Fall 2020

EVALUATING RECIRCULATING AQUACULTURE SYSTEM NUTRIENT PRODUCTION

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BY

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Biology BS, Mansfield University of Pennsylvania, 2014

THESIS

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Master of Science

In

Agricultural Sciences

September, 2020

This thesis has been examined and approved in partial fulfillment of the requirements for the degree of Biological Sciences: Integrative and Organismal Biology by:

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ABSTRACT

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While aquaculture production accounts for half of the world fish production, a growing problem emerges with the amount of effluent being produced. Waste treatment of aquaculture effluent is expensive and energy- intensive as conventional approaches to waste remediation have remained mostly unchanged. To improve the economic sustainability, the aquaculture industry needs to integrate with other production systems similarly as terrestrial animal agriculture has done with soil-based crop production. Integrating waste production in a wastes-to-resources approach as fertilizer for hydroponic cropping systems will allow aquaculture producers to monetize waste treatment. However, a full accounting of aquaculture nutrient production is necessary to develop a strategy to monetize costly effluent treatment. Capturing fish waste from aquaculture facilities provide an opportunity to offset operational costs by producing a naturally derived nutrient source as fertilizer.

Three replicate recirculating aquaculture systems (RAS) were designed and operated under pilot-scale production conditions to evaluate plant-available nutrient production from two commonly grown aquaculture species, tilapia (*Oreochromis* niloticus) and rainbow trout (*Oncorhynchus mykiss*). A nutrient mass balance was conducted while the research systems operated under “pseudo-steady state” conditions. Pseudo-steady state was defined as consistent

feeding and waste production activity during periods of fish growth and increasing feed demands while still accounting for fish growth and increasing feed demands. The macro-nutrients Ca, K, Mg, N, and P and micro-nutrients B, Cl, Cu, Fe, Mn, Mo, S, and Zn were analyzed over an 81-day period. Both the tilapia and trout nutrient production experiments revealed that all nutrients required for hydroponic crop production were present and available in the system culture water and effluent streams.

Macro-nutrients Ca, K, Mg, P, and N, and micro-nutrients, Cl, Mo, and S were observed primarily in the liquid portion of the wastewater and micro-nutrients B, Cu, Fe, Mn, and Zn were primarily observed in the particulate waste. The results of the first experiment indicated that tilapia excreted 3.39 ± 0.55 g Cu, 10.78 ± 1.90 g Fe, 5.61 ± 1.78 g Mn, 0.23 ± 0.08 g Mo, and 7.26 ± 0.89 Zn, per 100kg feed daily. Many of the tilapia nutrient production rates were determined to be statistically different between systems due to dilution and limits of measurement, notably -4.36 ± 4.78 g B, -76.71 ± 350.20 g Cl, -19.97 ± 163.60 g S, 1172.44 ± 706.72 g Ca, 405.27 ± 740.68 g K, 181.72 ± 196.13 g Mg, 704.34 ± 582.05 g P, and 2896.13 ± 4133.70 g Total Nitrogen (TN), per 100kg feed. The difficulties surrounding the accurate characterization of nutrient production from tilapia RAS were resolved and strict sampling procedures applied to the second nutrient mass balance experiment measuring nutrient production from rainbow trout in RAS.

Rainbow trout excreted nutrient production was 706.29 ± 49.58 g Cl, 1.01 ± 0.04 g Cu, 13.41 ± 0.51 g Fe, 7.08 ± 0.71 g Mn, 3.11 ± 0.57 g Mo, 312.95 ± 45.59 g S, 11.95 ± 0.58 g Zn. 2043.37 ± 29.18 g Ca, 659.48 ± 51.15 g K, 445.58 ± 7.61 g Mg, 690.11 ± 42.57 g P, and 5729.49 ± 540.33 g TN, per 100kg feed. It is important to distinguish if the nutrients would be directly available as a fertilizer. This study found that Cl, Mo, S, Ca, K, Mg, P, and N are nutrients

solubilized in liquid portion of rainbow trout waste rendering them immediately available for plant uptake. Alternately, B, Cu, Fe, Mn, and Zn, were retained in the solid particulate portion of the rainbow trout waste stream. Nutrients retained in the solid particulates require mineralization to make these nutrients plant available. Differences in nutrient production between the two species are due to variation in the feed composition and physiological distinctions such as gut length and muscle tissue composition.

The results from these experiments were inconsistent with the previous literature and differences are likely due to experimental design, system design, feed, fish species, and dilution effects. Experimental design is the key factor that limited the determination of nutrient production in this research because no tracer was used in the diet which would have allowed for a full accounting of nutrients assimilated and expelled by the fish.

This research supports the need to establish a predictive model for aquaculture-derived nutrient production for integration with other crop production systems. The results from this study demonstrate that nutrient reuse from RAS is possible for hydroponic crop production, but treatment of RAS effluent will be required to fully develop a valuable nutrient source as many of the nutrients are trapped in the solid particulate form.

ENABLING RECIRCULATING AQUACULTURE EFFLUENT REMEDIATION AND UTILIZATION: A REVIEW OF THE LITERATURE

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INTRODUCTION

Recirculating Aquaculture Systems (RAS), a form of land-based fish farming, maximizes fish production while efficiently reusing up to 99% of system water daily (Summerfelt et al., 1999; Rakocy et al., 2006). RAS shows potential to simultaneously decrease pressure on wild fisheries and improve the sustainability of farmed fish production (Somerville et al., 2014). In RAS fish are cultured in tanks and the water is treated and purified so that it can be reused with little discharge. The components required for water purification include mechanical filters that remove particulates, biological filters that treat dissolved wastes, and ozone or UV sterilization to disinfect water. These filtration processes to maintain water use efficiency, which increases production costs and therefore consumer market prices (Miller, 2002; Turcios and Papenbrock, 2014; Tsani and Koundouri, 2018). RAS effluent is a combination of solid and liquid (dissolved) waste products that both require treatment before discharge. Post-production effluent treatment is also required for waste remediation before effluent can be released into the environment (Black and Veatch, 1995; Chen et al., 1997; Mugg et al., 2007; Guerdat et al., 2013; Turcios and Papenbrock, 2014). In the United States, RAS requires expensive and energy-intensive treatments to remove excess nutrients and meet Environmental Protection Agency (EPA) wastewater discharge regulations (Yeo et al., 2004; EPA, 2006; Somerville et al., 2014).

Effectively, these same wastewater treatment processes could be used to capture and transform effluent into a fertilizer solution. Aquaculture waste management costs must be reduced or offset to improve the economic sustainability of RAS (Turcios and Papenbrock, 2014). Reusing waste as a fertilizer improves environmental and economic sustainability of RAS. It is important to understand the specific elements excreted, however, to determine the most efficient treatment process for collecting them.

RAS Water Treatment Processes

RAS uses physical, chemical, and biological filtration processes like those used in municipal waste treatment to remove solid and dissolved wastes. Physical filtration in RAS targets solids particulate matter greater than 40-100 μm in diameter (Summerfelt and Vinci, 2008; Timmons et al., 2018). These solid wastes contain high concentrations of total nitrogen (TN), total phosphorus (TP) and carbon (C) (Chen et al., 1993; Guerdat et al., 2013). RAS waste also includes high concentrations of dissolved nitrogen and phosphorus which are the primary nutrients regulated in wastewater discharge (Chen et al., 1993; Guerdat et al., 2013). Chemical and biological filtration convert nitrogenous wastes that are toxic to the fish (i.e. ammonia and nitrites) into non-toxic forms (nitrates). Specific combinations of physical, chemical and biological filtration are unique to individual systems and must be managed accordingly; however, a generalized understanding of nutrient production for individual species could offer foresight for future RAS waste treatment designs.

Physical filtration methods include sedimentation and capture techniques to remove solids (Summerfelt and Vinci, 2008; Turcios and Papenbrock, 2014; Timmons et al., 2018). Low-head swirl-separators alongside aerobic microbial digestion were the most common methods used to treat RAS culture water until the introduction of fluidized sand bed filters,

settling ponds, geotextile bags, belt feeders, micro-screen, and membrane filtration (Mugg et al., 2007; van Rijn, 2013; Turcios and Papenbrock, 2014). When these new technologies remove solids from the system water, a more concentrated waste stream is formed. However, more than 99% of this waste stream is water (Summerfelt, 2006; Sharrer et al., 2009). After removal from the system, further physical filters like a settling basin are used to dewater the waste stream. In turn, producers employ thickening, or the separation of solids from water portions, to treat the two waste streams independently (Mugg et al., 2007). Waste solids are better suited for land application whereas wastewater is easily remediated by any of the post-production treatment processes (Mugg et al., 2007; Turcios and Papenbrock, 2014).Chemical filtration has been shown to improve solids removal when used in conjunction with physical filters (Sharrer et al., 2009).

Chemical filtration techniques such as coagulants, flocculants, and oxidizers are widely used in municipal and aquaculture water and wastewater treatment. Coagulants and flocculants aid in solids and dissolved phosphorus removal but also have effects on alkalinity and pH (Sharrer et al., 2009). Ozone is an oxidizing agent used for disinfection and sterilization. It can be used to remove solids, nitrite, organic molecules, and inactivate microorganisms (Summerfelt and Hochheimer, 1997). Utilizing chemical filtration in RAS is expensive and is typically only used in large scale facilities. While chemicals can assist in solids removal and oxidation of organic matter, biological filters can also oxidize organic matter.

In RAS, biological filters are used in nitrification, denitrification, and oxidation of organic matter and their but require high energy costs (Turcios and Papenbrock, 2014). Filter types include moving bed bioreactors (MBBR), bubble bead filters (BBF), fluidized sand beds (FSB), trickling filter (TF), and rotating biological contactors (RBC) (Summerfelt and Vinci, 2008; Sharrer et al., 2009; Turcios and Papenbrock, 2014). In RAS biofilters, high concentrations

of nitrification bacteria, (*Nitrosomonas* and *Nitrobacter*,) responsible for nitrogen conversion (ammonia to nitrates) produce biofilm which sloughs off biofilter media and becomes a new source of sludge material (Timmons et al., 2018).The presence of solids in a biological filter can inhibit nitrification, therefore hybrid filters that pair physical and biological filtration have been developed to perform nitrification and capture solids simultaneously (Sastry et al., 1999).

Hybrid filters include bubble bead filters, fluidized sand beds, and trickling filters. While primarily classified as biological filters, the physical filtration performed by these units reduces solids injection into the culture system by capturing them. The solids captured by hybrid filters must be removed from the system requiring varied amounts of energy and effort. When hybrid filters are backwashed, water and air are injected into the filters to resuspend the captured solids followed by a flushing or skimming process to physically remove the solids from the unit. Hybrid filter efficiency is driven by system feed rates and requires regular backwashing to maintain adequate water quality within systems (Sastry et al., 1999). Regardless of type, water treatment and waste removal are the most important factor in the success of RAS.

