is used to determine difference between the three grow

systems against the multiple variables of RL, FW and DW. Variation in sample sizes was accounted for in a two-way ANOVA.

CHAPTER 3: RESULTS

3.1 Environmental and Water Conditions

The daily measurements of pH, total dissolved solids (TDS), electric conductivity (EC), dissolved oxygen (DO) and water temperature for Trial 1 and Trial 2 are shown in Table 1. The described metrics were measured for each replicate within each of the three treatment types. For the DRAPS treatment, water quality was measured in both the sump for the aquaculture unit and the sump for the hydroponic unit, as indicated by $DRAPS_F$ and $DRAPS_P$ respectively. The mechanical separation of water flow between the aquaculture and hydroponic unit validates the need for distinct measurements. Greater N values for Trial 2 compared to Trial 1 are a result of more frequent measurements taken throughout the grow period.

The metric of pH displayed a range in average values of 5.8 (±0.5) to 8.9 (±0.1). For both trials, HP treatment produced higher average pH values than the aquaponic treatments of the same trial. Mean (±SD) pH values for Trial 1 were as follows: HP, 8.9 (±0.1); SRAPS, 6.8 (±0.7); $DRAPS_F$, 6.6 (±0.8); $DRAPS_P$, 8.6 (±0.2) (Table 1). Mean (±SD) pH values for Trial 2 were as follows: HP, 7.1 (±1.0); SRAPS, 6.3 (±0.5); $DRAPS_F$, 5.8 (±0.5); $DRAPS_P$, 6.1 (±0.6).

Total dissolved solids (TDS) measures the total concentration of dissolved substances in water including minerals, salts and metals. TDS was measured in ppm and displayed as mean (±SD) values for Trial 1 and Trial 2 (Table 1). The HP treatment displayed the highest TDS values for both trials. Values for Trial 1 ranged from 233.1 (±23.3) in the $DRAPS_F$ treatment to a high range of 318.3 (±27.1) in the HP treatment. Following a similar pattern in Trial 2 measurements, however, with a range in values significantly higher ($p < 0.05$). Values for Trial 2 ranged from 369.1 (±39.8) in the $DRAPS_F$ treatment to a high range of 623.7 (±106.0) in the HP treatment.

Since electric conductivity (EC) is strongly correlated to TDS (Rusydi, 2018), a similar pattern is observed in EC measurements for Trial 1 and Trial 2. EC was measured in µS/cm and displayed as mean (±SD) for Trial 1 and Trial 2 (Table 1). Values for Trial 1 ranged from 445.6 (±51.3) in the $DRAPS_F$ treatment to a high range of 630.3 (96.8) in the HP treatment. Values for Trial 2 ranged from 796 (±82.9) in the $DRAPS_F$ treatment to a high range of 1295.6 (±211.1) in the HP treatment.

The measurements of dissolved oxygen (DO), measured in mg/L, represent the concentration of dissolved O_2 in the recirculating system. Mean (±SD) DO values for Trial 1 were as follows: HP, 6.6 mg/L (±0.5); SRAPS, 5.6 mg/L (±0.6); $DRAPS_F$, 5.9 mg/L (±0.7); $DRAPS_P$, 6.2 mg/L (±0.9) (Table 1). Mean (±SD) DO values for Trial 2 were as follows: HP, 8.2 mg/L (±0.4); SRAPS, 7.7 mg/L (±0.5); $DRAPS_F$, 8.0 mg/L (±0.3); $DRAPS_P$, 8.4 mg/L (±0.2) (Table 1). Trial 1 DO measurements are significantly lower ($p < 0.05$) than the Trial 2 measurements. Greatest DO measurements we observed in the HP treatment and lowest DO measurements were observed in the SRAPS and $DRAPS_F$ treatments, otherwise identified as the two treatments with aquaculture units impacting oxygen availability.

There was minimal variation between replicates and trials in regards to the variable of water temperature. Water temperature was recorded in degrees Celsius (°C) for SRAPS and $DRAPS_F$ treatments (Table 1). It can be noted that water temperatures were not measured for HP or $DRAPS_P$, but those environments remained equalized with the room temperature.

3.2 Lettuce Biomass Production

Biomass production of *Lactuca sativa* was assessed. Results are given both on a fresh mass basis (as customary in commercial practice) (Delaide et al., 2016a, 2016b; S Goddek et al.,

2016; Rakocy et al., 2006) and on a dry weight basis (to permit physiological comparisons) (Van Der Boon et al., 1990). The means (±SD) of lettuce fresh mass (FM, g), dry mass (DM, g), and root length (RL, mm) are presented in Table 2. Values represent the average mass/length of a single plant, averaged within its respective treatment. Tukey's HSD post hoc test was preformed, using a 95% confidence interval (α=0.05) to determine significant differences between the treatments and within each respective trial. DM to FM ratio was calculated as DM/FM (*100) and presented as a percentage (%). FM values for Trial 1 show significant ($p < 0.05$) differences between each of the three treatments with HP the lowest producing treatment at 0.54 g (±0.353), then DRAPS at 11.04 g (±8.178) and with the greatest biomass production, SRAPS at 26.05 g (±22.436) (Table 2). FM values for Trial 2 show the two aquaponic applications, SRAPS and DRAPS, with high significance ($p < 0.001$) greater FM production than the HP treatment. Trial 2 FM values are as follows: HP, 5.39 g (±6.799); SRAPS, 47.77 g (32.462); DRAPS, 41.51 g (±41.512) (Table 2).

