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Resource Recovery Through Halophyte Production in Marine Aquaponics: An Evaluation of the Nutrient Cycling and the Environmental Sustainability of Aquaponics

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Resource Recovery Through Halophyte Production in Marine Aquaponics: An Evaluation of the Nutrient Cycling and the Environmental Sustainability of Aquaponics

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
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Dedication

To my Mom, who celebrated with me on the good days and commiserated with me on the bad days. Who tirelessly edited papers throughout grade school and even sometimes in graduate school, despite my occasional defensiveness, providing me with the foundational writing skills to survive graduate school and all that it entails. And who, most importantly, taught me what it is to be a strong, capable woman. (Although, I'm still working on that comma thing and what are gerunds again?)

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Abstract

Aquaculture, the farming of aquatic animals and plants, is an important component of global food production, which supplies a nutritious protein source for millions of people. Interest in improving the sustainability of aquaculture has led to the development of aquaponics in which fish production is combined with plant production to create zero-discharge systems. A need for more fundamental science and engineering research on marine aquaculture and growing interest in production of halophytes motivated this novel research on marine aquaponics. One objective was to evaluate the growth and nutrient removal capacity of halophytes in marine aquaponics. Bench-scale studies were conducted to determine the best methodology to grow the halophytes sea purslane (*Sesuvium portulacastrum*) and saltwort (*Batis maritima*). The results indicated these species were important for nitrogen removal and function well under varying conditions of flow rate, species, or plant density. A prototype commercial-scale marine aquaponic system was evaluated through regular collection of water quality and plant growth data over a 9 month period. The system had a total volume of 50 m^3 and contained: a swirl separator, uplfow media filter, a moving bed bioreactor, 61.4 m^2 of hydroponic growing area, and a sand filter. Water quality parameters measured included: total ammonia nitrogen (TAN), nitrite (NO_2^-), nitrate (NO_3^-), total nitrogen (TN), total phosphorus (TP), chemical oxygen demand (COD), total suspended solids (TSS), and volatile suspended solids (VSS). TAN and nitrite concentrations in the fish tank effluent ranged from 0.04 to 2.42 mg/L TAN and 0.07 to 14.7 mg/L NO_2^--N, respectively. Nitrate concentrations increased to a maximum of 120 ± 5.7 mg/L NO_3^--N during the first 119 days of operation. To provide greater control over nitrate concentrations, the sand

filter was converted into a downflow submerged packed bed biofilter. This reduced concentrations to a mean of 27.5 ± 13.7 mg/L NO_3^--N during the last 3 months. Dried plant samples were analyzed for nitrogen and phosphorus content. Nutrient uptake by plants ranged from 0.06 to 0.87 g $N/m^2/d$ and 0.01 to 0.14 g $P/m^2/d$. It was estimated 0.55 kg/m^2 of plant biomass could be harvested every 28 days. Red drum (*Sciaenops ocellatus*) were initially stocked at an average weight of 0.047 kg and grew to a harvestable size of 0.91 kg in approximately 12 months. A mass balance indicated that plants contributed to less than 10% of nitrogen and phosphorus removal and passive denitrification was the dominant nitrogen removal process. The second objective was to evaluate the environmental impact of aquaponics through life cycle assessment (LCA). LCAs were completed on freshwater aquaponic systems at commercial- and residential-scales. The system expansion method was used address co-production of 1 ton live-weight fish, recovered solids, plants, and water treatment. The results indicated that aquaponics contributed to significant water savings; however, aquaponics is subject to trade-offs from high energy use and the addition of industrial fish feeds. The methodology developed for freshwater aquaponics was applied to the prototype commercial-scale marine aquaponic system and was compared with two alternative scenarios of maximized plant production and a denitrification reactor with no plant production. The results indicated that a system with a denitrification reactor had the lowest environmental impact. Alternatively in the system with maximized plant production, the use of renewable energy sources would reduce the environmental impact and would contribute to greater water savings, while realizing the economic benefits of dual products. This is the first study to complete an in-depth evaluation of a commercial-scale marine aquaponic system and to evaluate aquaponics using LCA while accounting for the potential environmental offsets of multiple co-products.

Chapter 1: Introduction

Current food production systems face divergent challenges of increasing food supplies for growing populations while simultaneously minimizing the use of scarce resources. Despite recent reductions, one in nine people are still undernourished and sufficient nourishment for an additional 2 billion people will be required by 2050 (FAO, 2015). Feeding these people must be done with increasingly limited land, water, nutrient, and energy resources (Cordell et al., 2009; Rosegrant et al., 2009; von Grebmer et al., 2012). In addition, shifting food preferences for more processed foods, meat, and dairy further tax resources (Godfray et al., 2010). Meanwhile, overshadowing these challenges are the anticipated regional and global impacts of climate change on crop productivity and food availability (Wheeler and von Braun, 2013).

Meeting these challenges will be accomplished through diverse and multifaceted avenues, in which aquaculture already plays a key role in providing people with a consistent, healthy protein source (Godfray et al., 2010). Aquaculture, the farming of aquatic animals and plants, is the fastest growing food production industry and is an important source of animal protein for over 16.6% of the global population (FAO, 2012b). Aquaculture is also extremely important to the global economy; farmed food fish alone represent a value of over US$137.7 billion (FAO, 2014). While aquaculture production still presents some ecological risks, significant improvements have been made. Aquaculture has even been proposed as a solution to mitigate pollution from agricultural or industrial sources (Subasinghe et al., 2009). Technological improvements to create more sustainable aquaculture production include integrated multi-trophic

aquaculture (IMTA) and aquaponic systems. Both types of systems use plants to assimilate excess nutrients and reduce potential ecological impacts.

IMTA and aquaponic systems have long histories, however, the recent emphasis on sustainable aquaculture systems has brought them to the forefront of aquaculture research. IMTA systems encompass both open water and land-based systems whereas aquaponic systems are predominately land-based. Both systems can be operated with freshwater or marine fish and plant species, although research on IMTA has focused on marine species (Barrington et al., 2009) and research on aquaponics has focused on freshwater species (Rakocy, 2012). A review of the literature (Chapter 3) revealed a general absence of information on the use of marine species in aquaponics.

Similarly there is a growing interest in the development of saltwater tolerant plant species, known as halophytes, for their potential to expand agricultural production. Halophytes also have potential uses as fuel, fodder, and fiber, each with respective economic values (Galvani, 2007). Despite growing interest in halophyte production, there is limited information on cultivation methods or mass production yields (Ventura and Sagi, 2013). Research on halophytes is further complicated by the number of potential species and the variable climatic and salinity tolerances of these species (Ahmad and Malik, 2002). In order to minimize production failure from increased plant stress in new climates, selection of regionally available species will aid domestication (Debez et al., 2011). The relative absence of research on halophytes or marine aquaponics and the requirement for saltwater tolerant plants in marine aquaponics distinguishes both topics as highly attractive areas for experimental and modeling studies.

Both freshwater and marine aquaponics have potential to become important components of global food production. For this reason, it is important not only to optimize these systems through experimental research, but also to evaluate potential ecological and environmental impacts of these systems. Life cycle assessment (LCA) is a tool used to quantitatively evaluate the environmental impact of a product or process and can provide metrics for sustainability (EPA, 2006). It has been used previously to evaluate a variety of food products and industries, including fisheries, aquaculture, and agriculture (Ayer and Tyedmers, 2009; Henriksson et al., 2012; Roy et al., 2009). Evaluating aquaponic systems in this way provides information on system components with the greatest impact and helps to identify areas for improvement. Joint collection of experimental and LCA data will aid the development of marine aquaponic systems which in turn will contribute to sustainable food production and strengthen global food security.

The research presented in this dissertation extended work previously completed on a marine land-based IMTA system at Mote Aquaculture Research Park (MAP) in Sarasota, FL. That IMTA system operated with 100% system water recirculation and had zero onsite waste discharge, which was facilitated by production of wetland plants for coastal restoration. More detail on this research can be found in Boxman (2013) and Boxman et al. (2015b). The successful operation of the initial marine IMTA system and broad interest in aquaponics motivated this innovative research on marine aquaponic systems.

The overarching goal of this dissertation was to understand the performance, nutrient cycling, and environmental sustainability of aquaponics within the context of sustainable food production. The two research questions that guided this dissertation are listed below along with the objectives needed to answer those questions:

1. How do halophytes, sea purslane and saltwort, perform in a marine aquaponics system in terms of halophyte growth and nutrient removal capability?
    a. (Chapter 2) Design and conduct bench-scale studies to determine: 1) the impact of plants on water quality; 2) the impact of planting medium selection on water quality and plant growth; 3) the impact of hydraulic loading rate (HLR), plant species, and plant density on water quality and plant growth; and 4) the best halophyte layout for a full-scale marine aquaponics system.
    b. (Chapter 3) Evaluate a full-scale marine aquaponic system for its operation and nutrient cycling through: 1) characterization of nitrogen and phosphorus transformations and removal; 2) determination of the nutrient removal capacity of the halophytes, sea purslane and saltwort; 3) evaluation of the growth and production of the halophytes sea purslane and saltwort; and 4) evaluation of the growth and production of the marine fish red drum and the relationship between water quality characteristics and fish health.
2. Using a LCA framework, what is the environmental impact of aquaponics at scales ranging from residential to commercial, for freshwater and marine systems?
    a. (Chapter 4) Conduct a literature review of LCAs on intensive and extensive aquaculture systems to develop: 1) a more complete picture of the environmental trade-offs incurred due to intensification of aquaculture systems and 2) provide background information on previously completed LCAs of aquaculture systems for perspective in the subsequent chapters.
    b. (Chapter 5) Complete a LCA of freshwater aquaponics to: 1) identify 'hot-spots' of environmental impact in a commercial-scale aquaponic system; 2)

determine the degree to which hydroponic plant production and recovered solids used as an agricultural amendment reduce the environmental impact of the whole system; 3) compare the commercial-scale system to a residential-scale system to determine if environmental impacts change with scale; and 4) develop a framework for use with LCA which accounted for the simultaneous production of multiple products in aquaponics.

c. (Chapter 6) Complete a LCA of a marine aquaponic system at MAP to: 1) complete a LCA on a marine aquaponic system that includes both plant production and denitrification to establish a baseline of environmental impact and 2) compare this baseline with alternative scenarios of high plant production or just denitrification in reactors(s) to evaluate trade-offs between the two water treatment approaches.

The objectives following each question correspond with a chapter of this dissertation. Each chapter is structured as a standalone research article complete with individual introduction, methods, results, discussion, and conclusion sections. Chapter 7 provides a summary of major findings from each chapter. General reflections on how aquaponics and halophytes can best contribute to sustainable food production and global food security and recommendations for future research are also included in Chapter 7. Following the conclusions, several appendices are included. Appendix A contains more detailed description of the experimental methodology used in Chapters 2 and 3. Appendix B contains life cycle inventory data on aquaculture feeds used for Chapters 5 and 6. Appendix C provides a more detailed explanation of the methods used to calculate nutrient budgets and inventory data for co-production in aquaponic systems used in Chapters 3, 5 and 6.

Chapter 2: Evaluation of Water Quality and Growth of Two Saltwater Vegetable Species in Bench-scale Marine Aquaponic Systems

2.1 Introduction

Freshwater aquaculture production has rapidly increased at an annual growth rate of 8.8% and now contributes to almost half of food fish production (FAO, 2012b). Conversely, marine aquaculture production has increased at a slower rate than freshwater aquaculture and currently contributes to about 15% of global aquaculture production (FAO, 2012a). Historically, aquaculture has been limited by the environmental impacts of waste discharges and associated nutrient loading to coastal and open water bodies (Chopin et al., 2001). Intensive land-based recirculating aquaculture systems (RAS) mitigate many of these problems by treating and recirculating 90-99% of system water, thereby minimizing waste discharges (Badiola et al., 2012). Efforts to further reduce water usage have led to the addition of an assimilative element to RAS, such as plant growth. The dual production in integrated multi-trophic aquaculture systems (IMTA) has the potential to improve resource use efficiency, minimize waste discharge, and improve economic returns.

In aquaponic systems, plants are produced simultaneously with fish in a RAS. The aquaponic industry has grown rapidly over the last 30 years resulting in research on a range of system designs and various combinations of aquatic animal and plant species (Endut et al., 2009; Lennard and Leonard, 2006; Rakocy et al., 2006; Trang and Brix, 2014). However, most aquaponic systems previously studied have used obligate freshwater aquatic animal and plant species, with little prior research on marine fish or plant species. Considering the limited growth

of marine aquaculture and advantages of IMTA, marine aquaponics could be a valuable technology to enhance production of marine fish and plant species.

Development of marine aquaponics requires use of halophytes. Recently, interest in methods to grow and produce these plants commercially has increased due to their potential as a food crop, forage crop, and oilseed crop in addition to their beneficial medicinal and chemical properties (Ventura and Sagi, 2013). In this study, two species, sea purslane (*Sesuvium portulcastrum*) and saltwort (*Batis maritima*), were selected due to local availability and potential value as vegetable crops (Panta et al., 2014). Saltwort grows in salt marshes across North and South America and has been shown to contain high concentrations of essential amino acids and tocopherol antioxidants (Debez et al., 2010). Sea purslane grows along coastlines in tropical and sub-tropical climates and is enjoyed as a wild vegetable due to the texture and salty taste in southern India (Kathiresan et al., 1997). At present little information is available that would aid development of commercial production of these halophytes, such as growth rates, methods for planting, and nutrient requirements.

Nutrient availability for plant growth in aquaponic systems is directly related to fish production rates and fish feeding rates. The appropriate ratio of fish feed to plant growing area has been reported to range from 15-180 g feed/m^2/day (Endut et al., 2010; Rakocy et al., 2006). The ideal ratio will vary with system design, fish species, and plant species. Nutrient availability also varies with plant density, a characteristic that is not fully captured by measures of total hydroponic growing area. Plant species such as barley can be grown at much higher densities than lettuce due to morphological differences (Rakocy et al., 2006; Snow and Ghaly, 2008). Halophyte species can also be grown at varying densities. *Salicornia europae* was grown at 10,000 plants/m^2 and 200 plants/m^2 in constructed wetlands with little difference in nutrient

removal (Webb et al., 2013). For the halophytic species used in this study, the feed/plant ratio and the impact of plant density on plant growth and nutrient removal was unknown.

Plant species selection is just one of several operational conditions that can influence the performance of aquaponic systems. High flow rates are typically maintained in RAS to achieve rapid removal of harmful nitrogen species. The high flow rates can translate into high hydraulic loading rates (HLR) in the hydroponic plant beds. These greater HLRs also contribute to greater pumping requirements and potentially greater energy costs. While HLRs of 0.018-0.3 m/day have been used successfully to treat aquaculture wastewater in constructed wetlands, at HLRs greater than 1 m/day nitrate removal is greatly reduced (Lin et al., 2005; Schulz et al., 2003). It remains in question whether hydroponic plant production in aquaponic systems has similar decreases in efficiency as constructed wetlands at higher HLRs or if the constant recirculation mitigates the reduced efficiency.

In addition to hydraulic or species variations in hydroponic plant beds, the type of planting media used can potentially impact nutrient removal (Xuan et al., 2010). A variety of media types can be used to support plant growth including light expanded clay aggregate (LECA), *Sphagnum* peat moss, and coconut fiber. LECA, also known as expanded clay, is a clay pellet formed by firing plastic clay in a kiln at high temperatures thereby forming an inert, porous, and sturdy material (Yaghi and Hartikainen, 2013). Peat moss is one of the most commonly used potting media for both soil and soilless horticulture; however, environmentalists have questioned whether current peat harvest rates are sustainable (Meerow, 1994). Alternatively, processing large quantities of coconuts has caused a mass accumulation of coconut coir waste making the coir, or fiber, a readily available potting medium (Bhatnagar et al., 2010).

In this study, bench-scale marine aquaponic systems were used to determine the feasibility of growing sea purslane and saltwort hydroponically, fertilized by fish waste. Replicate systems allowed for simultaneous testing of multiple operational parameters. The objectives of this study were to evaluate: 1) the impact of plants on water quality; 2) the impact of media selection on water quality and plant growth; and 3) the impact of hydraulic loading rate (HLR), plant species, and plant density on water quality and plant growth.

2.2 Materials and Methods

2.2.1 Aquaponic System Design

Twelve individual bench-scale aquaponic systems (Figure 2.1) were constructed indoors in thermostatically controlled rooms. Each system consisted of a 38 L rectangular glass fish tank and a 62 L plastic plant growth container. The plastic container was spray-painted black to eliminate algae growth. A biofilter was constructed from a 28 cm x 18 cm x 17 cm hard plastic box, which was filled with Kaldnes® K1 (Fureneset, Norway) biofilter media. A 4 cm thick piece of plastic mesh (Pentair Aquatic Eco-Systems, Apopka, FL) on the media's surface was used for solids removal. The media was initially seeded with media from an already operational moving bed bioreactor then acclimated for three weeks in a separate tank. An airlift pump was constructed with a piece of PVC pipe fitted over an air stone at the bottom of the pipe to move water to the biofilter. Additional aeration was provided in the fish tanks, with a 4 cm x 4 cm fine pore diffuser (Pentair Aquatic Eco-Systems, Apopka, FL).

Submersible pond pumps, designed to pump 0.53 m^3/min and 0.26 m^3/min (TotalPond®, FL, Model 11130 and 11060), were used to move water up to the hydroponic plant bed depending on the desired flow rate. Plastic ball-valves were used to adjust flow rates into the hydroponic plant beds. Flow rates were set manually at the start of each experiment with a

graduated cylinder and a stopwatch. An overflow pipe, set at 20 cm, maintained a constant water level in the plant bed. One-half inch thick extruded polystyrene board cut to 30 cm x 46 cm (0.13 m^2) was used as a floating raft to support the plants. Plants were placed in 2.54 cm net pots and supported with either coconut fiber or expanded clay (8-16 mm; brand name Hydroton®). Florescent grow lights were suspended at a height of 30 cm over the tanks to provide light for the plants. Light intensity was measured with an ExTech Easyview 30 light meter (ExTech Inc., Waltham, MA) and averaged 184 lx (23.9 lm) with a min of 139 lx (18.1 lm) and max of 200 lx (26.0 lm) at hydroponic raft height. Lights were set on timers to a 12 hour light : 12 hour dark photoperiod.

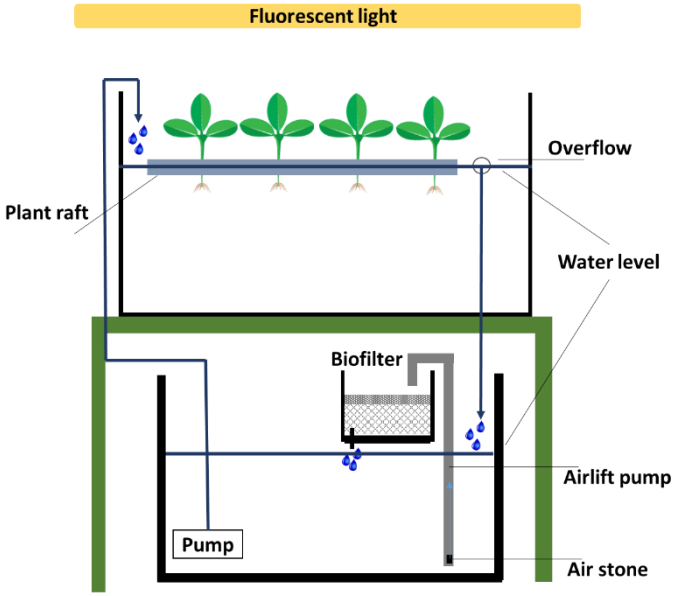

Figure 2.1: Schematic of the bench-scale aquaponic systems.

The aquaponic systems were filled with seawater from the Gulf of Mexico and diluted to a salinity of approximately 15 ppt with ground water. Ground water was also added as needed to account for evaporation. Plants were added to the aquaponic systems seven days before fish were stocked. During the study, both plant species were grown onsite in a subsurface flow constructed wetland similar to those described in Boxman et al. (2015b). Samples of both species were

harvested from the onsite constructed wetland to provide cuttings for this study. Each cutting had two nodes or meristems and was approximately 7-10 cm in length.

Platy fish (*Xiphophorus sp.*) were stocked in the fish tanks. This species was selected because it was readily available and would tolerate the low salinity conditions. Tanks were stocked to achieve an average total weight of 30 g (0.8 kg/m^3), which was equivalent to about 25-35 fish per tank. Fish were fed Skretting Classic Fry, 1.5 mm 45% protein pellets (Skretting USA, Utah). Fish were broadcast fed three times daily a total of 2 grams of feed per tank per day for the duration of the experiment. Mortalities were recorded during the study.

2.2.2 Experimental Design

Two full factorial experiments were completed to evaluate changes in water quality and plant growth (Montgomery, 2005). The first experiment was a 2^2 factorial, two factors and two levels, four combinations run with duplicates for a total of eight aquaponic systems (Figure 2.2a). It was designed to evaluate differences between plant presence and type of support media, and hereafter is referred to as 'media experiment'. The first factor was plant presence with the levels of plants and no plants. The second factor was support media with the levels of coconut fiber and expanded clay. During media experiments the number of plants, the plant species, and the flow rate were controlled. These factors were set such that all systems had a flow rate of 1 L/min. In the systems with plants, all had 24 plants and were planted with sea purslane.

The second experiment was a 2^3 full factorial, three factors each with two levels, eight combinations run with duplicates for a total of sixteen aquaponic systems (Figure 2b). The three factors evaluated were flow rate, plant species, and plant density. The levels of flow rate were high (1 L/min) and low (0.5 L/min). The levels for plant species were sea purslane and saltwort. The levels for plant density were high (24 plants) and low (12 plants). This experiment will

hereafter be referred to as the flow, species, density (FSD) experiment. Based on the results of the media experiment, coconut fiber was selected for the plant support media in the FSD experiment. Due to space limitations only twelve aquaponic systems were operated at one time. To complete the FSD experiment blocking was used to complete the full factorial. Since the media experiment only required eight systems the remaining four systems were used to run the FSD treatment combinations, where "first series" refers to all eight media experiment treatment combinations and four FSD experiment treatment combinations. The "second series" of testing refers to the second block of FSD experiments consisting of the remaining eight FSD experiment treatment combinations.

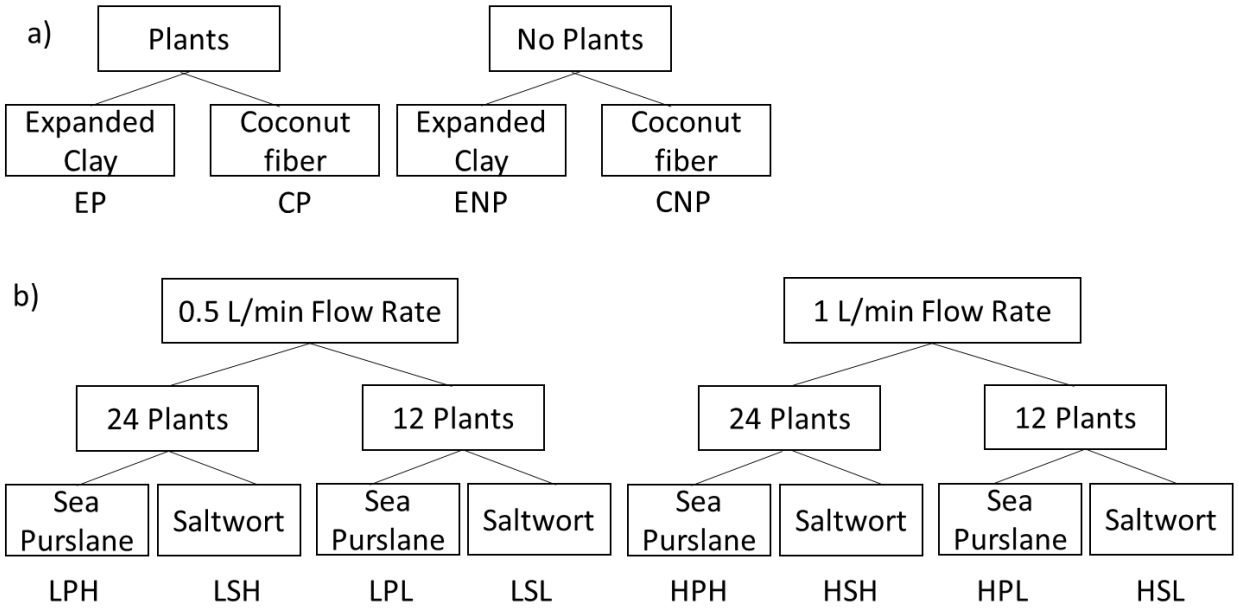

Figure 2.2: Experimental design. (a) media experiment treatments completed with sea purslane, at 1 L/min flow rate, and with 24 plants per system, EP: expanded clay/plants; CP: coconut fiber/plants; ENP: expanded clay/no plants; CNP: coconut fiber/no plants. (b) flow, species, density (FSD) experiment treatments completed with coconut fiber, HSH: high flow/saltwort/high density; LSH: low flow/saltwort/high density; HPH: high flow/sea purslane/high density; LPH: low flow/sea purslane/high density; HSL: high flow/saltwort/low density; LSL: low flow flow/saltwort/low density; HPL: high flow/sea purslane/low density; LPL: low flow/sea purslane/low density.

Both experiments were run for 30 days. After the first series of testing, the fish and plants were removed and all the systems were drained and cleaned. Fish were placed in a holding tank, while the systems were reestablished. Fresh cuttings were used for the second series of tests. The biofilter media was reserved and reused in the second series. The tanks were refilled with fresh saltwater and restocked with fish and plants as described above.

2.2.3 Sampling and Analysis

Temperature, salinity, pH, and dissolved oxygen (DO) were measured daily in the fish tanks with an YSI Probe (YSI Inc., Yellow Springs, OH). Twice weekly, 250 mL water samples were collected in triplicate from the plant beds. Samples were collected for 28 days and the first sample was taken prior to adding the fish. Samples were analyzed for total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2^--N), and nitrate-nitrogen (NO_3^--N). Standard curves were made with a background salinity concentration of 15 ppt. TAN was analyzed based on the method outlined in Bower and Holm-Hansen (1980) (method detection limit (MDL): 0.04 mg/L TAN); NO_2^- was analyzed using a combination of Standard Methods (method: 4500) and Strickland and Parsons (1972) (MDL: 0.01 mg/L NO_2^--N); NO_3^- was analyzed based on Zhang and Fischer (2006) (MDL: 0.15 mg/L NO_3^--N). More detail on the methods can be found in Appendix A.

Total fish weight, collective weight of all fish in tank, was taken at the start and end of each experiment. Individual plant weights were measured at the start and end of each experiment. Final weights were measured destructively as the whole plant was removed from the planting media. Plant fresh weights were used to determine the relative growth rate (RGR) which was calculated as:

$$RGR = \frac{\ln(w_2) - \ln(w_1)}{t_2 - t_1} \qquad \text{Eq. 2.1}$$

where w_1= initial wet weight; w_2 = final wet weight; t_1= start of experiment; t_2= end of experiment (Tylova-Munzarova et al., 2005).

2.2.4 Statistical Analysis

The statistical software Minitab 16 (Minitab, State College, PA) was used to carry out statistical analyses. The effect of plant presence and media type on water quality was tested with two-way analysis of variance (ANOVA). The effect of flow rate, plant species, and plant density on water quality and RGR was also tested with two-way ANOVA. One-way ANOVA was used to determine differences in plant RGR between media types. An incomplete block design was used for the FSD experiment. Tukey's test was used to determine differences between treatment means when significant ($\rho < 0.05$). If assumptions of normality and homogeneity of variance were not met, data were log transformed to meet assumptions.

2.3 Results

2.3.1 Daily Water Quality Measurements

Although the experiments were carried out in a thermostatically controlled room, the water temperatures fluctuated (Figure 2.3a). In the first series of tests, the mean temperature was 22.9 ± 1.9 ºC. The second series of tests had a slightly lower mean temperature of 22.0 ± 2.6 ºC. The mean salinity in both series of experiments fluctuated between a minimum of 13.1 ppt and a maximum of 17.1 ppt (Figure 2.3b). The DO concentration fluctuated between 6.1 and 8.6 mg/L for the first series of experiments, and was slightly higher for the second series of experiments, between 7.4 and 8.8 mg/L (Figure 2.3c). The pH remained quite stable during the first series of experiments and remained between 8.6 and 9.0.The pH was higher in the second series of experiments and ranged between 9.3 and 10.5 and generally increased over the experimental period (Figure 2.3d).

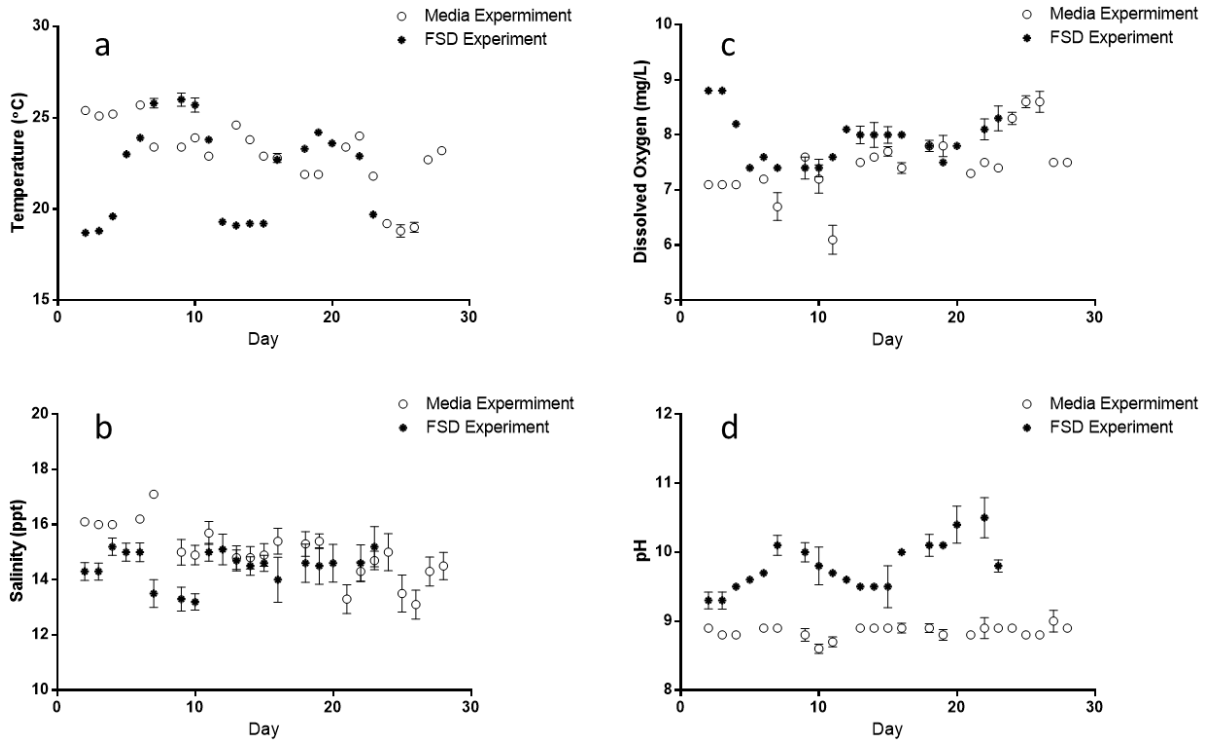

Figure 2.3: Daily water quality measurements. Data reflects average of all twelve aquaponic systems run simultaneously where: "first series" was media experiment systems and first block of FSD experiment systems and "second series" is the second block of FSD experiment systems. Error bars show standard deviations.

2.3.2 Media Experiment

The TAN concentration reached a maximum of 0.16 ± 0.01 and was within safe limits for fish over the entire experiment (Ebeling and Timmons, 2002) (Figure 2.4). No significant ($\rho < 0.05$) differences in TAN concentration were found between the expanded clay and coconut fiber or between the planted and unplanted treatments (Table 2.1).

Nitrite concentrations fluctuated over the duration of the experiment, with an increase in nitrite concentration observed over the first 15 days. On day 15, one ENP replicate had a concentration of 0.49 ± 0.04 mg/L NO_2^--N and the other of 0.08 ± 0.01 mg/L NO_2^--N, resulting in a high average concentration with a large standard deviation. Similar variations were also

observed for the prior sampling days. The coconut fiber treatment and planted treatment had significantly lower nitrite concentrations ($\rho < 0.05$); however, in all four treatments the concentrations were < 1.0 mg/L NO_2^--N and not a concern for fish health (Ebeling and Timmons, 2012) (Table 2.2).

Table 2.1: Results of two-way ANOVA on water quality for media experiment.

Effect	ρ		
	TAN	NO_2^--N	NO_3^--N
Media	0.160	0.013	0.002
Plants	0.169	0.012	0.016
Media x Plants	0.292	0.943	0.913

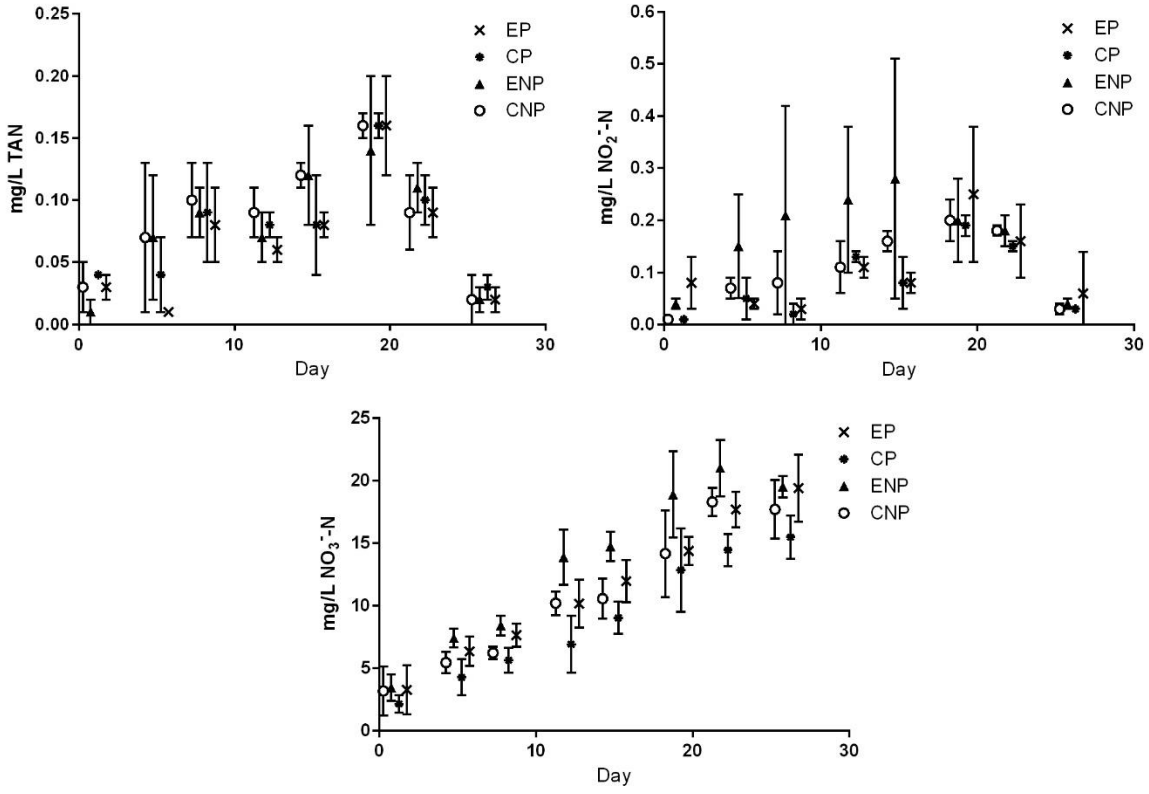

Figure 2.4: Concentrations of TAN, NO_2^--N, and NO_3^--N measured the media experiment. Points are average of two treatment replicates and bars show standard deviation. EP: expanded clay/plants; CP: coconut fiber/plants; ENP: expanded clay/no plants; CNP: coconut fiber/no plants.

Nitrate concentrations steadily increased in all four systems. Nitrate concentrations were significantly ($\rho < 0.05$) lower in the treatments with coconut fiber and treatments with plants.

The mean nitrate concentration followed the following order where ENP > EP > CNP > CP. An ANOVA comparing all four treatments showed that in the CP system the mean nitrate concentration was significantly ($\rho < 0.05$) less than that in the ENP system.

Table 2.2: Mean TAN, NO_2^--N, and NO_3^--N concentrations with standard deviations for each treatment condition in the media experiment.

mg/L	Plants		No Plants	
	Expanded Clay	Coconut Fiber	Expanded Clay	Coconut Fiber
TAN	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
NO_2^--N	0.10 ± 0.01	0.08 ± 0.10	0.17 ± 0.02	0.11 ± 0.01
NO_3^--N	11.4 ± 0.80	8.84 ± 0.72	13.4 ± 0.91	10.7 ± 0.81

No significant ($\rho < 0.05$) difference was found in plant RGR between the two media types. In both media types one replicate appeared to thrive and grow well, while the other replicate performed poorly with low biomass gains. The biomass gains per plant were 1.04 ± 1.5 g wet weight and 1.08 ± 1.4 g wet weight for the coconut fiber and expanded clay treatments, respectively. The harvest yield was 0.67 ± 0.26 kg/m^2 and 0.66 ± 0.24 kg/m^2 in the coconut fiber and expanded clay treatments, respectively.

2.3.3 FSD Experiment

In the FSD experiment, TAN concentrations reached a maximum of 0.30 ± 0.51 mg/L TAN. No significant ($\rho < 0.05$) differences were observed in TAN concentrations between flows, densities, or plant species nor were the interactions significant (Table 2.3).

Nitrite concentrations were high in the HPL system on days 19 and 22. For all other systems and sampling days nitrite concentrations did not surpass 0.2 mg/L NO_2^--N. No significant ($\rho < 0.05$) difference was found between flows, densities, or plant species for nitrite concentrations. There was a significant ($\rho < 0.05$) difference in nitrite concentrations between the two experimental blocks and there were significant interactions between all treatment conditions.

Table 2.3: Results of two-way ANOVA on water quality for FSD experiment.

Effect	ρ		
	TAN	NO_2^--N	NO_3^--N
Block	0.888	<0.05	<0.05
Flow	0.664	0.122	0.754
Species	0.565	0.253	0.227
Density	0.842	0.755	0.237
Flow x Species	0.646	0.000	0.341
Flow x Density	0.623	0.000	0.615
Species x Density	0.358	0.000	0.863

Similar to the media experiment, nitrate concentrations increased over the sampling period (Figure 2.5). The maximum nitrate concentration was 21.0 ± 2.3 mg/L NO_3^--N in the HPH system. The mean nitrate concentration was lower in the FSD experiment than the media experiment (Table 2.4). No significant difference ($\rho < 0.05$) in nitrate concentration was found between factors or interactions although, there was a significant difference between blocks. An ANOVA comparing mean nitrate concentration in all eight treatments showed that the LPL system was significantly ($\rho < 0.05$) lower than HPH and HSH treatments.

Table 2.4: Mean TAN, NO_2^--N, and NO_3^--N concentrations with standard deviations for each treatment condition in the FSD experiment.

	Flow		Density		Species	
mg/L	High	Low	High	Low	Sea purslane	Saltwort
TAN	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.00	0.09 ± 0.01
NO_2^--N	0.08 ± 0.01	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.09 ± 0.01	0.05 ± 0.00
NO_3^--N	5.2 ± 4.7	4.8 ± 4.2	4.8 ± 4.5	5.2 ± 4.5	6.5 ± 5.2	3.5 ± 3.1

No significant ($\rho < 0.05$) difference was found in the RGR between flows, densities, or plant species. All systems had an overall increase in plant biomass, although some plants lost weight and there were seven mortalities. Initial cuttings for all systems ranged from 1.19 ± 0.41 g wet weight to 2.94± 0.75 g wet weight. Systems with larger plant cuttings, indicated by a higher mean initial weight, had a greater mean increase in weight than the smaller plant cuttings.

The mean yields for sea purslane, 0.53 ± 0.09 kg/m^2, were greater than the saltwort at 0.32 ± 0.06 kg/m^2.

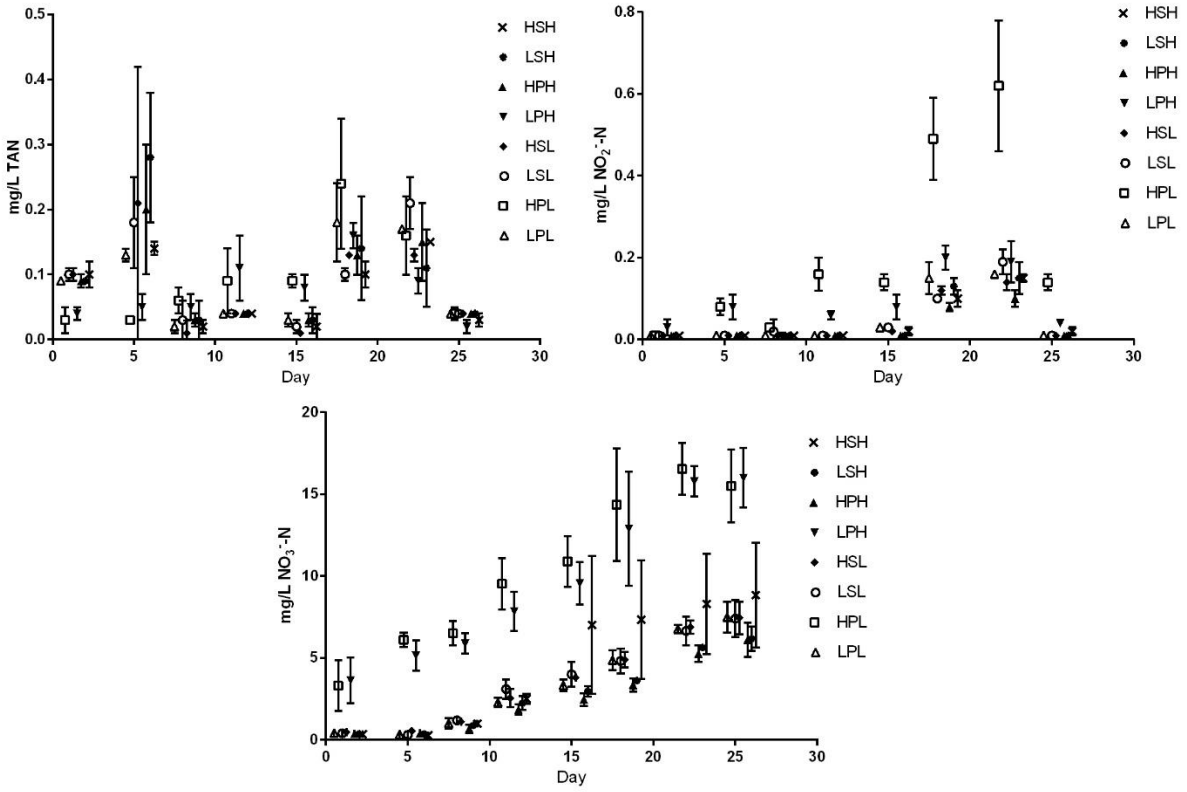

Figure 2.5: Concentrations of TAN, NO_2^--N, and NO_3^--N measured the FSD experiment. Points are averages of two treatment replicates and bars show standard deviation. HSH: high flow/saltwort/high density; LSH: low flow/saltwort/high density; HPH: high flow/sea purslane/high density; LPH: low flow/sea purslane/high density; HSL: high flow/saltwort/low density; LSL: low flow flow/saltwort/low density; HPL: high flow/sea purslane/low density; LPL: low flow/sea purslane/low density.

2.3.4 Fish

In both experiments adult fish were stocked based on an average total weight of 30 g per tank. At the end of the media experiment, average final biomass was 31.8 ± 6.1 g and 24 mortalities were recorded. At the end of the FSD experiment, the average final biomass was 28.7 ± 3.0 g and 1 mortality was recorded.

2.4 Discussion

2.4.1 Media Experiment

It is generally assumed in aquaponic systems that plant growth and nutrient uptake significantly contribute to the quantity of nutrients removed (Rakocy, 2012). While the body of published research on aquaponics supports this idea, the majority of studies were conducted in freshwater aquaponic systems. The purpose of the media experiment was to investigate whether similar results could be achieved in a marine aquaponic system with halophytes. Regardless of media type, treatments with plants had lower mean nitrate concentrations than the no plant treatments (Figure 2.4). Similarly, Snow and Ghaly (2008) produced barley hydroponically on aquaculture wastewater and found the presence of barley resulted in significantly lower nitrate concentrations. A similar study by Ghaly et al. (2005) suggested barley removed 95.9-99.8% of excess nitrate and attributed that removal to plant uptake.

Enhanced nitrogen removal has also been observed in constructed wetlands with plants (Gagnon et al., 2007). Instead of plant growth, denitrification is often considered the main nitrogen removal process in constructed wetlands (Vymazal, 2007). Plants contribute indirectly to denitrification by emitting oxygen from roots, which facilitates localized nitrification followed by denitrification in the predominantly anoxic wetlands soils (Gersberg et al., 1986). Due to variations in wetland designs, such as plant species, wastewater strength, nutrient loading rate, and environmental conditions, the reported contribution of plants to direct nitrogen uptake ranges from 3% to 47% (Gottschall et al., 2007; Koottatep and Polprasert, 1997). In hydroponic systems, plants are not subjected to the same level of resource competition found in soil systems where soil microorganisms are able to outcompete plant roots for inorganic nitrogen (Jones et al., 2005). As such, greater nitrogen uptake by plants in hydroponic systems may be due to a

combination of abundant access to dissolved inorganic nitrogen and limited microbial competition. Alternatively, studies on aquaponic systems may have underestimated the amount of nitrate removal due to denitrification and instead overestimated nitrogen removal through plant uptake.