Targeted water and waste treatment in RAS are necessary to maintain fish health and maximize growth. Specific filtration combinations are unique to each aquaculture facility and individual systems due to the variation of source water, species, feed, management practices, required level of filtration, and production goals (Mugg et al., 2007). Whether physical, chemical, biological or hybridized, all filter units require regular cleaning and maintenance. When any of the various types of filters are cleaned, backwashed, or purged, the resultant waste stream must also be treated before the effluent can be discharged to the environment. This is referred to as post-production treatment and can use the same filtration techniques used in RAS. The goal of post-production treatment is dewatering solids and mitigating any environmental

risks. After meeting EPA regulations, RAS effluent can be discharged to natural waterways, municipal treatment plants, constructed wetlands, or applied to fields (Reed, 1995; Mugg et al., 2007).

RAS Effluent Remediation

In the United States, RAS discharge is regulated by Environmental Protection Agency (EPA) to minimize the negative effects of effluent on receiving waters (Black and Veatch, 1995; Summerfelt et al., 1999; Mugg et al., 2007). Traditionally, RAS effluent is applied to fields or discharged directly into natural waters (Mugg et al., 2007). Recently, new strategies applied to RAS effluent remediation include municipal treatment and reusing nutrients to grow food plants.

RAS effluent is a combination of solid and dissolved waste products that both require treatment before discharge. Although RAS has a high level of water conservation, the effluent stream contains excess nutrients including nitrogen (N) and phosphorus (P), that contribute to eutrophication (Seawright et al., 1998; Summerfelt, 1998; Summerfelt, 1999).

Land application of raw RAS effluent is not a suitable choice due to a water content leading to excess runoff (Summerfelt et al., 1999; Rakocy et al., 2006). Land application of RAS effluent utilizes established protocols of terrestrial animal agriculture which directly injects waste into fields to generate crops (Nielson et al., 1999). However, through thickening, dewatering, and stabilization waste treatment processes, solids separated from the effluent stream can be effectively applied to fields. The high levels of sodium in commercial RAS waste solids make it nearly unusable as a field crop manure (Mugg et al., 2007; Turcios and Papenbrock, 2014). However, alternative sodium-free carbonate sources can be used to reduce the sodium load of waste solids. Land application remains a common method for RAS effluent discharge

even if it is not dewatered; however, dumping raw wastewater directly released to natural waters is illegal.

Discharging to natural waters and wetlands are also common methods for RAS effluent disposal. When land-based aquaculture facilities discharge to rivers or other natural waters effluent is regulated by costly discharge permits and subject to frequent testing (Black and Veatch, 1995; Summerfelt et al., 1999, Guerdat et al., 2013). Discharging to natural waterways including oceans, lakes, and rivers requires transportation and treatment to minimize negative environmental effects (Yeo et al., 2004; Mugg et al., 2007; Turcios and Papenbrock, 2014). Constructed wetlands have become a more popular on-site wastewater treatment for many RAS facilities due to environmental and economic advantages (Turcios and Papenbrock, 2014). Offering low maintenance inputs, wetlands act as natural filters for both solid and liquid wastes. The main drawback to constructed wetlands is the land area required to treat the high volumes of waste generated from a commercial scale RAS facility (Mugg et al., 2007; Turcios and Papenbrock, 2014). Discharging to natural waters and constructed wetlands is a cost-effective treatment strategy for RAS effluent, but location independence draws RAS producers into urban areas where there are few fields to accept discharge and little space to construct wetlands. RAS facilities in dense urban areas therefore have few options other than to outsource effluent treatment to their local municipalities.

When RAS facilities are located near target markets in urban areas, municipal treatment may be the best option for treatment but can quickly become costly due to the volume of wastewater requiring treatment (Mugg et al., 2007). RAS are generally lauded for their water conservation on a percent water reuse of the system volume; however, discharge is a function of farm scale and intensity of recirculation. Intensive RAS operations generates 1% of the waste

volume than a traditional flow through raceway system. At a scale of 500 metric tons (MT) of fish production, this discharge would exceed 1 million gallons per day (Bregnballe, 2010). This type of effluent remediation plan must be well executed because municipalities have their own maximum flow limitations and regulations. While the treatment process becomes simple for producers, they must negotiate contract costs for municipal services. For most urban RAS producers who elect to discharge directly to municipalities, the costs of paying someone to treat their effluent is more affordable than transporting such large volumes of water-based effluent (Mugg et al., 2007; Guerdat et al., 2013). Other urban fish farms take an alternative approach which converts the costly waste into a revenue stream.

Recirculating Aquaponics Systems

Some challenges regarding RAS waste can be addressed effectively and economically using a new type of system that combines fish farming with soil-free plant production. Such systems are termed recirculating aquaponic systems (RAqS). Instead of treating the waste for discharge, recirculating aquaponics captures, stores, and reuses waste nutrients to produce food plants. There are several advantages to aquaponic production including shared start-up, operating, and infrastructure costs, as well as high water efficiency, nutrient reuse by plants, and low volume discharge (Rakocy et al., 2006). RAqS also increase profit potential by simultaneous producing marketable fish and plants (Timmons & Ebeling, 2010). Using this strategy, plants rely on nutrients produced by the fish though some nutrients may be supplemented (Seawright et al., 1998; Summerfelt, 1998, Summerfelt, 1999). Previous RAS waste-solids research indicates that nutrients in the solid waste meet and exceed the levels of nutrients required for plant growth (Guerdat et al., 2013). The challenge then becomes liberating nutrients from solid waste into an available form for plant uptake. Examining nutrient production for various fish species in a

controlled setting allows farmers to better understand their effluent stream and how it can be effectively remediated or even monetized. The challenges facing the remediation of RAS effluent can be resolved through a better understanding of what is being produced. The implications of studying RAS effluent range from the development of more efficient treatment methods before discharge to monetizing the high cost of waste mitigation. Water quality is the driving force that determines the quantity and availability of nutrients from an aquaculture system (Seawright et al., 1998; Roosta, 2011; Timmons et al., 2018).

CONCLUSIONS

Understanding what elements and compounds are present in recirculating aquaculture systems provides an opportunity for producers to more effectively treat effluent streams and even profit from them. RAS are positioned to meet demands to improve environmental sustainability and the need for better food security (Gormaz et al., 2014; Summerfelt and Christianson, 2014). RAS minimizes water usage through physical, chemical, and biological filtration unit processes to remove solid and dissolved wastes. (Losordo et al., 2000; Guerdat et al., 2013; Timmons et al., 2018). Once discharged from the system, nutrient rich effluent with >99% water content typically goes through further waste management filtration before discharge to fields and natural waters (van Rijn, 1996; Summerfelt, 1999; Yeo et al., 2004; van Rijn, 2013). Municipal treatment is a costly option best suited for urban farms. Alternative strategies have been developed to capture, treat, and reuse nutrients to grow food crops using aquaponics. Regardless of the final treatment plan, nutrient mass balance studies provide RAS producers with insight on nutrient flows throughout their systems.

Nutrient mass balances are used to track all inputs, sinks, and outputs of a system. In the following studies, a nutrient mass balance will track nutrients added to RAS, assimilated by fish,

accrued in system water, and discharged from the system. Published studies on RAS nutrient production are often limited to individual nutrient production or interactions between water quality and individual nutrients within a system. The following studies show how RAS facilities can utilize nutrient mass balances to characterize their waste streams. Characterizing aquaculture nutrient production is necessary to produce a model for predicting effluent fractionation of liquid versus solid nutrient contents. This model may vary by facility due to differences in species, filtration, source water, and feed quality. Predicting culture-water treatment and wastewater treatment could simplify the wastewater discharge permit application by informing the EPA of exact nutrient profiles of the waste coming out of the systems and the final post-waste-treatment discharge to a river.

CHARACTERIZATION AND QUANTIFICATION OF *OREOCHROMIS NILOTICUS* NUTRIENT PRODUCTION IN RECIRCULATING AQUACULUTRE SYSTEMS

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Keywords: Aquaculture, Recirculating Aquaculture Systems, Nutrient Mass Balance, Nutrient Production

INTRODUCTION

Recirculating Aquaculture Systems

Recirculating Aquaculture Systems (RAS) are a popular land-based fish farming method that recycle up to 99% of system water volume each day (Summerfelt et al., 1999; Rakocy et al., 2006). However, as fish farming intensifies, it requires innovative solutions for waste remediation to improve economic success and environmental sustainability (Hochman et al., 2018). RAS are becoming more popular because they offer improved food security with a highly consistent product than terrestrial farming. They also show potential to simultaneously decrease pressure on wild fisheries and improve the economic and environmental sustainability of farmed fish production (Somerville et al., 2014). The challenge of waste remediation stems from RAS water treatment processes which increase production costs and therefore market prices for consumers. Studying the composition of the RAS waste effluent would give producers valuable insight to more efficiently treat effluent. One popular RAS species, tilapia, is often used as a model organism to determine the effectiveness of new RAS technology and strategies.

Tilapia

Tilapia (*Oreochromis sp.*) are a popular food fish used in aquaculture. While often grown in pond aquaculture, they have recently served as an important model organism in the development of RAS technology. Producers choose tilapia for their fast production cycles and overall hardiness. As a warmwater fish species, they can produce marketable fillets within six months of hatching. Tilapia are omnivores and tend to produce a well-formed fecal pellet that is ideal for physical filtration.

Tilapia Effluent

Tilapia produce a formed fecal pellet that is encased in a mucus membrane which makes it easy to remove from the system water via physical filtration. Most of the soluble waste produced from tilapia is unionized ammonia (NH^3) excreted from the gills. Exact nutrient contents for all waste products is not known, but several reports have focused on macronutrient production attributed to eutrophication (Seawright et al., 1998; Yeo et al., 2004; Timmons et al., 2018). Solid waste is removed within fifteen minutes of production while the gradual accumulation of nutrients within the system create a tea-colored tint to the water. Tilapia produce both a solid and liquid waste stream that is not completely understood, therefore producers only view waste treatment as a necessary expense to meet the wastewater discharge regulations.

RAS Effluent Remediation

In the United States, Environmental Protection Agency (EPA) regulations for wastewater discharge to natural waters require expensive and energy-intensive treatment to remove excess nutrients from RAS effluent (Yeo et al., 2004; EPA, 2006; Somerville et al., 2014). Currently, aquaculture effluent is disposed either by municipal treatment, discharge to natural waterways, or application to fields. RAS facilities are typically located close to their markets, leaving few

options for waste management (e.g. land application) other than discharge to municipal treatment systems and/or surface water systems (Yeo et al., 2004). Discharge to natural waterways may include lakes, rivers, and even constructed wetlands. It may also require transportation, and increases the potential for eutrophication (Yeo et al., 2004). Terrestrial application of aquaculture effluent is often inefficient, and results in runoff (Yeo et al., 2004). RAS waste treatment has traditionally been viewed as a cost but could become a secondary source of income through reintegration as a fertilizer for plants.