Dry mass (DM, g) values lack the significant ($p > 0.05$) differences between treatments due to the low n value of 3 for each treatment. DM mean is calculated from the total dry mass of all plants combined from its respective replicate, then divided by the number of plants within each dry mass measurement. The DM was measured after plants were combined by replicate and treatment which explains why the n value is 3 and not 90 as in the FM measurements. Trial 1 DM values are as follows: HP, 0.05 g (±0.006); SRAPS, 0.99 g (±0.820); DRAPS, 0.52 g (±0.322) (Table 2). Trial 2 DM values are as follows: HP, 0.36 g (±0.015); SRAPS, 1.64 g (±0.928); DRAPS, 1.44 g (±0.841) (Table 2).

To further evaluate the DM and FM data, DM to FM ratio was calculated as DM/FM (*100) and displayed as a percentage (%) (Table 2). The DM/FM percentages for HP treatments

in both Trial 1 and Trial 2 are significantly greater ($p < 0.05$) than the respective aquaponics, SRAPS and DRAPS treatments, of the same trial. Trial 1 DM/FM data is 9.26% for HP compared to 3.80% and 4.71% for SRAPS and DRAPS respectively (Table 2). Trial 2 DM/FM data is 6.68% for HP compared to 3.43% and 3.47% for SRAPS and DRAPS respectively (Table 2).

The numeric measurements of biomass show significant differences ($p < 0.05$) between HP systems and the comparable aquaponic systems, SRAPS and DRAPS. Figure 3 displays photographs of Trial 2 lettuce growth at day of harvest. The difference in biomass production between the HP treatment versus SRAPS and DRAPS treatment is visually apparent. Many net-pots from the HP system appear to be empty because 22 of 90 plants from the HP treatment were recorded to have had no growth at all. These unsuccessful plants were not accounted for in average FM and DM calculations.

3.3 Root Growth Response

Root length was measured for Trial 2 only and serves as a response variable for measuring the success of *Lactuca sativa* growth across the three treatments. Average root length (RL, mm) is displayed in Table 2. Values represent the average RL per plant in each respective treatment. Significant differences ($p < 0.0001$) in average RL were observed between treatments with DRAPS treatment producing the greatest average RL at 400 mm (±126), then SRAPS with 289 mm (±90) and then HP with an RL average of 70 mm (±40) (Table 2). While the SRAPS and DRAPS treatment produced statistically similar ($p > 0.05$) results in terms of FM, the RL data shows significantly greater RL production for the DRAPS treatment, producing an average RL 72% greater than SRAPS. Figure 4 displays the significance of these differences in a box-whisker plot. The box plot displays median, lower quartile range (Q1), upper quartile range

(Q2), and maximum and minimum RL measurements for each of the three treatments (HP, SRAPS and DRAPS). The lower and upper quartile range, also referred to as Interquartile Range (IQR) is the middle 50% of values when ordered from lowest to highest. This statistic gives insight into the spread of the data. The IQR for the all treatments are as follows: HP, 45-93 mm; SRAPS, 250-335 mm; and DRAPS, 291-491 mm (Figure 4).

Correlation between FM and RL is observed in Figure 5, a linear regression model with Fresh Mass (g) as a function of Root Length (mm). The coefficient of determination (R^2) in the model was found to be 0.43, meaning, 43% of FM can be determined by RL ($p < 0.0001$). The linear regression produced the equation, Y = -0.964 + 0.129 *Root Length. A slope of 0.129 translates as, for every 1 cm of root length, there is a predicted gain of 1.29 g FM per lettuce plant.

In addition to the quantitative depiction of root growth, Figure 6 displays photos of Trial 2 root condition at day of harvest. Photo of roots from HP treatment stand apart from SRAPS and DRAPS treatment because there is a buildup of organic matter surrounding the root fibers.

CHAPTER 4: DISCUSSION

Here, a new approach for aquaponics is presented, comparing a decoupled recirculating aquaponic system (DRAPS), coupled recirculating aquaponic system (SRAPS) and hydroponic (HP) system experimentally in a pilot study. There are some obvious reasons why decoupling of RAS and hydroponics in a commercial aquaponic facility is favorable compared to a classical coupled approach. The most important ones are to be discussed in the following section based on the results of this study and supplemented by some theoretical consideration.

4.1 Role of Treatment on Plant Biomass Production

Our study found that treatment does not have an impact on fresh mass (FM) production of *Lactuca sativa*. Harvest FM between SRAPS and DRAPS treatments for Trail 1 showed significantly greater ($p < 0.05$) FM production for SRAPS than DRAPS, total harvest equating to 2.31 kg and 0.95 kg respectively. This result, with low production coming from the DRAPS treatment, is most likely a result of low volumes of sludge being transferred into the mineralization chamber. For Trial 1, not all solids were effectively transferred from the aquaculture loop to the mineralization chamber. This operational error was corrected in Trial 2. It was observed for the Trial 2 harvest, there is no significant difference ($p > 0.05$) between the SRAPS and DRAPS in terms of FM. The HP treatment FM production is significantly less for both trials. For Trial 1, HP FM production is 98% less than SRAPS and 95% less than DRAPS. For Trial 2, HP FM production is 89% less than SRAPS and 87% less than DRAPS.