Media type also significantly impacted nitrogen removal, such that systems operated with coconut fiber as the potting media had lower nitrate concentrations. Studies by other researchers using scanning electron microscopy showed that coconut fiber has a high porosity, which corresponds with attachment surfaces for microbial populations (Fornes et al., 2003). In addition to surface area, coconut fiber can leach carbonaceous chemical oxygen demand (COD) and provide an organic carbon source for denitrifying bacteria (Weragoda et al., 2010). The added COD is important in dilute aquaculture wastewater in which denitrification can be limited by lack of an organic carbon source. Manoj and Vasudevan (2012) treated aquaculture wastewater with coconut coir in a packed column bioreactor and found it to successfully remove nitrate and COD through denitrification.

In wetlands, simultaneous nitrification-denitrification occurs even in oxygenated waters. Anaerobic microsites develop from microbial respiration and rapid aerobic degradation of organic carbon, which depletes pore space oxygen levels (Hamersley and Howes, 2002). In this study, development of anaerobic microsites and subsequent denitrification were aided by the large surface area and readily available organic carbon produced by the coconut fiber and plant roots. Despite bulk DO concentrations in this study, between 6.1 and 8.8 mg/L, the lower nitrate concentrations in systems with coconut fiber compared to expanded clay indicate coconut fiber aided denitrification. Considering the presence of anaerobic microsites, studies which have

assumed denitrification does not occur in aerated hydroponic plant beds likely overestimate nitrate removal through plant uptake (Snow and Ghaly, 2008).

As in freshwater systems, the results of this study indicate that plants significantly impact nitrogen removal in marine aquaponic systems. The results also indicate media selection can impact nitrogen removal, most likely by aiding denitrification, which has not been previously demonstrated in aquaponics. Coconut fiber was selected as the planting media for later experiments due to the lower nitrate concentrations observed in the media experiment. Furthermore, the results of this experiment show that coconut fiber can serve as an effective alternative to peat, which is important considering the vast availability of coconut coir and concerns over the future availability of peat moss.

2.4.2 FSD Experiment

In the FSD experiment, the impacts of plant density, plant species, and flow rate on nitrogen removal were evaluated. These parameters had no impact on TAN concentration, indicating that the biofilter provided sufficient nitrification. Similarly nitrite concentrations remained low throughout the experiments, indicating that there was complete biological oxidation. Marine biofilters often require a long acclimation period and the initial acclimation of the biofilter media and the reuse of media between experiments prevented incomplete biological oxidation and nitrite accumulation (Gutierrez-Wing and Malone, 2006).

In aquaponics, a careful balance between fish biomass and plant biomass is necessary to maintain adequate water quality and simultaneously provide plants with sufficient nutrients. In industry, some standard ratios of fish feed to plant growing area have been developed. Rakocy et al. (2006) recommended a range of 60-100 g feed/m^2 in a system that produced tilapia (*Oreochromis* sp.) and freshwater vegetable crops. Endut et al. (2010) recommended a range of

15-42 g feed/m^2 in a system that produced African catfish (*Clarias gariepinus*) and water spinach (*Ipomea aquatic*). Ratios of feed to hydroponic growing area did not account for variations in plant species and planting density in this study; 14 g feed/m^2 was used for both plant densities and this value was selected based on feasible fish density and expected plant growth rates.

Considering the equal nitrogen inputs from feed, the higher density systems (184 plants/m^2) maintained equivalent nitrate concentrations as the lower density (92 plants/m^2) without any visible detriment to plant health. Although the duration of the experiment was short and plants did not reach a harvestable size, the continuous increase in nitrate concentrations indicated that these systems have the potential to support even greater densities of sea purslane or saltwort at similar feeding rates. A cereal crop, such as barley, can be packed more densely as in Snow and Ghaly (2008), where barley seeds were broadcast covering the entire growing surface with vegetation. Densities of 200, 250, and 300 g of seeds per 0.15 m^2 tray maintained nitrate concentrations between 5.31 and 6.46 mg/L, with no difference in nitrate concentration at higher densities. Alternatively the aquaponic system in Rakocy et al. (2009) grew different varieties of lettuce at a planting density of 16 to 20 plants/m^2 and maintained a nitrate concentration of 0.4 to 69.4 mg/L NO_3^--N over three years. The required harvest size of lettuce heads prevents placing plants closer together. The final product size, value, and consumer expectations heavily influence the planting densities required for profitable hydroponic production.

When this study was initiated, there was no commercial market for the edible halophytes being tested and therefore, no consumer expectations for the product's appearance. As the halophyte market develops, the impact of planting density on consumers' preference for product appearance may change. A study on a similar edible halophyte species, *Salicornia*, found that

low-density planting increased the incidence of plant lateral growth and branching, while at higher densities the plants grew more vertically (Webb et al., 2013). Plant species that can tolerate higher densities could be advantageous to greenhouse aquaponic systems where space is limited.

Flow rates in conventional RAS are based on fish tank turnover rates, which are related to biofilter size and efficiency. Depending on the culture system, fish tank turnover rates can vary from 15-60 minutes (Ebeling and Timmons, 2012). In constructed wetlands slow flow rates and long HRTs are preferred for better nutrient removal (Kadlec and Wallace, 2009). Based on literature, the high flow rate of 1 L/min and tank turnover rate of 38 minutes was selected as appropriate to maintain fish tank turnover rates. The slower 0.5 L/min flow rate with a tank turnover of 76 minutes was expected to be better for nutrient removal. Neither flow rate had adverse effects on fish health, nor did flow rate impact nutrient concentrations indicating aquaponic systems can function without the low flow rates often required for treatment in constructed wetlands. While not necessary for plant growth, low flow rates offer the advantage of reduced electricity requirements and lower operational costs.

2.4.3 Plant Growth

Both plant species were successfully grown from cuttings. Plants were added to the systems six days before the fish to allow the plants to begin developing roots. After 7-10 days sea purslane had visible root structures. Saltwort typically took longer to become established and roots were visible in 10-14 days. The results of the media experiment indicated that expanded clay and coconut fiber were both adequate support media for sea purslane and resulted in no difference in the RGR.

The results of the FSD experiment indicated neither flow rate, plant density, or plant species impacted plant growth. Endut et al. (2009 and 2010) found that at a HLR of 1.28 m/day water spinach had significantly greater growth than the lower HLR of 0.64 m/day and suggested that at the lower HLR the plants may have been nitrogen limited. In this study, both HLRs were greater than in Endut et al.'s studies, which could have prevented the plants from being nitrogen limited. Plant density can also cause nutrient limitations, although deficiencies ultimately depend on the ratio of plants to fish. In Snow and Ghaly (2008), seed quantity was not found to impact plant height. However, when the plant to fish ratio was increased in Endut et al. (2010) the higher plant quantities were correlated with decreased plant growth. At the higher density of 184 plants/m^2 in this study, plants were not nutrient limited and greater densities could likely be sustained, raft space permitting.

Not all plant species are appropriate for hydroponic growth and some species do not perform well due to susceptibility to disease or micronutrient requirements (Ghaly et al., 2005; Waller et al. 2015). Saltwort and sea purslane have wide geographical ranges and both can survive in environments that experience periodic flooding (Hartmann, 2002; Lonard et al., 2011). Previous work with these species in our laboratory indicated that they could be grown constantly submerged, in 10-20 cm deep aquaculture effluent, while supported in soil (unpublished data). The successful growth of both species in these experiments answered two questions. First, that both species can be grown from cuttings and second, that they can be grown hydroponically.

The mean yields of 0.53 ± 0.09 kg/m^2 for sea purslane and 0.32 ± 0.06 kg/m^2 for saltwort were lower than many other studies. The short growth period of less than 30 days was one factor that contributed to low yields. Water spinach and mustard greens reach production size more quickly and in a month could produce 2.14 kg/m^2/month and 1.64 kg/m^2/month, respectively

(Endut et al., 2011). Barley can also be produced with a short 21 day period and yields of 2.5 to 5.9 kg/m^2 are achievable (Snow and Ghaly, 2008). Barley and water spinach are both species that can be densely packed, conversely sea purslane and saltwort may not have responded well to higher densities. In contrast to the results of this study, the halophyte *Salicornia dolichostachya* was grown hydroponically at a density of 19 plants/m^2 in an aquaponic system and after 35 days final plant weights were 20-44 times greater than the final weights in this experiment (Waller et al. 2015). Similarly, Shpigel et al. (2013) was also able to produce halophytes in wetlands at high densities of 90-100 plants/m^2 without limited growth. While increasing nitrate concentrations in this study indicate the plants were not nitrogen limited they may have been limited by physical factors such as space. The conflicting results indicate that more research needs to be done on these species to determine why their growth was limited in these bench-scale aquaponic systems and what conditions are needed to grow a commercially viable product.

2.4.4 Fish Growth

No increase in average weight was expected as all the fish stocked were adults and had reached a maximum size. The 24 mortalities in the first experiment were due to the design of the airlift system and were unlikely related to water quality. Most of the mortalities were found on the surface of the biofilter, indicating that the airlift pump had sucked up the fish. In the FSD experiment the aeration was reduced in the airlift pump, and fewer mortalities were recorded.

2.5 Conclusion

RAS are highly water and waste efficient production systems; however, further technological improvements have the potential to develop near-zero discharge systems in which dual products improve water treatment efficiency and increase overall product yields. The results of this research demonstrated that halophytes can improve water treatment in a marine aquaponic

system. Specifically, the species sea purslane and saltwort were successfully grown for the first time in a floating raft style aquaponic system. While plants contributed significantly to nitrogen removal, flow rate and by association the HLR did not impact nitrogen removal or plant growth. The ability to operate at a lower flow rate could translate into reduced operational costs due to lower pumping electricity requirements. Planting media were also evaluated and coconut fiber contributed to greater nitrogen removal when compared to expanded clay. Due to the greater nitrogen removal from coconut fiber and the advantages of co-opting what is otherwise a waste product, coconut fiber was selected as the media in a prototype commercial-scale marine aquaponic system (Boxman et al., 2015a).

Considering the limited information available on commercial production of halophytes, the bench-scale systems used in this study could be an effective screening tool to evaluate hydroponic production of halophytic plant species. In this study, information was quickly gathered about the ability to produce plants from cuttings, capacity for hydroponic growth, and production rates. While the results indicated lower growth rates than some other plant species, it is important to remember halophytes have potential as high-value luxury cash crops. Hydroponic production of cereal crops, such as barley, or a low-value crops, such as water spinach, need exceptionally high growth rates to compete with large-scale agricultural production and to be economically viable. Still, more research is needed to develop sea purslane and saltwort for commercial markets. The larger prototype system with an edible fish species described in Chapter 3 provides more information about commercial production of halophytes and the commercial viability of marine aquaponics.

Chapter 3: Evaluating Nitrogen and Phosphorus Transformations and Halophyte Production in a Marine Aquaponic System

3.1 Introduction

Aquaculture is the fastest growing food production industry, with an average growth rate of 8.8% annually over the last 30 years (FAO, 2012b). Growth of this industry has occurred predominately for freshwater species. As of 2012, farmed marine and brackish species accounted for only 15% of finfish aquaculture production and 3.5% of total finfish production from capture fisheries and aquaculture combined (FAO, 2012a). Considering that marine fish stocks are seriously threatened by overfishing and environmental pollution (Srinivasan et al., 2010), development of environmentally sustainable marine aquaculture systems can reduce pressure on threatened stocks and the environment while still providing marine fish products. Global aquaculture production has the potential to play a key role in eliminating hunger, improving health, and providing employment (FAO, 2014). Furthermore, marine aquaculture represents a yet untapped area for aquaculture growth.

Marine aquaculture often requires access to high-value, ecologically sensitive coastal areas that are in competition with other uses, such as real estate, navigation, industry, and recreation (Primavera, 2006). However, development of land-based marine aquaculture has been constrained by limited options for disposal of saline wastewater. While recirculating aquaculture systems (RAS) reduce discharges to the environment to less than 10% of total system volume per day, water exchanges are still required to prevent buildup of dissolved inorganic nitrogen species (Masser et al., 1999). As a result, marine RAS must be located adjacent to saline water bodies

for discharge. Therefore, development of 100% recirculating, zero-discharge aquaculture systems would aid expansion of marine aquaculture and provide greater flexibility in location.

The integration of plant production with aquaculture can further improve water treatment, potentially eliminating nutrient discharges. Plants are frequently used for water treatment in constructed wetlands where they remove nutrients through direct uptake and by facilitating microbial growth (Vymazal, 2005). Constructed wetlands are commonly applied as end-of-pipe treatments, although they have also been incorporated into RAS to improve recirculation rates (Boxman et al., 2015b; Lin et al. 2005; Tilley et al. 2002). Aquaculture water can also be treated through hydroponic plant production, where plants are grown without soil and roots are in constant contact with system water (Rakocy, 2012). This combination of aquaculture and hydroponics is known as aquaponics. Aquaponics most often combines freshwater fish (tilapia, trout, catfish) with production of edible plants (lettuce, basil, tomatoes). Floating raft aquaponics, in which plants are grown in net pots and suspended in polystyrene rafts over 20-40 cm of water, is most commonly used in commercial systems (Love et al., 2015)

To apply aquaponics to marine aquaculture, plants adapted to saline water must be produced. While some freshwater plants have been grown in saline conditions, the modification of freshwater plants to tolerate saltwater has been largely unsuccessful (Flowers, 2004). Alternatively, naturally salt tolerant halophytes do not require genetic modification to be grown in saline water. A number of halophytic plant species have been used successfully to treat marine aquaculture effluents (Buhmann and Papenbrock, 2013). In aquaponics, it is important that viable commercial species are selected. Halophytic wetland plants, such as mangroves, can be used for coastal restoration (Boxman et al., 2015b), but economic returns depend on how committed the location is to protecting sensitive coastal habitats. Ventura and Sagi (2013)

compiled a list of 14 edible halophytes which includes: *Salicornia* sp., *Sarcocornia* sp., *Batis maritima* (saltwort), and *Sesuvium portulacastrum* (sea purslane). While some research exists on the nutritional value and cultivation methods for these species, they are still relatively foreign to consumers, outside of some European countries were they are growing in popularity, and cultivation methods are ill-defined. Commercialization of edible halophytic plants will depend on the ability to market their nutritional qualities and the development of efficient cultivation methods.

The overall goal of this study was to collect detailed information on the operation and nutrient cycling in a marine aquaponic system in order to move beyond the prototype stage and develop a commercial-scale marine aquaponic system that produces edible halophytes. The specific goals were to: 1) characterize nitrogen and phosphorus transformations and removal in a marine aquaponic system; 2) determine the nutrient removal capacity of the halophytes, sea purslane and saltwort; and 3) evaluate the growth and production of the halophytes sea purslane and saltwort.

3.2 Brief Literature Review on Halophytes

Most terrestrial plants are considered glycophytes, or plants that are easily damaged when in contact with saltwater. In contrast, halophytes are adapted to saltwater, although the concentration of salt tolerance varies with species. The salt tolerance of halophytes is of growing importance for two reasons: 1) constricted freshwater supplies and increased prevalence of soil salinization has encouraged the development of food crops adapted to survive in saline soils or irrigation water; and 2) greater development of inland, marine aquaculture will require the capability to treat saline effluents. The purpose of this literature review is to provide general

background information on edible halophytes and highlight some of the literature specific to using edible halophytes species for water treatment.

3.2.1 Halophytes as a Food Crop

Coastal salt tolerant plants have been collected for food and medicinal purposes for thousands of years. These plants are typically harvested by foraging for wild plants rather than through cultivation of domesticated plants. Recently, interest has grown in developing domesticated varieties of halophytic plants that can be marketed for consumption on a larger scale.

Many halophytic species have the potential to be developed into products for human consumption, animal fodder, food oil, medicinal uses, or cosmetic uses (Debez et al., 2011). Some species that have been previously studied include: *Aster tripolium, Batis maritima, Portulaca oleraceae, Sesuvium portulcastrum, Salicornia sp.* The species most commonly cited for use as a potential food or forage crop is *Salicornia* sp. (Ventura and Sagi, 2013). Multiple species of *Salicornia* have been studied including: *Salicornia europaea*, *Salicornia bigelovii*, and *Salicornia herbacea*. Research on the nutritional value of *Salicornia* indicates that it is a good source of minerals, protein, and vitamins (Ventura et al., 2011). Additionally *Salicornia* contains more omega-3 fatty acids than spinach, lettuce, or mustard greens (Ventura et al., 2011). In Europe, markets for *Salicornia europaea* and *Salicornia bigelovii* have already been developed and these halophytes are sold as vegetables (Böer, 2006).

Ideally plants selected for domestication and commercialization should have high nutritional value as well as high productivity. Although many halophytes have been proposed as new food sources, little information is available on their nutritional content. Species, such as *Salicornia*, are an exception and are often used to justify the likelihood of similar nutritional

characteristics in other halophytes. Debez et al. (2011) suggested that the development of domesticated halophytes should begin with plants available locally as they are already adapted to the climate, which should aid production.

For the purpose of this dissertation, halophyte species with native ranges within the state of Florida were considered to be "local" and were assumed to be adapted to the climatic conditions found at the research site in Sarasota, FL. Two halophytic species that met these criteria that are also known to be edible were selected: *Sesuvium portulcastrum* and *Batis maritima* (Figure 3.1).

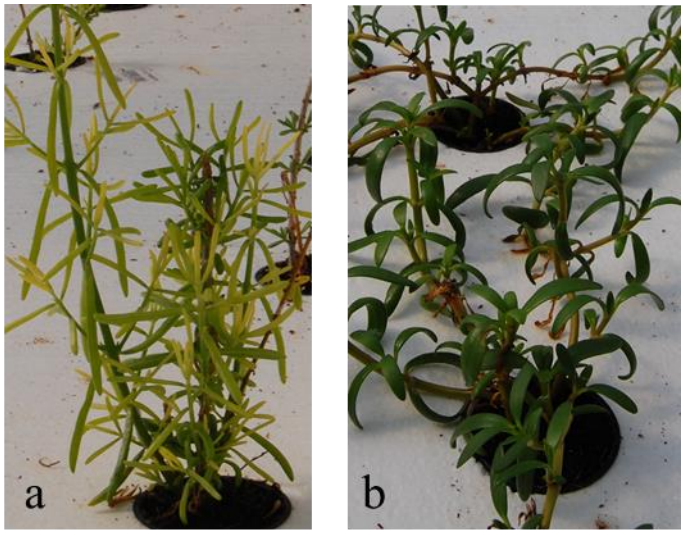

Figure 3.1: Plants selected for study. (a) saltwort (*Batis maritima*), (b) sea purslane (*Sesuvium portulcastrum*).

Sesuvium portulacastrum, also known as sea purslane, grows along coastal areas of Florida, and can also be found from Texas to North Carolina, in the Caribbean, Hawaii, and many other coastal areas globally (Duncan and Duncan, 1987). The plant is typically found along sand dunes at the high tide line (Gilman, 1999). It has fleshy succulent like leaves and small pink flowers.

Sesuvium portulacastrum has been used in traditional medicine throughout the world (Magwa et al., 2006). A study on essential oils found in *Sesuvium* sp. revealed that they have antibacterial, antifungal, and antioxidant properties (Magwa et al., 2006). Lokhande et al. (2013) provided an estimate of the nutritional content for some components, which are shown in Table 3.1. Additionally, *Sesuvium* sp. is a good source of phytoecdysteroids or insect molting hormones, which are used in the silk industry to regulate silkworm production (Lokhande et al., 2009). In the nutritional supplement industry, ecdysteroids have been shown to improve protein synthesis and help build muscle (Lokhande et al., 2013). *Sesuvium portulacastrum* also has the ability to remediate saline soils and can accumulate up to 872 mg Na^+ per plant (Rabhi et al., 2010). The ability to desalinate soils via plant uptake could be of great benefit to the estimated 6% of land area affected by soil salinization (Flowers and Yeo, 1995).

Batis maritimia is commonly called saltwort, although it is also known by the common names turtleweed, pickleweed, and barilla among others (Lonard et al., 2011). It can be found globally including in coastal areas in the southeastern United States as well as many parts of South America and the Caribbean (Lonard et al., 2011). The plant has woody stems and small green to green-yellow fleshy leaves.

Both the fleshy tissue of *Batis maritima* and the small fleshy seeds have potential commercial value. An in-depth analysis of the nutritional content of *Batis maritima* seed was completed by Marcone (2003) in which amino acids, fatty acids, vitamin E, carbohydrates, and other parameters were measured and compared with cereal crops. The seed of *Batis maritima* was found to have a high protein and oil content of 17.3% and 25.0%, respectively (Marcone, 2003). The fatty acid profile indicates it could be a viable oil seed and a nutritional supplement due to the high linoleic acid content. The phytosterol content, which has been shown to reduce

cholesterol levels in humans, is potentially valuable to the pharmaceutical industry (Marcone, 2003). These are just a small selection of potential applications for the seed and does not include any non-food applications.

Table 3.1: Nutritional content of two species selected. Information for *Sesuvium portulacastrum* from Lokhande et al. (2013); information for *Batis maritima* from Marcone (2003). The methods for analysis were not necessarily the same. N/A indicates information not available.

	Sesuvium portulacastrum	*Batis maritima* (seed)
Calories	223	N/A
Protein	10.2%	17.3%
Fat	0.24%	25%
Ash	33%	3.6%
Crude Fiber	9.9%	N/A
Carbohydrates	44.5%	46.5%

3.2.2 Halophytes for Water Treatment

Constructed wetlands are an established method for treating domestic, industrial, and agricultural wastewater. In constructed wetlands, macrophytes, or higher order plants, contribute to nitrogen removal directly through growth and uptake or indirectly by facilitating microbial growth, nitrification, and denitrification (Koottatep and Polprasert, 1997). Macrophytes also contribute directly to phosphorus removal through growth and uptake, although other important mechanisms include microbial uptake, sorption, and precipitation (Menon et al., 2013). Many of the plant species commonly used in constructed wetlands are freshwater or brackish water species that cannot tolerate high salinity conditions (Wu et al., 2008). The use of constructed wetlands for marine aquaculture requires the use of saltwater tolerant halophytes.

A variety of plant species have been used to treat saline water including mangroves, coastal grasses, and some of the edible halophytes mentioned above. Buhmann and Papenbrock (2013) provide a list of halophyte species used in constructed wetlands. In a prior study in our laboratory, Boxman et al. (2015b) used wetland plant species for treatment of marine aquaculture wastewaters. The species were harvested and sold for coastal restoration projects. However, for

the purposes of this review, only studies of constructed wetlands planted with halophytes that can be consumed by humans or animals will be included. Generally, the mangrove and grass species are used for water treatment and do not have secondary consumptive uses.

Constructed wetlands are often used for end-of-pipe treatment, where the wetland is the final step before discharge to the surrounding environment. A study by Webb et al. (2012) evaluated filter beds planted with *Salicornia europaea* to treat effluent from a commercial marine fish and shrimp RAS (Table 3.2). The plant filter beds removed most of the inorganic nitrogen species, with removals of 91 ± 12% to ~100%, 90 ± 9% to ~100%, and 91 ± 4 to ~100% for ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-), respectively. During the first 58 days, when operated at ambient loading conditions, the influent concentrations varied with overall mean influent concentrations for inorganic nitrogen species of 2.7 ± 1.1 mg/L NH_4^+-N, 0.24 ± 0.13 mg/L NO_2^--N, and 0.53 ± 0.43 mg/L NO_3^--N. The authors also measured nitrogen and phosphorous in plant tissue. Based on this information they estimated that 85% (15.3 g N/m^2) of the nitrogen was retained in plant tissue and 73% (2.48 g P/m^2) of dissolved inorganic phosphorous was retained in plant tissue.

In Webb et al. (2013), nine small constructed wetlands planted with *Salicornia europaea* were evaluated at different planting densities. On the first harvest date, significantly more fresh weight biomass was harvested per m^2 in the high density wetlands. Subsequent harvests showed no significant difference in harvested biomass, indicating that the beds equalized after planting. The pooled nitrogen uptake for both high and low density beds was 8.68 g N/m^2/d and there was no significant difference in removal efficiencies between the high, low, or unplanted beds. Typically ammonium was the major constituent of dissolved nitrogen in the influent, with concentrations ranging from 12 to 23.6 mg/L NH_4^+-N; nitrate was a minor constitutent with

Table 3.2: Summary of studies where edible halophytes were used to treat aquaculture effluent.

Reference	Species	Description of planting area	Planting area (m^2)	Planting density	Flow rate	Hydraulic loading rate	Recirculating
Webb et al. (2012)	*Salicornia europaea*	Constructed wetland style with lined bottom filled with sand and limestone	14.5 per bed (43.5 total)	90 plants/m^2	Flood and drain	Not specified	No
Webb et al. (2013)	*Salicornia europaea*	Constructed wetlands as described in Webb et al. (2012)	4 per bed	High density 10,000 plants/m^2 Low density 200 plants/m^2	Flood and drain	Not specified	No
Shpigel et al. (2013)	*Salicornia persica*	Constructed wetland with lined bottom and filled with graded gravel and sand	24.3 per bed	100 plants/m^2	0.5 m^3/hr	0.49 m/d	No
Lin et al. (2003)	*Phragmites australis*	Constructed wetlands, two systems operated in series with lined bottoms. One filled with soil and one filled with river gravel	5 per bed	>100 plants/m^2	0.16 m^3/hr	0.3 m/d	Yes
Waller et al. (2015)	*Salicornia dolichostachya*, *Tripolium pannonicum*, *Plantago coronopus* L.	Hydroponic bed with plants suspended in 0.35 m deep water	4.8 per bed (14.4 total)	38.5 plants/m^2	0.15 m^3/hr to hydroponic bed; 15 m^3/hr to biofilter	0.13 m/d	Yes

concentrations ranging from 0.5 to 33.7 mg/L NO_3^--N. On one sampling date the trend was reversed and the nitrate loading rate was much greater at 186.3 ± 6.4 mmol/m^2/d compared to the typical 0.1-12.8 mmol/m^2/d. At this time, the ammonium loading rate was 62.4 ± 3.3 mmol/m^2/d about one-third of the nitrate load. There was no significant reduction of nitrate which the authors suggested might be due to a preference for ammonium uptake by *Salicornia europaea*.

A study conducted by Shpigel et al. (2013) evaluated the species *Salicornia bigelovii* in a constructed wetland operated with two different flow regimes. A surface flow (SF) regime, where water was present above the substrate, and a subsurface flow (SSF) regime, where water flowed through a gravel and stone substrate. Effluent from a commercial, super-intensive, semi-recirculating aquaculture system was applied to the constructed wetlands at high and low nutrient loads. In the low loading conditions, TAN concentrations ranged from 1 µg/L to 99.8 µg/L and NO_x-N concentrations ranged from 11 µg/L to 253 µg/L over a 24 hour period. In the high loading conditions, TAN concentrations ranged from 3.3 mg/L to 3.9 mg/L and NO_x-N concentrations ranged from 5.7 mg/L to 9.4 mg/L over a 24 hour period. No significant differences were observed in plant yields with either flow regime or nitrogen load. In both flow regimes, a low nitrogen load resulted in greater nitrogen uptake by the *Salicornia* sp. This combined with a lower growth rate in the high loading conditions indicated that at higher loads the *Salicornia* sp. suffered from over fertilization. Unlike in Webb et al. (2012), the authors concluded that most of the nitrogen was removed by microbial activity, particularly in the high nitrogen loading experiments. Similar to Webb et al. (2013) they also concluded that the *Salicornia* sp. mainly contributed to TAN removal. Collectively the *Salicornia* sp. planted wetlands removed 6.57 g N/m^2/d and 7.94 g N/m^2/d for the surface flow and sub-surface flow, respectively.

Constructed wetlands can also be directly integrated into a RAS treatment train keeping treated water within the system. Published literature on the application of constructed wetlands for in-line treatment is less common for saline systems. Lin et al. (2003) provided an early example, operating free water surface (FWS) and SSF wetlands in-line to treat water from a shrimp RAS. The *Phragmites australis* used in that study was not an edible halophyte; however, the study is an important example of using constructed wetlands at the higher hydraulic loading rates necessitated by recirculation. A hydraulic loading rate of 0.3 m/d was applied to the constructed wetlands, which corresponded to hydraulic retention times of 0.5 and 0.26 days for the FWS and SSF wetlands, respectively. Typically constructed wetlands are designed to have low hydraulic loading rates of 0.0014-0.047 m/d (Metcalf & Eddy, 2014) to improve denitrification and total nitrogen removal (Schulz et al., 2003). Even with the higher loading rate in Lin et al. (2003) the constructed wetlands provided adequate treatment, reducing harmful inorganic nitrogen species to be within the safe ranges for fish health. The authors concluded that due to the low strength nature of aquaculture wastewater constructed wetlands can be operated at the higher hydraulic loading rates required by RAS and maintain sufficient nutrient removal.

More recently, Waller et al. (2015) grew edible saltwater halophytes on a side-stream of RAS water. Of the three species, the *Salicornia dolichostachya* had the greatest biomass at the end of the 35 day experiment and accumulated 167 mg N and 23 mg P per plant over that time. The authors calculated that the plants were able to uptake 24% of the excess nitrogen added daily through the fish feed. If the density was increased to 78 plants/m^2 100% of the excess nitrogen could be removed.

Based on the literature, halophytes are effective at treating saline aquaculture water. Halophytes have typically been used in constructed wetlands, although they can also be grown

hydroponically. Both Lin et al. (2003) and Waller et al. (2015) used halophytes as photosynthetic biofilters in RAS. When considering the production of halophytes as a secondary product an advantage to hydroponic plant beds as in Waller et al. (2015) could be more control over morphology and harvest regime. Webb et al. (2013) found that growing conditions impacted plant morphology and as the market for halophytes expands consumers will come to prefer and expect a specific plant form. Constructed wetlands might not allow the same consistent cultivation methods and predictable plant form as those that can be achieved in carefully controlled hydroponic environments.

It is clear that edible halophytes can simultaneously treat aquaculture system water, while increasing total caloric production per kg of fish feed in a RAS. However, several questions remain unanswered by the current literature. Waller et al. (2015) only extrapolated that hydroponically grown halophytes could remove all of the excess nitrogen from fish feed. Would hydroponically grown halophytes perform similarly when subject to the full flow of a RAS in a more aquaponic style system? Would the plants remove all of the excess nitrogen? Would they continue to do so over a longer period of time and as greater quantities of feed are added subsequently increasing nutrient levels? These are just a few of the questions that the commercial-scale marine aquaponic system was designed to help answer.

3.3 Materials and Methods

3.3.1 System Description

The study was conducted on an experimental marine aquaponic system located in Sarasota, FL. The system was housed in a polycarbonate greenhouse with two exhaust fans for ventilation. The hydroponic plant raceways were covered by a corrugated polycarbonate roof, which was rated at 30% shade. To provide shade and cooler temperatures for the fish tanks, a

55% shade cloth covered approximately 50% of the greenhouse for a total of 85% shading. A system diagram is shown in Figure 3.2 and a summary of system components and volumes are presented in Table 3.3.

Table 3.3: System components and volumes in the marine aquaponic system.

System Component	Volume (m^3)
Fish tanks (three 3.3 m^3 tanks)	9.9
Swirl separator	0.60
Upflow media filter	3.3
Backwashing sump	0.34
Moving bed bioreactor	5.4
Hydroponic beds (four 5.35 m^3 rectangular raceways)	21
Pumping sump	3.6
Partially submerged sand filter	4.6
Sand filter sump	1.3
Total	50

Three 3.3 m^3 fish culture tanks were stocked with red drum (*Sciaenops ocellatus*) on September 30, 2015, which was considered experiment day 0. Water flowed by gravity from the fish tanks to a 0.6 m^3 swirl separator (Wave Vortex, WLF36, Pentair, Apopka, FL) and then to an upflow media filter. After filtration, system water entered a moving bed bioreactor (MBBR). A target fish density of 21 kg/m^3 was used to determine both the MBBR media volume and system flow rate based on the recommendations given by Losordo and Hobbs (2000). To achieve sufficient TAN removal, the MBBR was packed with 1.8 m^3 Kaldnes media (Fureneset, Norway) to obtain a total surface area of 630 m^2 (Losordo and Hobbs, 2000). From the MBBR system, water was divided through four floating raft hydroponic plant beds operated in parallel. Four flow totalizers (Midwest Instruments, Model: 9002, Rice Lake, WI) were located in the influent pipes to the hydroponic plant beds and recorded flow rates of 38-61 L/min to each hydroponic plant bed. The hydroponic plant beds were constructed from wood and lined with polyethylene. Each hydroponic plant bed had dimensions 12.8 m x 1.2 m for a total growing area of 61.4 m^2. Aeration was added to the hydroponic plant beds using 3.8 cm x 3.8 cm fine-pore diffusers

placed every 1.2 m in the plant beds. A stand pipe located at the end of each plant bed maintained a water height of 40.6 cm. Effluent from the plant beds was recombined into one pipe and flowed to a 2.8 m diameter storage tank that contained a recirculation pump (Sweetwater High-Efficiency Pump, SHE2.4, Pentair, Apopka, FL). The system maintained a recirculation rate of 279 ± 26 L/min based on the requirements for TAN removal in the MBBR (Losordo and Hobbs, 2000). Fresh groundwater was added as needed to account for evaporation and evapotranspiration. It was added directly to the storage tank and monitored with a water meter (Badger Recordall®, Model 25, Badger Meter Inc. Milwaukee, WI).

The upflow media filter was constructed from a 2.3 m diameter tank packed with Kaldnes media (Fureneset, Norway) to a depth of 0.3 m. An aeration grid was located on the bottom of the upflow media filter, which was used to agitate the media for backwashing. Backwashing of both the swirl separator and upflow media filter was performed twice weekly until experiment day 157, after which time backwashing was performed three times weekly. System water removed during backwashing drained into a 1 m diameter sump tank, hereafter referred to as the solids sump. A submersible pump in the solids sump, operated by a float switch, pumped backwash water into a sand filter with dimensions of 12 m by 1.6 m. The sand filter was constructed in layers, with a bottom layer consisting of 15 cm depth of 3.8 cm gravel, a layer of polyethylene cloth, and a top layer consisting of 15 cm of 0.45-0.55 mm filter sand. A perforated pipe located underneath the sand collected filtered backwash, which then drained into a 1.8 diameter sump, hereafter referred to as the sand filter sump. Water in the sand filter sump was recirculated back to the upflow media filter via a submersible pump operated with a float switch. Initially unsaturated filtration was used, such that the sand filter dried out completely between backwashing events. On day 119 a 15.2 cm stand pipe was added to the sand filter, partially

submerging the filter with 10.2 cm of standing water and providing constant saturation. Solid matter (34 kg), which had accumulated on the surface of the sand filter, was removed on day 211. Prior to removal of solid matter, backwashing was suspended for six days, allowing the sand filter to dry out and facilitating harvest of the solids.

3.3.2 Fish Stocking and Measurement

Red drum were stocked at an initial density of 2.82 kg/m^3 with 200 fish per tank and an average weight of 46.5 g. On day 13 an additional 100 fish were added to each culture tank increasing the total biomass density to 4.23 kg/m^3. Fish were sampled monthly to determine average biomass and feed conversion ratio (FCR). All fish were removed from the aquaponic system and held in a separate RAS during experiment days 99 to 115. During this sixteen day period the fish tanks were elevated. When removed on day 99 the biomass density was 26.1 kg/m^3, which exceeded the MBBR design criteria. Therefore, when the fish were returned on day 115, the density was reduced to 22.04 kg/m^3. Fish were fed a manufactured diet (45% protein, 16% lipid) at 2.6% body weight/day (BWD).

3.3.3 Plant Stocking and Measurement

The saltwater vegetables, sea purslane (*Sesuvium portulacastrum*) and saltwort (*Batis maritima*), were stocked over two days on day 0 and day 2. Approximately 7-10 cm long cuttings of the two saltwater plant species were planted in net pots packed with coconut fiber and supported on polystyrene rafts floating in the raceways. Both plant species and coconut fiber media were selected based on the results of Chapter 2. Two to three cuttings per pot were added to obtain a total planting density of 47 plants/m^2 and a functional density of 19.5 net pots/m^2. Two hydroponic plant beds were stocked with saltwort and two with sea purslane. The saltwort was slow to adapt to the system, and one hydroponic plant bed of saltwort was replaced with sea

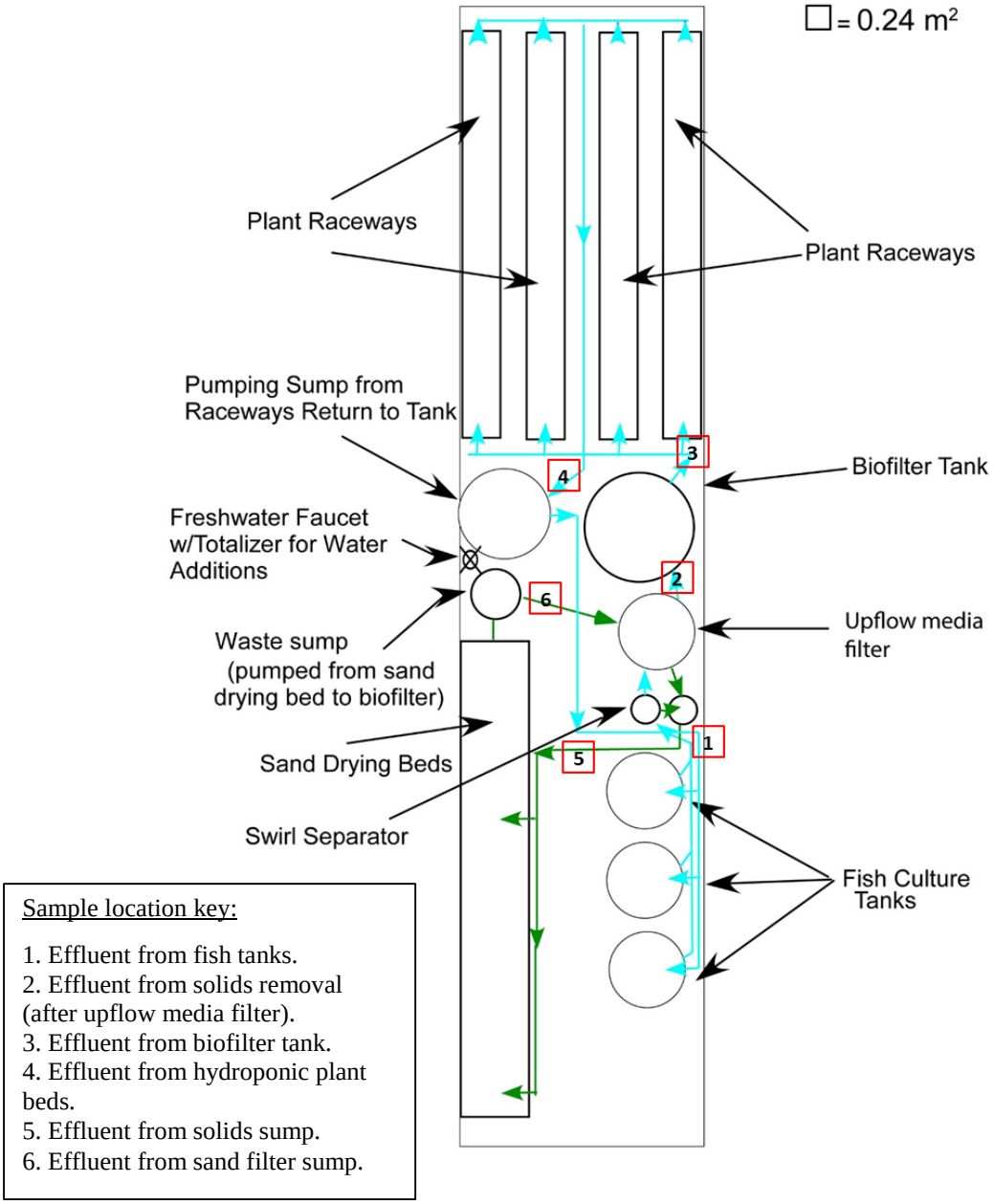

Figure 3.2: System diagram with sampling locations. Arrows indicate direction of system flows. Blue lines represent "main system" components and green lines represent "solids treatment" components.

purslane on day 129. Also at this time, in the remaining saltwort hydroponic plant bed, any dead or small saltwort plants were replaced with cuttings from the surviving saltwort plants.

Plant samples were collected twice monthly during the first 90 days of operation. Three net pots were randomly collected from each hydroponic plant bed using a coordinate grid system

and the random number generator in Microsoft Excel. Each contained two or three plant samples. On day 244 and 302, six additional net pot samples of each plant were collected randomly from among the hydroponic plant beds. Each plant was washed carefully to remove debris from the roots, separated into above and below ground weight, and dried at 80°C for 24-48 hours or until a constant weight. On day 108 harvesting of hydroponic plant beds began for sale and distribution where harvest is defined as trimming 15-40 cm pieces from the top of plants. The sea purslane was more productive and therefore it was harvested more regularly than the saltwort. In general, plant harvests were not structured and occurred when orders were placed by vendors or when labor was available, and at a sufficient frequency to maintain stable plant heights at the top of the hydroponic plant beds. During this period, high quality biomass was bundled into 113 g bunches (0.25 lb) for distribution and sale.

3.3.4 Water Sampling

Water samples were collected in triplicate, in 1 L acid washed (10%HCL) HDPE bottles from six points located throughout the system (Figure 3.2). During the first 83 days, samples were collected once weekly and analyzed for total ammonia nitrogen (TAN), nitrite (NO_2^-), total nitrogen (TN), total phosphorous (TP), chemical oxygen demand (COD), total suspended solids (TSS), and volatile suspended solids (VSS). Nitrate (NO_3^-) was analyzed twice weekly during this period. Beginning on day 118, sampling was reduced to twice monthly, for a total of six samples. At this point the biofilter was established and large variations in water quality were not expected. On day 188 sampling frequency was reduced to once monthly, for a total of three samples.

3.3.5 Analytical Methods

All water quality tests were adapted for use with saline water. Standard curves were made with a background salinity concentration of 15 ppt or 1.5 ppt depending on the chloride interference levels of the test. TAN was analyzed based on the method outlined in Bower and Holm-Hansen (1980) (method detection limit (MDL), 0.04 mg/L TAN); NO_2^- was analyzed using a combination of Standard Methods 4500-N B and Strickland and Parsons (1972) (MDL, 0.01 mg/L NO_2^--N); NO_3^- was analyzed based on Zhang and Fischer (2006) (MDL, 0.15 mg/L NO_3^--N); TN was analyzed based on a persulfate digestion in Standard Methods 4500-N C (MDL, 1.3 mg/L TN), TP was analyzed based on Standard Methods 4500-P B (MDL, 0.33 mg/L TP); COD was analyzed with Standard Methods 5220 D, an additional 0.5 g of mercury sulfate was added to sample vials to eliminate chloride interference (MDL, 3.1 mg/L COD); TSS and VSS were analyzed based on Standard Methods 2540 D & E. Total iron was measured once, on a grab sample collected from the fish tanks. The method was based on Standard Methods 3500-Fe B. No MDL was determined for this test, although the method suggested a MDL of 0.02 mg/L Fe. The off-flavor compounds geosmin and 2-methylisoborneol (MIB) were measured once, on the same grab samples collected to analyze Fe. Measurements were completed as described in Pettit et al. (2014).

Dried plant samples were finely ground with a burr grinder then analyzed for TN and TP. TN was analyzed on a TN 3000 Total Nitrogen Analyzer (Thermo Scientific, MA). The NIST standard reference material apple leaves (SRM #1515) were used to create the calibration line and peach leaves (SRM #1547) were used as an accuracy check. TP was analyzed using a persulfate digestion (Standard Methods 4500-P J) and an ascorbic acid colorimetric

determination (Standard Methods 4500-P E). A standard solution of potassium phosphate was used for the calibration line and apple leaves (SRM #1515) were used as an accuracy check.

3.3.6 Statistical Methods

Water quality data were presented as the mean of three replicate samples ± the standard deviation. Statistical analyses were completed with Minitab 16 Statistical Software (State College, PA) where $\rho < 0.05$ was considered significant for all analyses. Influent and effluent to system components such as the hydroponic plant beds were evaluated with paired t-tests. If necessary data was log transformed to meet assumptions for normality.

3.4 Calculations

In constructed wetlands, treatment efficiency is often calculated based on a mass or concentration removal efficiency for nutrients of interest (Chung et al., 2008). In this study, the concentration removal efficiency was calculated based on the following equation:

$$Removal\ efficiency = \frac{C_i - C_e}{C_i}\ x\ 100 \qquad \text{Eq. 3.1}$$

where C_i = concentration of influent, C_e = concentration of the effluent.

While concentration removal efficiencies are an important metric to evaluate treatment efficiency, they only give an indication of nutrient removal at a specific point in time. Furthermore, due to the recirculation within the aquaponic system and the short residence time within the hydroponic plant beds, it was important to consider removal over a greater time scale. For this reason, mass balances on nitrogen and phosphorus removal are presented for the whole system rather than for the hydroponic plant beds.