RAS Effluent Utilization

RAS waste can be utilized through waste treatment processing and reintegration with hydroponic plant production. Traditional, land-based animal agriculture utilizes existing models which are designed to capture, treat, and reuse nutrients to offset operational costs and generate revenue (USDA NRCS, 2009). However, RAS do not have effluent utilization strategies as used in other terrestrial agriculture systems. Research is needed to develop similar strategies for improving the economic viability of RAS (Yeo et al., 2004). The object of this research is to identify key factors, ranges, and system dynamics affecting nutrient production in RAS. Previous waste-solids research suggests that the macro- and micro-nutrients in the captured solids from RAS meet or exceed nutrient profiles required for crop production (Guerdat et al., 2013). Another study focused on how fish biomass affects nutrient production in a coupled aquaponic system found that nutrient ratios fluctuated dramatically (Seawright et al., 1998). Biotic differences between species are expected to influence quantity and availability of nutrients.

Effects on Nutrient Production

Fish species used for aquaculture are carnivorous or omnivorous. Therefore, they require diets with different protein, carbohydrate, and lipid contents (Cho and Bureau, 2001, Sarker et

al., 2018, 2020). They metabolize these diets differently and produce waste with varied nutrient compositions (Sarker et al., 2020). The waste composition and quantity will also be influenced by the amount of feed consumed, which is dependent on fish size, density, and behavioral factors. The combined effects of fish physiology on RAS nutrient production presents a unique opportunity to produce hydroponic fertilizer solutions with different nutrient profiles (Seawright et al., 1998; Yeo et al., 2004; Timmons et al., 2018). Environmental factors work in conjunction with the physiological effects factors to affect RAS nutrient production including day length, light intensity, and water quality. Beyond physically stressing the fish, chemical properties of water, pH, Electrical Conductivity (EC), and temperature cause feces breakdown (Seawright et al., 1998).

This study was conducted to characterize and quantify tilapia RAS nutrient production. It will focus on characterizing the liquid and solid effluent fractions to determine the viability of reusing tilapia effluent as a fertilizer.

MATERIALS AND METHODS

Facility Design

This study was conducted at the Anadromous Fish and Aquatic Invertebrate Research facility at the University of New Hampshire in Durham, New Hampshire, United States. The experimental design and fish protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of New Hampshire. Three replicate RAS were designed, constructed, and operated per intensive commercial aquaculture standards to maintain pseudo-steady-state system conditions (defined below).

Pseudo-Steady State

Pseudo-steady state was defined for this research as stable operating conditions in a dynamic production environment. Fish production operations are dynamic since they consistently increase feed rates as fish continue growing. Water treatment also remains consistent yet dynamic as a function of changing feed rates and waste production. Pseudo-steady state conditions in the three replicate systems were confirmed statistically based on system inputs and operation including feed rates, water usage, pH, dissolved oxygen (DO), temperature, EC, and alkalinity. During the pseudo-steady state study period, a mass balance was used to characterize nutrient production.

Nutrients were quantified using a mass balance analysis, characterizing and accounting for all nutrient inputs, sinks, and outputs. Nutrient inputs were defined as any nutrients entering the system, nutrient sinks as any unit process accruing nutrients, and nutrient outputs as nutrient exports from the system (Schneider, 2005). Inputs include all system additions in this experiment including incoming well water, chemical additions for pH adjustment, and feed. Sinks consume or accrue nutrients in a system; in this case, culture water and fish both sequester nutrients. Outputs include any nutrients that otherwise left the systems, which, in this study included wastewater and waste-solids. Inputs, sinks, and outputs terms in the mass balance were simplified for this study into system nutrient gain or loss terms to generate an understanding of a system wide nutrient flow. The dynamic nature of RAS contributes to the overall accumulation or release of nutrients. Under ideal conditions, RAS will have a maximum nutrient carrying capacity that can be maintained without compromising fish heath and growth. Nutrient production in any RAS is ultimately limited by system design and specifications.

System Specifications

Three laboratory-scale RAS were built to a harvest capacity of 75 kg/production tank (150 kg/system) at a maximum stocking density at harvest of 72 kg m^{-3}. Each 4,800 L system is comprised of two fish culture tanks (1.5 m^3), one standpipe well, one drum screen filter (Hydrotech 501, Veiolia North America, Boston, MA), one Moving Bed Bio Reactor (MBBR), and one pumping sump tank (

Figure *1*. Research recirculating aquaculture system schematic and water flow.

). Air was injected into the culture tanks using a regenerative air blower and medium-pore stone diffusers (Sweetwater, S45, Pentair AES, Apopka, FL). Biological filtration was achieved using an MBBR containing 0.7 m^3 of Kaldness K1 media. During the study, 93% of system water was retained and reused daily. This high recirculation rate facilitated nutrient retention in the system while producing a low volume and of concentrated effluent stream. Solid waste was removed from the system water using an inline rotary drum screen filter fitted with 40-micron screens (Hydrotech 501, Veiolia North America, Boston, MA). System water was used to remove solid wastes from the drum screen filter, creating a solid waste stream that was channeled out of the system for sampling and discharge.

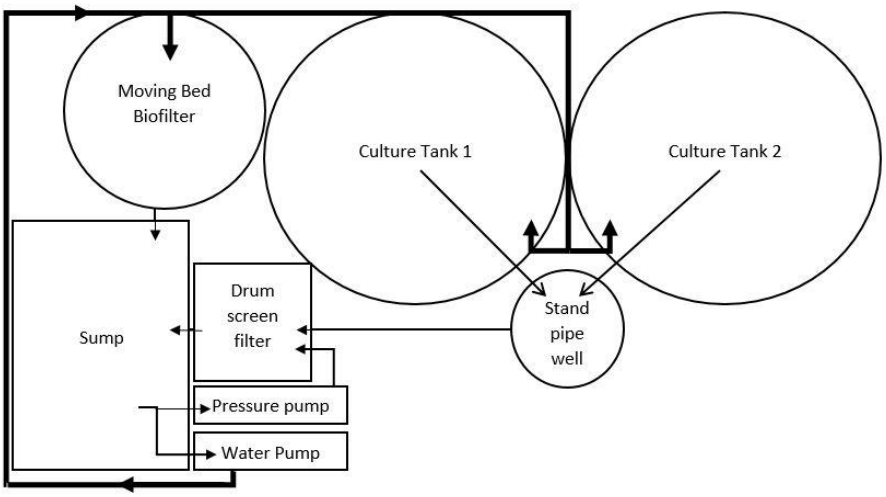

Figure 1. Research recirculating aquaculture system schematic and water flow.

System water volume was maintained using an auto-refill float valve supplied by well water and located in the pumping sump. Well water was filtered through a 5 µm floss filter as well as a carbon block filter to remove any nutrients and impurities. Water usage was monitored and recorded daily using an inline water meter (Flexible Axis Water Meter, Master Meter North America, Mansfield, TX) located immediately before each system's water refill valve. Flow rates to culture tanks were controlled using vertical injection manifolds with clear manometers used for visual verification of equal flow distribution between tanks. Culture tank flow rates were set at 94.6 L min^{-1} to maximize solids removal from the culture tanks. Besides refill water, the only additions to the system were sodium bicarbonate and feed.

Chemical Additions

Sodium bicarbonate was added to each system daily to buffer pH and alkalinity. Sodium bicarbonate is a common RAS reagent chosen for its affordability and high buffering capacity. Sodium, the constituent which would accumulate in the system and effluent, can also support fish health by bolstering slime coat production and mitigating harmful effects of unionized

ammonia (NH^3) (Soderberg and Meade, 1993). Sodium bicarbonate only contributes to the accumulation of sodium in RAS. Aquaculture feeds are the main source of nutrients that fish utilize, and RAS accumulate.

Aquafeed

Aquafeeds are the main sources contributing to RAS nutrient accumulation and discharge. Fish received 3.0 mm Zeigler Bros. Finfish Silver (40% protein, 10% lipid) floating feed. Feeding rates were determined using a percent body weight of the total fish biomass commensurate with predicted growth rates (DeLong et al. 2009). Lighting was applied 24 hrs day^{-1} to allow hourly feeding and therefore maximum fish production. Feed Conversion Ratios (FCR) are commonly used in aquaculture to determine the efficiency of feed inputs versus fish growth. Low FCRs indicate high efficiency of fish production. Preferred FCRs for tilapia are 1.5 or less (Soderberg 1994). (FCR) were determined using the following equation:

$$F_{in} \div M_{gain} = FCR \quad (1)$$

where F_{in} is the total amount of feed added over the course of the experiment (kg), M_{gain} is the mass gained by the fish during the same time period (kg), and FCR is the Food Conversion Ratio, a unit-less factor used to measure the efficiency of aquaculture production.

Water Quality Analysis

Water quality was monitored and adjusted daily according to conventional aquaculture standards (

Table 1). Daily analyses included dissolved oxygen, electrical conductivity, salinity, temperature, total ammoniacal nitrogen, and nitrite-nitrogen. Dissolved oxygen (DO), electrical conductivity (EC), and temperature were measured using a portable handheld meter (YSI Pro 2030, Yellow Springs Instruments, Ohio). Total ammoniacal nitrogen (TAN; sum of NH_3-N and NH_4^+-N), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) were analyzed using a HACH DR3900 spectrophotometer (HACH Methods: 8038 TAN, 8507 NO_2-N, 8507 NO_3-N, HACH Company, CO, USA) four times per week off-site at the UNH Agricultural Engineering Research and Analytical Lab. Alkalinity and pH were measured using a bench-top meter (Fisher Scientific Accumet AB250, MA, USA). Total alkalinity was determined via sulfuric acid titration to a pH 4.80 end point (APHA, 2012).

Table 1. Conventional aquaculture water quality standards for optimal tilapia culture.