4.2 Role of Treatment on pH

Of the abiotic variables measured, pH had the greatest variation between treatments. HP treatments operated well outside of optimal ranges for cultivation of *Lactuca sativa*, averaging

8.9 (±0.1) for Trial 1 and 7.1 (±1.0) for Trial 2. pH is arguably the most important variable to impact nutrient uptake of plants in hydroponic grow systems (Anderson et al., 2017; Pantanella et al., 2012; Seawright et al., 1998). pH is important to consider because it controls fish metabolism, the toxicity of ammonia and heavy metals to fish, microbial activities and biological oxidation of ammonium to nitrite and nitrate (Van Rijn et al., 2006; Zou et al., 2016). It is crucial to stabilize pH in the aquaponic system since it is critical to all living organisms within a recirculating system, i.e., fish, plants and microbes (Yildiz et al., 2017). Nitrifying bacteria function adequately within a pH range of 6.0–8.5 (Somerville et al., 2014) but are most active within a pH range of 6.8 to 7.5. *Lactuca sativa* is studied to have optimal nutrient absorption in aquatic environments at a pH of 5.8 (Anderson et al., 2017). In this experiment, aquaponic systems trended towards acidic. Average pH values in the SRAPS treatment were 6.8 (±0.7) and 6.3 (±0.5) for Trial 1 and Trial 2 respectively. Average pH values in the $DRAPS_P$ treatment were 8.6 (±0.2) and 6.1 (±0.6) for Trial 1 and Trial 2 respectively. A low pH correlates to carbon dioxide toxicity and a high pH correlates with ammonia toxicity in aquaculture systems. The HP treatment provided an alkaline environment which inhibited nutrient absorption at the roots, thus resulting in extremely stunted growth of all HP lettuce plants.

Most research suggests the electrical conductivity (EC) in aquaponic solution is typically between 300 and 1,100 µS/cm (Graber & Junge, 2009; Lennard & Leonard, 2006; Pantanella et al., 2012; Rakocy et al., 2006; Roosta, 2011). Ideal EC concentration in a hydroponic solution is slightly higher, typically between 1,000 and 3,000 µS/cm (Hashida et al., 2014; Rouphael & Colla, 2005; Sarooshi & Cresswell, 1994). Average EC measurements from this experiment fell within this range (Table 1). EC is a metric with a strong correlation to nutrient strength (Wortman,

2015). It would be predicted that the HP treatment with a higher EC would produce a greater yield compared to SRAPS and DRAPS. However, we find the opposite to be true; most likely the effects of pH outweigh the effects of EC (Pantanella et al., 2012).

Due to high TAN measurements during the experiment, it was necessary to perform several "flushes" of the system where approximately 50% of water was exchanged for fresh water. This resulted in possible dilution of nutrients, reducing nutrient availability for the plants. These findings demonstrate the importance of maintaining an intensive, high-density aquaculture system in aquaponics (with proportionately high feeding rates) to support sufficient nutrient levels. However, the intensity of aquaculture management required for successful aquaponic production described by (Rakocy et al., 2006) is rarely duplicated. Instead, many start-up aquaponic farms are "plant-centric" and maintain a low-density aquaculture sub-system that requires less intensive management. Unfortunately, the result is often nutrient deficiency, chlorosis, and reduced marketable yield, as observed in this study (See Appendix for an example of *Lactuca sativa* harvested from the STRAPS treatment). Yellowing is apparent on the outer edges of the oldest leaves. Similar observations were made for plants harvested from the DRAPS treatment.

4.3 Role of Treatment on Root Health

Root length of lettuce plants was measured in Trial 2 (Table 2). Statistically significant differences ($p < 0.05$) were observed between the three treatments. HP treatment with the least average root length of 70 mm (±40), then SRAPS, 289 mm (±90), and then DRAPS, 400 (± 126) with greatest average root length. DRAPS root length over five times greater than HP. There is strong correlation between root length and biomass (Bouteillé et al., 2012). This correlation

holds true to this experiment (Figure 5). Stunted growth of the HP roots may be due to organic matter accumulating around the roots, see Figure 6 for a visual. The buildup of organic matter may create an anoxic zone around the roots and inhibit nutrient absorption (Park & Kurata, 2009).

4.4 Closing the Loop

A criticism of the DRAPS method is that despite its many benefits, water from the hydroponic unit does not return to the fish. In other words, a discontinuity in the ecosystem model that aquaponics aims to replicate (Kloas et al., 2015). A solution for this has been developed, namely, the ASTAF-PRO decoupled aquaponics model in Berlin, Germany, Humboldt University (Kloas et al., 2015; Monsees, Kloas, et al., 2017). The ASTAF-PRO name is inspired by what it is, an Aquaponic System for (nearly) emission free Tomato And Fish PROduction in greenhouses. The system utilizes a novel approach to recycle water by capturing evapotranspiration from the plants and returning clean water to the aquaculture tanks, thus completing the water loop. Research produced from the ASTAF-PRO system is significant because it is the first DRAPS of its kind and first to be critically evaluated on the basis of nutrient availability, feed conversion ratio (FCR), fish and plant production, and water use (Kloas et al., 2015).

4.5 Refined Methods

The methods of this research study can be improved in several ways. First, data collection. It is necessary to collect nutrient measurements for both the recirculating water system and plant tissue. Water samples from each of the systems should be analyzed for dissolved nutrients including NO_3^--N, NO_2^--N, NH_4^+-N, PO_4^{3-}, K^+, Ca_2^+, Mg_2^+, SO_4^{2-}, Cl_2^- and Fe_2^+

(Monsees et al., 2017) Elemental analysis of the leaf tissue should be measured, periodically throughout the trial and at time of harvest. Elements to measure include Ca, K, Mg, Na, P, N, C, and C/N (Monsees et al., 2017). It would also be valuable to measure elemental composition of sludge. With this data, it is then possible to create a mass balance of nutrients and determine which engineered grow system best utilizes nutrients. This data will also give insight into determining if the growth of the plant is due to greater amounts of nutrients or if the type of grow system (i.e. DRAPS) can convert the nutrients into a more available form for absorption at the root.