3.4.1 Nitrogen Mass Balance

Nitrogen entered the system through the addition of fish feed. Dissolved nitrogen was removed through three mechanisms: plant growth, denitrification in the sand filter, and passive

denitrification throughout the system. Figure 3.3 is a conceptual diagram of the mass balance completed on the liquid phase nitrogen present in system. The mass balance was carried out to determine the quantity of nitrogen associated with each source and removal mechanism using experimentally collected data, literature values, and through calculations. The following assumptions were used to complete the nitrogen mass balance:

1. The system control volume was 45,400 L. The sand filter was not included in the main system control volume.
2. Mean TN concentrations measured in the plant bed effluent represented the liquid phase nitrogen concentrations for the aquaponic system.
3. Over the first 33 days, nitrogen added from the feed accumulated in system water, the concentration measured on day 34 included this accumulation. This concentration multiplied by the system volume represented the initial mass of nitrogen within the system.
4. In total, 6.5% of wet-weight feed was in the form of nitrogen. 3.5% of wet-weight feed was excreted by fish as dissolved nitrogen, specifically TAN. The remaining nitrogen from feed was either incorporated into biomass (1.95%) or present as particulate waste (1.04%) (Appendix C).
5. All TAN emitted by fish or produced from mineralization of solids was converted to NO_3^- in the biofilter.
6. All particulate nitrogen waste was captured in the solids removal system.
7. All NO_3^- present in the solids treatment backwash that was pumped to the sand filter was removed through denitrification. Once submerged on day 119, the sand filter was

anoxic and sufficient biodegradable organic carbon was available to allow complete denitrification in the sand filter.

8. All background nitrogen was present as dissolved nitrogen. This was supported by a low suspended solids concentration in collected samples and consequently a small number of binding sites were available for sorption of nitrogen species.

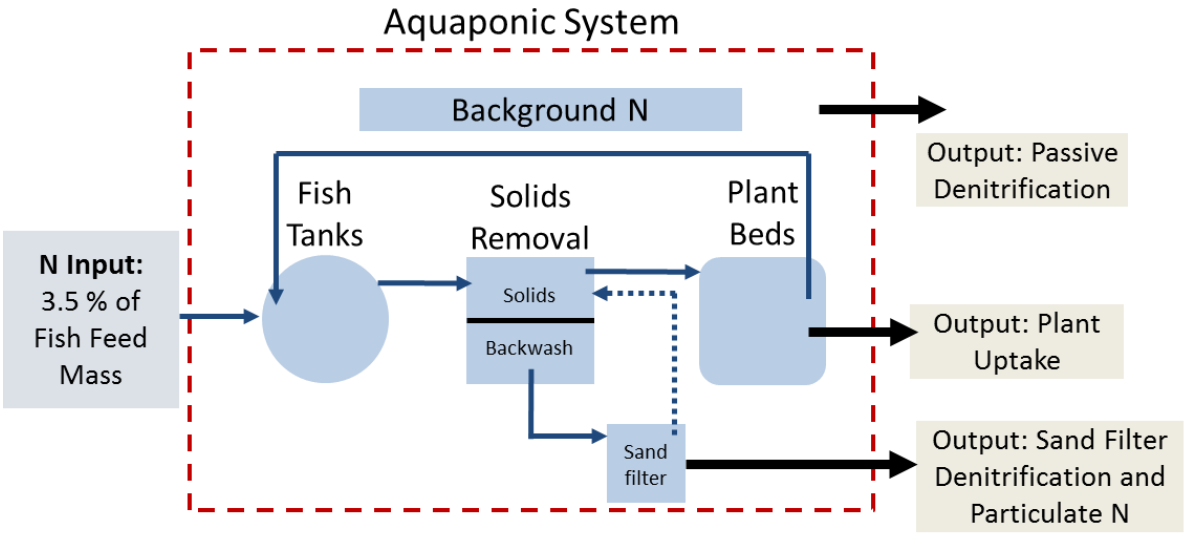

Figure 3.3: Simplified diagram of nitrogen source and removal mechanisms in the aquaponic system. Red dashed line shows the system boundary. Blue arrows show movement of nitrogen within the system boundary, black arrows show nitrogen exiting the system boundary, and the dashed blue line shows nitrogen-free return flow from the sand filter.

3.4.1.1 Feed Inputs and Total Daily Removal

To determine background mass of nitrogen in the system, the mean TN concentration measured in the plant bed effluent (Sample point 4) was multiplied by the system volume (Eq. 3.2).

$$CN_{t_i}\left(\frac{mg}{L}\right) x\, V_{system}\,(L)\; x\; \frac{1\,(g)}{1000\,(mg)} = N_{mass_{t_i}}\,(g) \qquad \text{Eq. 3.2}$$

where: CN_{t_i} was the TN concentration measured on a specific sampling day t_i, V_{system} is the total system volume, and $N_{mass_{t_i}}$ is the background mass of nitrogen on that specific day.

Nitrogen entered the system daily in the form of fish feed. It was assumed that 3.5% of the feed was converted to dissolved nitrogen (Appendix C), therefore daily dissolved nitrogen inputs were calculated as:

$$M_{feed}(g)x\ \frac{0.035\ (g\ dissolved\ N)}{(g\ feed)} = N_{added}(g) \qquad \text{Eq. 3.3}$$

where: M_{feed} was the mass of feed and N_{added} was the mass dissolved nitrogen added from fish feed.

The total quantity of dissolved nitrogen added from feed over a certain date range was calculated as:

$$\sum_{t_j - t_i} M_{feed}\ (g)\ x\ \frac{0.035\ (g\ dissolved\ N)}{(g\ feed)} = N_{added_{t_j - i}}(g) \qquad \text{Eq. 3.4}$$

where: t_j and t_i are specific water quality sampling dates and $N_{added_{t_j - i}}$is the total mass of nitrogen added from fish feed during that period.

The combination of Eq. 3.2 and Eq. 3.4 yielded the total quantity of dissolved nitrogen into the system and was calculated as:

$$N_{mass_{t_i}}\ (g) +\ N_{added_{t_j - i}}\ (g) = N_{total_{t_j - i}}\ (g) \qquad \text{Eq. 3.5}$$

where: $N_{total_{t_j - i}}$ includes the background mass of nitrogen and the total amount of feed added during that date range.

Since the quantity of dissolved nitrogen removed could not be measured directly it was based on the difference between the total quantity of nitrogen added to the system (Eq. 3.5) and the quantity of nitrogen remaining in the system. The quantity of nitrogen remaining was calculated similarly to Eq. 3.1 where the mean TN concentration measured in the fish tanks was multiplied by the system volume (Eq. 3.6).

$$CN_{t_j}\left(\frac{mg}{L}\right) x\ V_{system}\ (L)\ x\ \frac{1\ (g)}{1000\ (mg)} = N_{mass_{t_j}}\ (g) \qquad \text{Eq. 3.6}$$

where: CN_{t_j} is the TN concentration measured on specific sampling day t_j and $N_{mass_{t_j}}$ is the mass of nitrogen remaining at the end of the specific sampling day. Using Eq. 3.5 and Eq. 3.6, the quantity of nitrogen removed was calculated as:

$$N_{total_{t_{j-i}}}(g) - N_{mass_{t_j}}\ (g) = N_{removed_{t_{j-i}}}(g) \qquad \text{Eq. 3.7}$$

where: $N_{removed_{t_{j-i}}}$ is the total amount of nitrogen removed from the system during that date range.

Subsequently CN_{t_j} became the new background concentration at sample time $j + 1 = i$ and the series of calculations were continued for the total number of sampling dates. The percent removed of daily feed added was calculated for each date range as:

$$\frac{N_{removed_{t_{j-i}}}\ (g)}{N_{added_{t_j-i}}\ (g)}\ x\ 100\% = Percent\ removed\ of\ daily\ feed\ added \qquad \text{Eq. 3.8}$$

3.4.1.2 Plant Uptake

The quantity of nitrogen removed through plant uptake was calculated using experimentally derived plant uptake rates. Uptake rates were calculated as:

$$PN_{t_j}(\frac{g}{m^2}) -\ PN_{t_i}(\frac{g}{m^2})\ x\ plant\ bed\ area\ m^2 = N_{plant\ uptake_{t_{j-i}}}\ (\frac{g}{day}) \qquad \text{Eq. 3.9}$$

where PN_{t_j} and PN_{t_i} are the total mass of nitrogen in plant biomass per m^2 on day t_j and t_i and $N_{plant\ uptake_{t_{j-i}}}$ was the total mass of nitrogen removed by plants during the specific date range.

3.4.1.3 Sand Filter Removal

Prior to day 119, the sand filter was unsaturated and not considered a significant sink for nitrogen via denitrification. After day 119, the sand filter was saturated and complete

denitrification was observed. It was assumed that sufficient biodegradable organic carbon was available for denitrification and 100% of the nitrogen in the solids sump backwash was denitrified based on measurements of COD and calculations of the carbon/nitrogen (C/N) ratio (Section 3.5.3). Similarly, it was assumed that the solids sump, sand filter, and sand filter sump collectively functioned as a batch reactor. As such, an equivalent volume of water pumped from the solids sump into the sand filter was pumped into the main system from the sand filter sump. Therefore, the volume of nitrogen-rich backwash flowing into the sand filter was equal to an equivalent volume of nitrogen-free water flowing into the main system, resulting in a dilution of system water. The complete denitrification of backwash was supported by measurements completed by MAP staff on standing water in the sand filter in which nitrate concentrations were below detection limits 24 hours after backwashing (Appendix G). Similarly, measured TAN concentrations in sand filter effluent were low relative to the total mass of nitrogen entering the system and therefore were not considered as significant source of nitrogen.

The percentage of system water treated by the sand filter was estimated with sand filter sump pump run times and measured flow rates. The duration the sump pump was operational was measured with a T-CON-ACT-150 AC voltage transmitter (Onset, MA) and recorded with a Hobo U12-008 (Onset, MA) data logger. Flow rates were hand-measured with a stopwatch and bucket. The average number of minutes the pump was operational was multiplied by the flow rate to determine volume of water pumped and subsequently volume of system water treated in the sand filter daily. Due to changes in the backwashing frequency, 0.63% of system water was treated daily between days 119 and 148 and 0.88% was treated daily between days 149 and 272.

The mass of nitrogen removed by the sand filter was sensitive to the TN concentration. To account for fluctuations in the TN concentration between sampling periods the average of the

initial and final TN concentrations were used to calculate mass of nitrogen removed in the sand filter. This average concentration was multiplied by the percent of system water treated daily and the number of days in a specific date range. The product of these factors was the total quantity of nitrogen removed by the sand filter during that period.

$$\frac{CN_{t_i}\left(\frac{mg}{L}\right) + CN_{t_j}\left(\frac{mg}{L}\right)}{2} x\ \%\ water\ treated\ daily\ \left(\frac{L}{L}\right) x\ V_{system}\ (L)$$
$$x\ \frac{1\ (g)}{1000\ (mg)} x\ \{j-i\}\ day = N_{sand\ filter_{t_{j-i}}}\ (g) \qquad \text{Eq. 3.10}$$

where: $N_{sand\ filter_{t_{j-i}}}$ was the total mass of nitrogen removed by denitrification in the sand filter during that specific date range.

3.4.1.4 Other Mechanisms (Passive Denitrification)

To determine the mass of nitrogen removed by other mechanisms, the sum of plant uptake and denitrification in the sand filter was subtracted from the total mass of nitrogen removed during a specific date range.

$$N_{removed_{t_{j-i}}}(g) - \left\{N_{plant\ uptake_{t_{j-i}}}\ (g) + N_{sand\ filter_{t_{j-i}}}\ (g)\right\} = N_{other_{t_{j-i}}}(g) \qquad \text{Eq. 3.11}$$

where: $N_{other_{t_{j-i}}}$ was the mass of nitrogen lost from the system that could not be accounted for with plant uptake or denitrification alone.

3.4.2 Phosphorus Balance

Phosphorus entered the system through the addition of fish feed and was considered to be removed through four mechanisms: plant growth, precipitation, sedimentation, and sorption. With the data collected, it was not possible to distinguish between precipitation, sedimentation or sorption removal processes therefore these processes were aggregated under the term: other mechanisms. Figure 3.4 is a conceptual diagram of the mass balance completed on the liquid

phase phosphorus present in system. The mass balance was carried out to determine the quantity of phosphorus associated with each source and removal mechanisms using experimentally collected data, literature values, and through calculations. The phosphorus mass balance was calculated similarly to the nitrogen mass balance. The following assumptions were used to complete the phosphorus mass balance:

1. The system control volume was 45,400 L. The sand filter was not included in the main system control volume.
2. Mean TP concentrations measured in the plant bed effluent represented the liquid phase phosphorus concentrations for the aquaponic system.
3. Over the first 33 days phosphorus added from the feed accumulated in system water, the concentration measured on day 34 included this accumulation. When multiplied by the system volume it represented the initial background mass of phosphorus.
4. In total, 1.0% of wet-weight feed was in the form of phosphorus. 0.3% of wet-weight feed was excreted by fish as dissolved phosphorus. The remaining phosphorus from feed was either incorporated into biomass (0.3%) or present as particulate waste (0.4%) (Appendix C).
5. All particulate phosphorus waste was captured in the solids removal system.
6. No removal of dissolved phosphorus occurred in the sand filter. The measured TP concentrations in the sand filter effluent were not significantly lower than TP concentrations measured in the plant bed effluent used for the mass balance calculations, indicating limited dissolved TP removal (Appendix F).

7. All background phosphorus was present as dissolved P. This was supported by a low suspended solids concentration in collected samples and subsequently a small number of binding sites were available for sorption of phosphorus species.

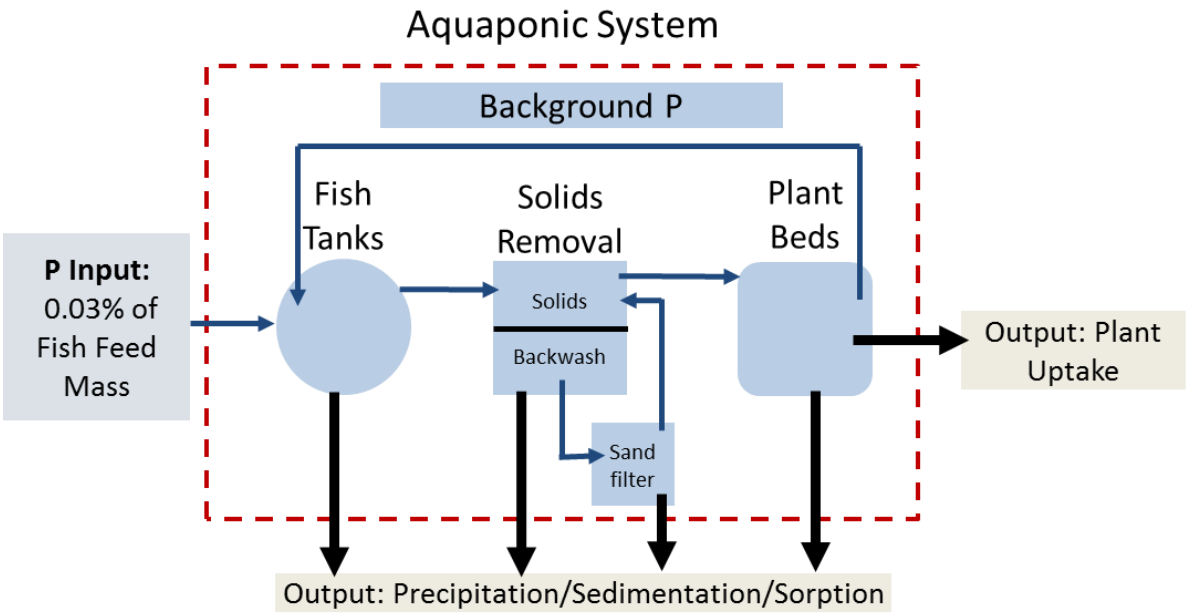

Figure 3.4: Simplified diagram of phosphorus source and removal mechanisms in the aquaponic system. Red dashed line shows the system boundary. Blue arrows show movement of phosphorus within system boundary and black arrows show phosphorus exiting system boundary.

3.4.2.1 Feed Inputs and Total Daily Removal

To determine background mass of phosphorus in the system, Eq. 3.12 was used with the mean TP concentration measured in the plant bed effluent (Sample point 4).

$$CP_{t_i}\left(\frac{mg}{L}\right) x\, V_{system}\,(L)\, x\, \frac{1\,(g)}{1000\,(mg)} = P_{mass_{t_i}}\,(g) \qquad \text{Eq. 3.12}$$

where: CP_{t_i} was the TP concentration measured on a specific sampling day t_i, V_{system} is the total system volume, and $P_{mass_{t_i}}$ is the background mass of phosphorus on that specific day.

Phosphorus entered the system daily in the form of fish feed. It was assumed 0.3% of the feed was converted to dissolved phosphorus (Appendix C).

$$M_{feed}(g)x\ \frac{0.003\ (g\ dissolved\ P)}{(g\ feed)} = P_{added}(g) \qquad \text{Eq. 3.13}$$

where: M_{feed} was the mass of feed and P_{added} was the mass dissolved phosphorus added from fish feed.

The total quantity of dissolved phosphorus added from feed over a certain date range was calculated as:

$$\sum_{t_j - t_i} M_{feed}\ (g)\ x\ \frac{0.003\ (g\ dissolved\ P)}{(g\ feed)} = P_{added_{t_j - i}}(g) \qquad \text{Eq. 3.14}$$

where: t_j and t_i are specific water quality sampling dates and $P_{added_{t_j - i}}$is the total mass of phosphorus added from fish feed during that period.

The combination Eq. 3.12 and Eq. 3.14 yielded the total quantity of dissolved phosphorus in the system and was calculated as:

$$P_{mass_{t_i}}\ (g) +\ P_{added_{t_j - i}}\ (g) = P_{total_{t_j - i}}\ (g) \qquad \text{Eq. 3.15}$$

where: $P_{total_{t_j - i}}$ includes the background mass of nitrogen and the total amount of feed added during that date range.

As in the nitrogen mass balance, the quantity of dissolved phosphorus removed could not be measured directly. The mass of dissolved phosphorus removed was calculated using the difference between total quantity of phosphorus in the system (Eq. 3.15) and the quantity of phosphorus remaining in the system. The quantity of phosphorus remaining was calculated similarly to Eq. 3.12, where the mean TP concentration measured in the fish tanks was multiplied by the system volume (Eq. 3.16).

$$CP_{t_j}(\frac{mg}{L})x\ V_{system}\ (L)\ x\ \frac{1\ (g)}{1000\ (mg)} = P_{mass_{t_j}}\ (g) \qquad \text{Eq. 3.16}$$

where: CP_{t_j} is the TP concentration measured on specific sampling day t_j and $P_{mass_{t_j}}$ is the mass of phosphorus remaining at the end of the specific sampling day. Using Eq. 3.15 and Eq. 3.16, the quantity of phosphorus removed was calculated as:

$$P_{total_{t_{j-i}}}(g) - P_{mass_{t_j}}(g) = P_{removed_{t_{j-i}}}(g) \quad \text{Eq. 3.17}$$

where: $P_{removed_{t_{j-i}}}$ is the total amount of phosphorus removed from the system during that date range.

Subsequently $P_{removed_{t_{j-i}}}$ became the new background concentration at sample time $j + 1 = i$ and the series of calculations were continued for the total number of sampling dates. The percent removed of daily feed added was calculated for each date range as:

$$\frac{P_{removed_{t_{j-i}}}(g)}{P_{added_{t_j-i}}(g)} \; x \; 100\% = Percent\ removed\ of\ daily\ feed\ added \quad \text{Eq. 3.18}$$

3.4.2.2 Plant Uptake

The quantity of phosphorus removed through plant uptake was calculated using experimentally derived phosphorus plant uptake rates. Uptake rates were calculated as:

$$PP_{t_j}(\frac{g}{m^2}) - PP_{t_i}(\frac{g}{m^2}) \; x \; plant\ bed\ area\ m^2 = P_{plant\ uptake_{t_{j-i}}}(\frac{g}{day}) \quad \text{Eq. 3.19}$$

where: PP_{t_j} and PP_{t_i} are the total mass of phosphorus in plant biomass per m^2 at day t_j and t_i and $P_{plant\ uptake_{t_{j-i}}}$ was the total mass of phosphorus removed by plants during the specific date range.

3.4.2.3 Other Mechanisms (Precipitation/Sedimentation/Sorption)

To determine the mass of phosphorus removal through other mechanisms, plant uptake was subtracted from the total mass of phosphorus removed during a specific date range.

$$P_{removed_{t_{j-i}}}(g) - P_{plant\ uptake_{t_{j-i}}}(g) = P_{other_{t_{j-i}}}(g) \quad \text{Eq. 3.20}$$

where: $P_{other_{t_{j-i}}}$ was the mass of phosphorus lost from the system that could not be accounted for with plant uptake alone.

3.5 Results and Discussion

The following sections present, data collected on water quality, plant and fish growth over a 9 month period. Overall the system performed well, maintaining water quality within safe ranges for fish health and hydroponically producing sea purslane and saltwort. Water quality data are presented in two sections: 1) inorganic nutrients and 2) total inorganic and organic nutrients. A discussion of the sand filter operation precedes discussions on the nitrogen and phosphorus mass balances. Following this, the capacity for plant production and a discussion of possible causes for poor plant growth are presented. The section concludes with fish production.

3.5.1 Inorganic Nutrients

3.5.1.1 Total Ammonia Nitrogen (TAN)

Concentrations of TAN fluctuated over the first 90 days, reaching a maximum concentration of 2.15 ± 0.14 mg/L TAN on day 62 (Figure 3.5). Fish growth measurements taken on day 70 revealed that biomass density was 22.40 kg/m^3, greater than the 21 kg/m^3 of biomass the MBBR was designed to support. Despite exceeding the biomass density and the high TAN levels, no change was observed in fish appetite and feeding behavior. Exposure to concentrations of 2.5-4.8 mg/L TAN can be tolerated by marine fish; however, long-term exposure at these concentrations should be avoided (Wajsbrot et al., 1993). By day 81 the TAN concentrations remained undesirably high and the feed ration was reduced to reduce TAN loading on the biofilter. Despite this, the TAN levels remained undesirably high and the biomass density

measurement on day 100 was 26.10 kg/m^3. On day 115, the biomass density was reduced to be 22.04 kg/m^3, which closer to the MBBR initial design criteria of 21 kg/m^3.

Two other factors were attributed to the high TAN levels: a flow rate lower than the MBBR design requirement of 265 L/min and insufficient alkalinity for nitrification. To account for the low flow rate the fish tanks were elevated to increase the system flow rate to 257-310 L/min. Sodium bicarbonate ($NaHCO_3$) additions began daily to increase the alkalinity. After these changes were made, the system maintained a stable TAN concentration of less than 0.5 mg/L TAN for the rest of the study period.

The TAN removal efficiency of the hydroponic plant beds ranged from 0% to 52%. If the samples collected during the first 80 days are removed from the analysis due to the variable ammonium concentrations during this period, the mean ammonium removal was 21% ± 18%. A paired t-test on the hydroponic bed concentrations showed a significant ($\rho < 0.05$) decrease between the influent and effluent TAN concentrations in the plant beds.

The TAN concentrations observed in this study were similar to the low N loading conditions used in Shpigel et al. (2013), which treated RAS effluent with constructed wetlands. Shpigel et al. (2013) reported TAN concentrations between 0.04 to 1.0 mg/L TAN over a 24 hour period with 100% removal of TAN in constructed wetlands planted with *Salicornia*. The system in Waller et al. (2015) was similar to the one in this study with a biofilter providing continuous nitrification and side-stream treatment through hydroponic plant beds planted with halophytes. The TAN concentrations were lower in Waller et al. (2015) than this study at 0 to 0.07 mg/L TAN.

3.5.1.2 Nitrite (NO_2^-)

With the exception of the first 35 days, nitrite concentrations remained low, less than 1 mg/L NO_2^--N (Figure 3.5). The biofilter was not fully acclimated when the fish were added on day 0, which resulted in higher nitrite concentrations as the microbiological community was being established during the first few weeks of operation. The development of nitrite oxidizing bacteria occurs more slowly in saltwater than freshwater systems, resulting in a longer acclimation period for biofilters (Díaz et al., 2012).

3.5.1.3 Nitrate (NO_3^-)

Nitrate concentrations increased steadily during the first 119 days of operation, reaching a maximum concentration of 120 ± 5.7 mg/L NO_3^--N (Figure 3.5). During the final sampling period the mean nitrate concentration was 25.6 ± 14 mg/L NO_3^--N in the fish tanks (min: 11.5 mg/L, max: 47.2 mg/L). A paired t-test showed no significant decrease in the influent and effluent concentrations to the hydroponic plant beds ($\rho < 0.05$).

Nitrate is less toxic to fish health than TAN and can be tolerated at higher concentrations (Piedrahita, 2003). In RAS, concentrations of 200-400 mg/L NO_3^--N are sometimes maintained (Otte and Rosenthal, 1979), although concentrations greater than 50 mg/L NO_3^--N are typically avoided in marine systems (Gutierrez-Wing and Malone, 2006). Water exchanges are used in RAS to maintain a constant nitrate concentration (Masser et al., 1999). Due to the use of saltwater in the land-based study system, discharge of system water and replacement with freshwater was not possible. Instead, to stabilize the nitrate concentrations, the sand filter was converted into a side-stream denitrification reactor on day 119 by partially submerging it and allowing anoxic conditions to develop. After the sand filter was modified the nitrate concentration began to decrease, as shown in Figure 3.5.

The steady increase in nitrate and the need to add a denitrification reactor was unexpected. In aquaponic systems the nitrate concentration typically stabilizes due to the large percentage of nitrogen removal through plant uptake and denitrification (Rakocy, 2012). For example, the marine system in Waller et al. (2015) maintained a stable nitrate concentration of 20.1 ± 3.4 mg/L NO_3^--N operating with a nitrifying biofilter and hydroponic plant growth.

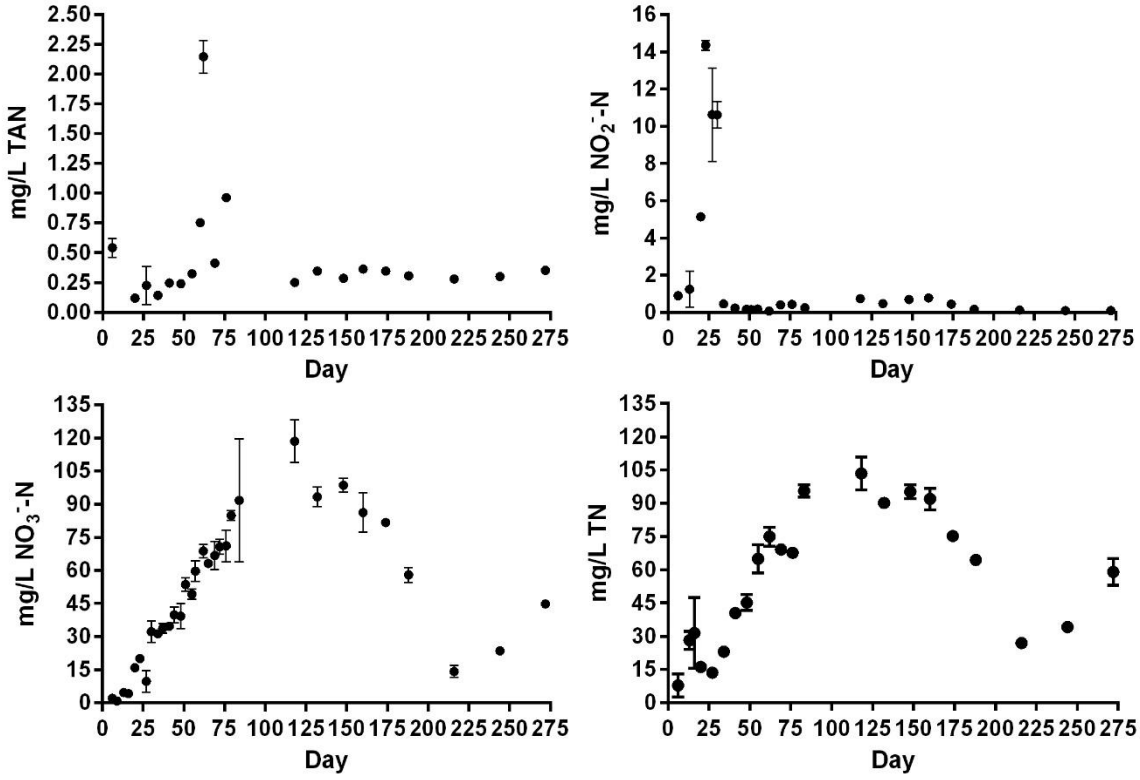

Figure 3.5: TAN, nitrite, nitrate, and TN concentrations measured over 272 day sampling period. Points are from sample point 1 (fish tank effluent) and error bars show standard deviations.

Alternatively, a long-term study of a freshwater aquaponic systems producing tilapia and basil by Rakocy et al. (2004) used plant growth and denitrification to maintain nitrate concentrations without water exchanges. Hydroponic plant growth was used predominantly to remove nitrogen; however, filter tanks were added that accumulated organic matter creating anaerobic zones in which denitrification could occur. Modification of filter tank cleaning

frequency provided control over nitrate concentrations where less cleaning allowed for more organic matter accumulation and more nitrate removal (Rakocy, 2012). That system maintained a nitrate concentration between 26.7 and 54.7 mg/L NO_3^--N.

The steady increase in nitrate at the start of the experiment indicated there was insufficient nitrogen uptake by the plants and insufficient passive denitrification. The combination of adding an anoxic zone to the system by partially submerging the sand filter and subsequent increased growth of the plants improved total nitrogen removal allowing nitrate to stabilize.

3.5.2 Total Inorganic and Organic Nutrients

3.5.2.1 Total Nitrogen (TN)

The total nitrogen concentration followed the same trend as nitrate, increasing during the first 100 days to a maximum of 103 mg/L TN and then decreasing once the sand filter was partially submerged to a minimum of 26.9 mg/L TN on day 216 (Figure 3.5). After this minimum value, the concentration again increased to 59.0 mg/L TN on the last sample date. The composition of nitrogen was roughly equally divided between inorganic and organic nitrogen species (Table 3.4). Day 244 was an exception in which 76.9% of the influent and 94.1% of the effluent were composed of inorganic nitrogen species. Of the inorganic nitrogen species, the majority consisted of nitrate with less than 1% from TAN or nitrite. A paired t-test showed no significant reduction of total nitrogen concentrations between the influent and effluent of the hydroponic plant beds ($\rho < 0.05$). Similarly, Waller et al. (2015) did not observe noticeable changes of TN in the influent and effluent of the hydroponic plant beds.

Dissolved inorganic nitrogen species are generally monitored more closely in RAS due to their potential health impacts on fish; however, mineralization of dissolved and particulate

organic nitrogen can contribute to increases in TAN (Piedrahita, 2003). Any TAN produced through mineralization in the hydroponic plant beds was likely nitrified or removed by plants as there were no significant increases in the effluent TAN concentration.

3.5.2.2 Total Phosphorus (TP)

The average total phosphorous concentration slowly increased over the entire study period reaching a maximum of 23.0 mg/L TP on Day 272 (Figure 3.6). Despite the general increase in concentration, on Days 118, 160, and 216 small decreases in total phosphorus occurred. A significant decrease was observed between the hydroponic plant bed influent and effluent concentrations ($\rho < 0.05$). There was also a significant decrease in total phosphorus concentration between the fish tank effluent and the solids removal effluent ($\rho < 0.05$). The mean percent removal of phosphorous in the hydroponic plant beds was 10% ± 21% (min: -49%, max: 55%). The swirl separator and the upflow media filter contributed to a mean percent removal of 0.73% ± 51% (min: -249%, max: 80%) from the fish tank effluent.

Table 3.4: Mean (± standard deviation) concentration of major nitrogen species during the last phase of sampling. Organic nitrogen was calculated as the total nitrogen minus the three inorganic nitrogen species. Percent of total nitrogen given in parentheses.

Day	188		216		244	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
Total Nitrogen mg/L TN	24.4 ± 1.4	24.7 ± 1.7	34.2 ±2.7	34.7 ± 0.8	57.2 ± 7.5	52.8 ± 4.0
Nitrate mg/L NO_3^--N	12.4 ± 0.83 (50.8%)	9.84 ± 0.61 (39.8%)	20.6 ±1.8 (60.2%)	21.1 ± 1.8 (60.8%)	43.7 ± 5.8 (76.4%)	49.5 ± 3.7 (93.8)
Ammonium mg/L TAN	0.16 ± 0.01 (0.66%)	0.09 ± 0.03 (0.36%)	0.07 ± 0.00 (0.20%)	0.00 ± 0.01 (0.00%)	0.21 ± 0.01 (0.37%)	0.10 ± 0.01 (0.19%)
Nitrite mg/L NO_2^--N	0.18 ± 0.01 (0.74%)	0.10 ± 0.00 (0.40%)	0.13 ± 0.00 (0.38%)	0.06 ± 0.01 (0.17%)	0.09 ± 0.01 (0.16%)	0.05 ± 0.00 (0.09%)
Organic N	47.8%	59.4%	39.2%	39.0%	23.1%	5.9%

Weekly and monthly fluctuations in total phosphorus can be attributed to variations in cumulative feed added, plant growth, and precipitation/sedimentation of phosphorus. Fish feed contains 1-2% phosphorous as wet-weight feed (Foy and Rosell, 1991) and fish retain about 17-40% of the phosphorus in feed (Piedrahita, 2003). In RAS, the remaining phosphorus can accumulate due to the constant recirculation (Barak et al., 2003). In this study, phosphorus accumulation in the main system was moderated by plant growth and uptake, filtration in the swirl separator and upflow media filter, and precipitation/sedimentation.

Table 3.5: Mean (± standard deviation) concentration of TP, COD, TSS, and VSS in the influent and effluent to the plant beds during the last phase of sampling.

Day	188		216		244	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
TP (mg/L)	18.0 ± 2.7	13.4 ± 1.3	16.2 ± 6.1	11.3 ± 0.18	19.3 ± 4.0	15.1 ± 1.1
COD (mg/L)	118 ± 2.1	119 ± 1.1	115 ± 5.7	134 ± 17	75.3 ± 4.6	79.6 ± 2.3
TSS (mg/L)	11.2 ± 0.70	10.8 ± 0.05	11.0 ± 0.08	9.93 ± 0.33	14.7 ± 0.5	14.4 ± 1.1
VSS (mg/L)	5.01 ± 0.29	3.13 ± 032	3.68 ± 0.19	3.77 ± 1.4	7.27 ± 0.11	6.43 ± 0.34

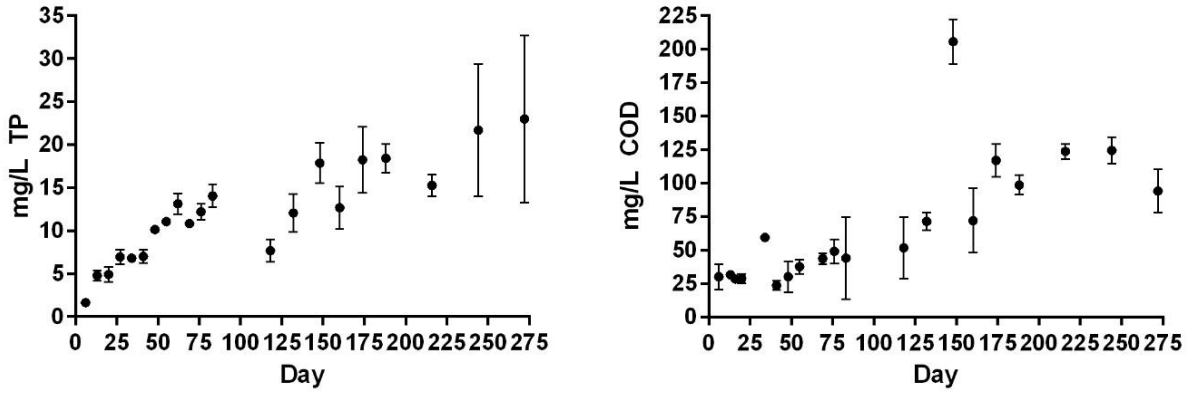

Figure 3.6: TP and COD concentrations measured over 272 day sampling period. Points are from sample point 1 (fish tank effluent) and error bars are standard deviation.

Of the 60-83% of phosphorus not retained in fish biomass, a majority is in the form of particulate phosphorus (Barak et al., 2003). This proportion of particulate phosphorus was not

effectively captured in the samples collected. The inefficient removal of TP from the swirl separator and upflow media filter, suggests the majority of the TP in collected samples was present as dissolved phosphorus.

Instead, the portion of wasted particulate phosphorus was not present at time of sampling, as sampling events occurred prior to fish feeding. The presence of these large particles, not captured in total phosphorus measurements, were illustrated by measurements of total phosphorus in the solids sump effluent (Figure 3.7). The sand filter effectively removed total phosphorus with a 56% ± 22% (min: 11%, max: 91%) removal capacity of total phosphorus in the solids sump effluent. The sand filter contributed to removal of particulate phosphorus by physically filtering out suspended solids (Urbonas, 1999). While the proportion of dissolved phosphorus present in the solids sump effluent was unknown, a portion of this was likely removed in the sand filter through precipitation followed by filtration or sedimentation. Over time the accumulated solids in the sand filter could contribute to phosphorus release; however, the absence of increasing TP concentrations in the sand filter sump effluent and the removal of sand filter solids on day 211 prevented phosphorus release during the study.

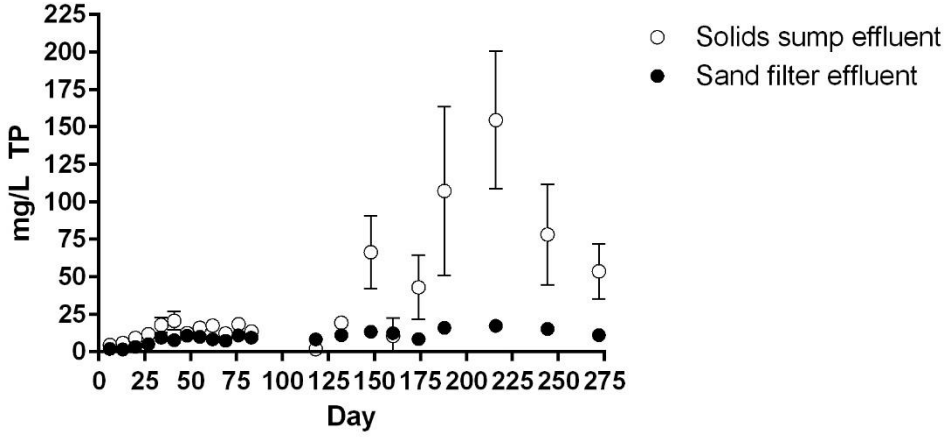

Figure 3.7: TP concentrations measured in the influent and effluent to the sand filter over 272 day sampling period. Points are from sample points 5 and 6. Error bars show standard deviations.

Compared to the swirl separator and the upflow media filter, the hydroponic plant beds were more efficient at total phosphorus removal based on the collected water samples. The significant decrease in total phosphorus observed in the influent and effluent concentrations of the hydroponic plant beds were due to the relatively fast removal process of sedimentation. Phosphorus measurements taken throughout a RAS indicated the majority of wasted phosphorus accumulated in a sedimentation basin located after the fish tanks (Barak et al., 2003). Sedimentation in aquaculture settling ponds occurs at velocities less than 1 m/s (Henderson and Bromage, 1988). In this study, the 0.2 m/s velocity in the hydroponic plant beds resulted in additional sedimentation of particulate waste remaining after the upflow media filter.

Plants contributed to phosphorus removal through uptake of dissolved inorganic phosphorus (Reddy et al., 1999). The large quantity of young plant biomass resulted in greater uptake of phosphorus and a gradual decrease in total phosphorus concentration. Prior studies have shown that phosphorus uptake rates are greatest in younger plants and that rates decrease as plants age (Edwards and Barber, 1976; Jungk and Barber, 1975). Despite recirculation, Waller et al. (2015) observed no change or net increase in the phosphate concentration between the plant bed influent and effluent, indicating the plants contributed to phosphorus removal. The gradual accumulation of phosphorus in system water in the current study was due to greater quantities of added feed than in Waller et al. (2015).

3.5.2.3 Chemical Oxygen Demand (COD)

In the main system, excluding the solids backwash and sand filter effluent, the COD concentration gradually increased over the duration of the study (Figure 3.6) reaching a maximum of 275 mg/L COD exiting the hydroponic plant beds. The mean concentration exiting the fish tanks was 67.5 ± 47.4 mg/L COD. Paired t-tests on influent and effluent concentrations

of the main system components indicated there were significant ($\rho<0.05$) decreases in the biofilter and the plant beds.

In fixed film biofilters, excess organic matter can inhibit nitrification due to competition for space and oxygen between autotrophic nitrifiers and heterotrophic bacteria (Zhu and Chen, 2001). High carbon to nitrogen (C/N) ratios result in greater proportions of heterotrophic bacteria resulting in decreased biofilter efficiency (Bovendeur et al., 1990; Ohashi et al., 1995). This is of particular importance in aquaculture systems, which must operate with low TAN concentrations reducing the C/N ratio. Zhu and Chen (2001) found C/N ratios as low as 1.0 to inhibit nitrification. In this study, a C/N of 1.2 and the significant decrease of COD in the biofilter suggests that heterotrophic bacteria may have decreased the efficiency of the biofilter; however, the presence of the hydroponic plant beds provided additional surface area for nitrification and ensured sufficient TAN removal.

The mean COD concentration in the solids sump, which collected backwash from the swirl separator and the upflow media filter, was 2280 ± 3590 mg/L COD. The mean concentration in the sand filter sump of 172 ± 190 mg/L COD was significantly lower than the solids sump indicating COD removal in the sand filter. Unlike biofilters where a high prevalence of organic matter is detrimental, in the sand filter the presence of organic matter was desirable to aid denitrification. Studies have shown that fish waste can be an effective carbon source for denitrification reactors in RAS (Arbiv and van Rijn, 1995; Gelfand et al., 2003 Phillips and Love, 1998). While the proportion of particulate to dissolved COD was not measured, the long retention time in the sand filter likely facilitated hydrolysis of some particulate COD to provide bioavailable carbon for denitrifying microorganisms (Conroy and Couturier, 2010). This added to the bioavailable carbon already available from dissolved COD present in system water. The

results of this study further support the use of fish waste as a carbon source for denitrification.

3.5.2.4 Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

The concentrations of both TSS and VSS were relatively stable in the main system, with slight increases as the quantity of feed added increased (Figure 3.8). The greatest mean TSS concentration measured in the fish tank effluent was 17.3 mg/L TSS on day 132. On average TSS concentrations were 9.7 ± 2.9 mg/L TSS. The greatest mean VSS concentration measured in the fish tank effluent was 9.4 mg/L VSS and the mean VSS concentration was 4.3 ± 1.9 mg/L VSS. Significant ($\rho<0.05$) reductions were observed after the biofilter and the hydroponic plant beds, with effluent concentrations of 9.88 ± 3.29 mg/L TSS and 8.61 ± 2.53 mg/L TSS, respectively. The mean percent removal of TSS in the hydroponic plant beds was 9.15% ± 22.6% (min: -86%, max: 55%). Similar trends were observed with VSS where significant ($\rho<0.05$) reductions also occurred after the biofilter and hydroponic plant beds. In the main system water, about 36% to 46% of the suspended solids were volatile with a significant ($\rho<0.05$) reduction in the volatile proportion after the hydroponic plant beds.

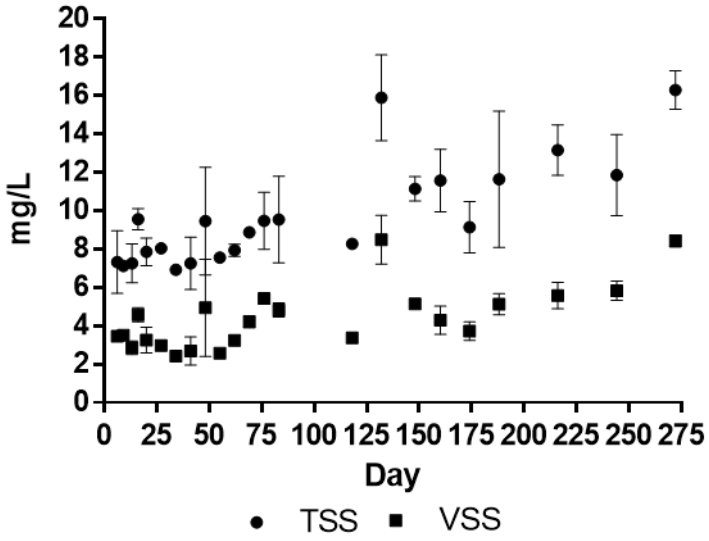

Figure 3.8: Concentrations of TSS and VSS measured over 272 day sampling period. Points are from sample point 1 (fish tank effluent) and error bars are standard deviation.

At the high biomass densities typical of RAS, passive solids removal systems may not provide sufficient TSS reduction. Suspended and volatile solids must be removed quickly from recirculating systems to prevent disease, gill damage, nutrient leaching, and increased oxygen demand, although specific thresholds have not been set for RAS (Davidson and Summerfelt, 2005; Ebeling and Timmons, 2012). Swirl separators are designed to capture large particles with high specific gravities and can be less effective capturing less dense fish fecal matter (Davidson and Summerfelt, 2005). Conversely, microscreen drum filters efficiently remove and concentrate fish and feed waste >60μm in size (Ebeling and Timmons, 2012). The disadvantage to microscreen drum filters are high capital costs, electricity, and water requirements (Summerfelt and Penne, 2005).