Parameter	Tilapia	Sources
Dissolved Oxygen (DO) mg/L	>5.0	Barton (1996)
pH	7-8	Wedemeyer (1996)
Electrical Conductivity (EC) µS/cm	>1.8	Barton (1996)
Alkalinity as $CaCO_3$ mg/L	80-100	Barton (1996)
Temperature (°C)	28-30	Barton (1996)
Ammonia Nitrogen (NH_3-N) mg/L	<1.0	Soderberg et al., (1983)
Nitrite Nitrogen (NO_2-N) mg/L	<0.1	Westin (1974)
Nitrate Nitrogen (NO_3-N) mg/L	<500	Westin (1974)

Fish Stocking

Nile tilapia (*Oreochromis niloticus*) were obtained from Aquasafra, FL, USA. Fish received at 28 days post-hatch were held in quarantine for 6 weeks before the start of the experiment, and then transferred into the RAS when they weighed 30 g each. Juvenile tilapia were stocked into each culture tank at a rate of 100 fish/tank with an expected harvest density of 42 kg/m^3. The average biomass for this study was 120 kg per system. Feed rates were determined

using a percent body weight of the total fish biomass commensurate with predicted growth rates (DeLong et al. 2009). After an initial biofilter startup, all systems ran for a three-month period to establish a baseline for fish growth, TAN and NO_2 conversion, as well as system water usage. An initial study determined the optimal protein content feed for RAS nutrient production. Subsequent experiments established a nutrient mass balance for tilapia in RAS.

Feed Protein Content Study

A preliminary study was used to test the effect of feed protein content on tilapia nutrient production. Three Zeigler Bros. 3.0 mm Finfish feeds with different protein content were used: Bronze (35% protein, 5% fat), Silver (40% protein, 10% fat), and Gold (42% protein, 16% fat). This study is considered preliminary due to only one replicate for each of the feeds. The Zeigler Silver diet represents the industry standard for commercial RAS tilapia aquafeeds. By bracketing the industry standard, growth and nutrient production were hypothesized to be significantly different between the three systems. This study was ended early due to lack to replication; however, the Silver diet was chosen for use in the next nutrient mass balance study because it led to the best water quality without sacrificing growth rates.

Nutrient Mass Balance Sampling

A new cohort of tilapia was then evaluated using a nutrient mass balance approach to quantify nutrient inputs, sources, sinks, and outputs. Samples were collected bi-weekly over the course of a 176-day period. Well water was collected directly from the building water supply and feed was sampled via grab sample from each feed bag in according with sampling dates. Whole fish were sampled once at the beginning and again at the end of the experiment to quantify nutrient assimilation. Culture water grab-samples were obtained from each system pumping sump reservoir after triple rinsing the bottles with the water to be sampled. Composite effluent

samples (approximately 20 L of wastewater and waste solids combined) were collected from the drum screen filter effluent pipe for a period of one hour. All samples were preserved at 4 °C for 24 hours prior to analysis per APHA (2012) sample storage protocols for nitrogen analysis (Method 4500).

Sample Preparation

Measured volumes of wastewater samples were filtered using binder-free, glass microfiber filters (Whatman 934-AH) to separate the liquid and solid portions for independent analyses. Well water, culture water, and filtered wastewater liquid were sent directly for analysis by a partnering laboratory (JR Peters, Allentown, PA, USA), while solid waste and tissue samples were first dried, ground, and mixed prior to analysis. Total Suspended Solids (TSS) of wastewater samples were determined by filtering and drying at 105°C. Complete dryness was achieved when dry mass changes over a 24-hour period were less than 5% of the total sample mass (Method 2540D, APHA, 2012). The same drying methodology was applied to feed and whole fish samples before nutrient analysis.

Initial analyses were carried out at the University of New Hampshire Environmental Research Group laboratory using a Varian Vista AX Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP-AES); however, this approach was unable to measure micronutrients Mo and B. Resh (2016) identifies essential nutrients that plants require with Mo and B both listed for their importance in redox reactions and energy storage reactions, respectively.

Samples were analyzed for 13 plant macro- and micro-nutrients, Table 2.. Samples were analyzed via inductively coupled plasma – optical emission spectrometry (ICP-OES) by a partnering laboratory (J.R. Peters, Inc. Laboratory, Allentown, PA, USA). ICP analysis of water,

waste, and fish was conducted per EPA method 200.7 using a Prodigy XP ICP-OES (Teledyne Leeman Labs, Hudson, NH, USA) (EPA, 1994).

Table 2. Nutrient List

Macronutrients	Micronutrients
Calcium (Ca)	Boron (B)
Potassium (K)	Chlorine (Cl)
Magnesium (Mg)	Copper (Cu)
Nitrogen (N)	Iron (Fe)
Phosphorus (P)	Manganese (Mn)
Sulfur (S)	Molybdenum (Mo)
	Zinc (Zn)

Nutrient Mass Estimation

The following nutrient mass balance equation was adapted from a general mass balance equation as described by Timmons et al. (2018):

$$\text{Accumulation} = \text{Input} - \text{Output} + \text{Production} - \text{Consumption} \quad (2)$$

where Accumulation was defined as the nutrients accumulated in the culture water/system (mg), Input was the sum of all nutrient masses introduced into the culture system (e.g. incoming well water and feed) (mg), Output was the sum of all nutrient masses exiting the system (e.g. effluent stream, fish) (mg), Production was the sum of all nutrient masses produced within the culture system (e.g. biofilm sloughing) (mg), and Consumption was the sum of all nutrient masses consumed within the culture system (e.g. nutrient assimilation into fish tissue) (mg).

All nutrient masses were calculated to reflect the total mass of each nutrient present for each sampling date then extrapolated between samples to calculate nutrient accrual over time.

Daily nutrient production was estimated using the sample data and operational data collected between sampling dates (e.g. water consumption, feed rates, etc.).

Culture water, well water, and wastewater nutrient masses were calculated using the following equation:

$$C \times V = M_{NL} \quad (3)$$

where C was the nutrient concentration in the liquid samples (mg L^{-1}), V was the volume of water added or system volume (L), and M_{NL} was the mass of nutrients in liquid (mg).

Whole fish, feed, and waste nutrient masses were calculated using the following equation:

$$C \times M = M_{NS} \quad (4)$$

where C was the concentration of nutrient in the dry matter samples (mg kg^{-1}), M was the mass of the dry matter sample (mg), and M_{NS} was the mass of nutrients in the dry matter solid sample (mg).

Daily Nutrient Production Rates were determined by comparing the nutrient loads over the duration of the experiment as:

$$\Delta NL \div \Delta t = NPD \quad (5)$$

where ΔNL was the change in nutrient load between sample at two separate sampling periods (mg), Δt was the number of days between the two sampling periods, and NPD was the nutrient production per day (mg day^{-1}).

Statistical Analysis

All analyses were carried out using JMP Pro 14 software package (JMP Pro 14, 2018). MANOVA tests were used to compare independent variables (feed rates, water usage, alkalinity, and pH) then the dependent nutrient production variables between systems. Significance was indicated when p-value ≤ 0.05.

RESULTS

Feed Protein Content

In the preliminary study on feed protein content, low protein feed (Bronze, 35%) resulted in very good water quality, but failed to meet the nutrient requirements for fish growth resulting in the highest FCR of 1.52 and smaller fish. High protein feed (Gold, 42% protein) resulted in poor water quality as a result of diarrhea-like feces from the fish and provided no significant benefit to fish growth or nutrient production with a 1.23 FCR. Feed protein content as well as stressful environmental conditions are known to induce enteritis leading to diarrhea (Penn et al., 2011; Sarker et al., 2018, 2020).) Medium protein content feed (Silver, 40% protein) provided the optimal amount of protein required for tilapia growth, yielding an FCR of 1.32 without reducing water quality. The results of this study identified the effects of dietary protein on RAS water quality and tilapia growth, 40% protein content feed was chosen for the nutrient mass balance study.

Feed Characterization

Tilapia aquafeed nutrient profiles were determined over the course of the experiment to provide a secondary confirmation of nutrient tracking within each system. The average nutrient profile on a dry mass basis for this experiment is shown in

Table 3. Tilapia aquafeed total nitrogen was 64.74±2.81 g kg^{-1} dry mass which is within the industry standard protein contents (Craig et al., 2017).

Table 3. Summary statistics for aquafeed nutrient characterization for basic nutrient inputs into a recirculating aquaculture system. Statistics based on the average nutrient concentrations of aquafeeds throughout the experiment.

	Tilapia Feed Zeigler Finfish Silver (40% PC, 10%F)
Micronutrients (mg/kg)	
B	5.63 ± 1.38
Cu	13.83 ± 4.35
Fe	62.35 ± 3.37
Mn	3.04 ± 0.81
Mo	0.33 ± 0.47
Zn	46.12 ± 10.25
Macronutrients (g/kg)	
Ca	17.06 ± 1.08
K	8.66 ± 0.62
Mg	1.86 ± 0.30
P	13.54 ± 0.51
S	514.00 ± 726.91
TN	64.74 ± 2.81

Nutrient Production

Data were collected over a period of 176 days with 81 consecutive days qualifying as pseudo-steady state conditions. The summary water quality conditions are presented in

Table 4 for each replicate system. Average dissolved oxygen concentrations during the length of the experiment were 6.35 ± 0.60 mg L^{-1}. Temperatures for all three systems averaged 25.47 ± 1.52°C. Average electrical conductivity among systems was 1.59 ± 0.30 mS cm^{-1}. The average pH during the experiment was 7.54 ± 0.21. Alkalinity among the three RAS averaged 67.85 ± 22.78 mg L^{-1} as $CaCO_3$. Tilapia received an average daily feed rate of 1.06 ± 0.48 kg d^{-1} of 3.0 mm Zeigler Bros. Finfish Silver (40% protein, 10% lipid) floating feed throughout the experiment. Average water usage rates for the tilapia experiment were 331 ± 591 L day^{-1}. The average Feed Conversion Ratio (FCR) during the tilapia experiment was 1.33 ± 0.05.

Table 4. Summary statistics of tilapia water quality for each of the recirculating aquaculture experimental systems. Statistics based on the pseudo-steady state experimental period of 81 days.