Second, the small-scale design of the grow systems had some complications. In theory, there should not be any solid waste matter recirculating through the SRAPS treatment. For two reasons; one, solid waste build up can clog the pipes and create anoxic zones in the grow system; and two, solid waste will naturally mineralize as it cycles through the NFT grow system. The aim of this experiment is to isolate the mineralization effect to only the DRAPS treatment. In sufficient solids removal in SRAPS diminished the mineralization effect in the DRAPS treatment. Additionally, the mineralization chamber in the DRAPS treatment should be designed with a cone bottom tank and greater air movement. This modification will allow for complete mineralization of solid waste and improved nutrient source for plants.

Third, the advantages of separation and control in the DRAPS treatment should be utilized in the experiment. Meaning, water quality conditions such as, pH, EC, TSS, aeration, and temperature should be manipulated to create ideal growing conditions for the lettuce. In contrast, this experiment took an “as-is” approach, allowing water conditions to stabilize naturally without outside interference. A more targeted experiment would compare DRAPS and

HP treatments with abiotic variables of the grow environment to be the same, then analyzing the microbial communities as the dependent variable.

CHAPTER 5: CONCLUSION

These results provide evidence that the incorporation of aquaculture systems to hydroponics, has a positive effect on the production of *Lactuca sativa* with respects to fresh mass and root length. This is evident by the greater fresh mass and root length production from SRAPS and DRAPS treatments compared to HP. This study also confirms that treatment type effects the root length of *Lactuca sativa*. The difference between SRAPS and DRAPS are not evident from the fresh mass and dry mass data, there is a significant difference in terms of root length. DRAPS treatment produced the greatest root length results compared to SRAPS and HP. There is evidence to suggest the DRAPS design provides a more hospitable environment for root growth. The treatment effects on pH reflect that SRAPS and DRAPS treatments provide the most optimal pH range for *Lactuca sativa* growth compared to the HP treatment. However, the main benefit of a HP system is the ability to manipulate pH and other environmental parameters manually, this advantage was not utilized in the experiment. Future experiments should maintain the HP treatment to meet optimal environmental conditions to support plant growth.

Further studies should focus on a better understanding of the factors that led to these results and utilize nutrient composition analysis to determine how the different treatments effect nutrient concentration and nutrient availability to the plant. The advantages of a decoupled aquaponics system are not obvious by the results of this experiment, rather, the experiment displays the advantages of increased control in a system with many variables. Previous research has already determined optimal conditions for producing lettuce crops, fin fish, and many other agricultural products. The present issue is the ability to control those variables and maintain ideal conditions for the fish and plants. Decoupled aquaponic systems are

engineered to allow for greater control of the grow environment with separate water flow between fish and plants, as well as, the utilization of solid fish waste. The decoupled system of aquaponics has the competitive benefits of commercial RAS and greenhouse hydroponic systems combined. The use of decoupled technology in the aquaponic system may improve the productivity but needs more studies done to refine the sludge mineralization process and define the environmental needs of plant growth promoting microorganisms. Moving forward with aquaponic technology, fish sludge needs to be considered more as a valuable source instead of a disposable waste.

5.1 Further Research

The most valuable information gained from this research project is a deep understanding and profound respect for the ecosystem services of our soil. The process of removing soil from agriculture systems is no easy feat (Bartelme et al., 2018; Hernandez & Engel, 2018). The justification that we can use alternative agriculture systems to feed the world after our soil has been depleted, is a dangerous state of mind. It is in the absence of soil that we realize how vital and irreplaceable the role of soil is in our ecosystem. Replicating the nutrients and minerals found in soil is no easy feat. Nutrients take on different chemical forms when in an aquatic environment versus organic soil environment. The nearly infinite array of microbes and fungi that inhabit the soil serve an essential role to the plants. Replicating this environment in hydroponic and aquaponic applications is the next challenge to overcome for the advancement of aquaponic food production.

Decoupled aquaponics provides greater control of the grow environment. Now research must focus on utilizing that control to create an ecosystem that can sustain the microbes, fungi

and mineral suspension needed for plants to thrive. Plant growth promoting rhizobacteria, fungi, and microbes are areas of study that deserve more attention.