In this study, a swirl separator and upflow media filter were used as opposed to more robust drum filters. The passive technologies were chosen to reduce capital and operational expenses in addition to minimizing design complexity. The concentration of TSS in the solids sump ranged from 102 to 7250 mg/L TSS with an average of 62% as volatile solids. About 0.34 ± 0.42 kg/d were removed by both the swirl separator and the upflow media filter, which is much lower than the 6.5 kg/d of TSS observed by Davidson and Summerfelt (2005) in swirl separator backwash in a rainbow trout RAS. Lower feed quantities likely resulted in the lower mass removal compared to Davidson and Summerflet (2005). Additional reductions in the biofilter and hydroponic plant beds further helped prevent a net accumulation of TSS or VSS.

After collection in the solids sump, backwash was pumped to the sand filter to dewater the solids and maximize water reuse. The sand filter reduced TSS and VSS concentrations by 76.9% ± 28.1% and 87.0% ± 13.9%, respectively. In general, sand filters remove suspended solids physically by filtering out particles based on the size of pore spaces (Urbonas, 1999). As

this was a marine system, it was imperative to collect the saline solids which should not be discharged into the inland environment.

3.5.3 Sand Filter

The sand filter was initially designed to filter and aggregate saline solid waste created by the aquaponic system. As stated previously, it was able to remove on average 76.9% ± 28.1% of TSS. In freshwater systems, the collected solids have a high concentration of valuable nutrients. When dewatered, the solids have a variety of potential applications including agricultural amendments, compost, and vermiculture (Cripps and Bergheim, 2000). Beneficial reuse or disposal of solids from marine aquaculture systems can be more challenging due to the salt content. A unique partnership with a commercial nursery, which produces halophytic plants for wetlands restoration, enabled collection and reuse of the saline solids in this study. On day 211, 34 kg of solids were removed from the sand filter by the commercial nursery. For many facilities other alternatives will be required for saline solids disposal. As the quantity of marine fishes produced in RAS increases the ultimate disposal of saline solids remains a largely unaddressed challenge and has encouraged research in USF laboratories on methods to anaerobically digest the saline fish waste.

More importantly, the sand filter provided a novel solution to the problem of nitrate accumulation. After over 100 days of operation and despite nitrate removal through plant uptake and passive denitrification, nitrate continued to accumulate. In freshwater RAS, water can simply be discharged to the environment and replaced with freshwater to prevent nitrate accumulation (Masser et al., 1999). Separate denitrification systems can be complicated to operate and expensive to build (Hamlin et al., 2008). For these reasons the use of denitrification reactors remains uncommon in RAS despite research demonstrating successful application in freshwater

and marine systems (Badiola et al., 2012; van Rijn et al., 2006). A variety of system designs have been used in both freshwater and marine systems including activated sludge, packed bed reactors, and fluidized bed reactors (van Rijn et al., 2006). Many studies use an exogenous electron donor (e.g. methanol, acetic acid) or system designs that require extensive maintenance for successful operation (Klas et al., 2006). This study was unique due to use of an endogenous carbon source (readily biodegradable COD in the fish waste), operation of the sand filter as a downflow submerged packed bed biofilter, and its use as a side-stream treatment system.

System nitrate concentrations began to decrease immediately after the sand filter was submerged. The sand filter removed between 8% and 36% of the total nitrogen removed daily. As it was a side-stream treatment process, only a small portion of the system flow passed through the sand filter on a weekly basis depending on the frequency of backwashing. On average 2390 L/week or 5% of the system volume was treated weekly. Klas et al. (2006) calculated that 4.0-6.0 g COD/g NO_3^- are required for complete denitrification using an experimentally derived formula for organic solids produced in a seawater RAS. Assuming an average load of 780 g COD/d and the C/N ratio given by Klas et al. (2006), an estimated 130-195 g N could be removed by the sand filter. On average, the daily load to the sand filter was 56 g N/d. Based on these calculations, denitrification in the sand filter was nitrogen limited unlike most RAS where organic matter is considered the limiting factor (Klas et al., 2006). With the surplus of carbon in this system, during periods of low plant growth or surplus fish production, additional nitrogen could be removed by modifying the backwashing frequency.

A side-stream denitrification reactor, such as the one in this study, provides numerous advantages in an aquaponic system. It allows operators the ability to control nitrate concentrations more precisely than possible with plant growth or passive denitrification alone.

The denitrification reactor can help control nitrate concentrations in situations where there are space limitations for plant growth, unexpected plant losses, or to support higher densities of fish.

3.5.4 Nitrogen Mass Balance

Nitrogen entered the system through the daily addition of fish feed at gradually greater quantities as the fish increased in size. The amount of nitrogen removed through plant uptake and denitrification must be equal to or greater than the amount of nitrogen added daily through feed to maintain a stable nitrogen concentration. At the start of this study, this did not occur and resulted in an accumulation of nitrogen in the system water, where the mass of nitrogen remaining was greater than the mass of nitrogen removed (Table 3.6). On average, 39% of the nitrogen added daily was removed before the sand filter was modified. After modification, of the nitrogen added daily, the quantity removed increased to 110% resulting in a net decrease of nitrogen in system water from 5187 g to 2386 g.

Plant uptake contributed to removal of 9.4 ± 11 g N/day before the sand filter was submerged and 9.8 ± 0.0 g N/day after (Table 3.7). The amount of nitrogen removed through plant uptake increased as the plants grew, but was limited by quantity of plant biomass. During the first sampling period the quantity of nitrogen removed by plant biomass gradually increased from 0.06 g $N/m^2/d$ to a max of 0.87 g $N/m^2/d$ (Table 3.9) Once harvesting began, the second plant sampling (days 244 and 272) indicated that the plants removed 0.28 g $N/m^2/d$. In constructed wetlands, plant uptake can contribute from 0.218 to 1.32 g $N/m^2/d$ (Burgoon et al., 1991; Hegedűs et al., 2010; Tanner, 1996). Burgoon et al. (1991) noted that at higher loading rates the plants did engage in some luxury uptake of nitrogen although plants only contributed to a maximum of 30% nitrogen removal.

Table 3.6: Mass of nitrogen added by feed and the mass removed once sand filter was flooded. Background N and removed N are based on measured TN concentrations multiplied by system volume.

Day range	Background N (g)	Added N (g)	Total N (g)	Removed N (g)	Percent removed of daily feed added	Remaining N (g)
Before sand filter was submerged						
35-41	1521	425	1947	238	56%	1709
42-48	1709	1089	2797	578	53%	2219
49-55	2219	539	2758	-253	-47%	3012
56-62	3012	551	3563	153	28%	3410
63-69	3410	551	3962	913	166%	3049
70-76	3049	495	3544	297	60%	3247
77-83	3247	557	3803	-390	-70%	4194
84-118	4194	1313	5507	867	66%	4640
After sand filter submerged						
119-132	4640	1596	6236	1049	66%	5187
133-148	5187	1964	7151	2646	135%	4505
149-160	4505	1580	6085	1914	121%	4171
161-174	4171	2254	6425	2753	122%	3672
175-188	3672	2254	5926	2990	133%	2936
189-216	2936	4042	6977	5860	145%	1117
216-244	1117	2940	4057	2489	84.7%	1568
245-272	1568	3035	4603	2217	73.1%	2386

Table 3.7: Mean dissolved nitrogen and phosphorus added from feed and mass removed through plant uptake, sand filter, or other mechanisms. Removal percentages are based on the total daily removal. Table shows data before the sand filter was submerged.

Variable	Nitrogen	Phosphorous
Feed (g/day)	1669 ± 963	
Dissolved nutrients from feed (g/day)	58 ± 34	5.0 ± 2.9
Plant removal (g/day)	9.4 ± 11 (43%)	1.5 ± 1.8 (3.0%)
Sand filter removal (g/day)	N/A	N/A
Other mechanisms (g/day)	12.6 ± 70 (57%)	-61.2 ± 23 (-103%)
Total daily removal (g/day)	22.0 ± 66 (100%)	-59.6 ± 23.4 (100%)

Similar to this study, Trang and Brix (2014) noted that plant uptake, in a freshwater system, only removed about 7% of the nitrogen added from feed. The authors suggested nitrification-denitrification in the gravel substrate and plant root zone removed the majority of nitrogen. In an aquaponic system producing halophytes without media, the plants assimilated 9%

of the nitrogen added from feed (Waller et al., 2015). Neither Waller et al. (2015) nor Trang and Brix (2014) observed increased nitrate concentrations; however the duration of both studies was short compared with this study. Waller et al.'s (2015) study was 35 days and Trang and Brix's (2014) study was 50 days.

Table 3.8: Mean dissolved nitrogen and phosphorus added from feed and mass removed through plant uptake, sand filter, or other mechanisms. Removal percentages are based on the total daily removal. Table shows data after the sand filter was submerged.

Variable	Nitrogen	Phosphorous
Feed (g/day)	3648 ± 1237	
Dissolved nutrients from feed (g/day)	128 ± 43	10.3 ± 4.3
Plant removal (g/day)	9.8 ± 0.0 (6%)	2.3 ± 0.0 (9.0%)
Sand filter removal (g/day)	26.3 ± 9.4 (17%)	N/A
Other mechanisms (g/day)	123 ± 59 (77%)	-28.5 ± 17.8 (-109%)
Total daily removal (g/day)	159 ± 62 (100%)	-26.2 ± 17.8 (100%)

Table 3.9: Total mass of nitrogen and phosphorus removed by plants between sampling periods. Data is combination of both sea purslane and saltwort uptake rates.

Day Range	Plant N Uptake (g N/m^2/d)	Plant P Uptake (g P/m^2/d)
9-23	0.06 ± 0.04	0.01 ± 0.02
23-37	0.07 ± 0.07	0.03 ± 0.02
37-51	0.05 ± 0.09	0.02 ± 0.03
51-65	0.23 ± 0.17	0.01 ± 0.04
65-79	0.87 ± 0.37	0.14 ± 0.05
244-272	0.28 ± 0.17	0.06 ± 0.04

Waller et al. (2015) calculated that to remove the 5.4 g N/d added to their system, *Salicornia dolichostachya* should be planted at a density of 78 plants/m^2. Assuming only sea purslane was used, a greater density of 231 net pots/m^2 would be required due to the 23 times greater nitrogen load in this system. Maintenance of the current net pot density with only sea purslane would require a 711 m^2 hydroponic plant bed area to remove 128 g N/d.

Ultimately there is a trade-off between nitrogen removal and system footprint with plant production in an aquaponic system. While plants are valuable by-products, they are less efficient at nitrogen removal than bacterial denitrification. At high fish densities, the plant densities or

hydroponic growing area required becomes greater and supplemental denitrification is required if land space is unavailable. With the production of halophytes of higher commercial value and with innovation in the design of inland systems that maximize use of space, it could be possible to better balance nutrient removal and plant production for maximum profit.

3.5.5 Phosphorous Mass Balance

Phosphorous is an essential mineral for bone development and other physiological processes; however, it is required in lower quantities than nitrogen and is present in lower quantities in fish feed (NRC, 1993). The quantity of phosphorus removed fluctuated widely. Removal was as low as -167% and as great as 310% of the quantity of dissolved phosphorus entering the system daily from feed (Table 3.10).

Several processes contributed to phosphorus removal including: plant uptake, precipitation, sedimentation, and sorption. Plant uptake was the only process to contribute to direct removal and removed between 1.5 and 2.3 g P/day (Table 3.7 & 3.8). Plant uptake accounted for 0.01 $P/m^2/d$ at the start of the study and up to 0.14 g $P/m^2/d$ before harvesting began (Table 3.9). During the second plant sampling, plant uptake accounted for 0.06 g $P/m^2/d$.

Precipitation, sedimentation, and sorption were aggregated under the term other mechanisms. This was actually responsible for a release of phosphorus between 61.2 and 28.5 g P/day. The wide variation in removal and the small quantity removed by plants, suggests the proportion of phosphorus removed was more dependent on the flux of phosphorus in the water column than direct removal from plant uptake. This fluctuation likely occurred due to a cycle of particulate phosphorus accumulation followed by mineralization in the hydroponic plant beds and tank bottoms (Reddy et al., 1999).

Table 3.10: Mass of phosphorus added by feed and the mass removed once sand filter was flooded. Background P and removed P are based on measured TP concentrations multiplied by system volume.

Day range	Background P (g)	Added P (g)	Total P(g)	Removed P (g)	Percent removed of daily feed added	Remaining P (g)
119-132	376	137	512	-200	-146	712
133-148	712	168	881	304	181	576
149-160	576	135	712	-38.6	-28.5	750
161-174	750	193	944	599	310	345
175-188	345	193	538	-134	-69.9	673
189-216	673	346	1019	415	120	604
216-244	604	252	856	345	137	512
245-272	512	260	772	87.9	33.8	684

In constructed wetlands, soil sorption, soil accretion, and plant uptake are considered the major processes that reduce aqueous phosphorus concentrations in effluent streams (Vymazal, 2007). In an aquaponic system, the absence of soil and the small quantity of potting media present in the system limit the mass of phosphorous associated with soil sorption. Mineral oxide precipitation from fish feed elements like iron could form surface coatings on the coconut potting media and other system components and increase the sites for phosphate sorption. Instead, soil accretion or sedimentation will be a temporary sink, as a portion of the organic matter will mineralize and release dissolved inorganic phosphorus (Reddy et al., 1999). This dissolved inorganic phosphorus is then considered available for plants and microorganisms (Reddy et al., 1999). As such, the permanent removal process of phosphorus, remaining after solids removal and subsequent backwashing to the sand filter, was plant uptake and harvesting.

Based on the amount of phosphorus available in the main system water, increasing the plant density and plant bed area could result in a phosphorus deficiency. All the dissolved phosphorus from feed would be removed with a density of 67 net pots/m^2 at the current 61.4 m^2. A 711 m^2 of hydroponic growing area, filled with sea purslane at the current 19.5 net pots/m^2, a

total of 43 g P/d would be required. Unlike nitrogen, in which the majority wasted nitrogen is dissolved, much of wasted phosphorus is particulate (Crips and Bergheim, 2000). Therefore the flux between accumulation and mineralization of phosphorus would be critical for providing the additional phosphorus the plants required. Over time the potential phosphorus release from mineralization of organic material in the sand filter could offset any phosphorus deficiencies.

3.5.6 Plant Production and Harvest

The sea purslane steadily increased in size during the first plant sampling period, with a total biomass of 684 ± 130 g DW/m^2 on day 79 (Table 3.11), where the mean weight of individual plants was 18.9 ± 6.7 g DW. A 100% survival rate for the sea purslane was estimated based our observations during the study. The saltwort did not perform as well, only reaching a maximum of 77.4 ± 14 g DW/m^2. Many saltwort cuttings did not survive planting in the hydroponic beds and the saltwort had an estimated 30% survival rate during the first sampling period. During this period, the surviving saltwort plants grew slower than the sea purslane. On day 79 the mean dry weight of individual saltwort plants was 0.70 ± 0.64 g DW.

Table 3.11: Mean dry weight, nitrogen content, and phosphorus content of total plant biomass during the first plant sampling period. Each point is average of 6 samples collected randomly (± standard deviation).

Day	9	23	37	51	65	79
Sea purslane						
g DW/m^2	78.9 ± 12	97.4 ± 9.7	124.2 ± 30	146 ± 37	222 ± 70	684 ± 130
g N/m^2	1.0 ± 0.3	1.9 ± 0.5	2.9 ± 0.9	3.4 ± 0.9	6.7 ± 2	18.8 ± 5
g P/m^2	0.13 ± 0.2	0.42 ± 0.2	0.81 ± 0.2	1.1 ± 0.4	1.2 ± 0.4	3.2 ± 0.6
Saltwort						
g DW/m^2	70.4 ± 4.4	71.9 ± 3.5	72.2 ± 3.0	77.4 ± 14	69.9 ± 5.4	73.1 ± 13
g N/m^2	1.1 ± 0.1	1.2 ± 0.2	1.4 ± 0.2	1.2 ± 0.5	1.7 ± 0.4	1.4 ± 0.8
g P/m^2	0.17 ± 0.1	0.22 ± 0.07	0.29 ± 0.09	0.21 ± 0.03	0.21 ± 0.03	0.33 ± 0.06

Because many of the saltwort plants did not survive in the system, the surviving plants were consolidated into one hydroponic plant bed on day 129. From the surviving plants new cuttings were made and planted in the same hydroponic plant bed. Most of these new cuttings

produced new growth. Due to the improved saltwort growth, reductions in the nitrate concentration, and potential impacts of plant harvesting a second series of samples were collected on days 244 and 272 (Table 3.12)

Table 3.12: Mean dry weight, nitrogen content, and phosphorus content of total plant biomass during the second plant sampling period. Each point is mean of 6 samples collected randomly (± standard deviation).

Day	244	272
Sea purslane		
g DW/m^2	285 ± 99	414 ± 200
g N/m^2	6.9 ± 2.4	10 ± 4.8
g P/m^2	2.1 ± 0.42	2.5 ± 1.2
Saltwort		
g DW/m^2	54.9 ± 40	172.1 ± 94
g N/m^2	1.3 ± 0.74	3.9 ± 2.0
g P/m^2	0.20 ± 0.21	0.62 ± 0.29

While a specific harvest regimen was not applied during this study, the information gathered can be used to estimate the potential annual production of sea purslane. The harvested plants were bundled in to approximately 113 g bundles in which sea purslane had about 21 pieces 23.3 cm long (Table 3.13). The saltwort bundles contained about 34 pieces that were 22.9 cm long. Due to the greater productivity of sea purslane and infrequent harvests of saltwort, only data from sea purslane harvests are presented here (Table 3.14). Large harvests, ranging from 34.7 to70.8 total kg, of sea purslane biomass were done on days 108, 174, 181, 188, and 234 in order to trim back growth. Over 164 days, 366 kg of sea purslane was harvested from the hydroponic plant beds.

Table 3.13: General information about plant bundles collected for sale.

Species	Number of pieces per bundle	Average piece length (cm)	Average piece weight (g)
Sea purslane	21 ± 1	23.3 ± 8.6	9.54 ± 5.2
Saltwort	34 ± 8	22.9 ± 7.0	5.36 ± 3.5

The plants collected on day 244 had been recently harvested and represent a baseline size for sea purslane. At this time the above ground biomass had a mean of 102 ± 30 g FW. After 28

days, the plants had approximately doubled in size to a mean above ground biomass of 269 ± 86 g FW. At this weight 113 grams could be harvested from individual net pots to produce one bundle, while leaving enough biomass to maintain the baseline size. After an initial 80 days to reach a harvestable size, sea purslane plants could be harvested every 28 days. Considering that there were 55 net pots per hydroponic raft and assuming a bundle was harvested from each pot, about 6.2 kg FW biomass can be harvested per raft and about 34 kg per raceway or 0.55 kg/m^2.

Table 3.14: Total quantity of fresh weight biomass harvested during specified day ranges. This includes biomass collected for sale and biomass discarded.

Day	Biomass (kg)
108-118	82.8
119-132	2.02
133-148	17.1
149-160	5.72
161-174	80.1
175-188	125
189-216	33.8
217-244	66.6
245-272	29.2

It is difficult to compare this estimated harvest data with other research due to variations in species and harvest regime. Comparison with available research indicates the harvest yields were low; however, the data presented above is a rough estimation and observations indicate greater harvest quantities could be sustained. A few studies that have employed a similar 28 day cropping regime were used for comparison. The study by Rakocy et al. (2004) looked at basil growth in a freshwater aquaponic system and reported basil production of 2.0 kg FW/m^2. The halophyte *Salicornia europae* was grown in a constructed wetland with greater harvest yields of 2.6 ± 1.1 kg FW/m^2 (Webb et al., 2013). Similarly, Ventura et al. (2011) produced mean harvest yields of 2.2-2.7 kg FW/m^2 of *Salicornia*.

Considering that the sea purslane did not appear to be nutrient limited and the harvest yields were lower than other studies, additional biomass could be sustained. If the same density

was maintained the size of the hydroponic plant beds would need to be increased. In this study, the size of the hydroponic plant beds was limited by the physical footprint of the greenhouse. Alternatively increased harvest yields could be obtained by increased plant densities. The functional density of 19.5 net pots/m^2 in this study was low compared to the higher densities of 184 and 92 plants/m^2 used in the bench-scale testing (Chapter 2). The results of the two studies are conflicting in that the high density bench-scale systems did not grow at a similar rate as the full-scale system during the first 28 days, possibly due to differences in planting density. This study provides a foundation on the production of sea purslane in aquaponic systems; however, more testing is needed to determine the ideal planting density for maximum harvest yields.

3.5.7 Possible Causes for Poor Plant Performance

Saltwort was not included in the harvest estimates due to its limited growth and unidentified deficiencies. Throughout the study a bleaching effect was noticed on the saltwort and to a minor extent on the sea purslane plants. Chlorosis of new growth can be an indicator of an iron deficiency (Marschner, 2011). In many aquaponic systems an iron chelate is added to prevent this deficiency. Rakocy et al. (2004) added enough of an iron chelate (13% EDTA Fe) to maintain a concentration of 1.8-3.0 mg/L Fe. Grab samples collected and analyzed for total iron after the study was completed indicated iron levels were below detection limits in the system water. Based on these rudimentary measurements, iron deficiency was a potential cause for bleaching.

Light stress can also cause a bleaching effect in plants. This is caused by the inability of plants to utilize all the energy accumulated by chlorophyll, resulting in photooxidative damage or chlorosis (Mullineaux and Karpinski, 2002). In this study, colorless plant tips were observed more frequently in plants that did not have the protection of the shade cloth and 85% shading.

While excess light possibly limited growth, the plants are native to Florida and should have been adapted to both the light intensity and temperatures of the greenhouse. Furthermore both species have been grown at the same facility with similar shading conditions.

High nitrate concentrations may have contributed to the reduced growth of saltwort. Growth was limited until the nitrate concentrations were reduced through modification of the sand filter. Prolonged exposure to excessive nitrate has been shown to reduce growth of some plant species (Reddy and Menary, 1990). Claussen and Lenz (1999) found Highbush blueberries grown in nitrate only solutions showed leaf chlorosis and hypothesized limited nitrate reductase activity was the cause. The ability of halophytes to uptake nitrate is documented (Stewart et al. 1973); however, there is limited information specifically about *Batis maritima* or about the potential for nitrate toxicity in halophytes.

Finally, it is possible the saltwort was not well suited to the soilless hydroponic culture. As a plant accustomed to growing in dense clusters in coastal marshy areas the absence of soil could have stressed plants triggering discoloration and limiting growth (Lonard et al., 2011). Due to the challenges in the study, further research should be completed on production of saltwort in hydroponic culture before it is considered for commercial production in an aquaponic system.

Sea purslane production was also temporarily disrupted by presence of a Hawaiian beet webworm. The caterpillar can be found throughout North America and commonly is found on vegetable crops (Capinera, 2001). In order to combat the caterpillar, netting was added over the hydroponic plant beds and foliar application of a biological insecticide containing *Bacillus thuringiesis* was performed as needed. These measures were moderately successful although the presence of the moths continued to be a problem.

3.5.8 Fish Production

Tanks were initially stocked with 200 red drum per tank, with a mean density of 2.8 kg/m^3 on day 0 (Table 3.15). An additional 100 fish were added to each tank after 10 days, bringing the mean density to 4.23 kg/m^3. Growth rates were excellent and on day 100 mean density had surpassed the 21.3 kg/m^3 that the MBBR was sized for, resulting in the culling of fish to reduce the density. Twelve months after the fish were hatched (9 months of system operation), the fish had reached harvest weight of 900 g (2 lb).

Table 3.15: Summarized data on fish production.

Parameter	Day				
	0	10	34	70	100
Number of fish	200	300	296	298	293
Mean fish weight (g)	46.5	46.5	183	248	294
Mean Density (kg/m^3)	2.82	4.23	16.42	22.4	26.1

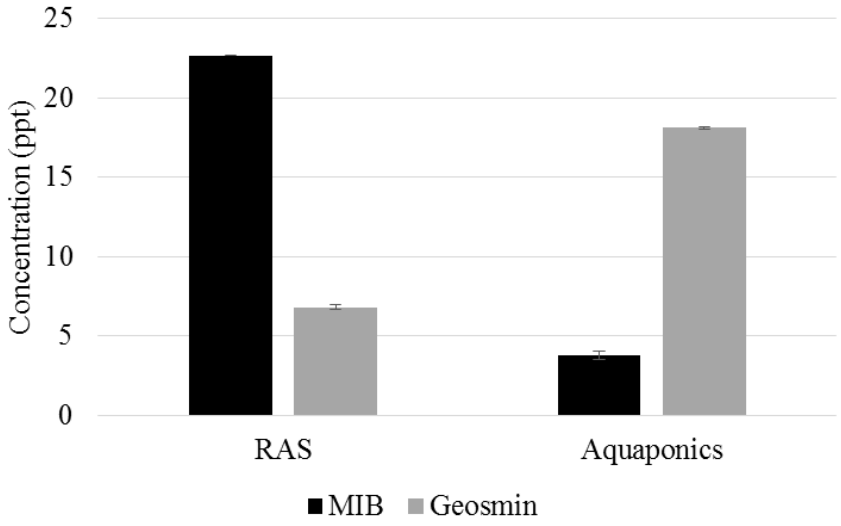

Figure 3.9: MIB and geosmin concentrations in conventional RAS and marine aquaponic system. Concentrations in parts per trillion.

Approximately 8 months after the fish were added to the aquaponic system, selected fish were harvested for taste testing to evaluate the presence of off-flavor compounds, such as geosmin and MIB. Taste test results indicated that the red drum produced in the aquaponic system were free from off-flavor compounds. This finding was in contrast to fish from the same

cohort produced in a conventional RAS at the same site. An analysis of water samples collected from both systems showed that the marine aquaponic system had a lower MIB concentration (Figure 3.9). The results for geosmin were opposite. The analytical tests were only completed once. The removal of off-flavor compounds can be a major operational expense in aquaculture (Schrader et al., 1998); therefore, future research is planned to complete a more in-depth analysis of the ability of aquaponic systems to remove off-flavor compounds.

Most of the literature on aquaponic systems is based on small-scale systems. The only information on larger or commercial-scale systems is from the University of the Virgin Islands system. In that system, Nile or Red tilapia are stocked at an initial weight of 79.2 g/fish and 58.8 g/fish, respectively (Rakocy, 2012). The maximum densities for the Nile and Red tilapia are 61.5 kg/m^3 and 70.7 kg/m^3, respectively (Rakocy, 2012). If the marine aquaponic system, in this study, maintained approximately 300 fish per tank, at a harvest weight of 0.9 kg per fish it would achieve a greater maximum biomass of 82 kg/m^3; however, culling was implemented on a weekly basis to prevent excessive fish densities. Tilapia when stocked at a commercial densities of 107 $fish/m^3$ resulted in an accumulation of nutrients in an aquaponic system (Rakocy, 2012). The addition of the sand filter, allowed this study to prevent accumulation of nutrients and successfully maintain fish densities greater than the 21 kg/m^3 the MBBR was designed to accommodate.

Red drum are conventionally produced in semi-intensive culture ponds or intensive land-based RAS (Davis, 1996). Some research on the production of red drum in land-based systems for stock enhancement has been conducted at MAP and Harbor Branch of Florida Atlantic University. Research conducted at Harbor Branch examined production of red drum in a RAS (Wills et al., 2010). Different culture densities of 365 $fish/m^3$ (20 kg/m^3) and 547 $fish/m^3$ (30

kg/m^3) were varied with feeding rates of 3% or 4% BWD. No differences in survival were found. Fish fed 4% BWD had significantly greater weight. Based on these results, it is possible that greater fish densities and feeding rates could be supported in the aquaponic system; however, the results from Wills et al. (2010) are for juvenile fish and may not be applicable for harvest size fish. If the greater densities could be supported, the additional surface area for nitrification in the hydroponic plant beds and the additional denitrification provided by the sand filter would prevent accumulation of nitrogen.

3.6 Conclusion

A marine aquaponic system successfully produced red drum and halophytes during a 9 month study period. Throughout the study, water quality was maintained within safe ranges for fish health, although several modifications were necessary to improve nutrient removal. Accumulation of nitrate early in the study prompted conversion of the side-stream sand filter to a partially submerged denitrification reactor. This change contributed to a system-wide decrease in total nitrogen concentrations. Although the sand filter was important for nitrogen removal, removing about 17% of nitrogen added daily, mass balances indicated nitrogen was predominantly removed through passive denitrification, removing about 77% of nitrogen added daily. Plants contributed to less than 10% of nitrogen removal. Phosphorus concentrations fluctuated mainly due to plant growth and mineralization/sedimentation equilibrium of phosphorus. Overall, phosphorus accumulated in the system and plants only removed a small portion of excess phosphorus of about 9%.

Fish were successfully grown to a harvestable size of 0.9 kg and greater fish densities could potentially be supported due to the increased nitrification capacity from the hydroponic plant beds and side-stream denitrification in the sand filter. Often in aquaponic systems the

economic value is considered to come from the production of plants as opposed to fish. This study shows that the addition of side-stream denitrification can increase the capability of an aquaponic system to produce fish at commercial quantities, while producing just enough plant product to meet a niche market demand.

The halophytes contributed to nutrient removal; however, the low total plant biomass compared to daily feed inputs limited total nutrient removal by plants. While the estimated halophyte yields were low compared to other studies, the mass balances indicated greater quantities of biomass could be supported. Due to the limited physical footprint of the prototype system, greater plant densities were not possible at this time. At present, the market for halophytes is being developed and the limited plant production was not a constraint. In the future, as the market for halophytes grows and the marketability as a luxury cash crop contributes to high economic value, an ideal system would have maximized plant production. Future work should build on the results presented here to develop optimized systems that maximize fish and plant production per unit of land area, start-up capital costs, and long-term operating costs to maximize the economic potential of marine aquaponics.

Chapter 4: Life Cycle Assessment of Aquaculture Systems: Does Burden Shifting Occur with an Increase in Production Intensity?

4.1 Introduction

Finfish and other aquatic animals are critical to providing a high-value protein source and important micronutrients for much of the world. According to the FAO State of World Fisheries and Aquaculture (2012b), in 2009, 16.6% of animal protein consumed was from finfish. While protein intake from fish consumption varies regionally and with a country's development status, fish protein is particularly important in many African and Asian countries where it contributes to greater than 20% of animal protein consumption (FAO, 2012b). These are also areas where population growth is anticipated to be greatest and food security is of concern (UN, 2013).

As yields from capture fisheries remain stable, aquaculture has become more important to increasing production of aquatic food products and improving food security. Aquaculture's critical role in meeting increased demands for aquatic food products is driving researchers to assess the sustainability of the industry. Consumers are also becoming increasingly concerned with the environmental and ethical impacts of their food choices (Andersson et al., 1994). Since aquaculture already has a major role contributing to global food supplies, it is important to evaluate the current environmental impacts associated with aquaculture.

Life cycle assessment (LCA) is a tool used to quantify local and global environmental impacts of systems and processes. It is considered a "cradle to grave" analysis, meaning that the assessment includes raw material extraction through the final disposal of all components (EPA 2006). LCA has become a valuable tool used to evaluate a variety of systems, including biofuel

production, wastewater treatment systems, agriculture, and aquaculture (Campbell et al., 2011; Stokes and Horvath, 2006; de Vries and de Boer, 2010).

Prior LCA studies have looked at environmental impacts from fishing vessels and fleets, fish feed, and aquaculture systems. Avadí and Freon (2013) reviewed 16 papers on LCAs of capture fisheries production. The review focused on differences in methodologies used to complete the LCAs. Henriksson et al. (2012) also completed a review focused on differences in LCA methodologies. Their review looked at different aquaculture production systems from 12 papers. Both review papers found variability in the methodologies used and suggested that their needs to be more standardization of methodology and aquaculture specific impact categories. Variations in reporting methodological and data choices hinder direct comparison of different studies; however, important industry trends can still be seen by reviewing different LCA studies on aquaculture.

Intensive aquaculture systems, such as recirculating aquaculture systems (RAS), in which 90 to 99 % of system water is recycled (Badiola et al., 2012), are commonly cited as a more sustainable option for aquaculture production due to localized reduction in water inputs and nutrient discharges. However, the high energy and material requirements for RAS, which can contribute to greater global impacts, such as global warming potential, are not usually included when discussing the sustainability of intensive systems. For this reason this review compares high input, high density intensive systems to low density, low input extensive systems. The aim of this review was to evaluate studies on intensive and extensive aquaculture systems, within a LCA framework, to develop a more complete picture of the environmental trade-offs incurred due to intensification of aquaculture systems.

4.2 Materials and Methods

Studies on aquaculture production were reviewed to compare differences in environmental impact. Papers were identified using web searches in the online database ScienceDirect and the internet search engine Google Scholar using combinations of the keywords: life cycle assessment, environmental impact, fisheries, aquaculture, recirculating aquaculture systems, and integrated aquaculture systems. Capture fisheries were neglected given the recently published review by Avadí and Freon (2013). Twelve papers were found that contained information on the pertinent aquaculture systems and are discussed in the results (Table 4.1).

The ISO 14040 four step methodology (goal and scope, life cycle inventory, life cycle assessment, interpretation) was used as a framework to compare aquaculture LCA studies. Specific processes commonly considered in the system boundaries and impact categories of interest were isolated for in-depth analysis. While the review is focused on variation in environmental impact of different aquaculture systems, an analysis of the goal and scope and life cycle inventory are necessary to establish a baseline and facilitate comparison of each studies' results.

4.3 Results

4.3.1 Goal and Scope

The goal and scope definition is the first step of an LCA. It should provide a clear statement of the study's purpose. Development of the scope is often comprised of an explanation of the system boundaries, functional unit, the impact assessment methodology, impact categories, and allocation used in the study. This step determines what information is included or excluded in the LCA and facilitates or hinders comparisons between studies.

Table 4.1: List of studies included in literature review and important characteristics of each study.

	Systems included	Location	Species	Functional unit	Impact assessment method[1]	FCRs	Infrastructure included	Integrated with other animals/plants
Aubin et al. (2009)	Flow through; sea cages; RAS	France, Greece	Rainbow trout; Sea-bass; Turbot	1 ton harvest ready live-weight fish	Papatryphon et al. (2004)	1.21, 1.77, 1.23	Yes	No
Aubin et al.(2006)	RAS	France	Turbot	1 ton live fish weight	Papatryphon et al. (2004)	1.23	Yes	No
Ayer and Tyedmers (2009)	Marine floating bag; land-based flow through; land-based RAS	Canada	Salmonids	1 ton harvest-ready live-weight fish	CML 2 Baseline 2000; CED v 1.03	Not reported	Yes	No
Efole Ewoukem et al. (2012)	Fish ponds integrated with pig manure, wheat bran, pig manure and crop by-products, or pig and chicken manure	Cameroon	Tilapia	1 ton fresh fish	CML 2 Baseline 2001; Aubin et al. (2009)	Not reported	Yes	Yes
Gronroos et al. (2006)	Net cage and land-based ponds	Finland	Rainbow trout	1 ton un-gutted rainbow trout after slaughtering	Individually calculated	1.255, 0.9, 1.53	No	No
Jerbi et al. (2012)	Traditional raceway, Cascade raceway	Tunisia	Sea bass, sea bream	1 ton live fish weight	CML 2 Baseline 2000; Papatryphon et al. (2004)	1.8, 2.1	Yes	No
Mungkung et al. (2013)	Net cage	Indonesia	Carp; tilapia	1 ton fresh fish to market	CML 2 Baseline 2000; CED v 1.03	1.7, 2.1	Yes	Yes

[1] Note: CML-Center for Environmental Studies, University of Leiden; CED- Cumulative Energy Demand Method

Table 4.1 (Continued)

	Systems included	Location	Species	Functional unit	Impact assessment method[1]	FCRs	Infrastructure included	Integrated with other animals/plants
Pelletier and Tydemers (2010)	Lake and pond	Indonesia	Tilapia	1 ton live-weight tilapia	CML 2 Baseline 2000; CED V1.03; Pelletier and Tydemers (2207)	1.7	No	No
Phong et al. (2011)	Fish ponds (high, medium, low intensity) integrated with rice fields or orchards	Vietnam	Fish	kilocalorie and kg per farm product	Individually calculated	Not reported	Not specified	Yes
Roque d'Orbcastel et al. (2009)	Flow through; low head RAS	France	Trout (various sp.), artic char	1 ton of fish	CML 2 Baseline 2001	1.1, 0.8	Yes	No
Samuel-Fitwi et al. (2013)	Extensive flow through; Intensive flow through; RAS	Denmark, Germany	Rainbow trout	1 ton live trout	CML 2 Baseline 2000	Not reported	No	No
Wilfart et al. (2013)	RAS; semi-intensive pond; extensive polyculture pond	France	Salmon; common carp; tench; roach; perch; sander; pike	1 ton live fish	CML 2 Baseline 2001; CED v 1.05	0.95, 1.29, 0.86	Yes	No

The organization of this information varied in the studies reviewed. Some studies included it all in one goal and scope section (Aubin et al., 2009; Ayer and Tydmeres, 2009; Phong et al., 2011), but most divided the goal and scope into additional sections. Only a few studies included a clearly expressed goal within the goal and scope definition (Jerbi et al., 2012; Samuel-Fitwi et al., 2013) many included a goal in the introduction (Aubin et al., 2006; Aubin et al., 2009; Ayer and Tydmeres, 2009; Efole Ewoukem et al., 2012; Gronroos et al., 2006; Phong et al., 2011; Roque d'Orbcastel et al., 2009; Wilfart et al., 2013). In general, the goals of the reviewed studies were to quantify or evaluate the environmental impacts of the studied systems while some included comparisons of different systems or operational scenarios. For example, Roque d'Orbcastel et al. (2009) stated "the aim of this study was to compare the LCA of two scenarios of trout production systems…"

4.3.2 System Boundaries

The system boundaries define what processes are included in the LCA. In its most basic form this includes all processes from cradle to grave. System boundaries of food product studies often stop at farm-gate and do not include processing, retail, or household use (Henriksson et al., 2012). Most of the reviewed studies used a boundary of cradle to farm-gate. Aubin et al. (2009) and Mungkung et al. (2013) only looked at hatchery to farm gate. Gronroos et al. (2006) used a system boundary that ended at delivery to additional processing or retailers and included packaging materials, production, and manufacture.

Within the defined boundary, each system was broken into different processes. The classification of these components is up to the author's discretion and varied among the papers reviewed. Feed, diet, or feed components were included in all studies. Energy carriers or electricity production were also commonly reported as a separate process. Where energy carriers

were not included as a separate process they were included within other processes (Gronroos et al., 2006; Pelletier and Tydemers, 2010). In the three studies where agriculture was integrated with aquaculture (Mungkung et al., 2013; Phong et al., 2011; Efole Ewoukem et al., 2012), energy was included in the system boundary but was not isolated as an individual process.

Across industries, infrastructure and capital goods have been excluded from LCAs based on the assumption that the impacts are relatively small (Frischknecht et al., 2007; Henriksson et al., 2012). Specifically within aquaculture, Ayer and Tyedmers (2009) reported that infrastructure's impacts were negligible in salmon production. Based on the results of Ayer and Tyedmers (2009), studies by Pelletier et al. (2009) and Pelletier and Tyedmers (2010) excluded infrastructure in their LCAs. The studies that were more likely to include infrastructure as a process were those that evaluated either land-based RAS or flow-through systems. Samuel-Fitwi et al. (2013) looked at RAS and flow-through systems, but provided no justification for excluding infrastructure in an LCA. Most studies that looked at ponds or net cages did not include infrastructure except Efole Ewoukem (2012).

4.3.3 Functional Unit

LCA relates the environmental impact to the production system through the functional unit (FU). The FU quantifies the intended purpose of the production system. Comparisons between different systems is only possible if they have the same FU. Typically the FU is based on the primary product produced but can be refined to include temporal and quality criteria for a more complete description of the system function (Avadí and Freon, 2013; Cooper, 2003).

The papers reviewed used similar functional units, in that they were mass quantities of fish. The amount of post-harvest processing, species, and quantity varied between papers. In general all the FUs were variations on 1 ton live-weight fish. Phong et al. (2011) studied an

integrated agriculture-aquaculture system with multiple products and therefore used two FU: kilocalorie and kg per individual farm product.

4.3.4 Allocation

Many systems have multiple products, which poses a problem when estimating the environmental impact. The environmental impact is not necessarily equally divided between the multiple outputs or co-products. Material and energy flows attributed to co-products must be allocated in a systematic way (Henriksson et al., 2012). The ISO (2006) describes a three step hierarchy to address allocation issues: 1) avoid allocation through subdivision or system expansion, 2) use allocation based physical relationships, 3) use allocation based on another non-physical relationship.

Four papers used economic allocation to divide environmental impacts between co-products where necessary. In Ayer and Tyedmers (2009) and Pelletier and Tyedmers (2010), the gross nutritional energy content was used to allocate environmental burdens. Allocation by gross nutritional energy content has been proposed as appropriate for seafood production because it incorporates the main function of aquaculture, chemical energy production in the form of food (Ayer, 2007). Ayer and Tyedmers (2009) also used system expansion to account for recovered fish waste in a RAS. To account for the use of fish waste as an organic fertilizer, an offset of an equivalent amount of chemical fertilizer was applied. In Gronroos et al. (2006), allocation was avoided by using whole fish as the functional unit to prevent allocation issues with co-products during processing.

4.3.5 Impact Assessment Methods

Life cycle impact assessment involves selecting impact categories and assigning characterization factors (Avadí and Freon 2013). A standardized method is often used to apply

the characterization factors to the life cycle inventory results; however, some methods are calculated independently (Avadí and Freon, 2013). A wide range of impact categories and characterization methods have been used for aquaculture studies. The diversity of impact categories used can impede comparison between studies, similar to difficulties with different system boundaries or functional units.

In total, twenty three different impact categories were used (Table 4.2). The CML baseline method was the only standardized method used to calculate common impact categories, such as eutrophication potential, acidification potential, and global warming potential. Studies that did not use the CML baseline method or had additional impact categories, used independent methods for characterization.

All studies included eutrophication and acidification potentials. Gronroos et al. (2006) considered eutrophication of aquatic and terrestrial systems individually. Gronroos et al. (2006) used characterization factors specific to Finland as opposed to using standardized impact assessment methods that do not incorporate regional effects. A measure of kg CO_2 equivalents was included in all the studies termed either greenhouse gas emissions or climate change. Energy use was considered in all but two of the papers; five different terms were used and three different units.

The above impact categories are all measures of abiotic resource use; however, in food production, biotic resources are also consumed. Net primary production (NPP) can be used as a quantifiable measure of biotic resource use. The calculation of NPP use (NPPU) is based on the principle that plants convert sunlight into chemical energy and store it as carbon complexes. These carbon complexes move between trophic levels losing efficiency as carbon is transferred to higher trophic levels. NPP is a finite resource, using it as an impact category can help identify

Table 4.2: Impact categories used in reviewed LCA studies with reporting units.

	AD	GWP	CC	HTP	MTP	AP	EP	CED	EU	NREU	TCED	FEU	NPPU	LC	LU	SU	LO	WU/ WD
	kg Sb eq	kg CO_2 eq	kg CO_2 eq	kg 1,4-DB eq	kg 1,4-DB eq	kg SO_2 eq	kg PO_4 eq	MJ	MJ	GJ and MJ	GJ	kJ	kg C	m^2a or m^2yr	m^2/ yr	m^2	m^2 yr	m^3
Aubin et al. (2009)			x			x	x		x				x					x
Aubin et al.(2006)		x				x	x			x (MJ)			x					
Ayer and Tyedmers (2009)	x	x		x	x	x	x	x										
Efole Ewoukem et al. (2012)						x	x			x (GJ)			x		x			x
Gronroos et al. (2006)			x			x	x											
Jerbi et al. (2012)		x				x	x		x				x			x (m^2/yr)		x
Mungkung et al. (2013)			x			x	x		x				x				x	x
Pelletier and Tydemers (2010)		x				x	x	x					x					
Phong et al. (2011)			x			x	x (NO_3 eq)					x			x (m^2)			

Table 4.2 (Continued)

	AD	GWP	CC	HTP	MTP	AP	EP	CED	EU	NREU	TCED	FEU	NPPU	LC	LU	SU	LO	WU/ WD
	kg Sb eq	kg CO_2 eq	kg CO_2 eq	kg 1,4-DB eq	kg 1,4-DB eq	kg SO_2 eq	kg PO_4 eq	MJ	MJ	GJ and MJ	GJ	kJ	kg C	m^2a or m^2yr	m^2/ yr	m^2	m^2 yr	m^3
Roque d'Orbcastel et al. (2009)		x				x	x		x				x			x		x
Samuel-Fitwi et al. (2013)		x				x	x							x				x
Wilfart et al. (2013)			x			x	x				x		x	x				x
Sum	1	6	5	1	1	12	12	2	4	2	1	1	8	2	2	2	1	7

AD: Abiotic Depletion; GWP: Global Warming Potential; CC: Climate Change; HTP: Human Toxicity Potential; MTP: Marine Toxicity Potential; AP: Acidification Potential; EP: Eutrophication Potential; CED: Cumulative Energy Demand; EU: Energy Use; NREU: Non Renewable Energy Use; TCED: Total Cumulative Energy Demand; FEU: Fossil Energy Use; NPPU: Net Primary Production Use; LC: Land Competition; LU: Land Use; SU: Surface Use; LO: Land Occupation; WU: Water Use; WD: Water Dependence

areas of inefficient resource allocation and can be used to improve the ecological efficiency of aquaculture (Pelletier and Tyedmers, 2007). NPPU measured as kg C was used as a characterization factor in eight of the papers reviewed. Most papers used the methodology described in Papatryhon et al. (2004). Only Pelletier and Tyedmers (2010) calculated biotic resource use with methods described in Pelletier and Tyedmers (2007).