	Mean ± Standard Deviation; (Minimum, Maximum)		
Parameter	RAS 1	RAS 2	RAS 3
Dissolved Oxygen (mg/L)	6.38 ± 0.67 (4.23,7.67)	6.26±0.57 (4.76, 7.39)	6.47±0.65 (4.70,7.75)
pH	7.55 ± 0.21 (6.76, 7.98)	7.54±0.19 (7.01, 7.97)	7.54±0.23 (6.73, 7.95)
Temperature (°C)	25.53 ± 1.44 (18.80, 28.20)	25.74±1.35 (22.00, 28.90)	25.17±1.67 (18.60, 28.40)
Feed Rate (kg/day)	1.05 ± 0.46 (0.00, 1.48)	1.08±0.47 (0.00, 1.67)	1.07±0.49 (0.00, 1.51)
Electrical Conductivity (mS/cm)	1.79 ± 0.11 (1.47, 2.04)	1.68±0.21 (1.30, 2.14)	1.28±0.28 (0.39, 1.84)
Alkalinity (mg/L as $CaCO_3$)	70.21 ± 23.14 (20.00, 196.00)	67.38±21.53 (24.00, 180.00)	66.01±23.74 (21.00, 170.00)
Water Usage (LPD)	211.05 ± 139.94 (27.93, 1008.06)	346.55±180.40 (0.00, 751.44)	327.83±227.67 (44.89, 1676.11)

Daily nutrient production from the tilapia production period of research are shown in Table 5. Nutrient production rates averaged Cu 3.39 ± 0.55 g per 100 kg feed, Fe 10.78 ± 1.90 g per 100 kg feed, Mn 5.61 ± 1.78 g per 100 kg feed, Mo 0.23 ± 0.08 g per 100 kg feed, and Zn 7.26 ± 0.89 g per 100 kg feed across all three systems and were not statistically different from one another ($p > 0.05$). The average production rates among the three systems for B -4.36 ± 4.78 g per 100kg feed, Cl -76.71 ± 350.20 g per 100 kg feed, S -19.97 ± 163.60 g per 100 kg feed, Ca 1172.44 ± 706.72 g per 100 kg feed, K 405.27 ± 740.68 g per 100 kg feed, Mg 181.72 ± 196.13 g per 100 kg feed, P 704.34 ± 582.05 g per 100 kg feed, and TN 2896.13 ± 4133.70 g per 100 kg feed, were highly variable and significantly different between all three systems.

Table 5. Summary statistics of tilapia average daily nutrient production rate in grams per 100 kg feed fed. Statistics based on the pseudo-steady state period of 81 days.

Micronutrients (g/100kg feed)	Mean ± Standard Deviation; (minimum, maximum)		
	RAS 1	RAS 2	RAS 3
B	2.15±14.09 (-6.56, 23.17)	-6.00±4.83 (-13.00, -1.99)	-9.21±6.92 (-17.99, -3.20)
Cl	-571.82±159.92 (-728.49, -392.06)	160.35±683.04 (-587.01, 1065.22)	181.32±388.62 (-244.79, 694.03)
Cu	2.87±1.09 (1.35, 3.88)	4.16±1.84 (1.45, 5.55)	3.15±2.06 (1.01, 5.58)
Fe	11.63±1.98 (9.45, 14.16)	12.56±7.21 (2.39, 18.94)	8.14±11.33 (-2.74, 17.99)
Mn	6.21±2.44 (3.18, 8.33)	7.43±2.80 (3.77, 10.00)	3.20±7.77 (-6.35, 10.41)
Mo	0.13±0.12 (-0.03, 0.23)	0.32±0.16 (0.18, 0.55)	0.23±0.07 (0.19, 0.33)
Zn	6.99±1.35 (5.19, 8.37)	8.47±3.46 (4.29, 12.62)	6.33±5.32 (1.64, 11.41)
Macronutrients (g/ 100kg feed)			
Ca	235.33±111.51 (159.17, 398.52)	1941.91±564.21 (1219.28, 2497.92)	1340.08±1111.72 (259.12, 2523.06)
K	-635.99±205.56 (-871.59, .432.22)	1024.57±918.94 (273.80, 2347.71)	287.23±248.02 (487.37, 1058.21)
Mg	-94.55±35.29 (-141.33, -55.98)	341.17±208.58 (142.02, 620.23)	298.55±113.72 (151.81, 411.55)
P	-97.64±32.86 (-142.35, -63.19)	1265.91±257.78 (945.64, 1503.57)	944.74±498.53 (386.27, 1548.75)
S	-244.51±86.63 (-323.98, -138.35)	140.66±408.34 (-162.89, 735.74)	43.94±133.27 (-115.24, 181.87)
Total Nitrogen (*constituents)	-2913.84±939.23 (-3886.16, -1750.37)	6361.82±4770.46 (2605.11, 13348.52)	5240.39±1424.42 (3176.63, 6323.99)
*NH4-N	5.29±20.52 (-19.46, 29.80)	48.39±207.27 (-155.45, 337.66)	-23.50±92.95 (-160.83, 44.99)
*NO3-N	-2963.57±978.66 (-3955.56, -1734.02)	79.03±4435.62 (-2775.44, 6571.43)	-732.98±1024.11 (-1628.35, 342.51)
*Urea	-5.91±3.18 (-9.43, -2.94)	-4.71±3.88 (-10.47, -2.32)	-6.02±5.67 (-12.15, -0.66)

Nutrient Fate

A key objective of this research was to examine nutrient allocation to liquid and solids phases of the waste stream. Table 6 details the effluent nutrient fate by species for each solid and liquid portion. Interestingly, 98% of macro-nutrients, Ca, K, Mg, P, S, and N and a micro-nutrient, Cl, were dissolved in the culture water. Conversely, a majority of B, Cu, Fe, Mn, and Zn were observed in the particulate fraction of the effluent stream.

Table 6. Summary of effluent nutrient mass fate for tilapia. Wastewater was filtered to 1.5-microns while waste solids were defined as anything captured on the 1.5-micron glass fiber filters.

	% of total wasted nutrients	
	Tilapia	
	Wastewater	Waste-solid
Micronutrients		
B	3%	97%
Cl	100%	0%
Cu	46%	54%
Fe	8%	92%
Mn	9%	91%
Mo	-	-
Zn	8%	92%
Macronutrients		
Ca	99%	1%
K	100%	0%
Mg	100%	0%
P	99%	1%
S	98%	2%
N	100%	0%

DISCUSSION

Aquafeed Protein Content

The preliminary study on the effects of protein content on nutrient production showed importance of tailoring aquafeed diets to the fish species. It was hypothesized that high protein feed would result in higher nutrient production than the commercial standard. While this was observed, it was also shown that the additional nutrient production was accompanied by poor

water quality. Low protein feeds performed as expected with reduced fish growth and nutrient production. The water quality in the low protein feed system was optimal with nearly undetectable levels of ammonia, nitrite, and nitrate. The drawback of this preliminary study was the lack of replication. Conducting nutrient production research under actual production conditions requires significant infrastructure. Initial plans to test three aquafeeds would require a 3x3 randomized complete block design to adequately compare nutrient production for three feeds. Experimental designs were limited to testing a single variable with three replications because the research facility can only hold three small commercial scale systems. In this regard, future research should use a minimum 3x3 randomized complete block experimental design to improve testing of multiple variables. Overall, the best option was to accept the nutrient production from the 40% protein commercial standard tilapia feed and begin a new study with one feed in replicated systems.

Pseudo-Steady State

Water quality measures including dissolved oxygen, pH, temperature, electrical conductivity, alkalinity, water usage, and feed rates were statistically similar between the three systems indicating that they were operating under pseudo-steady state conditions. High water recirculation, 93% reuse per day, in this experiment was attributed to strict feeding schedules and maintenance consistency during the 81-day study period. Feed rates and FCRs were comparable to commercial aquaculture production standards.

Feed Characterization

Feed nutrient characterization was consistent with the nutritional claims listed for the product. Zeigler Finfish Silver diets performed as expected with industry comparable FCRs.

During the 81-day study period, FCR averaged 1.3 meaning that it takes 1.3kg of feed for a fish to gain 1kg of mass.

Dietary protein influences the formation of feces. For example, high protein can lead to loosely formed feces (Moccia and Bevan, 2010). During this study, tilapia produced well-formed feces that were easily removed by the drum screen filter. These RAS were designed for tilapia production and therefore the water filtration, diet, and all other system specifications were tailored to their needs.

Nutrient Production

RAS nutrient production is a function of control exerted on an individual system. In this type of RAS during pseudo-steady state operation, nutrients are slowly and consistently released through drum filter backwash cycles. In this study, 93% daily water recirculation rates contributed to the observed accumulation of all measured nutrients. Throughout this study, tilapia assimilated most of the nutrients provided by the aquafeed. In this study, all nutrient production rates are representative only for systems of a similar design with comparable operating and maintenance routines. Negative nutrient production rates indicate a net loss of nutrients from the system. These negative values combined with large standard deviations indicate that ICP-OES may not be adequate for characterizing RAS nutrient production. Overall, the nutrient production observed in this study was consistent for two of the three systems. The nutrient production outlier saw a consistent release of nutrients throughout the 81-day study period. This indicated that the system either reached critical nutrient carrying capacity or that error is present from operational errors.

Nutrient Fate

Nutrient fate in this study revealed the system inputs and species effects on the physical state of RAS waste nutrients. Nearly all the macronutrients were dissolved in the wastewater and chlorine was the only micronutrient found mostly in the liquid portion. The remaining micronutrients, boron, copper, iron, manganese, and zinc were all sequestered in the solid wastes. Molybdenum was not presented because high sample variation and undetectable limits prevented further analysis. Nutrient fate is the direct result of combined effects of the water quality, chosen filters, operational procedures, feed, and fish species. Water quality parameters such as pH and temperature can affect the speciation of chemicals in the culture water therefore influencing the fate of nutrient solubility (Seawright et al., 1998; Schneider et al., 2005; Rakocy et al., 2006).

Challenges

Failure to maintain pseudo-steady state conditions throughout this experiment arose from multiple causes. Daily changes such as system water use, fish feed intake, fish stress, and equipment malfunction created problematic situations that negatively affected pseudo-steady-state conditions. Despite a replicated design, equipment malfunctions resulted in failure to meet pseudo-steady state conditions limiting the validity of the data. An equipment malfunction was realized during the experiment where the drum screen filter activation float switch was held in the “ON” position. This malfunction forced the drum screen to backwash the filter continuously overnight releasing more than 3,000 gallons of water from the system. This volume of makeup water diluted the nutrients and stressed the fish with the concurrent cool-water refilling the system.

Another problem with this study was the omission of nutrient data associated with the fishes' physiological processes during the experimental period. System wide calculations including whole fish starting and ending nutrient compositions were used to assess a general accounting of nutrients; however, fish nutrient uptake accounted for more nutrients than were supplied to the system. Whole fish samples, only taken at the start and end of the entire 176-day study period were later omitted because both samples were not representative of the 81-day pseudo-steady state period. After speaking with RAS researchers at Cornell University, the over-accounting of nutrients in fish was attributed to a phenomenon called the concentration effect.

The concentration effect is a phenomenon described as a disproportionate amount of nutrients that are expelled as waste versus the amount of nutrients in a fish's gut (Timmons et al., 2018). Measurements of expulsed nutrients in this study may also be skewed. The measured nutrients in the fish excrement exist in artificially higher percentages than in the gut leading to a misrepresentative sample (Timmons et al., 2018). The concentration effect is influenced by water quality and fish stress resulting in a variable effect in fish nutrient assimilation and release (Wendelaar, 1997; Sommerville et al., 2014). Aquafeed research often adds an indigestible marker to their feeds to calculate a concentration factor in the gut (Sarker et al., 2020). Under commercial production settings, specialized feed is manufactured by companies for each species and age class and can even be tailored to various types of systems (Sarker et al., 2016, 2018, 2020).