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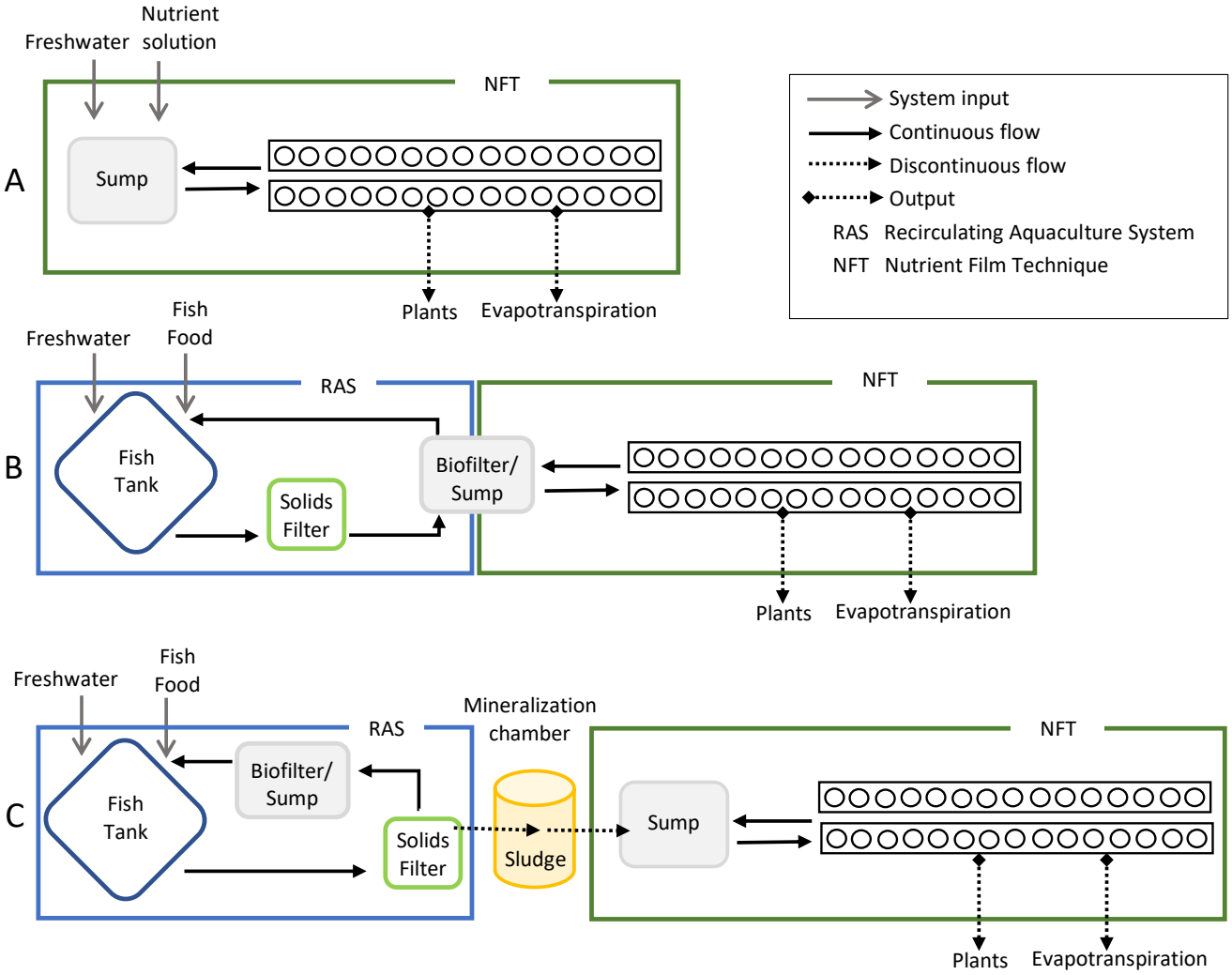
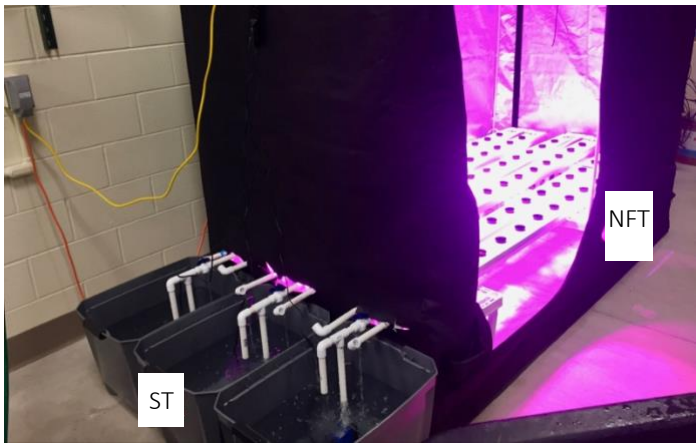


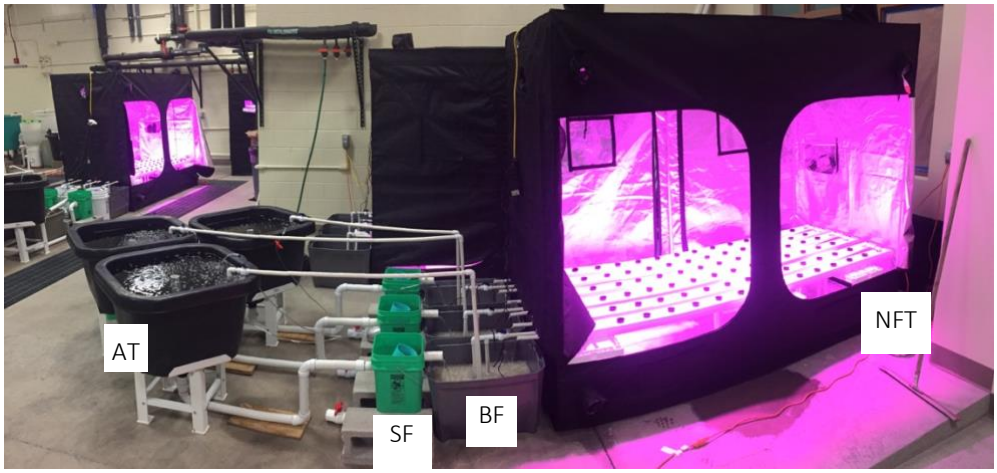
Figure 1. Schematic flow diagram of the three system designs (A) hydroponic (HP), (B) single recirculating aquaponics system (SRAPS), and (C) double recirculating aquaponics system (DRAPS). Blue lines mark the recirculating aquaculture system (RAS) with fish-rearing tanks, mechanical sedimentation filter, pump sump and biofilter; the green lines comprise the hydroponic unit which utilize nutrient film technique (NFT) to grow lettuce. Experiment consisted of three replicates for each system type.