In seven of the reviewed papers, land or surface use was used as an impact category. Land use encompasses the alteration of land directly through the removal of natural landscape due to deforestation, agricultural practices, or construction of impervious surfaces (Brentrup et al., 2002). The assumption is that land should be conserved and excessive loss of land due to human development, has negative impacts on the environment (Brentrup et al., 2002). Land use or land use occupation is typically measured as an area time, m^2a or m^2yr (Mattila et al., 2011) Each paper independently calculated land use and accounted for surface area occupied by crops for feed production and area occupied by physical aquaculture systems in m^2, m^2a, or m^2/year.

Land use is one method to connect natural resources with aquaculture, water use or water dependence are also measures of natural resource depletion. In aquaculture, water use is of particular importance because some production systems, like flow-through systems, are criticized for high volumes of water use, while others like RAS are commended for low water use. Incorporating this impact category can provide information about potential burden shifting of decreased water use. Six of the reviewed studies incorporated water use/water dependence as an impact category measuring m^3 of water flowing into production systems.

4.3.6 Impact Assessment Results and Interpretation

Interpreting the results from the impact assessment is the final step of a LCA. The purpose of the interpretation step is to translate the results from the impact assessment into

general conclusions about the type of environmental impact (global warming, eutrophication, etc.) and the system processes that contributed greatest (feed, energy, etc.). In the sections below, the results from three processes and three impact categories commonly included the reviewed papers are discussed.

4.3.6.1 Feed

In the papers reviewed, feed typically had the greatest environmental impact on NPPU and energy use. Several papers compared different types of aquaculture production systems. In Roque d'Orbcastel et al. (2009), a comparison between a RAS and a flow-through system for the production of trout showed that feed contributed greatest to NPPU (21,432 to 28,126 kg C) and energy (17,746 to 23,289 MJ). A sensitivity analysis on the feed conversion ratio (FCR) showed a reduction in NPPU and energy use could be achieved if the FCR of the RAS was decreased from 1.1 to 0.8. While the suggested 0.8 FCR was based on an experimental RAS, this level of efficiency is achievable in RAS producing various trout species (Buřič et al., 2014).

Similar results from a reduction in FCR were found in Jerbi et al. (2012) comparing two types of flow-through systems. Feed contributed approximately 40,000 kg C, which could be due to the higher FCRs of 1.89 and 2.11 in Jerbi et al. (2012). Estimates of energy use from feed for the systems of Jerbi et al. (2012) ranged from 29,000 MJ to 33,412 MJ and these were also likely higher than in Roque d'Orbcastel et al. (2009) due to the higher FCRs. Aubin et al. (2009) compared a trout flow-through system (FCR=1.21), sea-bass cages (FCR=1.77), and a turbot RAS (FCR=1.23). Similar as above, feed production contributed greatest to NPPU and energy use. The NPPU was 62,200, 71,400, and 60,900 kg C for the flow-through, cage, and RAS respectively. The values are similar to those found in Jerbi et al. (2012), but greater than those

found in Roque d'Orbcastel et al. (2009) possibly due to the variations in system boundaries despite similar FCRs.

The environmental impacts of feed can also change with intensity. In Samuel-Fitwi et al. (2013), three different system intensities were explored (extensive flow-through, intensive flow-through, and intensive RAS). Impacts from feed decreased with increasing intensity for all impact factors due to improved FCRs. As intensity increases FCRs typically improve, which results in decreased environmental impact, as shown with the sensitivity analysis in Roque d'Orbcastel et al. (2009). Mungkung et al. (2013) considered two net-cage systems with an intensive and semi-intensive stocking density. The systems were integrated the produce two species simultaneously. In the intensive, high density system the NPPU and energy use were 14,205 kg C and 28,645 MJ, respectively. These values are lower than in the semi-intensive, lower density system which had an NPPU and energy use of 16,462 kg C and 32,945 MJ, respectively. Mungkung et al. (2013) concluded that the cause of this difference was due to the greater feed efficiency in the intensive system.

In extensive systems the contribution of feed is decreased because fertilizer, often in the form of animal manure, is added to increase primary production of algae and microorganisms on which the fish feed. Wilfart et al. (2013) looked at a RAS and pond systems with two levels of intensity. The contribution of feed to NPPU was 333 kg C to 744 kg C because a lower quantity of the feed came from harvesting higher trophic level fishery resources. Feed as system process was not considered directly in Efole Ewoukem et al. (2012). Four Cameroonian ponds systems were studied that used manure or crop by-products as fertilizer with no additional commercial feed products. NPPU was greatest in the pond that integrated pig and fish production (8,600 kg C) and this system also had the greatest yield. The lower yield, systems using wheat bran, pig,

chicken, crop by-products or a combination of these fertilizers, had NPPU of 1,000 kg C to 1,700 kg C.

Two studies isolated the impact of feed components to environmental impact in addition to looking at system wide impacts. Gronroos et al. (2006) looked at variation in feed and found that improving the FCR or changing the feed composition, such as increasing the soy content, decreases the impact of feed for all categories. In Pelletier and Tyedmers (2010), crop and fisheries derived tilapia feeds were evaluated. The results from this assessment showed that the greatest contribution to NPPU was fish meal and fish oil used in pelleted feed. For example, fish oil uses over 40 times more kg C than palm oil. Cumulative energy demand was also greater from the fisheries derived components, however the margin was smaller. Fish oil was associated with 33,000 MJ and palm oil 4,580 MJ.

4.3.6.2 Energy

Energy was used as a system process in several of the reviewed papers and was typically reported as either electricity or energy carriers. In papers that did not consider energy directly as a process, the impact category cumulative energy demand or energy use was used to draw conclusions about the aquaculture system's energy consumption and associated environmental impacts.

Intensive flow-through systems and RAS require large quantities of electricity for operation. When comparing flow-through systems and RAS, RAS typically have higher energy requirements due to the pumping requirements for water recirculation. In Ayer and Tyedmers (2009), electricity for the RAS had an energy demand of 291,000 MJ, compared with a demand of 70,100 MJ for the flow-through system. The impacts of electricity are also seen in global warming potential (GWP). The RAS had a GWP of 23,700 kg CO_2 eq and the flow-through had

a GWP of 1,020 kg CO_2 eq associated with electricity. Other studies have found similar trends for energy in RAS and flow-through systems. Aubin et al. (2009) considered energy carriers as a process and compared three production systems, a cage system, flow-through system, and RAS. The energy use increased with higher on-farm energy consumption. The energy use for each system was 9,191 MJ, 37,132 MJ, and 290,985 MJ for the cage, flow-through, and RAS, respectively. The GWP followed the same trend; GWP was 163 kg CO_2 eq, 406 kg CO_2 eq, and 3670 kg CO_2 eq for the cage, flow-through, and RAS, respectively. The calculated GWP in Aubin et al. (2009) was low compared to the RAS in Ayer and Tyedmers (2009) despite similar energy use values because the system evaluated was located in France, where a higher proportion of electricity is produced by nuclear power plants. A sensitivity analysis in Ayer and Tyedmers (2009) illustrated the importance of the type of electricity generation. When the energy mix was varied to include less coal based production and more hydroelectricity, the GWP decreased from 23,700 kg CO_2 eq to 10,300 kg CO_2 eq.

The source of electricity is not the only factor that impacts the energy process. In Wilfart et al. (2013), a turbot RAS required more energy, due to water heating and cooling requirements, than a salmon RAS. The turbot RAS had an energy use of 250,010 MJ and the salmon RAS 55,530 MJ. The GWP followed the same trends. The turbot RAS had a GWP of 3,670 kg CO_2 eq and the salmon RAS had a GWP of 417 kg CO_2 eq A study comparing two flow-through systems also concluded that operational decisions influence environmental impacts (Jerbi et al., 2012). The flow-through systems with a cascade raceway had greater electricity use due to greater pumping requirements. The LCA results showed a higher total GWP of 17,500 kg CO_2 eq in the cascade raceway, with electricity contributing greatest to the GWP. Roque d'Orbcastel et al. (2009) also evaluated different operational characteristics of aquaculture systems. When two

different pumping scenarios were considered for flow-through systems, the high pumping scenario had a greater energy use and GWP.

Extensive systems have much lower energy requirements than the intensive systems discussed above. In Phong et al. (2011) electricity was included in the LCA, but not directly as a process. The contribution to impact categories was divided into on-farm and off-farm use. For the impact category of energy use, most of the use was attributed to off-farm activities, which includes inorganic fertilizer production, rice co-products, and feed. Since this study considered integrated agriculture and aquaculture, the authors also looked at the contribution of farm products to the impact categories. The on-farm energy use for pigs and fish were similar at 314 kJ/kg and 353 kJ/kg, respectively, and poultry was higher at 583 kJ/kg. GWP did not follow the same trend; instead pig and poultry had a high on-farm GWP of 6.5 kg CO_2 eq/kg and 7.0 kg CO_2 eq/kg and fish was slightly lower at 5.0 kg CO_2 eq/kg, but not significantly different. Mungkung et al. (2013) looked at extensive pond systems that produced multiple fish products. Energy was not considered directly as a process, but the impact category of energy use was used. As mentioned above, feed contributed most to energy use; the contribution of farm operation was negligible. Pelletier and Tyedmers (2010) considered the process of farm energy use for the pond and lake systems studied. The lake systems did not require aeration, as such they had low energy use and less of the GWP was due to farm energy use. In contrast the pond systems required more electricity for aeration and had higher energy use and GWP.

4.3.6.3 Infrastructure

In addition to energy, infrastructure is another factor that distinguishes intensive and extensive aquaculture systems. Intensive cage systems, flow-through systems, and RAS all have greater material requirements than extensive pond systems. In an LCA these material inputs are

occasionally considered, but more frequently they are considered negligible and are excluded from the life cycle inventory (Avadí and Freon, 2013).

Ayer and Tyedmers (2009) included infrastructure and provided tables showing their inventory data. Of the four systems compared, the RAS and net-pen systems typically had high impacts from infrastructure. Most of the impacts from infrastructure were seen in the marine toxicity potential and the second greatest impact was to cumulative energy demand/energy use. Focusing on the marine toxicity potential and cumulative energy demand/energy use impact categories, the impacts from infrastructure were consistently much lower than the impacts of electricity or feed production. For example, in the RAS that had the highest impact to marine toxicity potential, infrastructure only contributed 0.13%. In contrast, electricity production contributed 93% of the marine toxicity potential.

Other studies that included infrastructure also reported that it contributed to less than 10% of environmental impact for all impact categories included. Aubin et al. (2009) considered infrastructure impacts on three types of aquaculture systems. No trends were observed between production systems. The greatest impacts from infrastructure were to cumulative energy demand and climate change, but they were all less than 10%. The other papers reviewed which considered infrastructure were Wilfart et al. (2013), Jerbi et al. (2012), Mungkung et al. (2013), and Roque d'Orbcastel et al. (2009). In all cases the impacts of infrastructure were less than 10%.

4.3.6.4 Land Use

Land use (LU), land competition (LC), or surface use (SU) were impact categories considered in seven of the papers reviewed. Each term is associated with a different characterization method, since methods for inclusion of land use in LCAs are still debated (i

Canals et al., 2007). Most of the papers reviewed used the method outlined in the Handbook on Life Cycle Assessment by Guinée et al. (2002) developed by the Center for Environmental Studies, University of Leiden.

Collectively the results for the land use characterization factor, regardless of methodology or units used, indicated that feed production had the greatest impact on LU. Jerbi et al. (2009) investigated SU measured in m^2/yr and found that the tank surface area occupied by a flow-through system was negligible when compared to the surface area associated with fish feed. Roque d'Orbcastel et al. (2009) looked at SU in m^2 and also found feed contributed more to SU than any other process. Feed contributed 2,097 to 2,736 m^2 of SU, while other processes contributed 0.0-0.2 m^2. When FCR was decreased, the authors saw an associated decrease in SU. At an FCR of 1.1 SU from feed was 2,752 m^2. When FCR was decreased to 0.8, SU decreased to 2,097 m^2. Two pumping scenarios, a high and a low scenario, were also considered in this study. The changes in pumping requirements did not impact surface area, further indicating the importance of feed to SU.

A comparison of three different production system intensities in Samuel-Fitwi et al. (2013) found that electricity sources can also impact LC. For the RAS studied in Samuel-Fitwi et al. (2013) feed contributed to 62% of LC in m^2a and electricity contributed to 38% of LC. When electricity generation was changed to include wind power in a sensitivity analysis, the total LC dropped to 928 m^2a or about 37% less. The RAS had the greatest impact on LU followed by the extensive flow-through system, and the intensive flow-through system was last.

When compared to extensive systems, RAS had the lowest contribution to LC in m^2yr, the extensive pond was second, and the semi-extensive pond was greatest (Wilfart et al., 2013). Instead of feed production, the on-farm fish production contributed to most of the LC. Similar

results were found in Efole Ewoukem et al. (2012), which compared the intensive flow-through system from Aubin et al. (2009) to several Cameroonian pond systems. The integrated pig and fish pond system (4,369 m^2/year) had greater LU impacts than the flow-through system (2,351 m^2/year). When compared to the other extensive pond systems in Cameroon, the impacts to LU decreased with decreasing productivity. The extensive systems studied in Phong et al. (2011) did not find LU significantly impacted by any of the processes included. When assessed on an m^2/kcal basis, all LU impacts were 0.023 m^2/kcal with no differences between on and off farm use.

4.3.6.5 Water Use

Like LU, water use (WU) is a relatively new development in LCA characterization factors. It is important to consider in aquaculture production because one of the main benefits to developing RAS is the reduction in water use compared with extensive and semi-intensive production systems. In the papers reviewed, water use and water dependence (WD) was calculated based on direct water use, specifically the quantity of water flowing into the production systems. Mungkung et al. (2013) is an exception and also indicated that the quantity of water used for crop irrigation was included in the water use. None of the papers reviewed considered indirect water use.

Aubin et al. (2009) found an increase in water use efficiency with increasing intensity. The RAS was the most water efficient, using 4.8 m^3, the cages used 52.6 m^3, and least efficient was the flow-through system, which used 48,782.2 m^3. When feed and pumping requirements were varied in Roque d'Orbcastel et al. (2009), there was no change in the water use. A comparison of flow-through and RAS showed a 93% reduction in water use. In Jerbi et al. (2012), the cascaded flow-through systems had a WD of 396,000 m^3 compared to only 190,000

m^3 in the traditional flow-through system. A comparison of two types of flow-through systems in Samuel-Fitwi et al. (2013), showed that the intensive flow-through system used only 1% of the water required in the extensive flow-through system. A RAS was also included in this comparison and it had 0% water use relative to the two flow-through systems.

In extensive systems, water use will vary with size of the ponds and production practices. The comparison of four pond systems in Cameroon showed that despite similarly sized ponds the WD varied and was not related to yield (Efole Ewoukem et al., 2012). The integrated pig and fish system had a WD of 16,900 m^3, whereas the pond fertilized with pig manure and crop by-products had a WD of 51,000 m^3. In Wilfart et al. (2013), WD was related to the pond surface area. The extensive pond in this study had the greatest WD of more than 41,000 m^3, the semi-extensive pond had a WD of 7,500 m^3, and the RAS had a WD of 2,500 m^3. Mungkung et al. (2013) were the only authors to consider additional sources of WD. Irrigation for agriculture was included in particular water for rice production. When agricultural WD was considered, feed production contributed greatest to water dependence (71%). High and low stocking density farming practices were considered. The low stocking density system had a higher WD of 1,121 m^3 compared to 877 m^3 in the high stocking density system.

The papers reviewed consistently show RAS to have lower direct water requirements and flow-through systems to have high water requirements. The extensive pond systems will vary with farming practices and pond age (Efole Ewoukem et al., 2012). Extensive pond systems can have water use similar to a flow-through system, while others might be more conservative and have lower water requirements. However, even under the conservative water use conditions, the impact will still be approximately 500 times greater than RAS.

4.3.6.6 Eutrophication Potential

Eutrophication potential is based on nutrients, particularly nitrogen and phosphorous, emitted to environment. It is the one impact category that was included in all the papers reviewed. Like WU, the potential reduction in eutrophication potential is considered an advantage to RAS.

Several papers demonstrated lower eutrophication potential in RAS compared to flow-through or other production systems. Ayer and Tyedmers (2009), which compared four production systems, found RAS to have the lowest eutrophication potential. This was predominately attributed to feed and electricity processes. In the other systems the eutrophication was predominately due to growout emissions. In the sensitivity analysis, changing the electricity mix to incorporate more renewables reduced the eutrophication potential of the RAS from 20.1 kg PO_4 eq to 11.6 kg PO_4 eq Samuel-Fitwi et al. (2013) had similar results; the extensive flow-through system, the intensive flow-through system, and the RAS had eutrophication potentials of 60.36 kg PO_4 eq, 60.03 kg PO_4 eq, and 4.04 kg PO_4 eq, respectively. In the flow-through systems, most of the eutrophication potential was due to fish production processes and in the RAS it was mainly due to electricity and feed processes. When the electricity was produced from wind power, the eutrophication potential for the RAS decreased by about half.

Reduced water discharges in RAS due to recirculation contribute to the lower eutrophication potential, but does not guarantee a RAS will have a low eutrophication potential. In Aubin et al. (2009), the differences between the flow-through system and RAS were reversed. The flow-through and RAS had eutrophication potentials of 66 kg PO_4 eq and 77 kg PO_4 eq, respectively. The higher eutrophication potential of the RAS was due to a higher protein content in the feed of 55% compared to 45% in the flow-through system. In Roque d'Orbcastel et al.

(2009) a flow-through system was also compared to a RAS. The eutrophication potential was reduced by 26-38% in the RAS. The higher percent reduction was due to a lower FCR.

The eutrophication potential of a RAS will also vary depending on the facility. Wilfart et al. (2013) compared a RAS producing salmon and the turbot RAS studied in Aubin et al. (2009). The salmon producing RAS had an eutrophication potential of 34 kg PO_4 eq and the turbot RAS had an eutrophication potential of 77 kg PO_4 eq The difference could be attributed to the higher energy use in the turbot facility, from heating and cooling the water. When the salmon RAS was compared to an extensive and semi-extensive pond system, the pond systems had lower eutrophication potentials than the RAS. The authors suggested that the lower emissions in the pond systems were due to internal nutrient cycling within the ponds which was not present in the RAS.

In extensive systems the eutrophication potential will depend on farm management practices. In Mungkung et al. (2013) the extensive pond and cage system that used feed more efficiently had a lower eutrophication potential. In Gronroos et al. (2006) the eutrophication potential was divided into aquatic and terrestrial based impacts. The aquatic eutrophication was always greater than the terrestrial eutrophication. While fish production generally contributes greatest to eutrophication potential, feed type also impacts emissions. Decreasing the FCR can reduce the eutrophication potential as seen in Gronroos et al. (2006) and Mungkung et al. (2013). Over-fertilization of pond systems will also result in a high eutrophication potential (Efole Ewoukem et al., 2012). The eutrophication potential of the Cameroonian ponds ranged from 157 kg PO_4 eq to 908 kg PO_4 eq. These values are at least double the trout flow-through system, which had an eutrophication potential of 66 kg PO_4 eq. While pond systems have reductions in

some global environmental impacts locally they contribute to greater eutrophication potentials without the benefit of increased yields as in intensive systems.

4.4 Discussion

In the first three steps of the LCA methodology there are no specific patterns distinguishing intensive, semi-intensive, or extensive aquaculture production systems. The methodological choices are largely up to the author's discretion and intended goal. All the authors followed the guidelines developed by the ISO. The variation in functional units, system boundaries, allocation methods, and characterization factors does impede a direct comparison between LCA studies. As mentioned in Avadí and Freon (2013) more standardization for fisheries practices would aid future LCA fisheries research. The analysis of specific processes and impact categories did reveal a tendency for increased intensity to result in a shift from local to global impacts for some environmental burdens.

The impact of aquaculture feeds is well known to be one of the main impediments to development of sustainable aquaculture, which is further supported by this review. Both intensity level and FCR had clear impacts on the NPPU and cumulative energy demand/energy use of aquaculture systems (Gronroos et al., 2006; Mungkung et al., 2013; Pelletier and Tyedmers, 2010; Roque d'Orbcastel et al., 2009). However, there are confounding effects to the impacts of feed between intensive and extensive systems. Extensive systems benefit from reduced feed requirements and therefore global environmental impacts due to supplemental primary production from fertilizers. The jump from extensive to intensive systems resulted in a large increase in global impacts from feed; however, more intensive systems can have also lower feed impacts due to improved efficiency and FCRs. Further improving FCRs is one way to reduce the impacts of feed. Although, at present, even with a low FCR, fish only incorporate 12 to 25% of

the nutrients from feed into biomass (Lucas and Southgate, 2012). Alternatively, reducing the impacts from feed by improving the feed utilization of the whole systems through production of a secondary species that used excess nutrients could increase the total system production and improve efficiency (Neori et al., 2004). These integrated multi-trophic aquaculture (IMTA) systems are suggested to increase the environmental sustainability of RAS due to biomitigation of wastes. In addition, these systems have the potential to increase revenues (Barrington et al., 2009; Granada et al., 2015). The potential benefits of dual species production on feed could also extend to reductions in electricity and fuel use due to greater production per unit of energy.

As expected, the electricity and fuel use by intensive systems was consistently higher than in extensive systems. Intensive systems have greater pumping and aeration requirements resulting in greater global impacts of cumulative energy demand/energy use and GWP. In IMTA systems, greater production capacity can potentially moderate these impacts. This potential is illustrated by the reduced energy use at higher production densities with simultaneous production of two fish species in Mungkung et al. (2013). In addition, changing the electricity source can dramatically reduce the environmental impact of intensive RAS (Ayer and Tyedmers, 2009; Samuel Fitwi et al., 2013). Greater development and use of renewable energy sources will decrease the carbon emissions of intensive systems.

Unlike energy, the additional infrastructure attributed to intensive systems does not have a large environmental impact. In the studies that reported infrastructure as a separate process the environmental impacts were negligible. It is common for infrastructure or capital goods to be excluded from a LCA. Buildings are considered to have long lifespans and after their contribution is divided by the building's total lifespan the environmental impact is insignificant (Morais and Delerue-Matos, 2010). The inclusion or exclusion of capital goods is still debated.

Frischknecht et al. (2007) looked at the impacts of capital goods and found that they can have a significant impact on certain impact categories. Capital goods should not be excluded without consideration and proper justification for exclusion. The reviewed studies indicate that infrastructure did not contribute significantly; however, assumptions about infrastructure lifespan were not included. Exclusion of infrastructure in future aquaculture studies should be considered carefully and will depend on anticipated lifespan of the production system.

Similar to infrastructure, the impact category LU also had negligible impacts in intensive systems. The area occupied by tanks and water treatment equipment in intensive systems is much smaller than the area required to produce feed products. Extensive aquaculture requires more on-farm land use due to the increased area needed for pond construction and lower yields. When compared to other protein sources, intensive aquaculture production has fewer land use impacts on a kg live-weight basis. A comparison of pork, poultry, beef, and fish when normalized to m^2/kg edible product indicated fish in RAS to have the lowest land use (Table 4.3). Similar to intensive systems, off-farm land use requirements of other protein sources are attributed to feed production (Thomassen et al., 2008). Poultry, beef, and pork rely on similar agricultural feed products as those used to supplement fish meal in aquaculture feeds (Ellingsen and Aanondsen, 2006). Changing the aquaculture feed composition to include more plant derived ingredients could increase the land use requirements of aquaculture production. It could also increase competition for land use with other protein sources due to reliance on the same ingredients. In contrast, extensive aquaculture systems require less supplemental feed and indirectly compete less for plant derived feed ingredients; however, extensive systems could compete directly with other protein sources due to the large on-farm area requirements.

Table 4.3: Comparison of land use (m^2) results from LCA studies. Data on pork, poultry, and beef adapted from de Vries and de Boer (2010). Data on fish based on studies in this review.

Study	System	Functional Unit (FU)	m^2/FU	m^2/kg edible product*
Pork				
Williams et al. 2006	Heavier finishing	1 ton dead weight	6,900	9.8
Williams et al. 2006	Indoor breeding	1 ton dead weight	7,300	10.3
Williams et al. 2006	Outdoor breeding	1 ton dead weight	7,500	10.6
Williams et al. 2006	Conventional	1 ton dead weight	7,400	10.5
Poultry				
Williams et al. 2006	Conventional	1 ton dead weight	6,400	8.0
Williams et al. 2006	Free range	1 ton dead weight	7,300	11.9
Beef				
Williams et al. 2006	100% sucker	1 ton dead weight	38,500	49.2
Williams et al. 2006	Lowland	1 ton dead weight	22,800	29.2
Williams et al. 2006	Hill and upland	1 ton dead weight	24,100	30.8
Williams et al. 2006	Non-organic	1 ton dead weight	23,000	29.4
Fish				
Jerbi et al. 2012	Cascade flow-through	1 ton live fish weight	4,940	9.9
Jerbi et al. 2012	Traditional flow-through	1 ton live fish weight	4,260	8.5
Roque d'Orbcastel et al. 2009	RAS, FCR 0.8	1 ton fish	2,097	4.2
Roque d'Orbcastel et al. 2009	RAS, FCR 1.1	1 ton fish	2,752	5.5
Wilfart et al. 2013	RAS	1 ton fish	740	1.5
Wilfart et al. 2013	Semi-extensive pond	1 ton fish	30,897	61.8
Wilfart et al. 2013	Extensive pond	1 ton fish	56,750	113.5

*kg edible product for pork, poultry, and beef calculated based on information in de Vries and de Boer (2010); kg edible product for fish based on assumption of 0.5 kg edible product/ kg live weight (Iversen, 1996)

Water use is a unique impact factor considered in several of the reviewed papers. Intensive RAS systems utilize water more efficiently and therefore had lower water use impacts than flow-through or extensive aquaculture systems. Of the papers reviewed, one study accounted for agricultural irrigation and found irrigation contributed significantly to water use (Mungkung et al., 2009). The exclusion of irrigation for feed ingredients by studies on intensive aquaculture systems potentially ignores a large water requirement. Commercial feeds used in

intensive systems with a high quantity of plant derived ingredients will have lower NPPU impacts at the risk of greater water use impacts. The agricultural industry is one of the largest users of fresh water resources and most of the grains produced go into animal feeds (Goodland, 1997). If aquaculture feeds incorporate more agriculturally produced plant ingredients, it could potentially increase the water use of those systems placing more stress on limited water supplies. To properly compare water use of an intensive RAS and extensive pond system the water use in feed production must be considered. Incorporation of the irrigation water for feed production could result in a smaller difference in water use between intensive and extensive systems. For this reason, as with feed and energy, it could be beneficial to integrate aquaculture systems with additional products. Increased production per m^3 of water could mitigate indirect agriculture related water use.

While the assessment of water use in the reviewed papers is useful as a baseline comparison between systems, they are extremely simplified. The studies only consider direct quantity of water flowing into the system. As such, the assessments lack distinction between types of water used (blue, green, or grey), consumptive and non-consumptive uses, and spatially relevant scarcity (Ridoutt and Pfister, 2010; Ridoutt and Pfister, 2013). As of 2013, a new method to describe both consumptive and degradative water use, while incorporating an indicator of global water stress was developed for LCA (Ridoutt and Pfister, 2013). Future research on aquaculture should include this new method or even the commonly used Water Footprint Network method as described by Hoekstra et al. (2011), which includes indirect water use to provide more robust measures of water use.

Despite possible limitations in the water use category, increased water efficiency resulted in lower eutrophication potentials. Extensive systems that rely on pond fertilization have greater

direct emissions due to on-farm production. In addition to greater direct emissions, the lower yields in an extensive system resulted in a greater eutrophication potential per FU compared to the highly productive intensive systems (Thomassen et al., 2008). Furthermore, some extensive systems also supplement with commercial feeds thereby increasing indirect emissions from plant derived feed ingredients (Pelletier and Tyedmers, 2010). In contrast, intensive systems are the result of a historical focus on reducing local water quality and ecological impacts. The low eutrophication potential of RAS is evidence to support the success of this movement. Instead of direct emissions, eutrophication potential is largely due to the off-farm impacts of energy production and feed production. Therefore further reductions in eutrophication potential will come from reducing the impacts of feed and energy with better FCRs and alternative energy sources, or the elimination of all waste discharge. Such zero-emission RAS are currently being developed that include IMTA or additional treatment systems (van Rijn, 2013).

While zero-emission RAS, specifically IMTA, have great potential to reduce the environmental impact of aquaculture systems, future research is needed to quantitatively evaluate these new systems. At the time of this writing, no published literature on recirculating, land-based IMTA was identified. Due to this absence, it remains in question how the incorporation of additional products will change the environmental impact when evaluated through LCA. In addition, methods to address allocation in multi-output IMTA systems has yet to be studied. In this review, six papers included allocation and of those only two applied the system expansion method. Considering the inevitable allocation issues in IMTA and its limited use in aquaculture studies, the use of system expansion to address allocation in both IMTA and aquaculture are potential research areas. Another research gap identified of particular importance later in this dissertation (Chapter 5), was the limited availability of information on marine species and

absence of information on marine RAS. Future research should evaluate RAS designed to produce marine species due to variations in rearing requirements between freshwater and marine species.

Future LCAs on zero-emission aquaculture systems, freshwater and marine, will be needed to clarify the advantages and disadvantages of multiple products and its associated water treatment in terms of environmental impact. Just as there was a burden shift moving from extensive to intensive aquaculture systems a more in-depth assessment of zero-emission systems may uncover trade-offs to integration.

4.5 Conclusion

A comparison of different production systems, with a focus on the differences between intensive land-based systems and extensive pond systems, showed potential burden shifting when moving to more intensive aquaculture systems. Intensive systems are often considered to have fewer negative environmental impacts than extensive systems, specifically less water pollution and total water use. Exploration of these environmental impacts through the LCA lens provided support for these claims about intensive aquaculture. It also showed that other impacts, such as cumulative energy demand/energy use and NPPU, are greater. In areas where electricity is predominately supplied by fossil fuels the greater energy requirements correspond with greater carbon emissions. Facilities located in areas, such as Europe, that have access to renewable energy sources benefit from a reduction in carbon emissions despite greater energy requirements. The future of intensive land-based aquaculture development in the United States, which does not have a strong renewable energy market, nor has it established a federal renewable energy policy to encourage such a market (Delmas and Montes-Sancho, 2011), is at a distinct disadvantage due to the lack of renewable energy sources.

In addition to greater access to renewable energy sources, development of sustainable fish feed and better feed conversion efficiencies will reduce the environmental impacts of aquaculture. Aquaculture feed is well known to have large biotic resource and energy requirements. While the movement from extensive to intensive aquaculture resulted in an improvement of FCRs, fish can only incorporate a certain percentage of the nutrients in feed. IMTA systems could improve the total nutrient uptake and increase total yields thereby reducing impacts through greater production per unit of feed, water, and energy.

Intensive aquaculture systems have largely mitigated negative, local environmental impacts and IMTA systems could be the next step to further eliminate negative environmental impacts from aquaculture production, especially due to global factors. The achievement of sustainable aquaculture production will likely come from both improved technologies, such as IMTA, and also a careful balance between local and global environmental impacts through management of production intensities.

Chapter 5: Life Cycle Assessment of Residential- and Commercial-scale Freshwater Aquaponic Systems

5.1 Introduction

Aquaculture production has rapidly grown such that it now produces half of the fish for human consumption. These fish have a total value of approximately US$137.7 billion (FAO, 2014). Considering that our current food system fails to sufficiently support the nutritional needs of over 870 million people worldwide (FAO, 2012b), aquaculture is a valuable industry that can enhance economic and food security globally. Historically, the aquaculture industry was responsible for negative environmental impacts, particularly due to degradation of water quality. The industry has made significant progress reducing the negative environmental impacts of aquaculture through regulation and development of new technologies such as recirculating aquaculture systems (RAS) and integrated multi-trophic aquaculture (IMTA) systems.

RAS are land-based systems that employ physical, chemical, and biological water treatment processes to allow continual recirculation of system water. Depending on the RAS design, water and solids may still be discharged to the environment; however, RAS operators have more control over the location and method of disposal than conventional flow-through systems. Land-based IMTA systems reduce water use through recirculation and also recover nutrients through production of an aquatic plant product or second animal product (Chopin et al., 2001). Aquaponics, a specific type of IMTA system, combines recirculating aquaculture technologies with hydroponic plant production. Hydroponic plant production limits the accumulation of dissolved nutrients, specifically nitrate, in system water. The reduction in nitrate

eliminates the need for water exchanges to dilute nitrate concentrations in closed systems (Masser et al., 1999). In addition, the hydroponic plant beds remove ammonia and nitrite, potentially eliminating the need for a specific nitrification reactor and its associated capital cost and energy requirements (Rakocy, 2012). Rapidly gaining popularity over the last 10 years, aquaponics is frequently practiced on a small-scale in people's backyards. Only a few small, commercial facilities exist in the US, which mainly sell their products through farmer's markets and high-end restaurants; however, their numbers grow each year (Love et al., 2015).

Research on IMTA, including aquaponics, has focused on system functionality and performance parameters including fish health, in-system nutrient cycling, and fish and plant yields (Espinosa Moya et al., 2014; Rakocy, 2012; Roque d'Orbcastel et al., 2009). Several studies have examined the efficiency of water treatment processes in IMTA and associated local water quality impacts (Martins et al., 2010; Piedrahita, 2003; Troell et al., 2003; van Rijn, 2013). The performance research typically consists of large facilities producing between 4 and 100 metric tons of fish per year (Martins et al., 2010; Rakocy, 2012; Roque d'Orbcastel et al., 2009). Alternatively, small experimental systems have been evaluated which produced less than one metric ton over short-time periods (Espinosa Moya et al., 2014). Qualitative assessments have indicated that these systems reduce the spread of diseases and invasive species, prevent degradation of coastal areas, and reduce water use (Gutierrez-Wing and Malone, 2006; Wik et al., 2009). Missing from these studies is a quantitative evaluation of both the local ecological impacts and the potential global impacts of intensification.

Life cycle assessment (LCA) is a tool used to quantitatively evaluate the environmental impact of a product or process. The tool allows a side-by-side comparison of local impacts, such as eutrophication, and global impacts, such as greenhouse gas emissions. It has been used

previously to evaluate fisheries and aquaculture systems (Ayer and Tyedmers, 2009; Henriksson et al., 2012; Roy et al., 2009); however to the authors' knowledge no prior LCAs have been published on aquaponic systems or land-based IMTA systems (Chapter 4). Considering the growing number of commercial-scale aquaponic systems and the research focus on system performance, quantitative information on the environmental impact will aid the future development and enhancement of aquaponic systems. Similarly, limited information is available on smaller backyard aquaponic systems and the potential variation in environmental impact with scale. Quantitative evidence of the environmental benefits of aquaponics at multiple scales will further support its current designation as a sustainable aquaculture production technique. The goal of this study was to use LCA methods to 1) identify 'hot-spots' of environmental impact in a commercial-scale aquaponic system; 2) determine the degree to which hydroponic plant production and recovered solids used as an agricultural amendment reduce the environmental impact of the system; and 3) compare commercial- and residential-scale systems to determine the degree to which environmental impacts change with scale.

5.2 System Descriptions

The two systems studied, a commercial-scale and a residential-scale aquaponic system, were both designed with the same function, to produce fish and plants. For the purposes of this study the systems were designed to produce tilapia and basil. Scale determinations were based on the total system footprint. The residential-scale system had a footprint of 6 m^2, which could reasonably fit into someone's backyard for personal production. The commercial-scale system occupied a footprint of 500 m^2 and required at least one manager and one full-time staff member for production.

5.2.1 Residential Scale

The residential scale aquaponic system was operated between September 17 and December 9 in 2013 at the University of South Florida (USF), Botanical Gardens in Tampa, FL (Figure 5.1). The system had a fish tank, solids removal/nitrification tank, and floating raft hydroponic plant bed. Twenty-five blue tilapia (*Oreochromis aureus*) were stocked in the 0.34 m^3 fish tank. The tilapia were fed daily a commercial tilapia feed. Fifty basil plants were stocked in the 3 m^2 plant bed with a volume of 0.24 m^3. One 35 watt pump continuously recirculated the water. The fish tank and biofilter were aerated using a 6 watt aquarium air pump. Basil plants were grown hydroponically in polystyrene rafts. Ground water was added daily to supplement the water loss due to evaporation; there were no discharges to the environment during 83 days of operation. The fish density was not high enough to require draining accumulated solids in the solids removal/nitrification tank.

5.2.2 Commercial Scale

The University of the Virgin Islands (UVI) in St. Croix has operated an aquaponic system for over 30 years (Figure 5.2). The system consisted of four 3 meter diameter fish rearing tanks stocked with either Nile tilapia (*Oreochromis niloticusi*) or red tilapia (*Oreochromis sp.*). Water flowed from the fish tanks to two cylindro-conical clarifiers followed by four filter tanks filled with orchard netting for fine particulate removal. Solid wastes from the clarifiers were drained daily and the filter tanks were cleaned once or twice weekly. All solid wastes were routed into an aerated pond adjacent to the fish tanks and ultimately recovered as an agricultural amendment. Plants were grown in six raft hydroponic tanks with surface areas of 214 m^2 and a total volume of 11.4 m^3. A variety of vegetables have been grown in the UVI aquaponic system, and this study uses data collected during two basil production cycle (Rakocy et al., 2004).

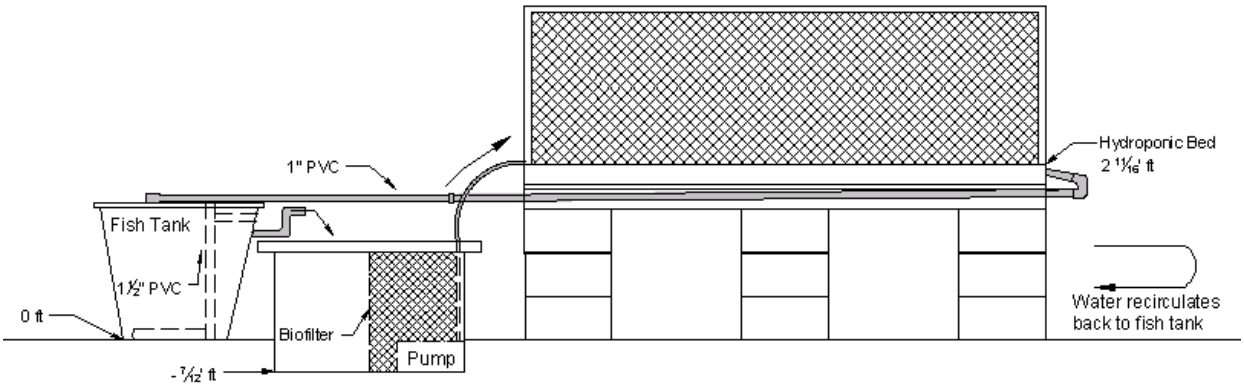

Figure 5.1 Schematic of the residential-scale aquaponic system.

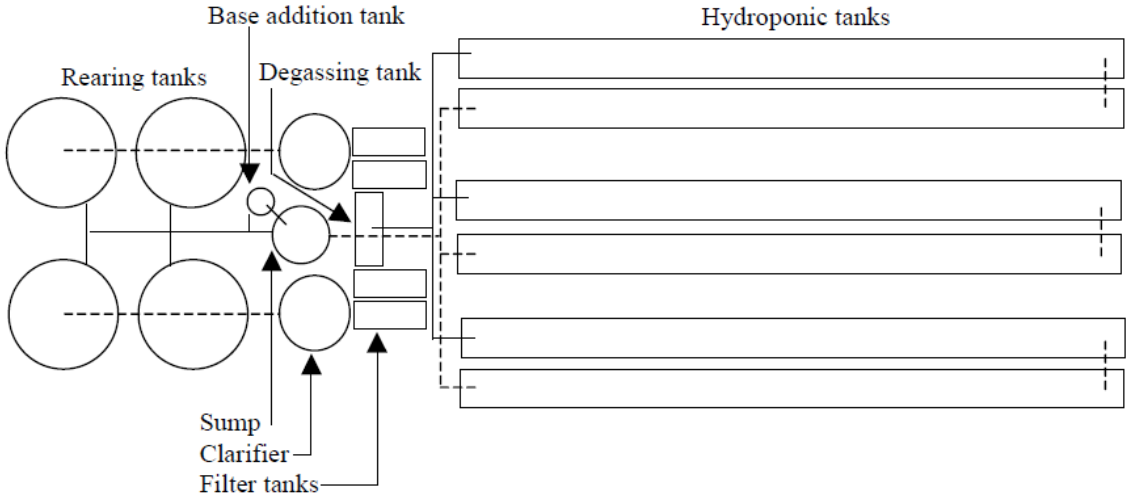

Figure 5.2: Schematic of the commercial-scale aquaponic system.

5.3 Methodology

A process-based LCA was conducted following the International Standard Organization (ISO) 14040 standards including four steps: goal and scope definition, inventory analysis, impact assessment, and interpretation.

5.3.1 Goal and Scope Definition

The goal of this LCA was described in Section 5.1. The scope of the LCA describes the system boundaries and functional unit (EPA, 2006). In this study, the system boundaries can broadly be described as cradle to farm-gate (Figure 5.3). The boundaries include raw materials

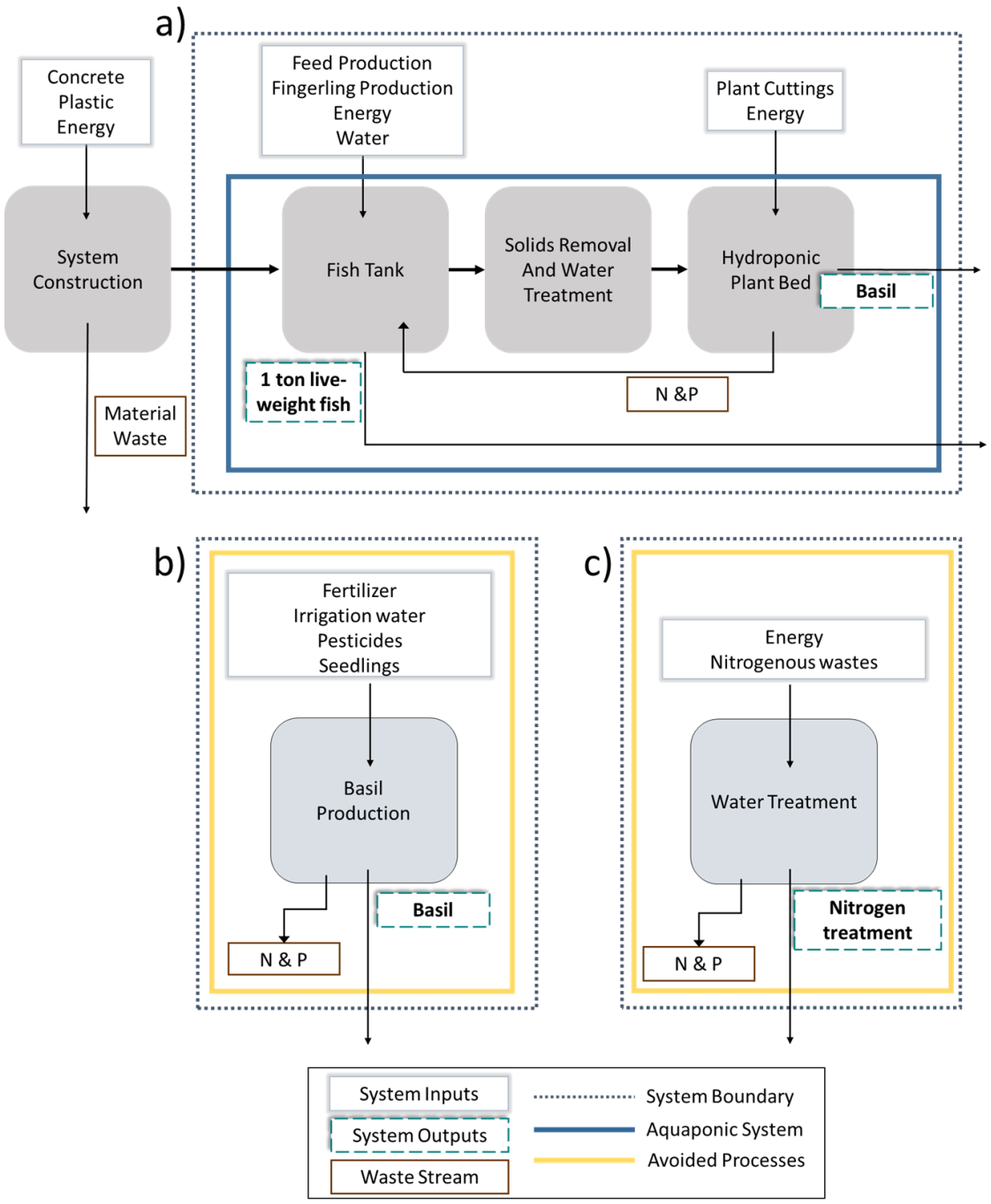

Figure 5.3: System diagrams for freshwater aquaponic systems. (a) system diagram and boundaries of the aquaponic system with inputs and outputs, (b) agricultural production of basil due to co-production of basil, (c) water treatment avoided due to co-production of water treatment in hydroponic plant bed.

and material processing to construct and operate the system. Transportation of feed and processed materials to the system site was considered outside the system boundaries as travel distances are highly variable and this study focused on the impacts of aquaponic system design not the source of materials. Based on a similar exclusion in Aubin et al. (2009), the hatchery phase was excluded from the system boundaries as fingerling production was not a function of the aquaponic system.