Conducting nutrient production research under actual production conditions requires significant infrastructure. This experimental design was ultimately limited because of the space and resource requirements. The limitation of replicate systems reduced the scope of the experiment. Initial plans to test three aquafeeds would require a 3x3 randomized complete block

design to adequately compare nutrient production for three feeds. Experimental designs were limited to testing a single variable with three replications because the research facility can only hold three small commercial scale systems. In this regard, future research should use a minimum 3x3 randomized complete block experimental design to improve testing of multiple variables.

CONCLUSIONS

Evaluating nutrient production in RAS at small-scale commercial production conditions reveals how RAS producers can analyze nutrient production to inform effluent treatment, system modification, and even profit from their waste stream. Complex biological systems that make up aquaculture systems are in a state of constant flux. The combination of each system can directly and indirectly affect the resultant effluent stream to make nutrient remediation easier. The inherent value of the nutrient rich aquaculture effluent has been overlooked and continues to cause problems with nutrient loading in the environment and costly treatment for aquaculture producers (Seawright et al., 1996; Mugg et al., 2007). In this study, lab- scale analyses of nutrient production were not representative of commercial RAS facilities and may not reflect true nutrient production levels.

This research supports the development of a nutrient mass balance strategy for the remediation and reuse of effluent by examining nutrient production at the small farm scale. By understanding what nutrients are produced and at what capacity, the larger implications of this research include the improvement of effluent treatment standards, potential cost savings, and the potential to convert a costly waste stream into revenue. This research supports the assumption that nutrient production and availability are dependent on operational standards and specific water quality conditions. Characterizing and quantifying aquaculture effluent as a potential nutrient source is most useful in the development of a nutrient mass balance method that

integrated-aquaculture farmers can apply to their own facilities. RAS are constantly changing and, to retain nutrients for optimal integration with hydroponics, systems must exert a high level of system control to minimize daily water usage to avoid dilution and loss of nutrients.

The nutrient fate discovered in this study indicates that further research on liberating nutrients from solid waste is needed. From this research, macro-nutrients, Ca, K, Mg, P, S, and N, and micro-nutrient, Cl were found to be sequestered the liquid portion of the waste with an immediate bioavailability. Alternatively, micro-nutrients, B, Cu, Fe, Mn, and Zn were retained in the solid portion of the waste and require liberation before they may be utilized. This reveals an opportunity for applying further waste treatment practices, to liberate and reintroduce reclaimed nutrients to a nutrient solution before reintegration in a hydroponic system. RAS already captures and treats nutrients, simply adapting waste treatment processes would allow for the storage and reuse of waste nutrients and therefore create an economically and environmentally friendly model of agricultural RAS production.

This study identifies a nutrient mass balance strategy for RAS producers by identifying key variables that influence nutrient production and retention in a system. Future research should include a feed tracer for better nutrient assimilation accounting. Solid nutrient reclamation should also be addressed to better assess the value of reusing these nutrients. Finally, studying the nutrient production of multiple commercial scale farms would contribute a better understanding of general RAS nutrient production.

CHARACTERIZATION AND QUANTIFICATION OF RECIRCULATING AQUACULTURE SYSTEM NUTRIENT PRODUCTION BY *ONCORHYNCHUS MYKISS*

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Keywords: Aquaculture, Recirculating Aquaculture Systems, Nutrient Mass Balance, Nutrient Production

INTRODUCTION

Recirculating Aquaculture Systems

While previous research suggests that Recirculating Aquaculture System (RAS) effluent nutrients are available as a fertilizer, physiological differences among aquaculture species are likely to influence the quantity and availability of those nutrients (Cho and Bureau, 2001). RAS can reuse 99% of its system water volume each day due to water treatment processes paired with fish production. Any combination of water treatment processes can be used to better suit an individual facility. The flexibility of RAS makes them an ideal choice for many intensive fish production operations. RAS are becoming more popular because they offer unprecedented levels of control and product consistency. However, the amount of waste requiring post-production treatment continues to increase. Aquaculture is the only animal agriculture sector which pay for on-site waste treatment, effectively increasing the costs of production and therefore market prices for consumers (Hochman et al., 2018; Tsani et al., 2018). Consumer demands for salmon require expensive protein rich feeds and produce nutrient rich effluent (Cho and Bureau, 2001).

The resultant waste stream from these water-efficient systems is a concentrated nutrient stream, which requires further treatment to mitigate environmental effects. In the United States, Environmental Protection Agency (EPA) wastewater discharge regulations require expensive and energy-intensive treatment to remove excess nutrients from aquaculture waste (Yeo et al., 2004; EPA, 2006; Somerville et al., 2014).

Rainbow Trout

Rainbow trout (*Oncorhynchus mykiss*) have become a major aquaculture species (FAO 2018). Rainbow trout were used in this study because of their value as a popular food fish, cold water requirements, and quick production cycles. Marketable rainbow trout can be grown in RAS within nine months. As a carnivorous species, rainbow trout require feed protein higher than 42%. Being a carnivore results in a shorter digestive tract and therefore inefficient carbohydrate usage. Diet and fish physiology greatly impact how feces are formed. Trout tend to produce pelleted feces. However, the protein rich feeds used in RAS can cause a diarrhea-like waste product.

Rainbow Trout Effluent

Under consistent operations, trout produce formed feces which are easily removed through physical filtration. Most of the ammonia (NH^3) produced by trout is directly released from the gills. Nutrient production rates for trout waste are not well established, however previous studies focused on nitrogen (N) and phosphorus (P) production have been modeled with variable results (Aubin et al., 2011).

RAS Effluent Utilization

RAS effluent is commonly applied to fields in terrestrial agriculture but remains a costly and inefficient practice due to the runoff potential (Yeo et al. 2004). RAS enables a waste

capture-and-utilization model by design, with many facilities utilizing on-site post-production treatment systems. Treated post-production RAS effluent has potential for reuse as a fertilizer in hydroponic systems. Previous waste-solids research suggests that the macro- and micro-nutrients in the captured solids from RAS meet or exceed nutrient profiles required for crop production (Guerdat et al., 2013). Terrestrial animal agriculture use practices which are designed to capture, treat, and reuse waste nutrients to offset operational costs and generate revenue (USDA NRCS, 2009). However, aquaculture does not have effluent utilization models as used in terrestrial agriculture. Research is needed to develop similar models for improving the economic viability of aquaculture (Yeo et al., 2004). This research supports a new model that utilizes a watery effluent in a water-based cropping system where the production medium, water, is the same.

Effects on Nutrients

Fish physiology and behavior are two major biotic drivers in RAS nutrient production. Differences in feeding strategies, diets, fish size, density, and behavioral factors drive RAS nutrient production (Cho and Bureau, 2001; Aubin et al., 2011). Rainbow trout, a carnivorous species, has shorter digestive tracts compared to an omnivore like tilapia (Sarker et al., 2018, 2020). Overall, physiological adaptations of fish differ between species and likely contribute to nutrient production. They also offer an opportunity to produce varied nutrient fertilizers simply by changing fish species (Seawright et al., 1998). Environmental factors affecting RAS nutrient production including, day length, light intensity, and water quality are directly linked to physical and chemical interactions that limit RAS nutrient production. Beyond physically stressing the fish, chemical properties of water, pH, Electrical Conductivity (EC), and temperature causes feces breakdown (Seawright et al., 1998).

This study was conducted to characterize and quantify rainbow trout RAS nutrient production. Both liquid and solid effluent fractions were characterized in terms of plant-required nutrients for the development of nutrient profiles relative to plant-availability.

MATERIALS AND METHODS

Facility Design

This study was conducted at the Anadromous Fish and Aquatic Invertebrate Research facility at the University of New Hampshire in Durham, New Hampshire, United States. The experimental design and fish protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of New Hampshire. Three replicate RAS were operated per intensive commercial aquaculture standards to investigate rainbow trout nutrient production under pseudo-steady-state system conditions.

Pseudo-Steady State

Pseudo-steady state was defined for this research as stable on-going operating conditions in a dynamic production environment. Fish production operations are dynamic since they consistently increase feed rates as fish continue growing. Water treatment also remains consistent yet dynamic as a function of changing feed rates and waste production. Pseudo-steady state determinations were supported using water quality data indicative of statistically similar system inputs and operation including feed rates, water usage, pH, dissolved oxygen (DO), temperature, EC, and alkalinity. During the pseudo-steady state study period, a mass balance was used to characterize nutrient production.

Nutrient Mass Balance

Nutrients were quantified using a mass balance analysis, characterizing and accounting for all nutrient inputs, sinks, and outputs. Nutrient inputs were defined as any nutrients entering

the system, nutrient sinks as any unit process accruing nutrients, and nutrient outputs as nutrient exports from the system (Schneider, 2005). Inputs included all system additions in this experiment including incoming well water, chemical additions for pH adjustment, and feed. Sinks consume or accrue nutrients in a system; in this case, culture water and fish both sequester nutrients. Outputs include any nutrients that otherwise left the systems, which, in this study included wastewater and waste-solids. For this study, inputs, sinks, and outputs terms in the mass balance were simplified into system nutrient gain or loss terms to generate an understanding of a system wide nutrient flow. The dynamic nature of RAS contributes to the overall accumulation or release of nutrients. Under ideal conditions, RAS will have a maximum nutrient carrying capacity that can be maintained without compromising fish heath and growth. Nutrient production in any RAS is ultimately limited by system design and specifications.

System Specifications:

Three RAS were built per industry production standards to a harvest capacity of 75 kg/production tank (150 kg/system) at a maximum stocking density at harvest of 72 kg m^{-3}. Each 4,800 L system is comprised of two fish culture tanks (1.5 m^3), one standpipe well, one drum screen filter (Hydrotech 501, Veiolia North America, Boston, MA), one Moving Bed Bio Reactor (MBBR), and one pumping sump tank (*Figure 2*). Forced air injection from a regenerative air blower (Sweetwater, S45, Pentair AES, Apopka, FL) injected air into the culture tanks through medium-pore stone diffusers. Biological filtration was achieved using an MBBR containing 0.7 m^3 of Kaldness K1 media. During the study, 98% of system water was retained and reused daily. This high recirculation rate facilitated nutrient retention in the system while producing a low volume and of concentrated effluent stream. Solid waste was removed from the system water using an inline rotary drum screen filter fitted with 40-micron screens (Hydrotech

501, Veiolia North America, Boston, MA). System water was used to remove solid wastes from the drum screen filter, creating a solid waste stream that was channeled out of the system for sampling and discharge. Systems were previously set up for warmwater tilapia production and were converted to cold water RAS production through purging with well water for one month while maintaining the biofilter with daily ammonium hydroxide additions. Chillers were added to maintain temperatures at 16 °C.