A



AT	Aquaculture Tank
BF	Biofiltration tank with submerged pump
MC	Mineralization Chamber
NFT	Nutrient Film Technique hydroponics
SF	Solids Filtration
ST	Sump Tank with submerged pump

B



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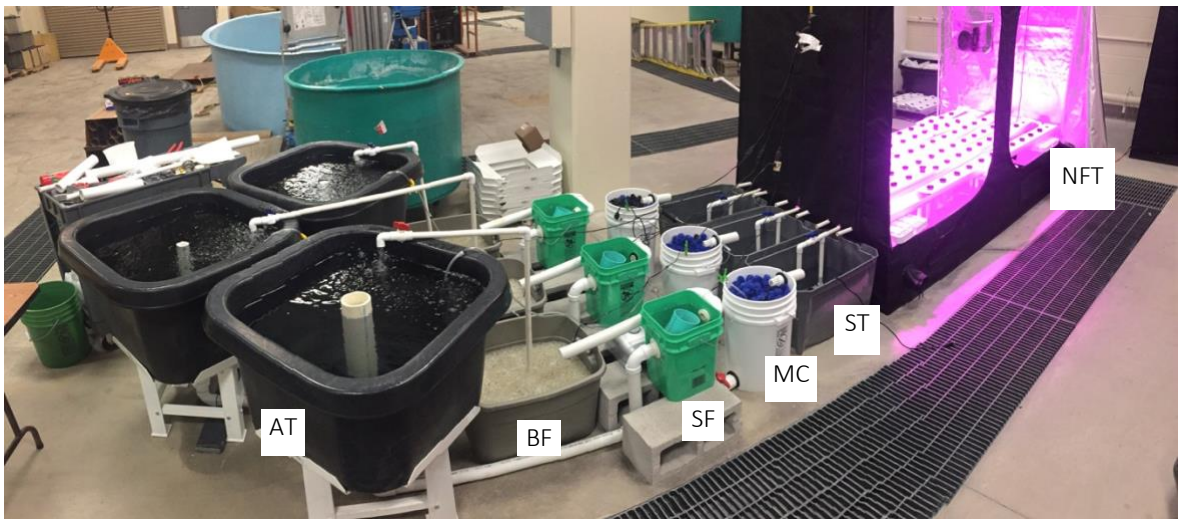

Figure 2. Photos of full experimental setup, displaying all three treatments with three independent replicates for each system. A, hydroponic (HP); B, single recirculating aquaponics system (SRAPS); and C, double recirculating aquaponics system (DRAPS). Grow tents with NFT hydroponics are identical for each treatment. There is no mixing of water between any replicate.

A B C

Figure 3. Trial 2 lettuce growth at day of harvest. (A) hydroponic (HP), (B) single recirculating aquaponics system (SRAPS), and (C) double recirculating aquaponics system (DRAPS). It can be observed that lettuce growth from treatment A is less than B and C. Treatment A shows plants to be smaller than the other treatments and many plants died completely after they were transferred from the germination tent. Treatments B and C show similar amounts of lettuce growth and both treatments have several plants that display yellowing of the leaf tips.