The functional unit is a quantitative measure of the system output, provides a baseline for analysis, and allows for comparison between systems producing the same functional unit (Avadí and Fréon, 2013). The functional unit selected for this study was 1 ton live-weight fish. This functional unit was chosen to allow comparison with other LCAs of aquaculture systems (Aubin et al., 2009; Ayer and Tyedmers, 2009; Jerbi et al., 2012).

5.3.2 Co-product Allocation Procedure

The co-products in this study were the 1 ton-live weight fish, recovered solids used as fertilizer, the quantity of wet-weight basil produced as a function of the total fish biomass, and the water treatment provided by the basil growth. The system expansion method was selected to address co-product allocation. In this method environmental impacts caused by conventional production of the co-product are subtracted from the impacts of the total system (Heijungs and Guinée, 2007).

The recovered solids were assumed to replace an equivalent mass of synthetic fertilizer (Ayer and Tyedmers, 2009). Conventional water treatment was assumed to consist of nitrification in a moving bed bioreactor (MBBR) followed by water exchanges to maintain a stable nitrate concentration. Due to the plant production both the electricity required for aeration in the MBBR and nutrient emissions associated with water exchanges were avoided. It was

assumed that the conventional growth of basil was through soil-based agriculture and required use of synthetic fertilizers, pesticides, and crop irrigation. To account for differences in basil production rates between aquaponic systems and agricultural systems the inputs required to produce an equivalent quantity of basil was used to calculate the agricultural inputs and was not based on equivalent production area (Naudin et al., 2014).

5.3.3 Life Cycle Inventory

The life cycle inventory is the collection of quantitative data on the inputs and outputs of a process as defined by the system boundaries and functional unit (EPA, 2006). Operational data on the commercial aquaponic system was collected from UVI through interviews with the facility manager and previous publications on the system (Rakocy et al., 2004; Rakocy et al., 2009). Infrastructure inputs were collected through facility records denoting materials required to replicate the system.

Nutrient budget modeling has been applied previously to determine emissions from fish production systems (Aubin et al., 2009). In nutrient budget modeling, the quantity of dissolved and particulate nitrogen and phosphorus emissions are calculated based on quantity of nutrients in fish feed that are not assimilated into fish biomass. As there were no direct emissions from the aquaponic systems, the quantities of the co-products recovered solids and water treatment were estimated based on the nutrient budget model developed for this project (Table 5.1) (Appendix C). It was assumed 100% of particulate nitrogen and phosphorus, estimated based on the nutrient budget model, were captured by the clarifiers and the solids captured replaced an equivalent mass of synthetic nitrogen and phosphorus fertilizer.

Water treatment is traditionally completed in two steps. First using a biofilter, in which an attached growth microbial nitrification process is used to oxidize ammonia to nitrate. In the

biofilter, aeration is added for nitrification and media mixing. Second, excess nitrate is typically discharged to local water bodies (Masser et al., 1999). The size and electricity requirements of the biofilter were based on ammonia excreted by fish and aeration requirements to fully mix media and oxidize ammonia to nitrate (Table 5.2). It was assumed 100% of excreted ammonia was oxidized to nitrate in the biofilter, therefore all nitrogen emissions were in the form of nitrate. Discharge of excess nitrate was based on the 13% of system water that must be discharged daily to maintain a stable nitrate concentration of 40 mg/L NO_3^--N in the commercial-scale system. Similarly, dissolved phosphorus emissions were calculated using the same daily discharge rate. Due to the small size of the residential-scale system, nitrate accumulation was negligible and it did not have dissolved nitrogen or phosphorus emissions.

Table 5.1: Inventory items calculated with nutrient budget model. Water treatment includes avoided nutrient and water discharges due to water exchange. Recovered solids shows mass of nutrients replaced with synthetic fertilizer. Quantity per ton of live-weight fish.

		Water treatment			Recovered solids	
	Feed added (kg/t)	Dissolved N[1] (kg/t)	Dissolved P[2] (kg/t)	Water (m^3/t)	Particulate N[3] (kg/t)	Particulate P[4] (kg/t)
Commercial	1680	58.8	5.04	1260	17.5	6.72
Residential	2570	0	0	0	26.7	10.3

[1] 3.5% of feed was excreted by fish as ammonia of which all was rapidly converted to nitrate by the biofilter (Piedrahita, 2003)
[2] 0.3% of feed was considered dissolved phosphorus (Cripps and Bergheim, 2000)
[3] 1.04% of feed was considered particulate nitrogen (Piedrahita, 2003)
[4] 0.4% of feed was considered particulate phosphorus (Cripps and Bergheim, 2000)

Data for the residential system was collected directly during the 83 days of operation in 2013. Feed added to the system was weighted daily. The total amount of feed required for one year was extrapolated based on expected fish growth rates and the feed measurments during the three month operational period. Expected harvest of basil was caluclated based on planting density in the residential system and mean harvest plant weight. The volume of freshwater added daily was recorded before addition to the system and averaged to determine total water additions for a year. All material inputs were recorded for the residential system as it was constructed.

Table 5.2: Sizing information for biofilter and energy required for the biofilter to convert ammonia emissions to nitrate.

	Maximum feed rate (kg/d)	Total ammonia nitrogen (TAN)[1] (kg/d)	Media volume[2] (m^3)	Air required (lpm)[3]	Electricity required (kWh/y)[4]
Commercial	35.9	1.1	6.3	895	3592
Residential	0.346	0.01	0.05	7.1	78.8

[1] TAN = maximum feed rate * 3.5% TAN from feed - passive nitrification - TAN concentration in water; where passive nitrification = 10% * maximum feed rate and TAN concentration in water = 0.5 mg/L (Losordo and Hobbs, 2000)

[2] Media volume = (TAN/nitrification rate)/media surface area; where media surface area = 350 m^2/m^3 and nitrification rate = 0.00051 kg TAN/m^2/d (Losordo and Hobbs, 2000)

[3] Based on industry ratio of 142 lpm required per 1 m^3 media volume (Michaels, 2015)

[4] Commercial: air provided by a 1/2 HP pump; residential: air provided by 9 watt diaphragm air pump

Table 5.3: Feed ingredients. Feed composition was based on order of ingredients on package and comparison of other feeds in literature (Mungkung et al., 2013; Pelletier and Tyedmers, 2010; Tacon et al., 2011).

Feed ingredients	32% protein fish food
Soybean meal	35%
Wheat middlings	15%
Maize/corn	15%
Fish meal	5%
Calcium carbonate	*
Corn gluten	*

* Indicates it was an ingredient listed in the food but was not considered in the analysis.

Background inventory data for the manufacture of infrastructure inputs and feed processing for both systems was obtained from Ecoinvent v 1.2 and LCA food databases available within the SimaPro 7.0 software (PréConsultant, Netherlands). Information on aquaculture feed components and the ingredients in the 32% protein aquaculture feed were estimated based on a literature review (Table 5.3) (Appendix B). The fertilizer and water requirements for basil production through agriculture were based on field experiments at the Agriculture Experiment Station at the UVI, St. Croix campus (Palada et al., 2008).

5.3.4 Life Cycle Impact Assessment

The impact categories selected were global warming potential (GWP), human toxicity potential (HTP), acidification potential (AP), eutrophication potential (EP), energy use (EU), land use (LU), and water use (WU). CLM 2 Baseline 2000 midpoint approach was used to estimate GWP, HTP, AP, and EP (Guinée, 2002). This method was selected because it was most commonly used in other aquaculture studies (Chapter 4). CED method v 1.02 was used to evaluate the energy use (Frischknecht et al., 2007). Land use encompasses the alteration of land and loss of biodiversity directly through the removal of natural landscape due to deforestation, agricultural practices, construction of impervious surfaces, etc. (Brentrup et al., 2002). The method outlined in the *Handbook on Life cycle Assessment* by Guinée et al. (2002) was used to calculate the impacts of land use in PDF*m^2yr. Water use is a relatively new development in LCA characterization factors. Direct water use was calculated based on the quantity of water m^3 flowing into the production systems (Roque d'Orbcastel et al., 2009).

5.4 Results

The system was divided into five processes and the contribution of each process to the selected impact categories was evaluated. A quantitative assessment of the impacts attributed to each process was estimated for the two systems and is presented in Table 5.4. The potential impacts from construction was small for both systems, with the exception of human toxicity potential and energy use in the residential system at 660 kg 1,4 DB eq and 32,200 MJ eq, respectively. In the residential-scale system, when compared to the other system processes, the relative impacts from construction on human toxicity potential were 31%. This was similar to the relative impacts from feed of 30%. The wood materials used in the residential-scale system contributed greatest to energy use and human toxicity potential. Specifically, energy associated

with wood drying and preservative treatments applied to protect wood contributed to energy use and human toxicity potential, respectively. Unlike construction, the potential impact from chemicals were negligible for both systems (Figure 5.4). The greatest contribution of chemicals was due to global warming potential and energy use in the commercial-scale system at 117 kg CO_2 eq and 1,820 MJ, respectively. When the impacts of construction are considered relative to the other system processes eutrophication potential had the greatest relative contribution. The large reduction in eutrophication potential from avoided water treatment contributed to the greater relative contribution from construction. Similarly to construction, the wood drying process contributed greatest to eutrophication potential.

Feed contributed greatest to eutrophication potential and was similar in both systems at 8.82 and 8.30 kg PO_4 eq for the commercial-scale and residential-scale, respectively. Feed also contributed greatest to the land use impacts at 5,330 and 5,020 PDF*m^2yr for the commercial-scale and residential-scale, respectively. Feed was the second highest contributor to the impact categories of acidification potential, global warming potential, and energy use, second only to electricity. The energy carriers, electricity and natural gas, required to process feed components, specifically the maize/corn feed component, contributed to these impact categories.

Electricity had the greatest contribution to all impact categories with the exception of land use and water use. In the category water use electricity required 0 m^3 of water due to the inclusion of only direct water use. Collectively the electricity requirements of the commercial-scale system contributed between 62% and 189% of the environmental impact. Of the categories impacted by electricity, impact from the residential-scale system was consistently greater than the commercial-scale system. The residential-scale system also had the greatest reduction in environmental impact from the co-products and associated avoided burdens for all categories

Table 5.4: Life cycle impacts for the production of 1 ton of live-weight tilapia for both commercial-scale and residential-scale aquaponic systems. The values for avoided burdens are negative because they were credited to the system. AP = Acidification potential, EP = Eutrophication potential, GWP = Global warming potential, HTP = Human toxicity potential, EU = Energy use, LU = Land use, WU = Water use.

Impact category	Construction		Chemicals		Water Additions		32% Protein feed		Electricity		Avoided burdens		Total	
AP (kg SO_2 eq)														
Commercial	0.81	2%	0.36	1%	0	0%	12.8	37%	26.3	77%	-6.00	-17%	34.3	100%
Residential	2.23	9%	0.00	0%	0	0%	12.1	47%	29.0	114%	-17.8	-70%	25.5	100%
EP (kg PO_4 eq)														
Commercial	0.29	8%	0.19	5%	0	0%	8.82	246%	6.63	185%	-12.4	-344%	3.59	100%
Residential	0.81	7%	0.00	0%	0	0%	8.30	69%	7.31	60%	-4.31	-36%	12.1	100%
GWP (kg CO_2 eq)														
Commercial	174	4%	117	2%	0	0%	1,940	39%	3,620	74%	-937	-19%	4,910	100%
Residential	487	13%	0.00	0%	0	0%	1,820	49%	3,990	108%	-2,590	-70%	3,700	100%
HTP (kg 1,4-DB eq)														
Commercial	265	12%	51.8	2%	0	0%	647	28%	1,730	76%	-413	-18%	2,290	100%
Residential	660	32%	0.00	0%	0	0%	609	30%	1,910	94%	-1,150	-57%	2,030	100%
EU (MJ)														
Commercial	3,660	4%	1820	2%	0	0%	43,300	45%	60,800	63%	-13,100	-14%	96,600	100%
Residential	32,200	32%	0.00	0%	0	0%	40,800	41%	67,000	67%	-40,100	-40%	100,000	100%
LU (PDF*m^2yr)														
Commercial	1.52	0%	1.02	0%	0	0%	5,330	100%	28.8	1%	-5.34	0%	5,360	100%
Residential	622	11%	0.00	0%	0	0%	5,020	89%	31.8	1%	-17.9	0%	5,650	100%
WU (m^3)														
Commercial	0.00	0%	0.00	0%	147	11%	0.00	0%	0.00	0%	-1,490	-111%	-1,340	100%
Residential	0.00	0%	0.00	0%	44.2	30%	0.00	0%	0.00	0%	-190	-130%	-146	100%

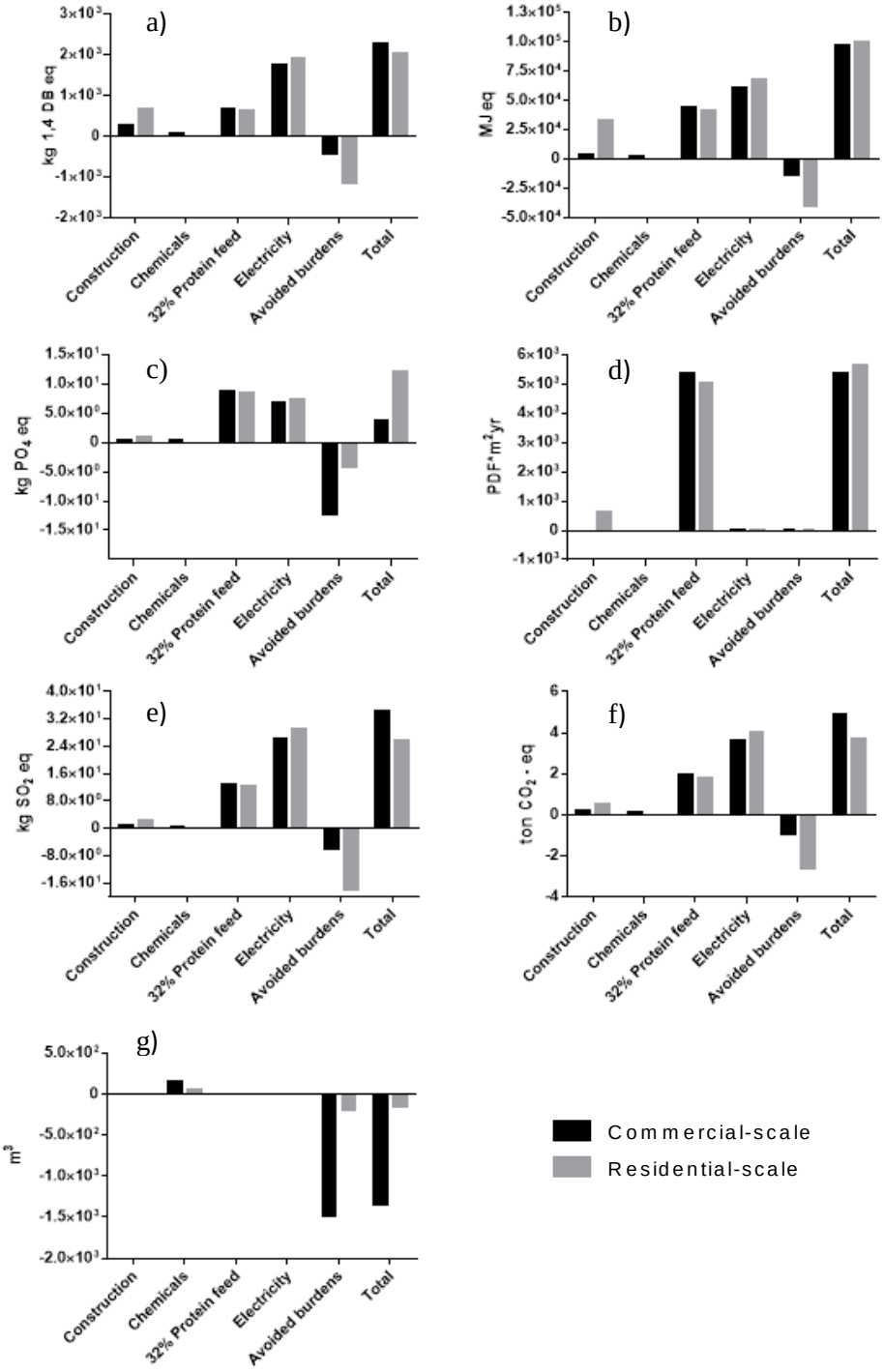

Figure 5.4: Impact of production of 1 ton live-weight fish in the commercial-scale and residential-scale aquaponic systems. Figure shows the impact categories of human toxicity potential (a), energy use (b), eutrophication potential (c), land use (d), acidification potential (e), global warming potential (f), and water use (g).

except eutrophication potential and water use. The total impacts from all five processes to each impact category varied such that human toxicity potential, acidification potential, and global warming potential were greatest in the commercial-scale system. In the residential-scale system energy use, eutrophication potential, land use, and water use had the greatest impact.

5.5 Discussion

5.5.1 Identification of Hot-spots

The first objective of this research was to identify hot-spots of environmental impact in a commercial-scale aquaponic system. Based on the results two hot-spots were identified: electricity and feed. Electricity requirements were large in the aquaponic system due to the water pumping and aeration requirements of RAS. The dominant impact of electricity in this study echo the trends found in previous LCA studies of intensive land-based aquaculture systems. Ayer and Tyedmers (2009) compared production of 1 ton live-weight salmon in four different aquaculture systems with increasing levels of intensification. The most intensive system, a RAS, consistently contributed greatest to the environmental impact categories selected due to the higher electricity requirements. Only in the category of eutrophication potential did the RAS contribute least to environmental impact when compared to other aquaculture systems. As an intensive land-based system it was not unexpected that aquaponic systems, like their RAS counterparts, are subject to the trade-off between electricity use and direct emissions to the water.

While electricity has a large environmental impact, the contribution of electricity was not equal across the system. The contribution of electricity to each impact category was proportional to the electrical consumption of the equipment used. In the commercial-scale system 61% of the electrical requirements were for aeration in the fish tanks and degassing immediately after the

fish tanks (Figure 5.5). Aeration in the plant beds contributed to 41% of the electricity requirement.

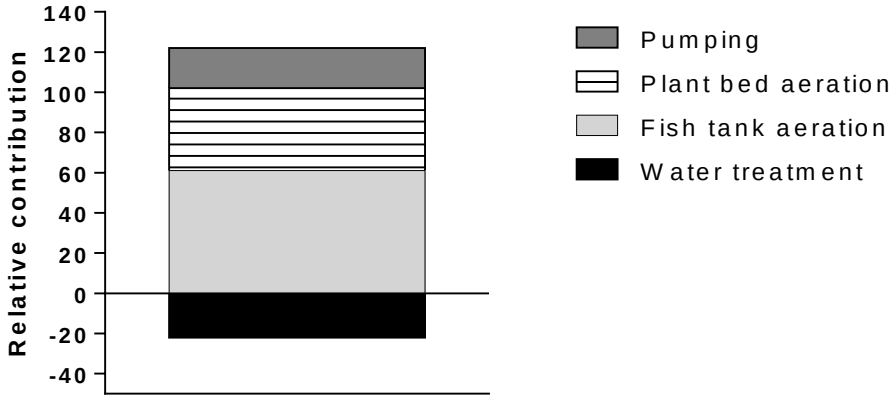

Figure 5.5: Relative contribution of electricity inputs in the commercial-scale aquaponic system.

Plant bed aeration is necessary to provide oxygen for root respiration and for nitrifying bacteria. Research on deep water hydroponic systems, like those used in the aquaponic systems in this study, demonstrated the necessity of adequate oxygen levels in system water (Morard et al. 2000). Failure to supply enough oxygen to roots results in limited plant growth and increased susceptibility to disease (Morard and Silvestre, 1996). Alternative hydroponic designs can potentially eliminate the need for constant aeration used in deep water hydroponic systems. The nutrient film technique (NFT) exposes roots to a thin layer of water and does not require additional aeration as plant roots are not subjected to complete submergence. This design has been successfully applied to aquaponics in research (Lennard and Leonard, 2006) and is commonly used in commercial or hobbyist aquaponic systems (Love et al., 2015; Reasons, 2015). In addition, NFT systems are compact and light-weight allowing for multiple levels of plant growth to maximize use of vertical space. The trade-off with NFT systems is the lack of passive nitrification on system walls. The absence of this nitrification mechanism could result in

the need for a separate biofilter and subsequently additional aeration thereby negating any electrical savings from operating a NFT plant bed.

It is clear that regardless of the system design the water treatment and recirculation of intensive aquaculture will result in greater electricity demands. In areas where the electricity is predominately provided by fossil fuels and non-renewable sources the environmental impacts will be greater due to the significant quantity of harmful emission produced by these energy sources (Ayer and Tyedmers, 2009). Use of renewable energy sources is therefore critical to reduce the environmental impact of land-based aquaculture systems, but this comes at a cost in places that do not encourage its introduction or growth in the marketplace.

The second hot-spot identified was feed, which is well known to be one of the main impediments to development of sustainable aquaculture. Other LCA studies on intensive aquaculture systems have found that feed was the main contributor to several impact categories including energy use, global warming potential, and land use (Aubin et al., 2009; Jerbi et al., 2012; Roque d'Orbcastel et al., 2009). In this study, feed contributed greatest to eutrophication potential relative to other categories which was unusual compared to other studies. The greater relative impact was due to the reductions in impact from the co-products. When compared based on kg PO_4 eq the eutrophication potential was similar to the 8.4 kg PO_4 eq found in Ayer and Tyedmers (2009).

Land use was particularly large due to the high percentage of agricultural ingredients in the feed. Rising prices for fish meal and fish oil combined with increased awareness of dwindling fisheries stocks has led to development of fish feeds with higher plant-based protein sources (Naylor et al. 2000). Soy meal, a common replacement for fish meal, was found to have a 30-44% reduction in environmental impact when compared to fish meal for all impact

categories considered in Pelletier and Tyedmers (2007). However, the reduction in environmental impact of plant-based feeds comes at the cost of higher land use impacts. In this study 99.5% of land use impacts were due to feed. Similar results were found in Roque d'Orbcastel et al. (2009), which suggested that a lower feed conversion ratio (FCR) was more effective at reducing environmental impact than feed composition.

Another option for reducing the environmental impact of aquaculture feeds could be the use of microalgae. Recent research has shown microalgae to be a suitable source of protein in animal feeds (Becker, 2007). Microalgae have several advantages over plant-based crops in that they can be grown on land unsuitable for agriculture, they have higher productivity than terrestrial plants, and they can utilize nutrients from various wastewater sources (Demirbas and Demirbas, 2011). A LCA of a microalgae cultivation system indicated microalgae-based feed could reduce impacts to land use and water use, although the system did not contribute to lower carbon emissions (Taelman et al., 2013). The current emphasis on microalgae biofuels and significant investment in algae cultivation for biofuels will potentially lead to improved technologies for production of microalgae for aquaculture feed.

5.5.2 Avoided Burdens

The second objective was to identify potential avoided burdens in the aquaponic system and determine if they reduced the environmental impact of the system as a whole. The first potential avoided burden was from nutrients contained in captured solid fish waste. The solid waste collected daily was assumed to offset production of similar quantities of synthetic nitrogen and phosphorus fertilizer. The greatest contribution of the solids to environmental impact was due to a 3.6% reduction in global warming potential. The reduction in this study was slightly greater than that in Ayer and Tydmers (2009) who found that captured fish waste in a RAS

contributed to a 0-1% reduction in environmental impact due to offset fertilizer production. The amount of solid waste captured in the present study was larger due to a greater quantity of feed per ton of live-weight fish and inclusion of waste feed in solid emissions. In both systems the quantity of captured solids was small relative to other inputs such that any offset was negligible.

Plant production in the aquaponic system theoretically contributes to reduced environmental impact in several ways. The first considered here was the avoidance of fertilizer, pesticides, and irrigation due to plant production. In aquaponic systems, fertilizer is provided by the fish waste such that no additional nitrogen or phosphorus are added (Bernstein, 2011). Similarly, pesticides cannot be used due to their potential negative impacts on fish health (Rakocy, 2012). The results indicated that the avoidance of synthetic fertilizer and pesticide production have negligible environmental impact. At its greatest, avoided synthetic fertilizer contributed a 1.8% reduction to global warming potential. Avoided pesticides contributed to a 4.1% reduction in human toxicity potential. While the average basil yields per square meter in the aquaponic system were greater than in agriculture, the impacts of feed and energy dominate any avoided burdens from fertilizer and pesticides.

Plant production did contribute to a large reduction in water use. Water use was reduced by 17% due to the avoided irrigation. Previous LCA studies of aquaculture systems have already demonstrated that RAS consume less water than other types of aquaculture. Samuel-Fitwi et al. (2013) compared a RAS to an extensive aquaculture system and found the water use in RAS was 99% lower. The results of the present study indicate that when plant production was added to the culture system there were additional water savings. The importance of those water savings should not be overshadowed by the high energy requirements. Agriculture faces conflicting challenges of substantially reducing water use while significantly increasing production to meet

food demands of a growing population (WWAP, 2015). Based on this LCA, aquaponics is one tool that can help increase water use efficiency in food production.

The second co-product considered to be derived from plant growth was water treatment. Plant production and associated nutrient uptake replaced the need for ammonia removal by means of a biofilter otherwise required in a RAS. The avoided electricity for aerating a biofilter contributed greatest to the reduction in environmental impact of the whole system for the categories of acidification potential, global warming potential, human toxicity potential, and cumulative energy demand. When compared to the other energy sources in the system, avoided biofilter aeration was 22% of the system's total energy use. It is difficult to isolate the impacts of water treatment in this study relative to other aquaculture systems which typically report aggregated energy use. If examined based on total energy usage, the energy use of 96,170 MJ in this study was less than the 353,000 MJ per ton live-weight fish in Ayer and Tydmers (2009). In Roque d'Orbcastel et al. (2009), a RAS operated with a biofilter had a lower energy use of 63,202 MJ per ton of live-weight fish. The lower energy use in Roque d'Orbcastel et al. (2009) was likely due to the use of airlift pumps which require less electricity than the centrifugal pumps used in this study.

In addition to the impacts on energy use, water treatment through plant production resulted in a reduction in water use and eutrophication potential. Plant production replaced the need for these water exchanges and reduced the water use by 94%. This avoided discharge contributed to a large reduction in eutrophication potential of 313% in the commercial-scale aquaponic system. A comparison of a flow-through, off-shore cage, and RAS showed that the eutrophication potential from fish production of the flow-through and RAS were 60.8 and 69.9 kg PO_4 eq, respectively (Aubin et al., 2009). The reduction in eutrophication potential found in

this study and the flow-through and RAS in Aubin et al. (2009) demonstrate the importance of post-treatment of RAS effluents. Constructed wetlands can be employed to treat water discharged from RAS (Zhong et al., 2011), although the dilute concentrations of aquaculture effluents reduces the treatment efficiency (Martins et al., 2010). Heterotrophic denitrification, algal ponds, and periphyton systems have also been used successfully to improve recirculation rates in RAS (van Rijn, 2013). These alternative nutrient removal mechanisms would result in similar water use reductions, although only algal ponds have the potential to provide a secondary product as in aquaponic systems (Merz and Main, 2014). The water treatment provided by plant production is a key advantage to aquaponic systems, allowing for 100% recirculation of system water, a demonstrated reduction in environmental impact, and a secondary income source.

Plant production had the potential to reduce the environmental impact of the aquaponic system in two ways: through avoided conventional plant production and through avoided conventional nutrient removal. Of the two options, avoided nutrient removal contributed to a greater reduction in environmental impact than avoided agricultural production. The main advantages were a small reduction in energy use and a large reduction in water use; however, these advantages are not isolated to an aquaponic system. A RAS in Roque d'Orbcastel et al. (2009) using a biofilter for nutrient removal had an even lower energy use than the commercial-scale aquaponic system. Ultimately, energy efficient system design with secondary nutrient removal to achieve 100% recirculation is more critical to the environmental impact of an intensive land-based aquaculture system than the presence or absence of plants.

5.5.3 Impacts of Scale

At present, small residential type aquaponic systems are far more prevalent than larger commercial operations (Reasons, 2015). Due to their popularity and purported environmental

and socio-economic benefits it is important to evaluate the life cycle impacts on the type of system most likely constructed. As such, the final objective of this study was to determine how the environmental impacts of the commercial-scale system compare with a residential-scale system.

Similar to the commercial-scale system, electricity contributed greatly to the environmental impact. It contributed between 60% and 114% of the environmental impact for the categories of acidification potential, eutrophication potential, global warming potential, human toxicity potential, and cumulative energy demand. When compared to the commercial-scale system, the residential-scale system used in this study, had a higher energy use. One cause for the higher energy use was due to inefficiencies in pumping, which was responsible for 63% of the electricity use. Raft designs are the most common scheme for commercial-scale aquaponic systems (Love et al., 2015) and frequently used for residential-scale systems (Reasons, 2015); however, even within the raft configuration electricity use could be highly variable for residential-scale systems.

The second major contribution to energy use was due to construction. Production of wood materials are relatively energy intensive particularly for composite wood products like the plywood used in the residential-scale system (Werner and Richter, 2007). Modifying the system design to rely less on first-use wood resources and instead using recycled materials could decrease the environmental impacts of the residential-scale system.

Similar to the design of many aquaponic systems, the system in this study was based on widely available informal internet resources (Ako and Baker, 2009; Reasons, 2015). Due to the vast quantity of information available, the ultimate design of residential systems can vary widely. Assuming this specific design was representative of the many possible iterations, there was a

small economy of scale effect on electricity use with the larger system. While scale has not been considered previously in LCAs of aquaculture systems it has been considered in assessments on wastewater treatment plants (Cornejo, 2015). Operational electricity requirements per functional unit were lower for larger wastewater treatment systems benefiting from economies of scale (Lundin et al., 2000). This resulted in a higher contribution of electricity to the environmental impact of the residential-scale system. Alternatively, the residential-scale system benefited from economies of scale in the avoided burdens process. In both systems, avoided electricity for water treatment contributed greatest to the avoided burdens process. Due to the inefficiencies of a smaller water treatment system in the residential-scale there was a greater offset from avoided water treatment contributing to lower global warming potential. In contrast, the energy use was greater in the residential-scale system, due to the energy requirements associated with construction compared to the commercial-scale system. Similar trends were seen for acidification potential and human toxicity potential.

The residential-scale system did not benefit from the same reduction in eutrophication potential or water use. Due to the small quantity of dissolved wastes it was assumed water exchanges were not necessary in the residential-scale system. Water exchanges contributed greatest to the reduction in the eutrophication potential and water use of the commercial-scale system and the absence of this in the residential-scale system resulted in lower total offsets for these categories.

The contribution of feed to environmental impact in the residential-scale system was slightly less than in the commercial-scale system. The same FCR of 1.7% was used for both the systems but the final harvest size of 500g instead of 813g reduced amount of feed per ton of fish. Feed had the greatest impact to eutrophication potential and land use feed for both systems.

5.5.4 Sustainable Aquaponics

Historical concerns about water quality have often taken precedence when evaluating sustainability of aquaculture systems; however, sustainability is a measure of more than environmental impact. Both commercial-scale and residential-scale systems have social and economic benefits not captured in LCA. Development of more commercial-scale aquaponic systems could provide communities with additional jobs, food security, and can help mitigate food deserts similar to community gardens (Corrigan, 2011). Smaller residential-scale systems are becoming a popular addition to schools where they function to educate students about core science, technology, engineering and mathematics (STEM) topics and help fulfill the growing need to educate students about the origins of their food (Hess and Trexler, 2011). Economically, the dual product system likely reduces the cost to produce fish through intensive aquaculture, which is advantageous at both scales. Considering these benefits and many others, aquaponics should play a role in developing small agriculture and intensive aquaculture industries.

5.6 Conclusion

LCA was used to evaluate the environmental impacts of aquaponic systems at commercial and residential scales. Two hot-spots of environmental impact were identified from the contribution analysis: electricity and feed. Similar to previous LCA studies on RAS and intensive aquaculture the electricity requirements of the aquaponic systems contributed greatest to six of seven environmental impact categories. Considering the large environmental impact of electricity, reducing the electricity requirements or using renewable energy sources would contribute to large reductions of the environmental impacts for aquaponic systems. Specifically, electricity use in the plant beds was identified as a potential location for optimization. Feed was identified as the second hot-spot and is well established as an impediment to sustainable

aquaculture. Better FCRs, plant-based feeds, and the development of new feed sources will all likely contribute to reductions in environmental impact from feed.

In addition to fish, aquaponic systems produce several co-products of which vegetable products and captured solids are most frequently considered due to their tangibility and economic value. Equally important, is the contribution of vegetable production as a water treatment process. Calculation of the avoided burdens through system expansion indicated avoided water treatment from vegetable production contributed greatest to a total reduction in environmental impacts. Vegetable products alone contributed to large reductions in water use. As such, it is important to consider the avoided burdens from plant production and water treatment collectively. Only then the aquaponic system resulted in a lower environmental impact than intensive fish production alone.

Quantitatively the environmental impact of commercial-scale and residential-scale aquaponic systems are similar. Residential-scale systems had slightly greater environmental impacts, although not enough to discourage continued development of smaller aquaponic systems. While this study represents one of many possible configurations, residential-scale systems will benefit from careful material selection and avoidance of first-use wood in the construction design. Ultimately, aquaponic systems at any scale are exceptional at reducing local impacts from nutrient discharges. As the aquaculture industry expands, it is time to shift from a focus on reducing local impacts to creating highly productive, intensive systems with similarly low global impacts. Joint reductions in local and global impacts will lead to better aquaponic systems and help guide the next phase of sustainable aquaculture.

Chapter 6: Life Cycle Assessment of a Marine Aquaponic System with Different Degrees of Plant Production

6.1 Introduction

The stagnation of capture fisheries combined with competition for coastal land, constricted freshwater resources, and increased concerns over local water quality has led to increased development of land-based recirculating aquaculture systems (RAS). The compact water treatment systems used in RAS minimize water use and nutrient discharges, while providing the potential for year-round seafood production located close to markets (Masser et al., 1999; Wik et al., 2009).

Despite benefits of RAS and growing production quantities, RAS remain less prevalent than other aquaculture systems. High capital investments and operational costs are one of the largest impediments to RAS production (Dalsgaard et al., 2013). In addition, sludge disposal (Mirzoyan et al., 2010) and nitrate removal (van Rijn et al., 2006) still present environmental risks. One technique to manage dissolved nitrogenous wastes while improving system revenues is the use of integrated multi-trophic aquaculture (IMTA) systems. These systems combine the production of fish with additional animal or plant species to promote nutrient uptake and biotransformation, thereby providing a secondary source of income (Troell et al., 2003). Aquaponics is a type of land-based aquaculture system within the IMTA classification and tends to strictly refer to systems using edible plants as the secondary product.

The use of denitrification reactors in RAS can potentially manage nitrate and sludge disposal simultaneously (van Rijn et al., 2006). In denitrification reactors, heterotrophic bacteria

convert nitrate to nitrogen gas in the presence of an organic carbon source under anoxic conditions (van Rijn and Barak, 1998). Denitrification reactors have the potential to use aquaculture sludge as the carbon source thereby efficiently minimizing both dissolved and solid wastes simultaneously (van Rijn et al., 2006). Denitrification reactors do not produce a secondary source of income; however, they require a smaller system footprint than that required for plant production with the floating rafts most frequently used in commercial aquaponics (Love et al., 2015).

In anticipation of more stringent water quality regulations and greater water scarcity, it is necessary to further reduce emissions and water use in aquaculture. Both aquaponics and denitrification reactors are viable solutions to further reduce local ecological impacts of aquaculture, such as eutrophication from waste discharges. While local impacts are important to evaluating the sustainability of these systems, global impacts resulting from industrialization and intensification should also be considered. Life cycle assessment (LCA) is a tool used to quantitatively evaluate the environmental impacts of a product or process. It facilitates the simultaneous comparison of various environmental impact indicators with both local and global implications (EPA, 2006). A LCA on aquaponics and denitrification reactors can show the trade-offs between local and global impacts of both technologies.

The aim of this study was to: 1) complete a LCA on a marine aquaponic system that includes both plant production and denitrification to establish a baseline of environmental impact and 2) compare this baseline with alternative scenarios of high plant production or just denitrification in reactor(s) to evaluate trade-offs between the two water treatment approaches.

6.2 System Description and Scenarios

The marine aquaponic system as described in Chapter 3 was used as the baseline system. Briefly, the system was stocked with red drum (*Sciaenops ocellatus*) and two species of saltwater vegetables: sea purslane (*Sesuvium portulacastrum*) and saltwort (*Batis maritima*). Water quality was maintained through a series of treatment devices including a swirl separator and upflow media filter for solids removal, a biofilter for nitrification, hydroponic plant beds for dissolved nutrient removal, and a downflow submerged denitrification reactor for solids capture and denitrification. The biofilter was a moving bed bioreactor (MBBR) that contained 1.8 m^3 Kaldnes media (Fureneset, Norway) to obtain a total surface area of 630 m^2. About 900 fish were initially stocked in three 3.3 m^3 fish tanks at a density of 4.23 kg/m^3 and about 1,200 net pots were added to obtain 47 plants/m^2 and 19.5 net pots/m^2. During the study, all solid wastes were captured in the sand filter and no water was discharged from the system; however, some local groundwater was used to make up for evaporative losses.

The second alternative scenario to the baseline, Scenario 1, assumes a maximum planting density based on the calculations presented in Chapter 3, In Scenario 1, no biofilter was present as the saltwater vegetable production provided all the necessary water treatment. Scenario 2 assumes no plant production. In this scenario, a downflow submerged denitrification reactor was sized to provide complete denitrification of all excess nitrogen added daily from fish feed. Due to the absence of plant beds, a full-size biofilter was required for nitrification. The MBBR biofilter design was used for the hypothetical biofilter in Scenario 2 and the co-product water treatment in the baseline and Scenario 1. All scenarios were 100% recirculating and eliminated any need for water exchanges to reduce nitrate concentrations therefore eliminating local emissions of nitrogen or phosphorus. Collected solids, although not discharged during this study, would

eventually require disposal to a landfill or as a soil amendment for saltwater tolerant macrophytes.

6.3 Methodology

A process-based LCA was conducted with a similar methodology to that outlined in Chapter 5 and only brief explanations will be provided here.

6.3.1 Goal and Scope Definition

The system boundaries were considered cradle to farm-gate (Figure 6.1). They include raw materials and material processing. Based on the results of Chapters 4 & 5, infrastructure was excluded as a process due to the low contribution to environmental impact (Morais and Delerue-Matos, 2010). The functional unit selected for this study was 1 ton live-weight fish. This functional unit was chosen to allow comparison with other LCAs of aquaculture systems.

6.3.2 Co-product Allocation Procedure

The co-products in this study were 1 ton-live weight fish, the quantity of wet-weight vegetables produced as a function of the total fish biomass, and the water treatment provided by the vegetable growth. Recovered solids were not accounted for as agricultural amendment based the results of Chapter 5 in which recovered solids contributed to less than a 10% offset to environmental impact, nor were they considered an emission as they were not directly discharged by the aquaponic system. The system expansion method was selected to address co-product allocation.

As required for the system expansion method, a substitute product was selected for the saltwater vegetables; however, since the saltwater vegetables, at present, are not produced commercially a similar vegetable product was selected. To estimate the avoided burdens from co-production of the saltwater vegetables, spinach was selected as a surrogate crop. While

limited nutrition research was available on the saltwater vegetables used in this study, they were considered to have a high nutritional value (Chapter 3). Based on consumer expectations of nutritional value, spinach was considered a likely substitute product.

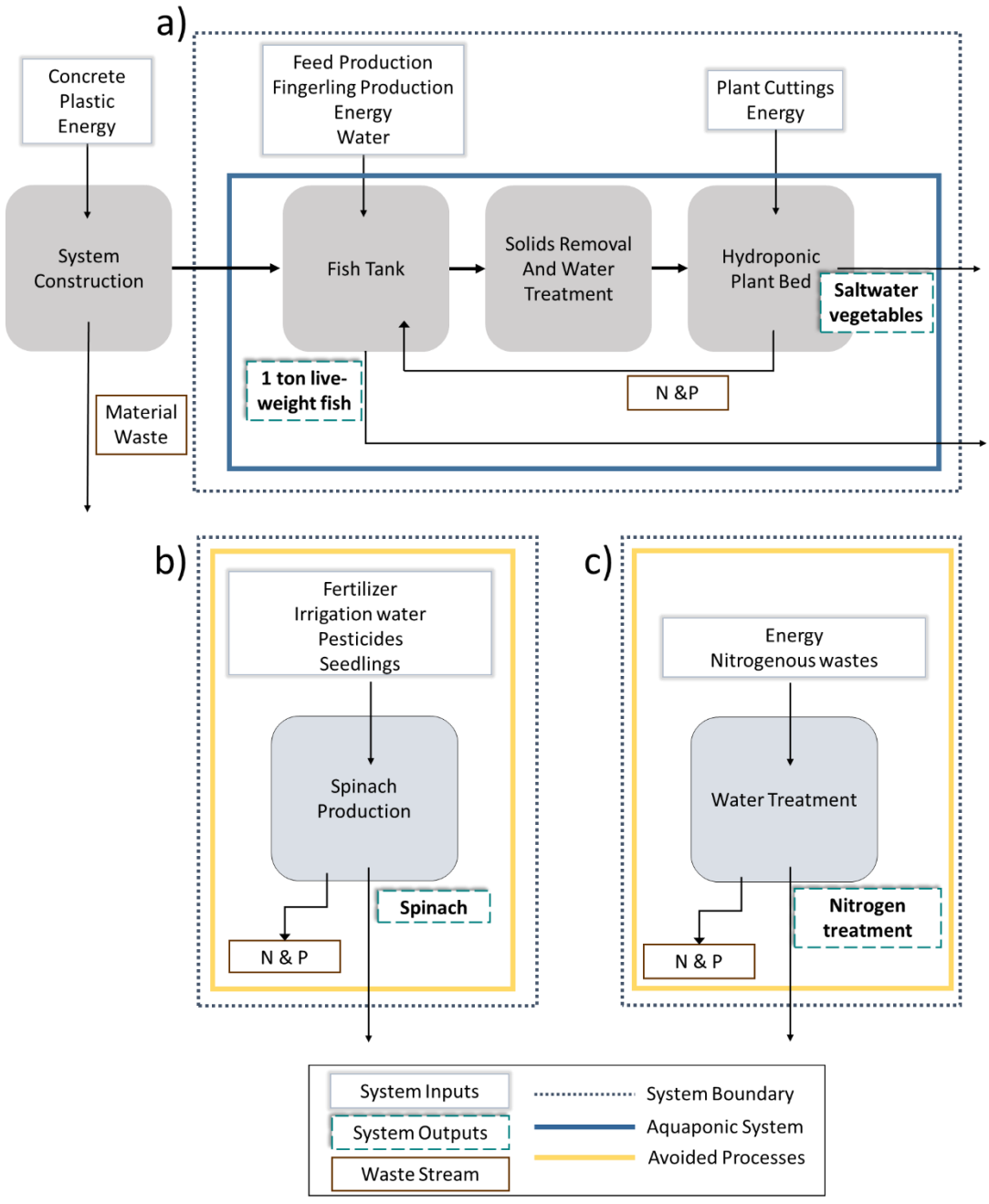

Figure 6.1: System diagrams for marine aquaponic system. (a) system diagram and boundaries of the aquaponic system with inputs and outputs, (b) agricultural production of vegetables due to co-production of vegetables, (c) water treatment avoided due to co-production of water treatment in hydroponic plant bed.

6.3.3 Life Cycle Inventory

Background inventory data were obtained from Ecoinvent v 1.2 and LCA food databases available within the SimaPro 7.0 software (PréConsultant, Netherlands). Data collected on site at Mote Aquaculture Research Park were used to estimate electricity requirements, feed added, expected fish and vegetable harvests, freshwater additions, and chemical additions (Table 6.1). Data were collected through interviews with the facility manager. Information on aquaculture feed components and the ingredients in the 45% protein aquaculture feed were estimated based on literature review (Table 6.2) (Appendix B). The fertilizer and water requirements for spinach production were based on information provided by the University of California, Vegetable Research & Information Center on spinach production in California (Koike et al., 2011).

The co-product water treatment was assumed to be completed in two steps, as described in Chapter 5: nitrification followed by water exchanges. In the baseline system, the avoided nitrification due to saltwater vegetable production was estimated based on the difference between the fish density recorded during year one of operation and the theoretical density used to initially size the biofilter. Fish densities were used to calculate ammonia emissions, which were used to determine the volume of media and subsequently aeration required for nitrification. In Scenarios 1 and 2, the recorded fish densities were also used to size the biofilter and estimate the energy requirements. In Scenario 1, no biofilter was present and 100% of the biofilter aeration was avoided. In Scenario 2, the plant beds were not present and 100% of the biofilter aeration was required.