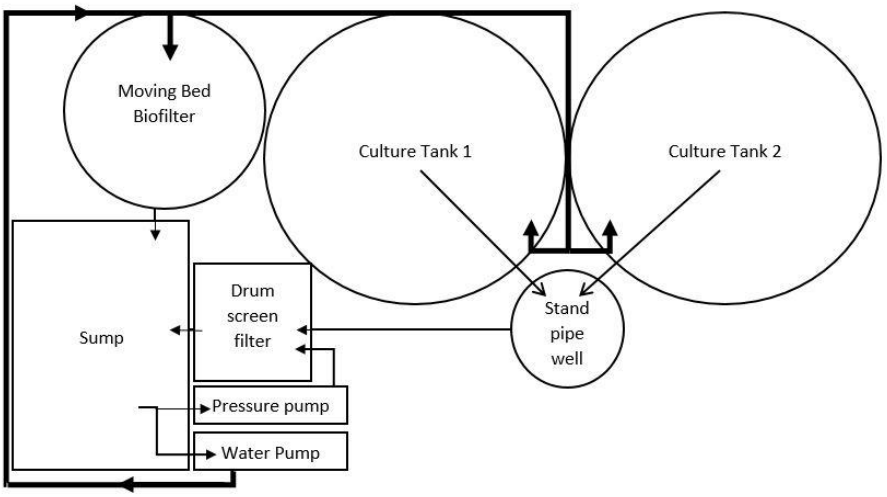

Figure 2. Research recirculating aquaculture system schematic and water flow.

System Water

System water volume was maintained using an auto-refill float valve supplied by well water and located in the pumping sump. Well water was filtered through a 5 µm floss filter as well as a carbon block filter to remove any nutrients and impurities. Water usage was monitored and recorded daily using an inline water meter (Flexible Axis Water Meter, Master Meter North America, Mansfield, TX) located immediately before each system's water refill valve. Flow rates to culture tanks were controlled using vertical injection manifolds with clear manometers used for visual verification of equal flow distribution between tanks. Culture tank flow rates were set

at 94.6 L min^{-1} to maximize solids removal from the culture tanks. The only additions to the system were well water, sodium bicarbonate and feed.

Chemical Additions

Sodium bicarbonate was added to each system daily to buffer pH and alkalinity. Sodium bicarbonate is a common RAS addition chosen for its affordability and high buffering capacity. Sodium, the constituent which would accumulate in the system and effluent, can also support fish health by bolstering slime coat production and mitigating harmful effects of unionized ammonia (NH^3) (Soderberg and Meade, 1993). Sodium bicarbonate only contributes to the accumulation of sodium in RAS; however, aquaculture feeds are the main source of nutrients that fish utilize, and RAS accumulate.

Aquafeed and Food Conversion Ratio

Aquafeeds are the main sources contributing to RAS nutrient accumulation and discharge. Fish received 5 mm Bio-Oregon Bio Trout feed (45% Protein, 24% Fat) throughout the study. Feed rates based on biomass were determined using a standard aquaculture feed rate guide provided by Bio-Oregon, OR, USA. Fish biomass was sampled biweekly using 10% of a tank population. Feed rates were adjusted for individual tanks based on the sampling. Lighting was applied 24 hrs day^{-1} to allow hourly feeding and therefore maximum fish production. Food Conversion Ratios (FCR) are commonly used in aquaculture to determine the efficiency of feed inputs versus fish growth. Low FCRs indicate high efficiency of fish production. Preferred FCRs for tilapia are 1.5 or less (Soderberg 1994). (FCR) were determined using the following equation:

$$F_{in} \div M_{gain} = FCR \quad (1)$$

where F_{in} is the total amount of feed added over the course of the experiment (kg), M_{gain} is the mass gained by the fish during the same time period (kg), and FCR is the Food Conversion Ratio, a unit-less factor used to measure the efficiency of aquaculture production.

Water Quality

Conventional aquaculture standards,

Table 7. Conventional aquaculture water quality standards for rainbow trout culture according to literature.

Table 7, were applied for the management of water quality parameters. Water quality was monitored and adjusted daily. Daily water quality analyses included dissolved oxygen, electrical conductivity, salinity, temperature, total ammoniacal nitrogen, and nitrite-nitrogen. Dissolved oxygen (DO), electrical conductivity (EC), and temperature were obtained using a portable

handheld meter (YSI Pro 2030, Yellow Springs Instruments, Ohio). Total ammoniacal nitrogen (TAN; sum of NH_3-N and NH_4^+-N), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) were analyzed using a HACH DR3900 spectrophotometer (HACH Methods: 8038 TAN, 8507 NO_2-N, 8507 NO_3-N, HACH Company, CO, USA) four times per week off-site at the UNH Agricultural Engineering Research and Analytical Lab (AERAL) to ensure fish health and proper system operation. Alkalinity and pH were measured using a bench-top meter (Fisher Scientific Accumet AB250, MA, USA). Total alkalinity was determined via sulfuric acid titration to a pH 4.80 end point (APHA, 2012).

Table 7. Conventional aquaculture water quality standards for rainbow trout culture according to literature.

Parameter	Rainbow Trout	Sources
Dissolved Oxygen (DO) mg/L	>6.0	Good et al., (2011)
pH	7-8	Good et al., (2011)
Electrical Conductivity (EC) µS/cm	>1.8	Good et al., (2011)
Alkalinity as $CaCO_3$ mg/L	5	Good et al., (2011)
Temperature (°C)	10-20	Good et al., (2011)
Ammonia Nitrogen (NH_3-N) mg/L	<1.0	Soderberg et al., (1983)
Nitrite Nitrogen (NO_2-N) mg/L	<0.23	Good et al., (2011)
Nitrate Nitrogen (NO_3-N) mg/L	150	Good et al., (2011)

Fish Stocking

Rainbow trout were obtained from Danaher Fishery (Shrewsbury, Vermont). Fish were received at an average mass of 100 g, quarantined for 6 weeks before transfer into the study systems. Ninety-one fish were initially stocked into each culture tank for a total of 182 fish in each study system. In the two weeks following the initial stocking, water quality declined and was identified as the cause of multiple mortalities in each system. Upon seeing a visible change in water clarity, turbidity was added to daily water quality monitoring procedures. During this two-week period, the three RAS maintained a 99% water recirculation rate leading to high total suspended solids (TSS) and potential nutrient toxicity. Previous rainbow trout RAS experiments found that exchanging water at a minimum of 0.26% of the total culture tank flow rate reduced accumulated nutrients and suspended solids, improving fish health (Good et al., 2009; Davidson et al. 2011). Implementing the low flow-through element to the experimental system design alleviated mortality events and stabilized water quality. Surviving fish stocks were reduced to 75

fish per tank and held for one month before the start of the study to stabilize pseudo-steady-state system operation.

Nutrient Mass Balance Sampling

Samples were collected biweekly over 183 days. Well water was collected directly from the building water line and feed was sampled via grab sample from each feed bag biweekly. Whole fish were sampled once at the beginning and again at the end of the experiment to quantify nutrient assimilation. Culture water samples were obtained from each system pumping sump reservoir after triple rinsing the bottles with the water to be sampled. Composite effluent samples (approximately 20 L of wastewater and waste solids combined) were collected from the drum screen filter effluent pipe for a period of one hour. All samples were preserved at 4 °C for 24 hours prior to analysis per APHA (2012) sample storage protocols for nitrogen analysis (Method 4500).

Sample Preparation

Measured volumes of wastewater samples were filtered using binder-free, glass microfiber filters (Whatman 934-AH) to separate the liquid and solid portions for independent analyses. Well water, culture water, and filtered wastewater liquid were sent directly for analysis, while solid waste and tissue samples were first dried, ground, and mixed prior to analysis. Total Suspended Solids (TSS) of wastewater samples were determined by APHA (2012) Method 2540D. Complete dryness was achieved when dry mass changes over a 24-hour period were less than 5% of the total sample mass (Method 2540D, APHA, 2012). The same drying methodology was applied to feed and whole fish samples before nutrient analysis. The system flow-through element was account through calculations of culture water nutrients as a consistent culture water stream leaving the system.

Nutrient Sample Analysis

Samples were analyzed for 13 plant macro- and micro-nutrients

Table 8. Samples were analyzed via inductively coupled plasma – optical emission spectrometry (ICP-OES) by a partnering laboratory (J.R. Peters, Inc. Laboratory, Allentown, PA, USA). ICP analysis of water, waste, and fish was conducted per EPA method 200.7 using a Prodigy XP ICP-OES (Teledyne Leeman Labs, Hudson, NH, USA) (EPA, 1994).

Table 8. Nutrient List

Macronutrients	Micronutrients
Calcium (Ca)	Boron (B)
Potassium (K)	Chlorine (Cl)
Magnesium (Mg)	Copper (Cu)
Nitrogen (N)	Iron (Fe)
Phosphorus (P)	Manganese (Mn)
Sulfur (S)	Molybdenum (Mo)
	Zinc (Zn)

Nutrient Mass Estimation

The following nutrient mass balance equation was adapted from a general mass balance equation as described by Timmons et al. (2018):

$$\text{Accumulation} = \text{Input} - \text{Output} + \text{Production} - \text{Consumption} \quad (2)$$

where Accumulation was defined as the nutrients accumulated in the culture water/system (mg), Input was the sum of all nutrient masses introduced into the culture system (e.g. incoming well water and feed) (mg), Output was the sum of all nutrient masses exiting the system (e.g. effluent stream, fish) (mg), Production was the sum of all nutrient masses produced within the culture

system (e.g. biofilm sloughing) (mg), and Consumption was the sum of all nutrient masses consumed within the culture system (e.g. nutrient assimilation into fish tissue) (mg).

All nutrient masses were calculated to reflect the total mass of each nutrient present for each sampling date then extrapolated between samples to calculate nutrient accrual over time. Daily nutrient production was estimated using the sample data and operational data collected between sampling dates (e.g. water consumption, feed rates, etc.).

Culture water, well water, and wastewater nutrient masses were calculated using the following equation:

$$C \times V = M_{NL} \quad (3)$$

where C was the nutrient concentration in the liquid samples (mg L^{-1}), V was the volume of water added or system volume (L), and M_{NL} was the mass of nutrients in liquid (mg).

Whole fish, feed, and waste nutrient masses were calculated using the following equation:

$$C \times M = M_{NS} \quad (4)$$

where C was the concentration of nutrient in the dry matter samples (mg kg^{-1}), M was the mass of the dry matter sample (mg), and M_{NS} was the mass of nutrients in the dry matter solid sample (mg).