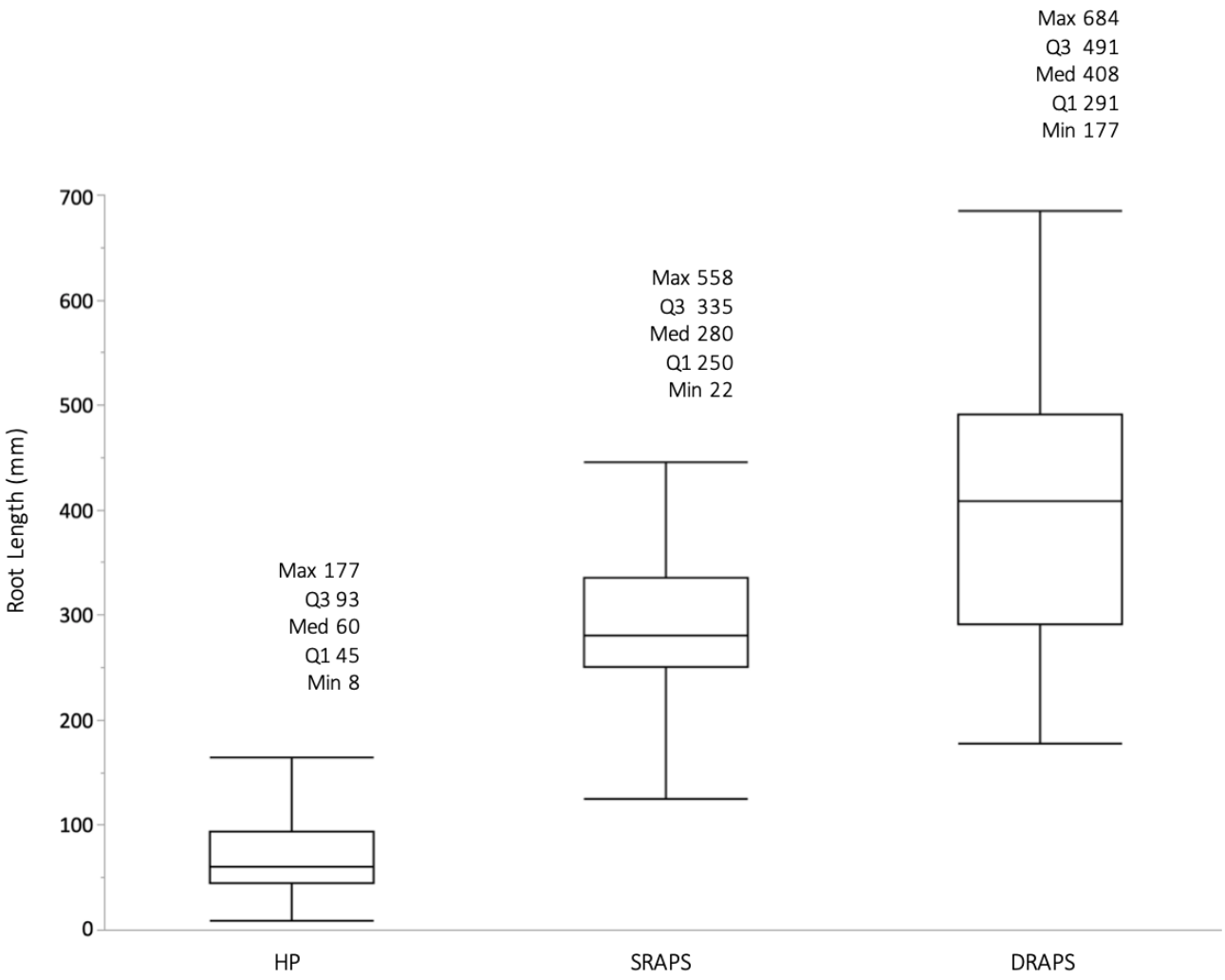

Figure 4. Box plots of root length measurements against treatment type for Trial 2 lettuce at time of harvest. Treatments displayed include hydroponics (HP), single recirculating aquaponics system (SRAPS) and decoupled recirculating aquaponics system (DRAPS). The box plot displays median, lower (Q1) and upper (Q3) quartile ranges, and maximum (Max) and minimum (Min) root length measurements. Displayed above each treatment are the summary statistics. Interquartile range (IQR) is the middle 50% of values when ordered from lowest to highest, quartile 3 (Q3) represents the high end of the IQR and quartile 1 (Q1) represents the low end of the IQR. Tukey's HSD post hoc test determined that root length means for each treatment are significantly different ($p<.0001$).

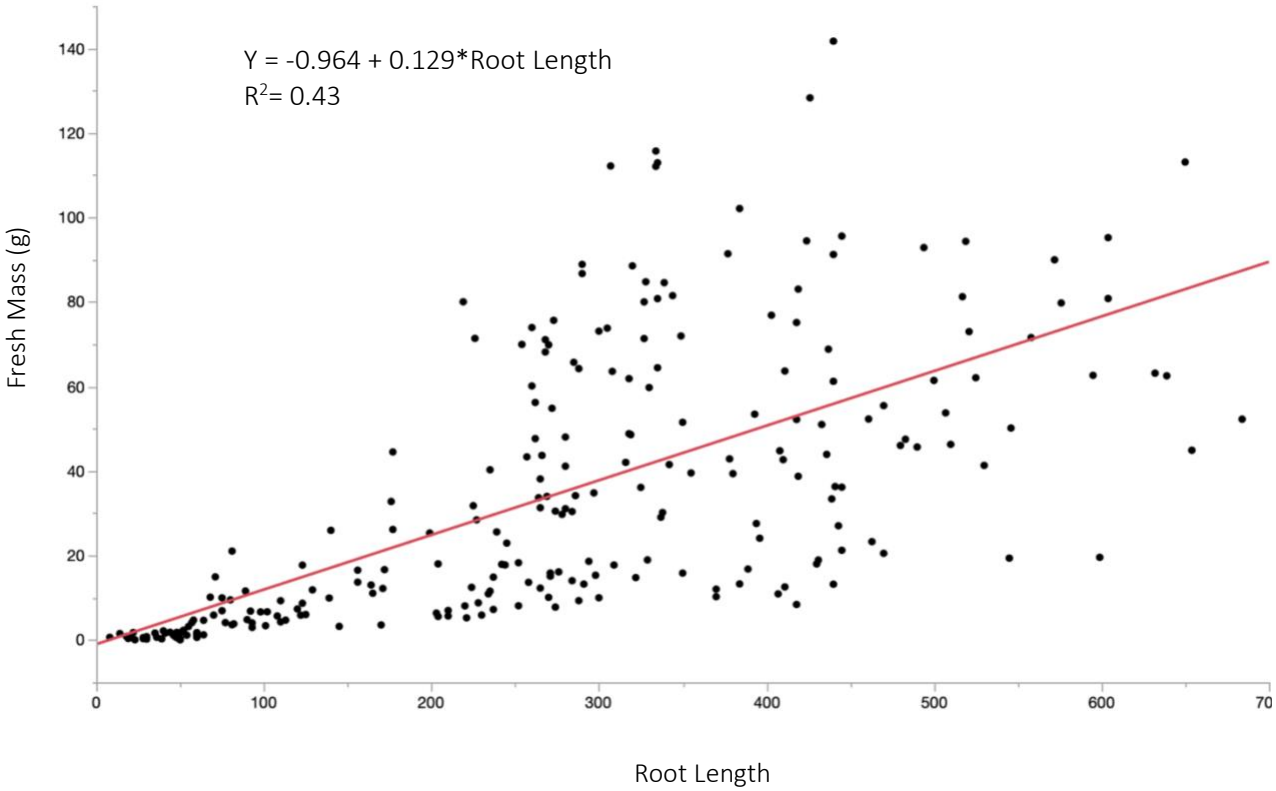

Figure 5. Linear regression model with fresh mass (g) as a function of root length (mm), using Trail 2 harvest data. The coefficient of determination (R^2) in the model was found to be 0.43. Meaning, 43% of the variance of fresh mass can be determined by root length ($p<.0001$). The slope of the fit model translates as, for every 1 cm of root length, there is a predicted gain of 1.29 grams biomass per lettuce plant.