The same nutrient budget model developed for Chapter 5 was used to estimate nutrient emissions from water exchanges associated with the co-product water treatment for all three scenarios (Appendix C). Water exchange volume and nitrate emissions were based on the 8% of

system water, which must be discharged daily to maintain a stable nitrate concentration of 25.6 mg/L NO_3^--N. The nitrate concentration was based on the mean concentrations measured on days 188, 216, and 244 (Chapter 3). Similarly, dissolved phosphorus emissions from water exchanges were calculated using the same 8% daily discharge rate required for nitrate removal.

Table 6.1: Inputs and outputs for the baseline system and two hypothetical scenarios.

	Baseline system	Scenario 1	Scenario 2
Inputs			
Electricity			
Aeration (kWh/ton)	2,240	3,490	1,510
Pumping (kWh/ton)	813	813	813
Feed (kg/ton)	453	453	453
Sodium bicarbonate (kg/ton)	31.0	31.0	31.0
Saltwater (m^3/system)	54.0	308	29.0
Freshwater (m^3/ton)	4.14	23	2.18
Outputs			
Harvest weight (kg)	0.900	0.900	0.900
Co-products			
Saltwater Vegetables (kg/ton)	312	3,580	0
N Fertilizer (kg/ton)	0.324	3.72	0
P Fertilizer (kg/ton)	0.386	4.42	0
K Fertilizer (kg/ton)	1.16	13.3	0
Irrigation (m^3/ton)	23.8	273	0
Water treatment			
Aeration (kWh/ton)	377	1,130	0
Water exchange (m^3/ton)	580	614	534
Nitrogen (kg/ton)	16.8	16.8	16.8
Phosphorus (kg/ton)	1.43	1.43	1.43

6.3.4 Life Cycle Impact Assessment

The life cycle impact assessment was performed as described in Chapter 5. The impact categories selected were global warming potential (GWP), human toxicity potential (HTP),

acidification potential (AP), eutrophication potential (EP), energy use (EU), land use (LU), and water use (WU).

Table 6.2: Feed ingredients. Simplified feed composition was based on comparison of other feeds in literature (Mungkung et al., 2013; Pelletier and Tyedmers, 2010; Tacon et al., 2011).

Feed ingredients	45% protein fish food
Soybean meal	25 %
Wheat middlings	15 %
Maize/corn	24 %
Fish meal	36 %

6.4 Results

A quantitative assessment of the impacts attributed to each process is presented in Table 6.3. The feed and chemical inputs were identical for each scenario, as such the contribution to the impact categories considered were constant. When considered relative to the other impact categories, feed contributed greatest to eutrophication potential and land use (Figure 6.2). The mean relative contribution of feed for all scenarios was 16% and 95% for eutrophication and land use, respectively. Chemical inputs (sodium bicarbonate) did not have a large environmental impact on any category. At the greatest chemicals contributed to 2.6% of the life cycle impacts to human toxicity potential in Scenario 2.

Electricity contributed greatest to all impact categories considered, with the exception of land use and water use. Scenario 1, with expanded plant production, had the greatest electricity requirements of the three scenarios and therefore quantitatively the greatest acidification potential, eutrophication potential, global warming potential, and human toxicity potential. Scenario 2 had the lowest electricity requirements and therefore quantitatively the lowest contribution to acidification potential, eutrophication potential, global warming potential, and human toxicity potential.

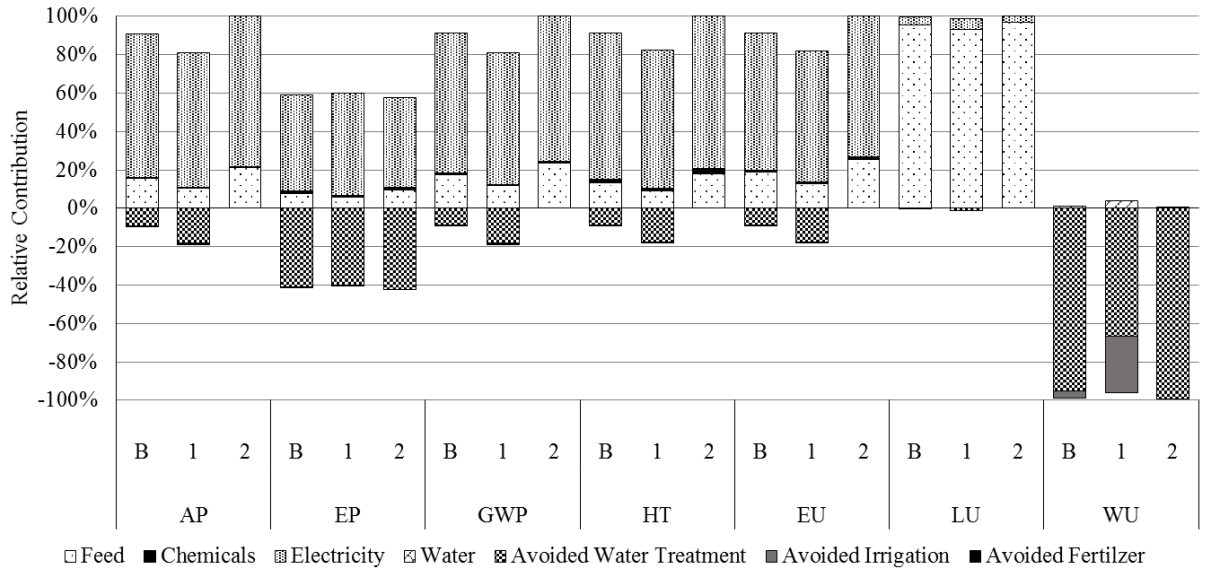

Figure 6.2: Relative contribution from system processes in three aquaculture system scenarios. Where B = Baseline, 1 = Scenario 1, 2 = Scenario 2 and impact categories are AP = Acidification potential, EP = Eutrophication potential, GWP = Global warming potential, HTP = Human toxicity potential, EU = Energy use, LU = Land use, WU = Water use.

Water use depended on the total volume of the system and the amount of water that was replaced for evaporation. Ignoring potential avoided water use, Scenario 1 had greater water use due to the increased system volume from additional plant beds. In Scenario 2, the absence of plant beds resulted in the smallest total system volume and therefore the lowest water use.

Avoided water treatment contributed to a reduction in all impact categories for the baseline and Scenario 1. In Scenario 2, avoided water treatment only contributed to a reduction in eutrophication and water use due to the absence of avoided electricity. Scenario 1 had the greatest reduction in impacts from avoided water treatment for the categories acidification potential, global warming potential, human toxicity potential, and energy use. In the baseline scenario the reduction in water use from avoided water treatment was 95%. In contrast, in Scenario 1, only 67% of the avoided water use was due to avoided water treatment.

Table 6.3: Life cycle impacts for the production of 1 ton live-weight red drum for three scenarios. The scenarios were: the system described in Chapter 3 (baseline), a maximum planting density and no biofilter present (scenario 1), no plant production, only a downflow submerged denitrification (scenario 2). The values for avoided burdens are negative because they were credited to the system. AP = Acidification potential, EP = Eutrophication potential, GWP = Global warming potential, HTP = Human toxicity potential, EU = Energy use, LU = Land use, WU = Water use.

	Feed	Chemicals	Electricity	Water	Avoided Water Treatment	Avoided Irrigation	Avoided Fertilizer	Total
AP (kg SO_2 eq)								
Baseline	20.4	0.70	99.1	0.00	-12.0	0.00	-0.10	108
Scenario 1	20.4	0.70	139	0.00	-35.8	0.00	-1.13	123
Scenario 2	20.4	0.70	76.0	0.00	0.00	0.00	0.00	97.1
EP (kg PO_4^- eq)								
Baseline	3.91	0.56	25.3	0.00	-20.6	0.00	-0.01	9.15
Scenario 1	3.91	0.56	35.3	0.00	-26.7	0.00	-0.14	13.0
Scenario 2	3.91	0.56	19.5	0.00	-17.6	0.00	0.00	6.36
GWP (kg CO_2 eq)								
Baseline	3,230	181	13,600	0.00	-1,640	0.00	-24.6	15,300
Scenario 1	3,230	181	19,000	0.00	-4,930	0.00	-282	17,200
Scenario 2	3,230	181	10,400	0.00	0.00	0.00	0.00	13,800
HTP (kg 1,4-DB eq)								
Baseline	1,230	176	6,950	0.00	-788	0.00	-0.17	7,560
Scenario 1	1,230	176	9560	0.00	-2,360	0.00	-1.95	8,600
Scenario 2	1,230	176	5420	0.00	0.00	0.00	0.00	6,830
EU (MJ)								
Baseline	60,500	3,420	228,000	0.00	-27,600	0.00	-213	264,000
Scenario 1	60,500	3,420	320,000	0.00	-82,800	0.00	-2,440	299,000
Scenario 2	60,500	3,420	175,000	0.00	0.00	0.00	0.00	100,000
Land use (PDF*m^2yr)								
Baseline	2,539	2.14	109	0.00	-13.1	0.00	0.00	2,637
Scenario 1	2,539	2.14	153	0.00	-39.3	0.00	0.00	2,660
Scenario 2	2,539	2.14	83.9	0.00	0.00	0.00	0.00	2,630
Water Use (m^3)								
Baseline	0.00	0.00	0.00	6.00	-580	-23.8	0.00	-598
Scenario 1	0.00	0.00	0.00	36.0	-614	-273	0.00	-851
Scenario 2	0.00	0.00	0.00	3.00	-534	0.00	0.00	-531

In the baseline scenario, avoided irrigation contributed to a 4% relative reduction in water use. Scenario 1 had a relative reduction of 30% from avoided irrigation due to the greater

production of plant biomass. Scenario 2 did not result in avoided water use from avoided irrigation due to the absence of plant growth.

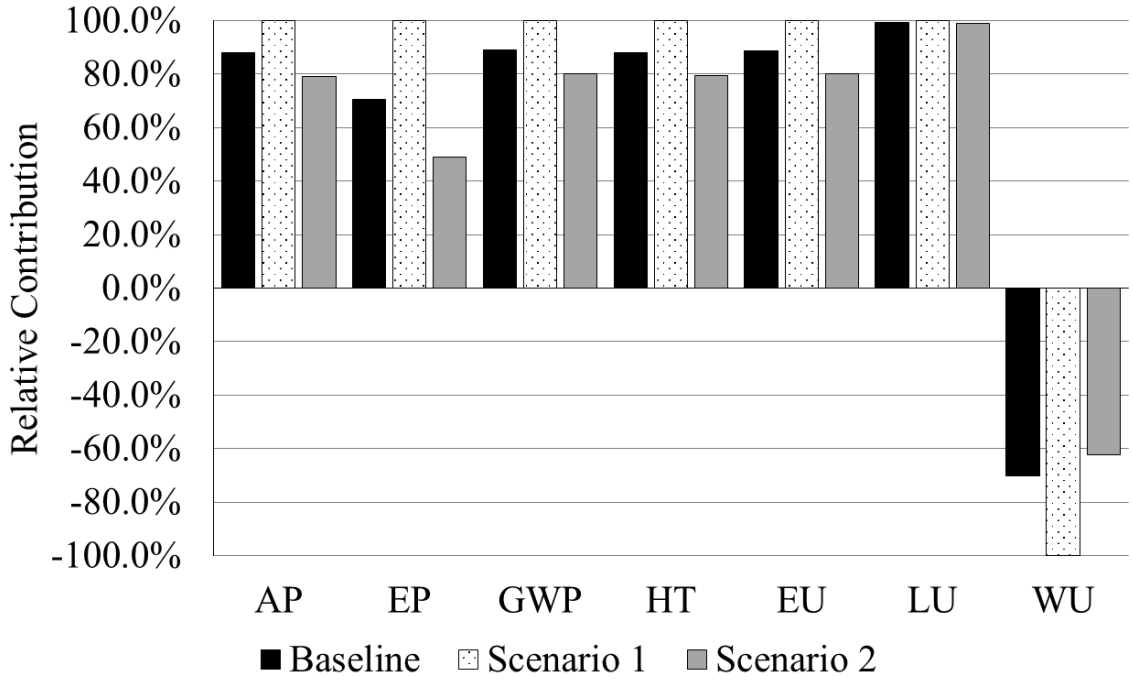

Figure 6.3: Comparative analysis of three scenarios. Impact categories are AP = Acidification potential, EP = Eutrophication potential, GWP = Global warming potential, HTP = Human toxicity potential, EU = Energy use, LU = Land use, WU = Water use.

The contribution of avoided fertilizer to reductions in the impact categories considered was small. At the greatest, Scenario 1, with maximized plant capacity, had a reduction of 1% for the category of global warming potential. Similarly, in all other categories, Scenario 1 had greater reductions from avoided fertilizers due to the greater plant production.

When the three scenarios are compared, Scenario 1 consistently had a greater environmental impact (Figure 6.3). Quantitatively Scenario 1 had the highest reductions due to avoided water treatment, avoided irrigation, and avoided fertilizer; however, the amount of energy required was also higher. Scenario 2 consistently had the lowest environmental impact due to the lowest energy requirements. In particular, Scenario 2 had the lowest eutrophication impacts due to lower electricity inputs despite the lowest reductions from avoided water

treatment. For the category of water use, Scenario 1 also had the greatest contribution although in this instance it was a positive impact. The avoided water treatment and avoided irrigation combined resulted in greater reductions than in the baseline or Scenario 2.

6.5 Discussion

When evaluating the life cycle impacts of aquaculture systems it is important to remember that one important purpose of intensifying systems is to reduce nutrient discharge (Ebeling and Timmons, 2012). Through the life cycle lens this corresponds to reductions in local impacts, such as eutrophication. Consequently to achieve reduced local impacts systems often have greater energy requirements, which correspond with a potential increase in global impacts, such as global warming potential. Considering this potential trade-off, it was the goal of this study to evaluate how variations in supplemental water treatment from plant growth and denitrification combined or each process independently contributed to the environmental impact of an aquaponics.

Also, it is important to note that the baseline scenario represents a prototype commercial-scale system and does not reflect economies of scale possible with larger fully commercialized systems (Carter and Keeler, 2008). Therefore, it potentially reflects higher costs and energy requirements than achievable in more streamlined systems. Similarly the fish and plant production capacities are based on one year of experimental data and parameters, such as yield and total harvest, and should be further evaluated over several growth seasons.

6.5.1 Comparison of Scenarios

All three systems were designed to eliminate local nutrient discharge and by association had low eutrophication impacts. The total eutrophication potential increased such that Scenario 2 < baseline scenario < Scenario 1 with respective values of 6.36, 9.15, 13.0 kg PO_4 eq. The

quantity of nitrate and phosphate discharged was based on the fish biomass and therefore was a constant reduction of 17.6 kg PO_4 eq for all systems. Variations in eutrophication potential between scenarios was due to differences in electricity requirements.

When compared to a turbot RAS, the eutrophication potential of Scenario 1 was about 80% lower (Aubin et al., 2009). Alternatively the salmon RAS in Ayer and Tyedmers (2009) had a lower eutrophication potential of 20.1 kg PO_4 eq Similar to this study the RAS in Ayer and Tyedmers (2009) was assumed to have zero discharge, with all emissions routed to a wastewater treatment plant. The lower eutrophication potential was largely due to differences in electricity source. As discussed in Chapter 4, the eutrophication potential is not inherently reduced due to intensification and recirculation. Choices of electricity source, electricity quantity, and feed source will also impact emissions (Aubin et al., 2009; Wilfart et al., 2013).

Based on this study, aquaponics can further reduce environmental impacts from local nutrient emissions; however, as with RAS, this reduction comes at the cost of higher global impacts due to electricity use. Ultimately, the need for additional water treatment should be evaluated contextually. In areas highly sensitive to nutrient discharges, reducing eutrophication impacts is a priority. Alternatively, in less ecologically sensitive areas with predominately fossil fuel based energy sources, reducing electricity requirements or adding renewable energy technologies could be a better use of resources.

When energy use is considered independently it followed the same trend as eutrophication. Between the scenarios, the energy use due to electricity requirements for all three systems increased such that Scenario 2 < baseline scenario < Scenario 1, with respective values of 175,000, 228,000, and 320,000 MJ. LCA studies on RAS have found energy usage from electricity to be in a similar range at 291,000 MJ (Ayer and Tyedmers, 2009) and 250,010 MJ

(Aubin et al., 2009). The similarity of energy use attributed to electricity indicates that despite the potential over-estimation of electricity in this study due to inefficiencies of scale, the total electricity estimates were reasonable when compared to previous studies.

When the energy use from avoided water treatment and avoided fertilizer were considered, Scenario 1 had the greatest reduction in energy use. Collectively both avoided burdens resulted in a 30% reduction to energy use. However, despite this reduction, Scenario 1 had the greatest energy requirements of the three scenarios. As reported in Chapter 5, aeration in the plant beds can contribute to as much as 41% of the energy requirements. In the baseline scenario plant bed aeration contributed to 25% of the energy requirements and in Scenario 1 it contributed 64% of the energy requirements. Considering that Scenario 1 is a hypothetical system it is possible that an actual system would require less plant bed aeration, which could reduce the overall energy use to be more similar to Scenario 2.

Regardless of the exact energy requirements for aerating the plant beds, it is clear that additional research is needed in this area to reduce the environmental impact of aquaponics. While some research has demonstrated the need for hydroponic plant bed aeration (Morard and Silvestre, 1996; Morard et al., 2000), it remains unclear the exact quantity of aeration required for plant roots in deep water hydroponics. Zeroni et al. (1983) suggested that 65% O_2 saturation was the minimum required for consistent vegetative growth and fruit production of tomatoes in deep water culture hydroponics. While hydroponic research can be used to establish baseline requirements, difference between hydroponic and aquaponic system designs, such as flow rate and water depth, could impact oxygen availability. For this reason, more research should be conducted to improve aeration efficiency in aquaponics especially considering the environmental impact associated with the predominately fossil fuel based sources as used in this study.

Alternatively a total reduction in fossil fuel based energy sources can also reduce the environmental impact as demonstrated in previous LCA studies of intensive aquaculture systems (Aubin et al., 2009; Ayer and Tyedmers, 2009).

The area where aquaponics showed the greatest advantage environmentally was in terms of water use. The elimination of water exchanges to manage nitrate concentrations resulted in a large reduction in water use, such that the quantity of water use was negative. While it is well established that RAS reduce water use (Aubin et al., 2009; Wilfart et al., 2013), it is unclear if previous LCA studies included freshwater additions for nitrate control in their water use and water dependence impact categories. Despite the lack of this information, the results of this LCA indicate that the reduction in water use from avoided water treatment is significant.

Due to the additional reduction in water use from avoided irrigation, Scenario 1 had the lowest water use. The avoided irrigation in Scenario 1 contributed to 40% greater water savings than Scenario 2 with denitrification and no plant production. The baseline scenario also benefited from a reduction in water use, to a smaller extent. As freshwater supplies are increasingly constricted by greater irrigation demands, water intensive food preferences, nonagricultural water demands, and global climate change, any improvements in water use efficiency could be beneficial (Rosegrant et al., 2009). Similar to reductions of nutrient emissions, the need for water use reductions should be evaluated based on context. In areas of water scarcity the reduction in water use could be worth potential trade-offs with increased energy demands.

6.5.2 Co-product Decisions

In LCA, the selection of substitute products can be highly subjective resulting in high levels of uncertainty (Eady et al., 2012). While spinach was selected as the vegetable crop, production inputs can vary greatly with location, season, and farm management practices.

Similarly, selection of a different vegetable co-product would have resulted in different inputs. When blue water inputs are compared, where blue water is a measure of surface and groundwater consumed, spinach requires 14 m^3/ton (Mekonnen and Hoekstra, 2011). If broccoli or asparagus had been substituted instead, with their respective blue water requirements of 21 and 119 m^3/ton, a greater quantity of irrigation water would have been avoided. Considering the results of this LCA, to maximize water savings it could be advantageous to focus on producing water intensive crops in aquaponics.

Similarly, the energy requirements for Scenarios 1 and 2 and from avoided water treatment were also susceptible to uncertainty. The quantity of aeration required for nitrification in the biofilter can vary between types of biofilters, whole system design, and with operator preference. At present, there are not well established guidelines for aeration in the MBBR selected for use as the biofilter in all three scenarios. As discussed above the quantity of aeration required for hydroponic plant bed production is unknown and requires additional research.

6.5.3 Other Protein Sources

Consumption of protein, especially from animal sources, potentially contributes to a greater dietary carbon footprint (Scarborough et al., 2014). When carbon emissions from different protein sources are compared there are large variations in emissions, which hinder identification of clear trends.

A review of LCA studies on different protein sources found the carbon footprint of aquaculture products ranges from 3-15 kg CO_2 eq/kg product (Nijdam et al., 2012). Only one RAS was considered in the study, which had the highest value of 15 kg CO_2 eq/kg product. An assessment of carbon footprints of additional aquaculture systems indicates values as high as beef and as low as poultry and pork (Table 6.4).

Table 6.4: Comparison of carbon emission results from LCA studies. Data on pork, poultry, and beef adapted from de Vries and de Boer (2010). Data on fish based on studies in Chapter 4 and this study.

Study	System	Functional Unit	kg CO_2 eq/FU	kg CO_2 eq/kg edible product[1]
Pork				
Williams et al. 2006	Heavier finishing	1 ton dead weight	6,080	8.60
Williams et al. 2006	Indoor breeding	1 ton dead weight	6,420	9.08
Williams et al. 2006	Outdoor breeding	1 ton dead weight	6,330	8.69
Williams et al. 2006	Conventional	1 ton dead weight	6,360	9.00
Poultry				
Williams et al. 2006	Conventional	1 ton dead weight	4,570	5.71
Williams et al. 2006	Free range	1 ton dead weight	5,480	6.85
Beef				
Williams et al. 2006	100% sucker	1 ton dead weight	25,300	32.4
Williams et al. 2006	Lowland	1 ton dead weight	15,600	20.0
Williams et al. 2006	Hill and upland	1 ton dead weight	16,400	21.0
Williams et al. 2006	Non-organic	1 ton dead weight	15,800	20.2
Fish				
Jerbi et al. 2012	Cascade flow-through	1 ton live fish weight	17,400	43.6
Jerbi et al. 2012	Traditional flow-through	1 ton live fish weight	11,100	27.7
Roque d'Orbcastel et al. 2009	RAS, FCR 0.8	1 ton fish	1,600	4.01
Roque d'Orbcastel et al. 2009	RAS, FCR 1.1	1 ton fish	2,040	5.11
Ayer and Tyedmers 2009	RAS	1 ton live fish weight	28,200	70.5
Aubin et al., 2009	RAS	1 ton fish	6,020	15.0
This study baseline	Aquaponic system plants and denitrification	1 ton live fish weight	15,300	38.3
This study scenario 1	Aquaponic system plants only	1 ton live fish weight	17,200	43.0
This study scenario 2	RAS with denitrification, no plants	1 ton live fish weight	13,800	34.5

[1]kg edible product for pork, poultry, and beef calculated based on information in de Vries and de Boer (2010); kg edible product for fish based on assumption of 0.5 kg edible product/ kg live weight (Iversen 1996)

While the aquaponic systems assessed in this study had carbon emissions slightly higher than beef, these numbers do not account for transportation or processing impacts, which can have

a significant impact on emissions (Carlsson-Kanyama et al., 2003). Commercial aquaponics facilities in the United States frequently sell goods to local, direct markets such as farmers markets, farm stands, and community supported agriculture (Love et al., 2015). As a result products have shorter transport distances and minimal food processing, potentially contributing to a reduced environmental impact if system boundaries are expanded past the farm-gate. More research should be conducted to determine if localized production in aquaponics mitigates impacts from high energy use.

In terms of carbon emissions, poultry and pork typically have the lowest emissions; however, this does not account for other environmental impacts, such as nitrogen and phosphorus emissions or land requirements. Xue and Landis (2010) compared eutrophication potentials of different protein sources and found red meat to have the highest eutrophication potential and fish the lowest. Also, as discussed in Chapter 4, the land use requirements of intensive aquaculture were smaller than poultry or pork.

Water use is also an important resource consumed in variable quantities by different food products. Research on water use in food production has been completed at global and national scales (Wallace and Gregory, 2002); however, limited research on water use for specific food product life cycles is available (Foster et al., 2007; Ruviaro et al., 2012). Using average crop yields and water requirements for select crops in California, Renault and Wallender (2000) examined water productivity (the production per unit water) and nutritional water productivity (nutritional value per unit water). The nutritional water productivity of a subset of products evaluated by Renault and Wallender (2000) were converted to water use per unit of nutritional energy (kcal) (Table 6.5). Similarly, water use from several LCAs of aquaculture systems were

converted to water use per kcal. The standardized numbers indicated water use in aquaculture system was much lower than other foods, excepting only flow-through aquaculture systems.

Table 6.5: Comparison of water use for different food products.

Study	Product/System	L/kg product	L/kcal[1]
Renault and Wallender, 2000	Wheat	1,159	0.439
Renault and Wallendee, 2000	Orange	378	1.51
Renault and Wallender, 2000	Bovine meat	13,500	9.80
Renault and Wallender, 2000	Pork meat	4,600	2.45
Renault and Wallender, 2000	Poultry	4,100	3.03
Aubin et al., 2009	Fish/Trout flow-through	97,600	128
Aubin et al., 2009	Fish/Sea-bass cages	105	0.138
Aubin et al., 2009	Fish/Turbot RAS	9.6	0.013
This study baseline scenario[2]	Fish/Aquaponic system plants and denitrification	12.0	0.016
This study scenario 1[2]	Fish/Aquaponic system plants only	72.0	0.095
This study scenario 2[2]	Fish/RAS with denitrification, no plants	6	0.008

[1] kg edible product for fish based on assumption of 0.5 kg edible product/ kg live weight (Iversen, 1996); 0.19 kg protein/kg edible product (Lawrie and Ledward, 2006); 4000 kcal/kg protein (Southgate, 1981)
[2] Only the fish products are included in analysis.

As discussed above, the water use in the aquaponic systems was 111-177 times lower than conventional RAS when avoided water treatment and agricultural irrigation are included. Due to the uncertainty of how other studies handled potential water discharge and treatment, the avoided burdens were excluded from comparison with other products. Even with the exclusion, the water use in the aquaponics was many times lower than other food products. If the kcals from vegetable production were also included the quantity of water use per kcal would be reduced even further. The analysis presented here was extremely simplified and future studies should be conducted in which the methods of data collection, system boundaries, and classification of water inputs are standardized. However, based on these rough estimations, aquaponics contribute to substantial water savings, which will be increasingly important as water supplies are further constricted.

Equally important as environmental impact considerations, is the variable nutritional content of different protein sources. While the quantity of protein in meat is roughly consistent at 19%, the availability of amino acids and minerals can vary between species (Lawrie and Ledward, 2006). For example seafood is known to be high in omega-3 fatty acids, vitamin D, and vitamin B12, which are all important for human health (Lund et al., 2013). Alternatively, red meat is known be a good source of iron, zinc, and niacin (Higgs, 2000). Considering that all protein sources inherently have advantages and disadvantages, consumers should vary their purchases based on health benefits and taste preferences in addition to reducing carbon footprints and maximizing water savings.

6.6 Conclusion

In this study three aquaculture systems were evaluated to determine potential advantages and disadvantages to supplemental water treatment through plant production or denitrification and no plant production. In order to accomplish this, a LCA on a prototype commercial-scale marine aquaponic system was conducted along with LCAs on two hypothetical systems designed based on data collected from the prototype system. The results indicated a zero-water-discharge system operated with a denitrification reactor and no plant production had the lowest environmental impact in six of the seven categories considered. In contrast, the aquaponic system with maximized plant growth had the greatest impact due to high energy requirements, particularly from aeration in the plant beds. These results are concurrent with previous LCAs on intensive aquaculture systems which have established that energy use in intensive aquaculture systems can be substantial due to pumping, aeration, and temperature regulation. The higher energy requirements also contributed to higher carbon footprints relative to other protein sources.

Despite the high energy requirements, these results also show water treatment from hydroponic plant production contributed to large reductions in energy and water use. The potential reduction in energy use from avoided water treatment due to plant production was a unique conclusion of this study. In addition to avoided energy for water treatment, aquaponics contributed to substantial water savings. While previous studies on RAS have shown reduced water use compared to other types of aquaculture, this study demonstrated a net water saving from additional water treatment processes. Furthermore, the avoided irrigation from maximum hydroponic plant production contributed to 40% greater water savings compared to denitrification alone. When compared to other food products the quantity of water used for aquaponic fish production was lower than all other fish or protein sources.

Considering that criticism of aquaculture systems has conventionally focused on nutrient discharges, both RAS and aquaponic technologies inherently increase the sustainability of these systems in the eye of the consumer. The reduction in these local impacts come at the cost of greater global impacts, such as carbon emissions, which have relatively recently emerged as a concern for consumers. To the benefit of consumers and the aquaponics industry, the unfavorable increase in carbon emissions was offset by confirmation of substantial water savings. As consumers become more critical of all environmental impacts associated with production of various protein sources, both aquaponics and RAS with denitrification will play a part in the development of sustainable protein from aquaculture. In addition, marine aquaponics has the potential to advance commercialized halophyte production. Expansion of this market will enhance the economic viability of marine aquaponics and contribute to food security as climate change and population growth necessitate a transformation of the current food production system.

To realize the greatest benefits from these systems, the advantages and disadvantages of each system should be considered along with contextual factors of proposed site locations, such as areas most likely to be impacted by climate change, water and food scarcity. It is in these areas where the ability to produce highly nutritious protein and vegetable products simultaneously with saline water resources will be critical for ensuring food security.

Chapter 7: Conclusions and Recommendations

Aquaculture production of aquatic animal and plant products is a critical component of global food and economic security. However, similar to other food production industries, aquaculture is now confronted with the challenge of feeding 9 billion people sustainably. Diverse solutions must be integrated to achieve the ultimate goals of increasing productivity, reducing environmental impacts, and improving resiliency to climate change. As the aquaculture industry continues to grow rapidly, new technologies are being developed to meet these challenges. It is important that these new systems and technologies are optimized for maximum production and that their environmental sustainability is assured as they become permanent components of food production.

Building upon the concepts of recirculating aquaculture systems (RAS) and integrated multi-trophic aquaculture (IMTA), aquaponic systems are one component to creating a new era of sustainable aquaculture. Freshwater aquaponics has already been established as an efficient way to produce freshwater fish and vegetables on non-arable land and in areas with constricted water supplies. Considering the success of freshwater aquaponics, the need for expanded production of marine fish species, and growing interest in halophytes there is strong potential for development of marine aquaponics. This dissertation sought to complete an in-depth evaluation of marine aquaponics through two research questions: 1) How do halophytes, sea purslane and saltwort, perform in a marine aquaponics system in terms of halophyte growth and nutrient removal capability? and 2) using a life cycle assessment (LCA) framework, what is the

environmental impact of freshwater and marine aquaponics at scales ranging from residential to commercial?

The first question was addressed in Chapter 2 through a series of bench-scale experiments conducted to demonstrate the potential to grow the halophytes sea purslane and saltwort hydroponically and explore specific design parameters. The specific conclusions were:

1. Sea purslane and saltwort can be grown in a floating raft style aquaponic system and contribute significantly to nitrogen removal.
2. Flow rate, plant density, and plant species did not significantly impact nutrient removal or plant growth.
3. Coconut fiber contributed to greater nitrogen removal than light-weight expanded clay media.

The first question was also addressed in Chapter 3, in which a prototype commercial-scale aquaponic system was evaluated. The specific conclusions were:

1. The zero-discharge marine aquaponic system successfully produced both halophytes and fish for commercial sale and maintained water quality within safe ranges for fish production.
2. A denitrification reactor was needed to manage nitrate concentrations due to insufficient plant biomass or passive denitrification.
3. A side-stream denitrification reactor provides operators with flexibility in system operation, facilitating maximum fish production independent of plant production.
4. Greater quantities of plants could be supported such that the hydroponic plant beds could be increased to a size of 711 m^2.

5. Phosphorus accumulated in system water although daily measurements varied due to plant growth and the mineralization/precipitation/sedimentation of phosphorus in tank and plant bed bottoms

In order to address the issue of sustainable production with aquaponics, the second question of this dissertation examined the environmental impacts of aquaponics through the application of LCA. First a literature review of LCAs on intensive and extensive aquaculture systems was conducted and presented in Chapter 4. The specific conclusions were:

1. The movement from extensive to intensive aquaculture systems contributed to a shift from local to global environmental impacts.
2. Intensive systems had less water pollution and lower total water use than extensive systems and had lower land use requirements than other protein sources.
3. The greatest contribution to environmental impact in intensive systems was due to energy requirements, although renewable energy can mitigate these impacts.
4. Intensive systems had greater environmental impacts from feed, although the feed conversion ratio (FCR) and feed ingredients had greater influence on environmental impact than intensity.
5. Integrated multi-trophic aquaculture systems could improve the total nutrient uptake and increase total yields thereby reducing impacts through greater production per unit of feed, water, and energy.

In Chapter 5, the environmental impacts of aquaponics were evaluated through LCA of two freshwater aquaponic systems at a commercial- and residential-scale. The specific conclusions were:

1. Electricity use was a hot-spot of environmental impact and the aeration of hydroponic plant beds was identified as a place to reduce total electricity requirements, alternatively use of renewable energy would reduce environmental impacts.
2. Feed was a hot-spot and improved FCRs or use of less resource intensive ingredients, like microalgae, are a potential way to reduce the environmental impact from feed.
3. The co-product water treatment, provided by hydroponic plant growth, contributed to the greatest reduction in environmental impacts.
4. The co-products plants and recovered solids contributed to a less than 10% reduction in environmental impacts, with the exception of water use in which plants contributed to a 17% reduction.
5. The impacts of scale were inconsistent across impact categories and both systems had similar environmental impacts.

Finally in Chapter 6, the environmental impact of the prototype commercial-scale aquaponic system and two alternative scenarios were evaluated though LCA. The specific conclusions were:

1. The environmental impact of a RAS with a denitrification reactor and no plant growth < an aquaponic system with both plant growth and a denitrification reactor < an aquaponic system with maximized plant production in six of the seven environmental impact categories considered.
2. Scenario 1, maximized plant production, had a 40% reduction in water use due to co-production of plants.
3. Carbon emissions were greater when compared to other protein sources and water use was substantially lower than other protein sources and conventional RAS.

Unique to the results of Chapters 5 & 6 was a demonstration of the water savings achievable in aquaponics due to avoided water exchanges and co-production of plants. While, the results on water use in these chapters provide a foundation, more research should be completed to evaluate water use coupled with type of water (e.g. green, gray, blue), direct versus indirect uses, and spatially relevant water scarcity.

In addition to the environmental impacts, future research is needed to quantify the economic feasibility, particularly of simultaneous fish and plant production in marine aquaponics. Dual products are often cited as an advantage to aquaponics; however, few studies have quantified the economic potential. The few existing economic studies on aquaponics focused on freshwater systems with an emphasis on plant production. The marine aquaponic system evaluated in this dissertation was unique due to the novel edible halophytes and also the focus on fish production. Designs with an emphasis on maximizing production of high-value fish species over plants should be evaluated economically as the potential economic returns are unknown. Furthermore, as market demand for halophytes expands, the value of commercially produced halophytes should be evaluated in relation to the value of marine fish.

Aquaculture has already distinguished itself as a critical component of global food security and as an important income source in developed and developing nations. As the industry grows, freshwater and marine aquaponics will play an important role in advancing the development of sustainable aquaculture. Ultimately, the intersection of non-arable land, water scarcity, and access to renewable energy is highly advantageous in an environmental context for aquaponics. In time, the most desirable economic context for aquaponics will also be established. Meeting the challenge of creating a sustainable food system capable of eliminating hunger

requires a multifaceted approach in which aquaculture and marine aquaponics will play an important role.

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Appendix A: Supplementary Methods Information

A.1 Ammonia Method

Solution Preparation

Solution #1: Ammonia-N Stock Solution (1.0 mL = 0.1 mg NH_3-N or 100 mg NH_3-N/L)
Dissolve 0.3819 g of dried Ammonium Chloride (NH_4Cl) into 15 ppt salt water and dilute to 1 L in a volumetric flask. Mix.

Solution #2: Ammonia-N Standard Solution (1.0 mL = 0.001 mg NH_3-N or 1 mg NH_3-N/L)
Add 10 mL of the Ammonia-N Stock Solution (Solution #1) to a 1 L volumetric flask. Dilute to 1 L with 15 ppt saltwater. Mix. This is the solution used for the calibration curve.

Solution #3: Ammonia-N Calibration Curve
The calibration curve is determined using dilutions made from the Ammonia-N Standard Solution (Solution #2). Aliquots of this solution are added to different 50 ml volumetric flasks and then diluted to 50 ml with saltwater. The following samples are generated for use in the calibration process.

mg NH_3-N /L	mL solution #2
0.02	1
0.05	2.5
0.08	4
0.10	5
0.20	10

Solution #4: Salicylate Catalyst Solution

a) Add 0.14 g of sodium nitroprisside to a 500 ml volumetric flask.
b) Add 220 g of sodium salicylate to the same 500 ml volumetric flask.
c) Dilute to 500 ml with DI water.
d) Store in the refrigerator in brown bottles.

Solution #5: Sodium Citrate Solution

Method B:

a) Add 9.25 g of sodium hydroxide to a 500 ml volumetric flask.
b) Add 57 g of sodium citrate ($2H_2O$).
c) Dilute to 500 ml with DI water.

Solution #6: Alkaline–hypochlorite Solution
To a 100 ml beaker, add the following:

a) 5 ml of Chlorox bleach (1 part)
b) 45 ml of Solution #5 (9 parts)

This solution should be made just before use and any unused solution should be discarded.

Sample Analysis

This test should be run under subdued light and then put under black plastic during the one hour testing time.

1. Set up a rack of screw top vials and add 7.5 ml of sample to each vial

   Include saltwater blank (15ppt saltwater) and 5 calibration standards for total of 6 additional samples (0, 0.02, 0.05, 0.08, 0.1, and 0.2 mg NH_3-N/L)

3. Add 0.9 ml of the salicylate catalyst solution (Solution #4) to each vial

4. Add 1.5 ml of the alkaline-hypochlorite solution (Solution #6).

5. Invert samples 1-2 times to fully mix.

6. Let react for 1 hour in the dark. (You can place samples in a hood and then cover with a dark lab coat or plastic bag)

7. Measurement: Read absorbance using Hach DR 2800 Spectrophotometer at 640 nm. (Check samples to make sure solution has not separated, mix again if separation is seen before reading in spectrophotometer.)

A.2 Nitrite Method

Solution Preparation

Solution #1: Nitrite-N Stock Solution (1.0 mL = 0.1 mg NO_2^--N or 100 mg NO_2^--N/L)
Add 50 ml of a purchased standard nitrite solution (1.0 ml = 1 mg of NO_2-N) to a 500 mL volumetric flask. Add saltwater to bring the volume to 500 mL.

Solution #2: Nitrite-N Standard Solution (1.0 mL = 0.001 mg NO_2-N or 1 mg NO_2^--N/L
Add 10 mL of the Nitrite-N Stock solution (Solution #1) to a 1 L volumetric flask. Dilute to 1 L with 15 ppt saltwater. Mix. This is the solution used for the calibration curve.

Solution #3: Nitrite-N Calibration Curve
The calibration curve is determined using dilutions made from the Nitrite-N Standard Solution (Solution #2). Aliquots of this solution are added to different 50 ml volumetric flasks and then diluted to 50 ml with saltwater. The following samples are generated for use in the calibration process.

mg NO_3^--N /L	mL solution #2
0.02	1
0.08	4
0.1	5
0.2	10
0.5	25

Solution #4: Sulphanilamide Solution
Add 25 ml of conc. hydrochloric acid HCl (12 N) (or 50 ml of 6N) to about 150 ml of DI water. Dissolve 2.5 g of crystalline sulphanilamide in the acidic solution and transfer the contents to a 250 ml volumetric flask. Dilute this solution to 250 ml with DI water and mix thoroughly.

Solution #5: 2% N-(1-naphthyl) ethylenediamine dihydrochloride Solution
Dissolve 0.25 g of N-(1-naphthyl) ethylenediamine dihydrochloride in 250 ml of DI water. Store the solution in an amber glass bottle in a refrigerator. This solution is stable for 1 month only and should be discarded if the solution turns brown at any time.

Sample Analysis

1. Set up as many sample cuvettes as needed. Pipet 10 mL of sample or calibration standard into vial.

   Include saltwater blank (15 ppt saltwater) and 5 calibration standards for total of 6 additional samples (0, 0.02, 0.08, 0.01, 0.2, and 0.5 NO_3^--N/L)

3. Add 0.4 ml of the sulphanilamide solution (Solution #4) to each vial.
4. Wait 15 minutes.
5. Add 0.4 ml of the N-(1-naphthyl) ethylenediamine dihydrochloride solution (Solution #5) and mix well by inverting vials up and down several times.
6. Measurement: Read absorbance using Hach DR 2800 Spectrophotometer at 540 nm

A.3 Nitrate Method

Solution Preparation

Solution #1: Nitrate-N Stock/Standard Solution (1.0 mL = 0.1 mg NO_3^--N or 100 mg NO_3^--N/L)
Dissolve 0.30357 g of dried Sodium Nitrate ($NaNO_3$) into 15 ppt salt water and dilute to 500 mL in a volumetric flask. Mix.

Solution #2: Nitrate-N Calibration Curve
The calibration curve is determined using dilutions made from the Nitrate-N Standard Solution (Solution #1). Aliquots of this solution are added to different 50 ml volumetric flasks and then diluted to 50 ml with saltwater. The following samples are generated for use in the calibration process.

mg NO_3^--N /L	mL solution #2
1	0.5
2	1.0
4	2.0
8	4.0
10	5.0

Solution #3: Concentrated Sulfuric Acid H_2SO_4

Solution #4: 2% Resorcinol Solution
Add 2 g of resorcinol to a 100 mL volumetric flask dilute to line with DI water. Mix until crystals are dissolved.

Sample Analysis

This test should be run under subdued light and then put under black plastic during the one hour testing time.

1. Set up as many 25 mL volumetric flasks as needed. Pipet 5 mL of sample or calibration standard into vial.

   Include saltwater blank (15ppt saltwater) and 5 calibration standards for total of 6 additional samples (0, 1, 2, 4, 8, and 10 NO_3^--N/L)

3. Add 0.6 ml of the resorcinol solution (Solution #4) to each vial; swirl flask to mix

4. Add 5 ml of concentrated sulfuric acid (Solution #3) to each vial; swirl flask to mix.

5. Cover flasks with Parafilm (squares can be cut from the roll then cut into four pieces).

6. Let react for 30 minutes in the dark. (You should place samples in a hood and then cover with a dark lab coat or plastic bag)

7. Place flasks in water bath for 5 minutes to bring to room temperature.

8. Make up volume on flasks to the line with DI water.

7. Measurement: Read absorbance using Hach DR 2800 Spectrophotometer at 505 nm. (Samples should be poured from volumetric flasks into curvettes)

A.4 Chemical Oxygen Demand (COD) Method

Solution Preparation

Solution #1: KHP Stock/Standard Solution (1.0 mL = 1 mg COD or 1000 mg COD/L)
Dissolve 0.850 g of dried Potassium hydrogen phthalate (KHP) into 15 ppt salt water and dilute to 1 liter in a volumetric flask. Mix.

Solution #2: KHP Calibration Curve
The calibration curve is determined using dilutions made from the KHP Stock/Standard Solution (Solution #1). Aliquots of this solution are added to different 50 ml volumetric flasks and then diluted to 50 ml with saltwater. The following samples are generated for use in the calibration process.

mg COD/L	mL solution #1
25	1.25
50	2.5
75	3.75
100	5
150	7.5

Sample Analysis

Follow steps outlined in Hach Method 8000. See summarized steps below.
***Note: All steps except reading absorbance should be performed in a hood.**

1. Digestion: Turn on hot block, set temperature to 150°C.
2. Pipet 2 mL of sample or calibration standard into vial.

   Include saltwater blank (15ppt saltwater) and 5 calibration standards for total of 6 additional samples (0, 25, 50, 75, 100, and 150 mg COD/L)
3. Invert gently several times to mix.
4. Heat vials for 2 hours.
5. Invert vials several times while still warm, then wait until vials have cooled to room temperature.
6. Measurement: Read absorbance using Hach DR 2800 Spectrophotometer at 420 nm.

A.5 Total Nitrogen Method

Solution Preparation

Solution #1: Ammonia-N Stock Solution (1.0 mL = 0.1 mg NH_3-N or 100 mg TN/L)
Dissolve 0.3819 g of dried Ammonium Chloride (NH_4Cl) into 15 ppt salt water and dilute to 1 liter in a volumetric flask. Mix.

Solution #2: TN Calibration Curve
The calibration curve is determined using dilutions made from the Ammonia-N Standard Solution (Solution #1). Aliquots of this solution are added to different 50 ml volumetric flasks and then diluted to 50 ml with saltwater. The following samples are generated for use in the calibration process.

mg TN/L	mL solution #2
5	2.5
10	5.0
15	7.5
20	10.0
25	12.5

Sample Analysis

Follow steps outlined in Hach Method 10071. See summarized steps below.