A B C

Figure 6. Photos of Trial 2 root condition at day of harvest. (A) hydroponic (HP), (B) single recirculating aquaponics system (SRAPS), and (C) double recirculating aquaponics system (DRAPS). Roots from treatment A standout compared to the roots from treatments B and C because there is a buildup of organic matter surrounding the root fibers.

Table 1. Environmental growth conditions for Trial 1 and Trial 2. Mean ± standard deviation (SD) are displayed for each measured variable. Abiotic variables described in the table include pH, total dissolved solids (TDS, ppm), electric conductivity (EC, µS/cm), dissolved oxygen (DO, mg/L) and water temperature (°C). The treatments measured include hydroponics (HP), single recirculating aquaponic system (SRAPS) and decoupled recirculating aquaponic system (DRAPS). The DRAPS system, due to the mechanical separation of water flow between fish and plants, was measured as independent systems. Thus, $DRAPS_F$ indicates measurements from the fish sump tank and $DRAPS_P$ indicates measurements from the plant sump tank. N represents number of measurements. Greater N values for Trial 2 data are a result of greater frequency in taking water quality measurements throughout the trial. Water temperatures were not measured for HP or $DRAPS_P$, but it can be noted that those environments remained equalized with the room temperature.

		pH		TDS (ppm)		EC (µS/cm)		DO (mg/L)		Water Temp. (°C)	
		(N)	Mean ± SD	(N)	Mean ± SD	(N)	Mean ± SD	(N)	Mean ± SD	(N)	Mean ± SD
Trial 1	HP	51	8.9 ± 0.1	45	318.3 ± 27.1	45	630.3 ± 96.8	34	6.6 ± 0.5	-	-
	SRAPS	51	6.8 ± 0.7	45	250.5 ± 34.7	45	508.2 ± 67.9	36	5.6 ±0.6	66	20.4 ± 0.5
	$DRAPS_F$	51	6.6 ± 0.8	45	233.1 ± 23.3	45	445.6 ± 51.3	36	5.9 ± 0.7	66	19.6 ± 0.6
	$DRAPS_P$	51	8.6 ± 0.2	45	249.6 ± 21.8	45	498.1 ± 44.4	34	6.2 ± 0.9	-	-
Trial 2	HP	102	7.1 ± 1.0	78	623.7 ± 106.0	99	1295.6 ± 211.1	102	8.2 ± 0.4	-	-
	SRAPS	102	6.3 ± 0.5	78	456.3 ± 83.2	99	972.8 ± 163.6	102	7.7 ± 0.5	93	19.2 ± 0.8
	$DRAPS_F$	102	5.8 ± 0.5	78	369.1 ± 39.8	99	796.0 ± 82.9	102	8.0 ± 0.3	96	18.4 ± 0.7
	$DRAPS_P$	102	6.1 ± 0.6	78	412.7 ± 29.2	99	868.1 ± 70.0	102	8.4 ± 0.2	-	-

Table 2. Lactuca sativa growth measurements of Fresh Mass (FM, g), Dry Mass (DM,g), ratio of DM/FM as percent (%) and Root Length (RL, mm). Displayed as mean values ± standard deviation (SD) for each treatment hydroponics (HP), single recirculating aquaponic system (SRAPS) and decoupled recirculating aquaponic system (DRAPS). Different letter superscript indicate significant differences within treatment type and trial. Significant differences were determined using ANOVA with Tukey's HSD post hoc test, α=0.05. N = sample size. Fresh Mass data was measured from each individual plant, thus, the N value is equal to the total number of survived plants from each treatment. Dry Mass mean is calculated from the total dry mass of all lettuce plants combined from its respective replicate, then divided by the number of plants within each dry mass measurement. This explains why the N value is a low value of 3, representing one measurement from each replicate. Root Length measurements were only collected for Trial 2.

		FM (g)		DM (g)		DM/FM (%)	RL (mm)	
		N	Mean ± SD	N	Mean ± SD		N	Mean ± SD
Trial 1	HP	82	0.54 ± 0.353^{C}	3	0.05 ± 0.006^{A}	9.26^{A}	-	-
	SRAPS	89	26.05 ± 22.436^{A}	3	0.99 ± 0.820^{A}	3.80^{B}	-	-
	DRAPS	86	11.04 ± 8.178^{B}	3	0.52 ± 0.322^{A}	4.71^{B}	-	-
Trial 2	HP	68	5.39 ± 6.799^{B}	3	0.36 ± 0.015^{A}	6.68^{A}	68	70 ± 40^{C}
	SRAPS	90	47.77 ± 32.462^{A}	3	1.64 ± 0.928^{A}	3.43^{B}	90	289 ± 90^{B}
	DRAPS	90	41.51 ± 41.512^{A}	3	1.44 ± 0.841^{A}	3.47^{B}	90	400 ± 126^{A}

APPENDIX

Close-up photo of *Lactuca sativa*, harvested from SRAPS treatment. Yellowing is apparent on the outer edges of the oldest leaves with slight browning behind the yellowing. Similar observations were made for plants harvested from the DRAPS treatment.