1. Digestion: Heat hot block to 100°C. Add Total Nitrogen Persulfate Power Pack to number of required vials (Total Nitrogen Hydroxide Reagent).
2. Pipet 2 mL of sample or calibration standard into vial.

   Include saltwater blank (15ppt saltwater) and 5 calibration standards for total of 6 additional samples (0, 5, 10, 15, 20, and 25 mg TN/L)
3. Cap vials and shake vigorously for more than 30 seconds
4. Heat vials for 30 minutes.
5. Remove vials immediately after 30 minutes.
6. Remove caps of digestion vials (Total Nitrogen Hydroxide Reagent vials) and add one Reagent A Powder Pack to each vial. Cap tubes and shake vigorously for 15 seconds. Wait 3 minutes.
7. Remove caps of digestion vials (Total Nitrogen Hydroxide Reagent vials) and add one Reagent B Powder Pack to each vial. Cap tubes and shake vigorously for 15 seconds. Wait 2 minutes.
8. Remove caps and extract 2 mL of digested sample (Total Nitrogen Hydroxide Reagent vials) and add to H/L TN Acid vial.

9. Important: Cap and invert 10 times slowly.

10. Wait 5 minutes.

11. Measurement: Read absorbance using Hach DR 2800 Spectrophotometer at 410 nm.

A.6 Total Phosphorus Method

Solution Preparation

Solution #1: Phosphate-P Stock Solution (1.0 mL = 1 mg PO_4^{3-}-P or 1000 mg TP/L)
Dissolve 4.3871 g Potassium Phosphate Monobasic KH_2PO_4 into 15 ppt salt water and dilute to 1 liter in a volumetric flask. Mix.

Solution #2: Phosphate-P Standard Solution (1.0 mL = 0.01 mg PO_4^{3-}-P or 10 mg TP/L)
Add 1 mL of the Phophate-P Stock Solution (Solution #1) to a 1 L volumetric flask. Dilute to 1 L with 15 ppt saltwater. Mix. This is the solution used for the calibration curve.

Solution #3: TP Calibration Curve
The calibration curve is determined using dilutions made from the Phosphate-P Standard Solution (Solution #2). Aliquots of this solution are added to different 50 ml volumetric flasks and then diluted to 50 ml with saltwater. The following samples are generated for use in the calibration process.

mg TP/L	mL solution #2
0.25	1.25
0.5	2.5
1	5
1.5	7.5
2.0	10

Sample Analysis

Follow steps outlined in Hach Method 8000. See summarized steps below.

1. Digestion: Turn on Hot Bloc, set temperature to 100°C.

2. Pipet 5 mL of sample or calibration standard into vial.

   Include saltwater blank (15ppt saltwater) and 5 calibration standards for total of 6 additional samples (0, 0.25, 0.50, 1.0, 1.5, and 2.0 mg TP/L)

3. Add one Potassium Persulfate powder pack to each vial. Cap and shake to mix.

4. Heat vials for 30 minutes. Remove from Hot Bloc and cool to room temperature.

5. Add 2 mL of 1.54 N Sodium Hydroxide solution to each vial. Cap and mix.

Note: Perform steps 6-8 in batches. After addition of Phosphate Reagent powder pack samples must be read in 2-8 minutes. Completing in batches of 6-8 vials ensures samples are read in the appropriate time frame.

6. Add Phosphate Reagent pack to vials. Shake for 10-15 seconds.

7. Wait 2 minutes, but no more than 8 minutes.

8. Measurement: Read absorbance using Hach DR 2800 Spectrophotometer at 890 nm.

A.7 Modified TN/TP Protocol for the Digestion of Plant and Soil Samples

Method modified from:

Standard methods for the examination of water and wastewater. 20th Edition. Prepared and Published jointly by American Public Health Association, American Water Works Association, Water Environment Federation. 1998. Franson, M.A.H. managing editor. Persulfate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorous and Ascorbic Acid Method for Phosphorous Determination. APHA.

C.L. Langner, P.F. Hendrix, Evaluation of a persulfate digestion method for particulate nitrogen and phosphorus, Water Research, Volume 16, Issue 10, 1982, Pages 1451-1454.

Reagents:

1. Sodium hydroxide 3N	• Dissolve 120g NaOH in 800mL DI water in a 1000mL volumetric flask. Cool and dilute to volume
2. Oxidizing Reagent	• 64g potassium persulfate, $K_2S_2O_8$, in 500mL DI water, warm to dissolve • Add 80mL of 3N NaOH and dilute to 1000mL • Store in a brown bottle at room temperature
3. Dilute H_2SO_4	• Dilute 300mL concentrated sulfuric acid into 1000mL total with DI water

4. Phenolphthalein Indicator	• 1g per 100mL ethanol
5. Sodium hydroxide 2N	• Dissolve 8g in 100mL total volume DI water
6. H_2SO_4 ~ 1N	• Dilute 10 mL of solution number 3 (dilute H_2SO_4) to 100 mL
7. Phosphate Reagent: 100mL total, stable for 4 hours, mix in exact order as listed. (All reagents can be kept at room temperature except for Ascorbic Acid which should be stored at 4ºC)	
• 5N sulfuric acid (70mL conc. brought to 500mL using DI water) • Potassium Antimony Tartrate (0.2743g/100mL water) • Ammonium molybdate·$4H_2O$ (4g/100mL) • Ascorbic Acid (1.76g/100mL) stable one week	50 mL 5 mL 15 mL 30 mL 100mL

Procedure (*for 50 mL prep)*:

1. Place dry weight sample in clean, acid washed (soaked in 10% HCl or HNO_3 solution for at least 15 minutes) 125 mL digestion vials. Weigh between 10 and 15 mg of sample directly into vial.
2. Prepare standards. Weigh or pipette standards from 50 mg/L P standard solution. I typically use 0, 0.12, 0.25, 0.50, and 1 mL of solution. As necessary add DI water to standards to achieve final volume of 1 mL (e.g. add 0.88 mL DI to 0.12 mL standard). To avoid dilution use a small acid washed beaker, rinsed with DI, then pour a small amount of standard into the beaker swirl and discard. Then pour a small amount into the beaker and use that to measure out the standards.
3. Prepare a NIST reference material to run with sample. Weight approximately a similar quantity to samples, about 10-15 mg of reference material. Apple leaves (NIST #1515 0.159%P) are what I tend to use. All reference materials should be pre-dried according to NIST instructions (Drying in a desiccator at room temperature for 120 hours over fresh anhydrous magnesium perchlorate, depth should not exceed 1 cm. Note: avoid oven trying at elevated temperatures this could result in weight losses).
4. Add 1 mL of DI to all samples and NIST reference standard.
5. Add 9 mL of Oxidizing Reagent to all vials.
6. Heat on Environmental Express 100 mL Hot Block at 120ºC for 60 minutes. Cover vials with disposable digestion watch glasses and monitor to be sure all of the sample does not evaporate. After digestion crystalline solid should have formed in the vial and the solution/solid should be colorless or nearly so.
7. Cool to room temperature. At this point you may cap vials and continue the next day if desired.
8. Add approximately 10 mL of DI water to each vial.

9. Add a drop of phenolphthalein indicator.
10. If necessary titrate to a faint pink color with 2N NaOH. When digesting with the watch glasses I found that if there is very little or no liquid remaining adding NaOH is not necessary.
11. Add dilute sulfuric acid (conc. ~1N) until color just clears. This typically takes 1 to 2 drops.
12. Turn on spectrophotometer, allow it to warm up.
13. Add 8 mL of Phosphate reagent.
14. Add DI water to bring to 50 mL total volume. Mix vials carefully swirling by hand.
15. Allow color to develop for 15-30 minutes and solids to settle.
16. Read absorbance at 880nm. I pipette about 3-4 mL into a 10 mL round sample tube.

Appendix B: Supplementary Material for Life Cycle Inventory of Aquaculture Feeds

The following tables were used to create feed processes in SimaPro for the aquaculture feeds used in Chapter 5 and 6. For more detailed equations contact Suzie Boxman at boxmansuz@gmail.com.

Table B.1: Comparison of feed ingredients from two LCA studies and the two theoretical feeds used in this study.

Feed ingredients	Mungkung et al. (2013)	Pelletier and Tydemers (2010)	32% protein fish feed[1]	45% protein fish feed[2]
Soybean meal	22-30	50	35%	25%
Wheat middlings		32	15%	15%
Maize/corn	10		15%	24%
Fish meal	8-17	3	5%	36%
Poultry by-product meal				
Calcium carbonate		2.5	*	
Corn gluten		3	*	
Palm oil		2		
Fish oil	2-5	2		
Rice meal	22-30			
Soy lecithin		1		
Wheat bran	11-30			
Corn distiller dried grains		4		

[1]The percentage of ingredients was determined from the ingredient list on Purina Mills® Aquamax Pond Fish 2000
[2] The percentage of ingredients was determined with the Pearson Square Method to calculate animal feeds (Wagner and Stanton, 2012).

Table B.2: Inventory data for the processing of 1 kg of soybeans (source: Pelltier, 2004).

Description	Input	Unit	Amount
Industrial Energy for Processing	Electricity	kJ	244.8
	Natural gas (for steam production)	kJ	812.0
Outputs			
Processed Product	Soy Meal	g	812.0
	Soy Oil	g	188.0

Table B.3: Inventory data for the processing of 1 kg of wheat.

Description	Input	Unit	Amount
Industrial Energy for Processing	Electricity	kJ	306[3]-418[1]
Outputs			
Processed Product[2]	Flour	g	750
	Wheat middlings	g	250

[1]Source: Pelltier, 2004

[2]Division of outputs based on Blasi et al. (1998)

[3]Calculated based on assumptions that: 0.059 kWh/800 g loaf (Espinoza-Orias et al. 2011); 473 g flour/loaf (Espinoza-Orias et al. 2011); 0.45kg flour/0.66 kg wheat (National Association of Wheat Growers, 2015)

Table B.4: Inventory data for the wet milling of 1 kg of corn (source: Galitsky et al., 2003).

Description	Input	Unit	Amount	Allocation percent
Industrial Energy for Processing	Electricity	kJ	450	
	Fuel	kJ	2340	
	Natural gas (for steam production)	kJ	1084	
Outputs				
Processed Product	Corn starch	g	571	68%
	Corn gluten meal	g	44	5%
	Corn gluten feed	g	196	23%
	Corn oil	g	31	4%

Table B.5: Calculations for allocation of wet milling outputs (source: Galitsky et al., 2003).

	lb/bushel	kg/bushel	Allocation percent
Corn starch	32	14.5	68%
Corn gluten meal	2.5	1.13	5%
Corn gluten feed	11	4.99	23%
Corn oil	1.75	0.797	4%
	Sum	21.4	

Appendix C: Supplementary Material for Nutrient Budget Calculations

For more detailed equations contact Suzie Boxman at boxmansuz@gmail.com.

C.1 Nutrient Breakdown of Fish Feed

The amount of nitrogen in fish feed varies depending on the protein content. According to Brunty et al. (1997) protein is about 16% nitrogen. On average the total amount of nitrogen in fish feed is reported to vary from 6.5% (Nash, 2001) to 7.7% wet weight (Bromley and Smart, 1981). Of the total amount of nitrogen in feed, typically 25-30% is considered to be incorporated into fish biomass (McCarthy, 2013). Dissolved nitrogen ranges from 37-72% and particulate nitrogen ranges from 3.6-35% (Piedrahita, 2003). Feed waste also contributes to particulate nitrogen. The amount of feed waste varies from 3-20% (Reid et al., 2009) depending on feeding technique and species.

Table C.1: Breakdown of nitrogen species from feed.

Nitrogen Species	Percentage dry weight
Feed total nitrogen	6.5%
Of nitrogen in feed	
Dissolved N	54%
Biomass	30%
Particulate N	
Feces	10%
Feed waste	6%

Phosphorus can be present in fish feed in different forms depending on the ingredients. In fishmeal the phosphorous typically comes from bone. Feeds high in fish meal can contribute to excessive concentrations of phosphorus in the feed (Satoh et al., 2003). Plant components supply phosphorus in the form of phytic acid which is not as easily digestible by fish (Riche and Brown, 1996). Phosphorus typically comprises 1-2% of feed (Foy and Rosell, 1991) but can

contain as much as 5% (Cho and Bureau, 2001). Fish retain 17-40% of phosphorus in feed (Piedrahita, 2003). Waste particulate phosphorus ranges from 30-84% of phosphorus in waste feed and feces; waste dissolved phosphorus ranges from 26-70% (Cripps and Bergheim, 2000).

Table C.2: Breakdown of phosphorus species from feed.

Phosphorus Species	Percentage dry weight
Feed total phosphorus	1.0%
Of phosphorus in feed	
Dissolved P	30%
Biomass	30%
Particulate P	
Feces	25%
Feed waste	15%

C.2 Water Treatment: Avoided Water and Nutrient Discharges

Nitrogen enters aquaculture systems in the form of feed. The majority of nitrogen excreted from fish is in the form of dissolved ammonia and urea and a small portion is lost as feces. The feces are captured through various solids removal mechanisms and will be addressed later. In a RAS, a biofilter oxidizes total ammonia nitrogen (TAN) to nitrate. To prevent nitrate accumulation in a RAS, a percentage of the system water must be discharged. In aquaponics this discharge is avoided due to the water treatment provided by plants in aquaponics. The percentage of water discharged was based on the assumption that maintenance of a stable nitrate concentration requires all of the nitrogen added daily from feed to be removed daily. Therefore the percent of system water discharged was calculated as a ratio of mass of nitrogen added daily to total mass of nitrogen present in system water. In this study, the amount of nitrogen was assumed to be equal to dissolved nitrogen excreted (3.5%) and that all of particulate nitrogen from waste feed and feces were removed through solids capture.

$$\frac{Feed\ \left(\frac{g}{d}\right) x\ \frac{0.035\ (g\ dissolved\ N)}{(g\ feed)}}{[C_{NO_3^-}\left(\frac{mg}{L}\right) x\ V_{system}(L)\ x\ \frac{1\ g}{1000\ mg}] + [Feed\ (\frac{g}{d}) x\ \frac{0.035\ (g\ dissolved\ N)}{(g\ feed)}]}\ x\ 100 = \%\ discharge \quad \text{Eq. C1}$$

Table C.3: Information used to determine percent of system volume discharged to maintain stable nitrate concentrations.

System	Feed input (kg/d)	Dissolved N input (kg/d)	Total system volume (L)	Average NO_3^- concentration (mg/L)	Mass of N (kg)	% discharge
Commercial (Chapter 5)	19.3	0.676	111,196	40.0	5.12	13%
Residential (Chapter 5)	0.190	0.007	0.865	40.0	34.6	0.02%
Baseline (Chapter 6)	3.10	0.109	50,000	25.6	1.39	8%

The quantity of nutrient discharges are similarly based on the amount water discharged to maintain a stable nitrate concentration. The quantity of nitrogen discharged corresponds with the mass of dissolved nitrogen entering the system daily with feed. The quantity of phosphorus discharged was similarly based on the amount of dissolved phosphorus that entered the system daily through feed. Of the 1% of phosphorus in feed, assuming that all particulate phosphorous is removed, 30% of the phosphorus in feed would be in the form of dissolved phosphorus.

Table C.4: Amount of dissolved nitrogen and phosphorus discharged avoided by having plant growth.

System	Feed Input (kg/day)	Dissolved N (kg/day)	N discharged (kg/year)	Dissolved P (kg/day)	P discharged (kg/year)
Commercial (Chapter 5)	19.3	0.676	247	0.058	21.1
Residential (Chapter 5)	0.190	0.009	0.00	0.0006	0.00
Baseline (Chapter 6)	3.10	0.109	39.6	0.009	3.39

In the residential-scale system, due to the small quantity of discharge required to maintain nitrate concentrations it was assumed that water exchanges were not required therefore no dissolved nitrogen or phosphorus discharge was avoided.

C.3 Recovered Solids: Avoided Solid Discharge

As mentioned previously, a portion of the nitrogen and phosphorus entering the system was in the form of wasted feed and feces. These particulate wastes are typically removed by sedimentation or filtration. In freshwater aquaculture, captured solids can be used as an agricultural amendment and are considered a secondary product. The nitrogen and phosphorous associated with the solids were assumed to replace an equivalent amount of commercially produced synthetic fertilizer. Assuming 100% of wasted solids were captured the particulate nitrogen and phosphorus percentages given in Tables C1 and C2 were used to calculate the quantity of nitrogen and phosphorus fertilizer avoided. No fertilizer was avoided in the systems described in Chapter 6 due to the salt content of the solids.

Table C.5: Quantity of nitrogen and phosphorus present in solid fish waste replaced by an equivalent amount of fertilizer.

System	Feed Input (kg/day)	Particulate N (kg/day)	N Fertilizer (kg/year)	Particulate P (kg/day)	P Fertilizer (kg/year)
Commercial (Chapter 5)	19.3	0.201	73.4	0.077	28.2
Residential (Chapter 5)	0.190	0.002	0.721	0.0008	0.277

C.4 Water Treatment: Avoided Energy for Biofilter

The TAN that enters aquaculture systems must be removed immediately to prevent fish mortalities. In RAS, biofilters are used to oxidize TAN to nitrate. Several types of biofilters can be used in RAS including moving bed bioreactors (MBBR), fluidized-bed biofilters, and trickling biofilters (Ebeling and Timmons, 2012). In this study, a MBBR was selected as the

mechanism for TAN removal. Due to the presence of plants, any associated impacts from using a MBBR to oxidize the TAN were considered avoided in an aquaponic system, therefore the impacts from an MBBR were considered a credit to operating an aquaponic system over a conventional RAS.

Commercial MBBRs are sized based on the amount of TAN entering the system from feed and surface area of media required to support growth of nitrifying microorganisms. Aeration is added to provide oxygen for the microorganisms and to provide constant mixing of the media, which eliminates the need for backwashing and removes excess biofilm growth (Michaels, 2015). The quantity of aeration required to provide constant mixing was based on an industry ratio of 142 lpm/m^3 media volume (Michaels, 2015). The smallest air blower in the Pentair Aquatic Eco-Systems® 2015 catalogue which provided the required air flow was used to determine electricity requirements. If appropriate the electricity for the air blower was split between MBBR requirements other system components such as the fish tanks and hydroponic plant bed.

Table C.6: Sizing information for biofilter and electricity required for aeration.

System	Maximum feed rate (kg/d)	TAN (kg/d)	Media volume[2] (m^3)	Air required (lpm)	Air Blower Model	Electricity required (kWh/y)
Commercial (Chapter 5)	35.9	1.1	6.3	895	S31	3592
Residential (Chapter 5)	0.346	0.01	0.05	7.1	SL14	78.8
Baseline[1] (Chapter 6)	16.2	0.57	2.9	404	S313	898
Scenario 1[2] (Chapter 6)	16.2	0.57	2.9	361	S313	2694
Scenario 2[3] (Chapter 6)	16.2	0.57	2.9	361	S313	2694

[1]25% additional aeration required, where actual biofilter already used 50% of energy from S313
[2]75% of aeration was used for biofilter, 25% used for fish tanks, avoided
[3]75% of aeration was used for biofilter, 25% used for fish tanks, not avoided

In the Baseline scenario presented in Chapter 6 a MBBR was already present. Due to the presence of greater fish densities than the biofilter was initially sized for, a larger biofilter would have been needed. The difference between the biofilter actually present and the theoretical biofilter needed was used to calculate the electricity requirements. In Scenario 1 the electricity from the biofilter was avoided in its entirety due to full plant production. In Scenario 2 the electricity from the biofilter was not avoided and aeration was required for operation of the biofilter.

C.5 Plant Production: Avoided Fertilizer

In Chapter 5 basil was considered the plant product co-produced. The amount of fertilizer needed for basil grown in soil conditions was estimated based on data from Palada et al. (2008). The study looked at basil grown at the University of the Virgin Islands on St. Croix. The plants were fertilized with 100 kg N/ha, 50 kg P/ha, and 40 kg K/ha and used 2823 m^3/ha of irrigation water. It was assumed that three harvests of basil per year occurred. Based on the average plant fresh weight production of the two years studied about 31,000 kg/ha fresh weight basil can be produced per harvest or about 93,300 kg/ha/year.

In Chapter 6 spinach was considered the plant product co-produced. The fertilizer and water requirements for spinach production were based on information provided by the University of California, Vegetable Research & Information Center on spinach production in California (Koike et al., 2011). The plants were fertilized with 84 kg N/ha, 100 kg P/ha, and 300 kg K/ha and used 6165 m^3/ha of irrigation water. It was assumed that three harvests of spinach per year occurred.

Greater yields were produced in the aquaponic systems, therefore the quantity of avoided fertilizer was determined based on agricultural area required to produce an equivalent quantity of

plant product. For example, the commercial-scale aquaponic system described in Chapter 5, produces about 50 kg/m^2/year and the agricultural system produces about 9.3 kg/m^2/year. The agricultural system requires about 5.4 times more area to produce the same yields of basil. This ratio was used to estimate the amount of fertilizer and irrigation water avoided to produce equivalent yield of basil.

Table C.7: Plant yields in aquaponic and agricultural systems considered and the area required to produce equivalent yields in the agricultural system.

System	Aquaponic plant yields (kg/m^2/yr)	Agricultural system plant yields (kg/m^2/yr)	Area required to produce equivalent basil yields in the agricultural system
Commercial (Chapter 5)	50	9.3	5.4
Residential (Chapter 5)	19	9.3	2.0
Baseline (Chapter 6)	12	8.1	1.5
Scenario 1 (Chapter 6)	12	8.1	1.5

Appendix D: List of Acronyms

AD	Abiotic Depletion
ANOVA	Analysis of Variance
AP	Acidification Potential
BWD	Body weight/day
C/N	Carbon Nitrogen Ratio
CC	Climate Change
CED	Cumulative Energy Demand
CML	Center for Environmental Studies, University of Leiden
CNP	Coconut fiber/no plants
COD	Chemical Oxygen Demand
CP	Coconut fiber/plants
DO	Dissolved Oxygen
DW	Dry Weight
ENP	Expanded clay/no plants
EP	Eutrophication Potential
EP	Expanded clay/plants
EU	Energy Use
FCR	Feed Conversion Ratio
FEU	Fossil Energy Use
FSD	Flow Species Density
FU	Functional Unit
FW	Fresh Weight
FWS	Free Water Surface
GWP	Global Warming Potential
HLR	Hydraulic Loading Rate
HPH	High flow/sea purslane/high density
HPL	High flow/sea purslane/low density
HSH	High flow/saltwort/high density
HSL	High flow/saltwort/low density
HTP	Human Toxicity Potential
IMTA	Integrated Multi-trophic Aquaculture
LC	Land Competition
LCA	Life Cycle Assessment
LECA	Light Expanded Clay Aggregate
LO	Land Occupation
LPH	Low flow/sea purslane/high density

LPL	Low flow/sea purslane/low density
LSH	Low flow/saltwort/high density
LSL	Low flow/saltwort/low density
LU	Land Use
MAP	Mote Aquaculture Research Park
MBBR	Moving Bed Bioreactor
MDL	Method Detection Limit
MIB	2-methylisoborneol
MTP	Marine Toxicity Potential
N	Nitrogen
NFT	Nutrient Film Technique
NPP	Net Primary Production
NPPU	Net Primary Production Use
NREU	Non Renewable Energy Use
P	Phosphorus
RAS	Recirculating Aquaculture System
RGR	Relative Growth Rate
SD	Standard Deviation
SF	Surface Flow
SSF	Subsurface Flow
SU	Surface Use
TAN	Total Ammonia Nitrogen
TCED	Total Cumulative Energy Demand
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
USF	University of South Florida
UVI	University of the Virgin Islands
VSS	Volatile Suspended Solids
WD	Water Dependence
WU	Water Use

Appendix E: List of Symbols

C_e	Concentration of Effluent (mg/L)
C_i	Concentration of Influent (mg/L)
$N_{mass_{t_j}}$	Mass Nitrogen Day j (g)
$N_{mass_{t_i}}$	Mass Nitrogen on Day i (g)
$P_{mass_{t_j}}$	Mass Phosphorus Day j (g)
$P_{mass_{t_i}}$	Mass Phosphorus on Day i (g)
$N_{removed_{t_{j-i}}}$	Mass Nitrogen Removed Between Day j and i (g)
M_{feed}	Mass of Feed (g)
N_{added}	Mass of Nitrogen Added (g)
$N_{added_{t_j-i}}$	Mass of Nitrogen Added Between Day j and i (g)
PN_{t_i}	Mass of Nitrogen in Plant Biomass on Day i (g)
PN_{t_j}	Mass of Nitrogen in Plant Biomass on Day j (g)
$N_{other_{t_{j-i}}}$	Mass of Nitrogen Removed by Other Between Day j and i (g)
$N_{plant\ uptake_{t_{j-i}}}$	Mass of Nitrogen Removed by Plants Between Day j and i (g)
$N_{sand\ filter_{t_{j-i}}}$	Mass of Nitrogen Removed in Sand Filter Between Day j and i (g)
P_{added}	Mass of Phosphorus Added (g)
$P_{added_{t_j-i}}$	Mass of Phosphorus Added between Day j and i (g)
PP_{t_i}	Mass of Phosphorus in Plant Biomass on Day i (g)
PP_{t_j}	Mass of Phosphorus in Plant Biomass on Day j (g)
$P_{other_{t_{j-i}}}$	Mass of Phosphorus Removed by Other Between Day j and i (g)
$P_{plant\ uptake_{t_{j-i}}}$	Mass of Phosphorus Removed by Plants Between Day j and i (g)
$P_{removed_{t_{j-i}}}$	Mass Phosphorus Removed Between Day j and i (g)
NO_3^-	Nitrate
NO_2^-	Nitrite
$V_{\frac{backwash}{day}}$	Volume of System water Treated Daily in Sand Filter (L)
CN_{t_i}	TN Concentration on Day i (g)
CN_{t_j}	TN Concentration on Day j (g)
$N_{total_{t_j-i}}$	Total Mass of Nitrogen in System Between Day j and i (g)
$P_{total_{t_j-i}}$	Total Mass of Phosphorus in System Between Day j and i (g)
V_{system}	Total System Volume (L)
V_{system}	Total System Volume (L)

CP_{t_i}	TP Concentration on Day i (mg/L)
CP_{t_j}	TP Concentration on Day j (mg/L)

Appendix F: Additional Water Quality Data

Table F.1: Nitrate (mg/L NO_3^--N) water quality data for sample points 2, 3, and 4.

Date	2		3		4	
	Mean	SD	Mean	SD	Mean	SD
10/6/2014	1.8	0.1	1.8	0.1	2.1	0.1
10/9/2014	5.3	0.4	7.4	1.4	6.9	1.3
10/13/2014	4.6	0.2	4.5	0.2	4.7	0.3
10/16/2014	4.7	0.1	4.3	0.4	4.4	0.6
10/20/2014	15.3	1.3	15.5	0.4	15.3	0.8
10/23/2014	19.3	0.7	18.9	0.9	20.4	0.5
10/27/2014	10.7	5.8	13.8	1.2	16.6	2.5
10/30/2014	31.4	4.7	34.3	1.2	35.7	1.0
11/3/2014	29.8	0.6	30.6	1.1	30.6	0.5
11/6/2014	35.6	2.6	36.1	2.2	36.8	1.5
11/10/2014	33.9	2.6	34.5	1.8	36.0	1.1
11/13/2014	56.4	4.3	67.0	4.8	39.6	2.6
11/17/2014	35.0	3.1	41.6	8.5	38.9	5.6
11/20/2014	52.8	0.6	49.3	1.5	46.0	2.8
11/24/2014	54.2	4.3	52.0	5.0	44.6	6.2
11/26/2014	61.7	1.8	51.6	3.3	53.7	9.1
12/1/2014	67.2	0.5	63.9	3.9	59.9	0.1
12/4/2014	59.9	3.6	58.9	5.4	62.8	3.1
12/8/2014	56.9	6.4	62.8	4.2	61.8	2.1
12/11/2014	65.0	2.9	64.9	2.5	70.3	0.4
12/15/2014	66.8	5.2	72.7	4.3	72.7	2.0
12/18/2014	80.3	5.5	76.8	6.2	77.4	6.5
12/23/2014	85.8	5.3	79.9	12.3	79.2	10.0
1/26/2015	124.7	2.9	119.0	7.8	120.2	5.7
2/9/2015	89.9	1.2	81.2	4.5	84.2	6.0
2/25/2015	96.9	0.6	93.2	3.5	88.0	9.8
3/9/2015	89.7	7.0	78.7	6.4	78.1	3.6
3/23/2015	74.6	2.1	79.1	2.3	76.2	4.0
4/6/2015	53.8	2.9	55.2	8.0	50.5	7.0
5/4/2015	11.4	1.0	12.4	0.8	9.8	0.6
6/1/2015	22.5	0.5	20.6	1.8	21.1	1.8
6/29/2015	47.3	5.8	43.7	5.8	49.5	3.7

Table F.2: Nitrate (mg/L NO_3^--N) water quality data for sample points 5 and 6.

Date	5		6	
	Mean	SD	Mean	SD
10/6/2014	0.9	0.0	0.9	0.0
10/9/2014	0.9	0.0	0.9	0.1
10/13/2014	1.8	0.2	2.7	0.1
10/16/2014	0.7	0.5	1.3	0.7
10/20/2014	3.4	0.2	2.3	0.1
10/23/2014	8.1	1.2	10.9	0.5
10/27/2014	0.9	0.0	5.5	0.4
10/30/2014	7.6	1.6	4.3	1.4
11/3/2014	3.7	1.8	17.0	1.7
11/6/2014	0.4	0.0	13.6	3.9
11/10/2014	1.0	0.9	20.8	0.9
11/13/2014	13.1	2.1	42.1	20.8
11/17/2014	28.8	1.7	28.7	3.1
11/20/2014	26.7	2.1	34.1	2.4
11/24/2014	24.1	1.5	22.3	3.1
11/26/2014	31.3	3.6	33.1	2.4
12/1/2014	13.0	8.2	50.6	1.4
12/4/2014	48.0	2.9	39.6	26.9
12/8/2014	39.9	3.5	55.8	0.5
12/11/2014	45.2	3.9	60.4	3.0
12/15/2014	36.0	11.1	59.3	9.4
12/18/2014	56.1	19.6	69.0	7.1
12/23/2014	87.0	26.5	73.7	5.9
1/26/2015	43.9	10.3	107.5	30.3
2/9/2015	21.5	0.0	54.2	16.6
2/25/2015	25.8	6.9	80.6	1.6
3/9/2015	20.3	8.7	37.0	5.6
3/23/2015	21.7	9.3	44.0	3.1
4/6/2015	12.7	4.6	3.3	4.6
5/4/2015	0.4	0.0	0.5	0.1
6/1/2015	0.4	0.0	0.4	0.0
6/29/2015	15.6	1.9	9.8	1.4

Table F.3: Total nitrogen (mg/L TN) water quality data for sample points 2-6.

Date	2		3		4		5		6	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10/6/2014	6.9	0.8	8.6	2.2	4.3	1.6	8.4	4.9	2.7	0.0
10/13/2014	16.0	2.9	13.4	0.0	13.4	0.0	17.9	6.0	13.4	0.0
10/16/2014	31.5	16.1	29.5	13.9	46.5	20.6	34.9	15.2	15.0	2.2
10/20/2014	22.7	9.7	19.5	4.0	20.1	3.1	24.7	7.4	13.8	0.6
10/27/2014	13.4	0.0	13.4	0.0	13.7	0.5	28.5	6.6	13.4	0.0
11/3/2014	26.3	0.0	26.5	3.8	23.3	8.6	35.7	15.1	17.4	2.6
11/10/2014	37.1	1.3	41.5	3.5	37.3	1.3	44.1	12.8	26.6	0.6
11/17/2014	45.8	3.8	47.2	1.0	49.9	2.0	47.5	5.2	39.7	2.1
11/24/2014	59.6	6.5	67.7	4.7	66.6	6.7	67.6	4.7	46.6	4.7
12/1/2014	82.8	2.6	77.5	2.6	75.5	4.6	59.7	12.0	70.3	3.7
12/8/2014	67.3	1.9	66.5	0.8	67.5	0.5	58.3	5.5	59.9	1.7
12/15/2014	70.6	3.6	73.6	3.3	71.8	3.8	68.4	5.0	62.8	0.4
12/22/2014	88.0	9.4	95.5	3.8	92.8	4.8	82.5	9.4	81.2	6.7
1/26/2015	101.2	0.7	94.5	4.6	102.7	8.3	168	74.6	97.4	10.2
2/9/2015	100.9	7.6	108.9	9.5	114.8	2.4	445	177.1	109.9	2.4
2/25/2015	98.5	3.4	98.7	2.5	99.7	5.2	529	207.0	105.4	3.9
3/9/2015	91.4	1.0	88.4	3.2	92.3	3.0	311	52.8	113.1	2.2
3/23/2015	71.5	7.0	69.7	0.7	81.2	2.9	361	100.7	100.3	1.0
4/6/2015	61.4	2.8	57.1	1.7	65.0	4.8	225	37.1	95.5	3.2
5/4/2015	24.1	0.5	24.4	1.4	24.7	1.7	676	81.7	24.9	2.1
6/1/2015	32.6	0.8	34.2	2.7	34.7	0.8	236	53.9	78.5	2.4
6/29/2015	57.6	2.7	57.2	7.5	52.8	4.0	89.3	30.7	37.5	2.1

Table F.4: Total phosphorus (mg/L TP) water quality data for sample points 2-6.

Date	2		3		4		5		6	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10/6/2014	4.76	0.95	4.06	0.61	4.76	0.22	4.54	1.06	1.90	0.44
10/13/2014	2.96	0.26	5.66	1.43	3.60	0.65	5.95	2.93	1.65	0.00
10/20/2014	4.70	0.41	5.85	0.60	5.26	0.66	9.27	0.64	3.25	1.39
10/27/2014	5.21	0.54	6.83	0.84	5.78	0.69	11.77	1.84	5.07	0.47
11/3/2014	7.12	0.57	8.15	0.22	7.44	1.03	17.97	5.06	9.38	1.20
11/10/2014	6.70	0.80	6.94	0.72	6.77	0.84	20.67	6.16	7.78	1.43
11/17/2014	10.23	0.37	10.51	0.24	10.40	0.08	12.40	0.36	10.73	0.46
11/24/2014	10.41	0.16	10.62	0.03	10.31	0.08	15.97	1.74	10.11	0.35
12/1/2014	10.39	1.36	13.71	1.04	10.75	0.69	17.60	4.25	8.41	0.66
12/8/2014	8.76	0.82	11.46	1.43	8.99	1.01	12.21	1.41	7.35	1.02
12/15/2014	11.02	0.22	12.55	0.60	11.30	0.68	18.47	1.28	10.89	0.24
12/22/2014	12.92	1.83	13.15	0.89	10.56	0.17	13.61	1.27	9.41	0.35
1/26/2015	7.05	3.41	7.16	3.39	8.31	2.01	3.94	1.12	8.38	0.79
2/9/2015	16.57	1.59	12.99	2.59	15.76	1.45	19.36	3.00	11.22	0.49
2/25/2015	14.82	1.50	16.08	1.26	12.75	0.18	66.56	24.54	13.35	1.70
3/9/2015	17.44	0.47	12.89	1.80	16.60	2.64	19.16	4.61	12.42	0.90
3/23/2015	8.83	6.76	12.01	4.64	7.63	0.23	43.07	21.41	8.51	0.44
4/6/2015	14.47	0.50	19.67	4.10	14.88	2.17	107.4	56.36	15.99	1.82
5/4/2015	14.16	1.86	18.01	2.66	13.37	1.29	154.6	45.93	17.34	0.93
6/1/2015	18.83	6.31	16.24	6.06	11.32	0.17	78.33	33.65	15.33	2.51
6/29/2015	13.19	3.89	19.28	3.99	15.13	1.06	53.77	18.34	11.13	2.53

Table F.5: COD (mg/L COD) water quality data for sample points 2, 3, and 4.

Date	2		3		4	
	Mean	SD	Mean	SD	Mean	SD
10/6/2014	24.6	14.6	22.0	3.7	20.0	6.6
10/13/2014	48.1	16.0	32.7	3.6	19.1	3.9
10/16/2014	25.8	6.0	21.5	0.0	38.0	4.6
10/20/2014	29.8	1.7	20.2	9.2	44.4	3.4
11/3/2014	61.2	6.2	57.5	2.4	31.3	2.8
11/10/2014	19.1	4.4	19.6	3.6	11.5	9.3
11/17/2014	21.0	3.0	19.1	6.4	33.7	18.8
11/24/2014	39.2	11.1	39.2	16.7	68.4	24.2
12/8/2014	46.8	0.4	54.6	15.8	86.4	5.9
12/15/2014	43.0	5.1	44.6	21.9	33.7	3.3
12/22/2014	6.1	0.0	6.1	0.0	159.7	20.2
1/26/2015	24.7	6.4	24.7	6.6	268.0	6.5
2/9/2015	94.0	3.4	67.6	3.2	118.8	1.1
2/25/2015	244.6	9.7	82.2	5.7	133.5	17.7
3/9/2015	65.2	6.9	73.2	38.2	79.6	2.2
3/23/2015	133.1	6.1	127.0	9.4	16.6	2.6
4/6/2015	109.7	6.9	105.2	2.4	7.6	0.2
5/4/2015	116.2	9.7	118.6	2.1	14.9	2.2
6/1/2015	139.0	9.8	114.6	5.7	13.4	1.3
6/29/2015	82.9	10.9	75.3	4.5	11.3	0.2

Table F.6: COD (mg/L COD) water quality for sample points 5 and 6.

Date	5		6	
	Mean	SD	Mean	SD
10/6/2014	134.5	62.6	56.0	31.9
10/13/2014	89.6	51.7	30.6	0.0
10/16/2014	220.3	94.6	38.8	38.2
10/20/2014	325.8	5.8	63.1	3.1
11/3/2014	109.5	23.1	84.1	37.9
11/10/2014	489.9	196.9	67.6	4.8
11/17/2014	31.5	1.3	61.4	6.9
11/24/2014	196.2	118.3	66.9	8.7
12/8/2014	214.8	147.1	56.6	0.5
12/15/2014	426.9	244.4	153.4	43.3
12/22/2014	30.6	0.0	6.1	0.0
1/26/2015	1987.1	172.7	333.2	5.5
2/9/2015	4226.3	853.8	233.5	46.2
2/25/2015	7338.3	882.4	309.6	155.1
3/9/2015	1872.7	461.9	327.9	50.0
3/23/2015	9796.8	4186.8	440.4	121.9
4/6/2015	2916.0	662.9	297.8	98.2
5/4/2015	12247.6	1232.3	729.8	45.2
6/1/2015	1646.3	131.3	230.8	173.8
6/29/2015	869.4	255.3	59.2	31.2

Table F.7: TSS (mg/L) water quality for sample points 2-6.

Date	2		3		4		5		6	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10/6/2014	5.2	0.4	4.6	0.2	3.5	0.3	62.4	17.9	10.4	1.4
10/9/2014	4.2	3.7	3.3	1.4	3.6	0.8	82.6	3.2	7.0	0.6
10/13/2014	3.4	0.4	2.5	0.2	2.6	0.1	51.3	1.6	5.4	0.6
10/16/2014	2.7	2.8	4.0	1.0	2.9	0.1	10	12.7	32.6	4.9
10/20/2014	3.7	1.5	2.1	0.1	2.0	0.1	94.7	7.3	6.9	1.4
10/27/2014	3.3	0.2	2.6	0.3	1.9	0.2	316.5	106	14.4	1.7
11/3/2014	3.7	1.6	4.2	0.3	1.9	0.6	192.5	72.9	28.3	20.3
11/10/2014	2.1	0.1	2.4	0.2	1.8	0.1	194.3	132	14.0	1.9
11/17/2014	3.1	0.1	2.4	0.1	2.4	0.1	80.5	0.6	34.0	25.2
11/24/2014	2.9	0.6	3.0	0.9	2.3	0.4	106.8	52.1	18.9	10.3
12/1/2014	3.5	0.7	6.0	3.0	2.6	0.9	95.5	48.0	22.2	9.7
12/8/2014	3.8	0.2	3.7	0.3	2.9	0.1	95.8	19.6	24.1	1.0
12/15/2014	5.3	0.4	3.5	1.9	2.8	2.2	245.5	53.2	21.4	9.2
12/22/2014	9.3	2.6	7.1	0.6	4.9	0.4	60.9	5.6	17.9	1.5
1/26/2015	7.3	0.7	4.7	0.5	3.1	0.0	1902	1367	89.3	23.4
2/9/2015	11.2	0.3	8.2	1.6	7.8	0.8	2343	983	47.8	21.2
2/25/2015	10.2	0.9	4.8	1.1	3.0	0.2	4200	2179	37.6	1.4
3/9/2015	6.7	0.6	6.2	0.1	2.2	0.5	1248	109	46.2	4.3
3/23/2015	5.9	0.3	3.9	0.2	2.3	0.2	1492	190	52.4	5.9
4/6/2015	9.9	0.8	6.3	0.1	3.4	0.3	1117	267	48.4	21.5
5/4/2015	4.8	0.8	5.0	0.3	3.1	0.3	2078	304	61.5	6.3
6/1/2015	4.3	0.8	3.7	0.2	3.8	1.4	945.3	88.8	55.3	4.3
6/29/2015	8.4	0.2	7.3	0.1	6.4	0.3	604.0	36.2	22.3	2.3

Table F.8: VSS (mg/L) water quality for sample points 2, 3, and 4.

Date	2		3		4	
	Mean	SD	Mean	SD	Mean	SD
10/6/2014	14.6	1.5	11.4	0.6	7.6	0.4
10/9/2014	3.6	9.8	7.0	0.4	8.1	1.8
10/13/2014	8.9	1.4	7.2	0.2	6.7	0.2
10/16/2014	8.5	3.2	9.4	2.1	7.5	0.2
10/20/2014	7.5	0.0	6.8	0.1	6.4	0.2
10/27/2014	7.9	0.6	7.6	0.9	6.8	0.2
11/3/2014	8.4	2.0	9.2	0.6	6.7	0.3
11/10/2014	6.5	0.2	6.5	0.6	6.3	0.2
11/17/2014	7.9	0.9	6.9	0.5	6.3	0.2
11/24/2014	7.7	0.8	7.7	0.8	7.2	0.5
12/1/2014	9.4	1.0	12.9	4.8	7.5	0.2
12/8/2014	8.6	0.4	8.1	0.2	8.1	0.3
12/15/2014	9.6	0.7	9.1	0.2	8.6	0.6
12/22/2014	13.7	1.9	14.6	7.6	10.2	0.4
1/26/2015	13.6	1.1	7.3	0.8	7.6	0.3
2/9/2015	20.8	0.7	14.3	4.5	15.5	1.6
2/25/2015	19.5	1.7	10.9	2.6	9.0	0.2
3/9/2015	15.4	2.1	13.8	0.5	7.9	0.7
3/23/2015	13.5	0.5	9.1	0.6	7.4	1.8
4/6/2015	15.2	3.6	10.6	2.1	11.7	1.5
5/4/2015	11.6	0.8	11.2	1.2	10.8	0.1
6/1/2015	12.1	1.2	11.0	0.1	9.9	0.3
6/29/2015	15.9	0.8	14.7	0.9	14.4	1.1

Table F.9: VSS (mg/L) water quality for sample points 5 and 6.

Date	5		6	
	Mean	SD	Mean	SD
10/6/2014	179.4	69.0	55.3	0.9
10/9/2014	139.1	4.5	33.9	5.2
10/13/2014	105.6	0.7	25.0	2.4
10/16/2014	178.7	19.7	186.3	25.2
10/20/2014	175.5	14.5	31.5	3.5
10/27/2014	585.7	169.3	34.1	3.4
11/3/2014	378.2	153.7	80.1	71.9
11/10/2014	369.6	213.3	34.2	2.4
11/17/2014	135.4	11.3	94.3	70.7
11/24/2014	176.2	77.4	41.4	22.3
12/1/2014	176.6	80.0	64.6	29.2
12/8/2014	166.6	47.1	76.6	2.8
12/15/2014	363.4	59.6	45.0	22.8
12/22/2014	112.4	11.2	37.7	2.9
1/26/2015	2198.2	1529.1	114.8	25.6
2/9/2015	2869.5	1159.5	72.4	30.0
2/25/2015	4701.6	2206.6	57.6	2.5
3/9/2015	1649.0	13.7	79.0	12.2
3/23/2015	1995.0	232.2	91.8	7.8
4/6/2015	1564.0	337.0	98.3	32.7
5/4/2015	3020.7	521.2	99.0	9.3
6/1/2015	1422.3	195.8	139.6	13.2
6/29/2015	926.0	96.2	56.2	1.5

Appendix G: Nitrate Water Quality Data Collected by Staff at MAP

Table G.1: Nitrate (mg/L NO_3^--N) data collected on sand filter. Samples were filtered and acidified before analysis.

		Sample Location			
Date	Day	From standing water within stand pipe in sand filter	From standing water within stand pipe in sand filter immediately after backwashing	Solids sump effluent (Sample point 5)	Sand filter effluent (Sample point 6)
2/25/15	148	3.1		66.1	39.1
2/27/15	150	1.45	34.2	56.7	
2/28/15	151	<1.0			
3/1/15	152	<1.0			
3/2/15	153	<1.0	1.7		
3/3/15	154	<1.0			
3/4/15	155	<1.0	1.2		