Daily Nutrient Production Rates were determined by comparing the nutrient loads over the duration of the experiment as:

$$\Delta NL \div \Delta t = NPD \quad (5)$$

where ΔNL was the change in nutrient load between sample at two separate sampling periods (mg), Δt was the number of days between the two sampling periods, and NPD was the nutrient production per day (mg day^{-1}).

Statistical Analysis

All analyses were carried out using JMP Pro 14 software package (JMP Pro 14, 2018). MANOVA tests were used to compare independent variables (feed rates, water usage, alkalinity, and pH) then the dependent nutrient production variables between systems. Significance was indicated when p-value ≤ 0.05.

RESULTS

Pseudo-Steady State

Data were collected over 183 days. Pseudo-steady state operating conditions were achieved during the final 26 consecutive days of the study. Data from this 26-day period were used in the reporting of water quality, water usage, nutrient production rates and nutrient fate throughout this study. Daily water quality for all three systems is summarized in Table 9. The average concentration of dissolved oxygen between all three systems was 7.02 ± 0.56 mg L^{-1}. The average temperature was 15.90 ± 1.59 °C. Electrical conductivity (EC) averaged 0.47 ± 0.07 mS cm^{-1}. Salinity remained stable at 0.57 ± 0.08 mg L^{-1} during the experiment. Average pH was 7.67 ± 0.17 and alkalinity averaged 83.96 ± 10.83 mg L^{-1}.

The average water usage rate between all three systems during the RBT experiment was 981 ± 445 L day^{-1}. The recirculating aquaculture systems were operated with a 0.26% culture tank flow-through design to maintain adequate fish health during the study. While daily water usages were higher in systems 1 and 2 as compared to system 3, 1023.86 ± 295.83 L day^{-1}, 1152.36 ± 561.73 L day^{-1}, 767.34 ± 365.83 L day^{-1} respectively, pseudo-steady-state was achieved and nutrient production results were more conclusive than the previous experiment using tilapia.

Table 9. Summary statistics of rainbow trout water quality for each experimental recirculating aquaculture system. Statistics based on the pseudo-steady state period of 26-days.

	Mean ± standard deviation; (minimum, maximum)		
Parameter	RAS 1	RAS 2	RAS 3
Dissolved Oxygen (mg/L)	6.99±0.56 (5.92,7.94)	7.19±0.49 (6.20, 8.16)	6.89±0.60 (5.25, 7.89)
pH	7.68±0.19 (7.33, 8.02)	7.67±0.18 (7.37, 7.90)	7.67±0.16 (7.38, 8.00)
Temperature (°C)	17.17±1.86 (14.10,21.90)	15.32±1.01 (14.30, 18.80)	15.21±0.95 (14.30, 18.60)
Feed Rate (kg/day)	0.99±0.45 (0.00, 1.59)	1.12±0.56 (0.00, 1.95)	1.11±0.51 (0.00, 1.82)
Electrical Conductivity (mS/cm)	0.44±0.05 (0.35, 0.53)	0.48±0.08 (0.33, 0.59)	0.49±0.06 (0.40, 0.58)
Salinity (ppt)	0.52±0.06 (0.43, 0.602)	0.58±0.10 (0.41, 0.69)	0.60±0.07 (0.50, 0.60)
Alkalinity (mg/L as $CaCO_3$)	80.04±10.82 (56.00, 100.00)	87.20±9.67 (73.00, 103.00)	84.64±11.35 (63.00, 100.00)
Water Usage (LPD)	1023.86±295.83 (741.75, 1666.80)	1152.36±561.73 (603.86, 2219.15)	767.34±365.83 (371.69, 1440.08)

Feed Nutrient Characterization

During the 26-day pseudo-steady state period, the trout received an average daily feed rate of 1.07 ± 0.50 kg system^{-1} day^{-1} of Bio-Oregon Bio Trout feed (47% Protein, 24% Fat). Trout averaged 450.0 ± 32.2 g at the beginning of the pseudo-steady state period and grew to an average of 681.7 ± 66.2 g by the end of the study. FCRs during the 26-day period averaged 0.92 ± 0.07. Rainbow trout aquafeed nutrient profiles were determined during the 26-day study period to confirm nutrient inputs for each system. Average nutrient profiles on a dry mass basis for the experiment are shown in Table 10. Trout aquafeed total nitrogen was 73.50±3.51 g kg^{-1} dry mass.

Table 10. Summary for aquafeed nutrient characterization based on average nutrient concentrations of aquafeed throughout the experiment.

	Rainbow Trout Feed Bio-Oregon Bio-Trout (45% PC, 24%F)
Micronutrients (mg/kg)	
B	3.51 ± 1.56
Cu	13.09 ± 1.71
Fe	169.90 ± 18.78
Mn	34.19 ± 6.13
Mo	0.89 ± 0.11
Zn	151.55 ± 15.81
Macronutrients (g/kg)	
Ca	13.92 ± 3.33
K	5.23 ± 0.28
Mg	1.30 ± 0.07
P	9.55 ± 1.69
S	624.09 ± 95.27
TN	73.50 ± 3.51

Nutrient Production

Rainbow trout daily nutrient production rates are shown in Table 11. Daily micronutrient production was measured as follows, 706.29 ± 49.58 g per 100 kg feed Cl, 1.01 ± 0.04 g per 100 kg feed Cu, 13.41 ± 0.51 g per 100 kg feed Fe, 7.08 ± 0.71 g per 100 kg feed Mn, 3.11 ± 0.57 g per 100kg feed Mo, 11.95 ± 0.58 g per 100 kg feed Zn. Macronutrient production during this experiment averages were 2043.37 ± 29.18 g per 100 kg feed Ca, 659.48 ± 51.15 g per 100 kg feed K, 445.58 ± 7.61 g per 100 kg feed Mg, 690.11 ± 42.57 g per 100 kg feed P, 312.95 ± 45.59 g per 100 kg feed S, and 5729.49 ± 540.33 g per 100 kg feed TN.

Table 11. Summary statistics of rainbow trout average daily nutrient production rate for each system. Statistics based on the pseudo-steady state conditions during a 26-day period.

Micronutrients (g/100kg feed)	Mean ± Standard Deviation; (minimum, maximum)

	RAS 1	RAS 2	RAS 3
B	-0.70±0.69 (-1.44, -0.07)	-0.64±0.29 (-0.86, -0.30)	-0.46±0.98 (-1.59, 0.21)
Cl	838.33±113.88 (717.76, 944.06)	824.14±123.22 (712.57, 956.39)	960.88±110.82 (855.49, 1076.43)
Cu	1.19±0.44 (0.89, 1.70)	1.30±0.44 (1.00, 1.81)	1.28±0.47 (0.99, 1.82)
Fe	15.82±4.47 (10.91, 19.65)	17.37±3.51 (13.73, 20.74)	16.62±4.00 (12.62, 20.62)
Mn	8.68±4.40 (3.70, 12.05)	9.87±6.18 (3.18, 15.36)	7.74±4.26 (2.99, 11.23)
Mo	2.96±3.68 (-0.34, 6.94)	4.67±4.12 (2.13, 9.43)	3.92±2.93 (2.11, 7.30)
Zn	13.83±4.25 (10.95, 18.71)	15.54±3.85 (12.87, 19.95)	15.02±4.39 (12.28, 20.08)
Micronutrients (g/100kg feed)			
Ca	2490.33±351.82 (2144.23, 2847.61)	2577.68±319.07 (2377.30, 2945.62)	2521.66±338.50 (2294.70, 2910.73)
K	749.26±165.00 (617.52, 934.33)	798.87±252.89 (526.71, 1026.60)	901.34±252.06 (647.68, 1151.77)
Mg	541.01±49.95 (486.26, 584.10)	550.09±60.86 (484.05, 603.92)	563.92±63.77 (495.54, 621.78)
P	781.38±345.07 (504.60, 1168.00)	877.99±358.23 (561.87, 1267.09)	903.87±377.37 (580.12, 1318.32)
S	336.15±73.82 (261.56, 409.18)	360.15± 128.69 (215.16, 460.83)	466.08±135.08 (315.42, 576.40)
Total Nitrogen (*constituents)	6211.95±1867.30 (4507.92, 8208.08)	7237.43±2174.78 (4891.65, 9186.62)	7831.57±2699.31 (5008.74, 10387.52)
*NH4-N	50.42±35.94 (9.03, 73.63)	44.65±30.76 (10.14, 69.16)	47.06±28.12 (17.80, 73.89)
*NO3-N	-700.14±390.50 (-1143.52, -407.38)	-434.21±614.82 (-1140.82, -21.50)	427.63±901.57 (-610.16, 1017.70)
*Urea	0.17±5.27 (-5.38, 5.09)	-0.36±2.56 (-2.33, 2.54)	1.53±5.39 (-3.83, 6.94)

Nutrient Fate

Table 12 details the effluent nutrient fate for the solid and liquid portions of the waste. Macronutrients Ca, K, Mg, P, S, and N as well as micro-nutrients Cl and Mo were nearly fully solubilized in the liquid waste. The majority of micronutrients B, Cu, Fe, Mn, and Zn were observed in the particulate fraction of the effluent.

Table 12. Summary of effluent nutrient mass fate for rainbow trout. Wastewater was filtered to 1.5 µm while waste solids were defined as anything captured on the 1.5-micron glass fiber filters.

	Rainbow Trout	
	Wastewater	Waste-solid
Micronutrients		
B	8%	92%
Cl	100%	0%
Cu	18%	82%
Fe	22%	78%
Mn	7%	93%
Mo	100%	0%
Zn	4%	96%
Macronutrients		
Ca	98%	2%
K	100%	0%
Mg	100%	0%
P	95%	5%
S	100%	0%
N	99%	1%

DISCUSSION

Pseudo-Steady State

Daily water quality values, dissolved oxygen, temperature, electrical conductivity, salinity, pH, alkalinity, and water usage were non-significant from one another indicating that pseudo-steady-state conditions were achieved during the experiment. Feed rates and FCRs were comparable to commercial aquaculture production standards. Water usage in this experiment was higher than the previous experiment with tilapia likely because trout require a flow-through

system element. Rainbow trout have a low tolerance to the buildup of nutrients which is resolved through a water exchange at 0.26% of the culture tank flow rate (Davidson et al., 2011).

Feed Nutrient Characterization

Feed nutrient characterization was consistent with the nutritional claims listed for the product. The Bio Oregon Trout feed was an excellent performing feed regarding fish growth. The FCRs achieved during the final 26-day pseudo-steady state period were <1.0 indicating that less than 1kg of feed was required for 1.0 kg of fish growth. Rainbow trout are carnivorous, requiring higher protein content feed than omnivorous species like tilapia (Wilson and Halver, 1986).

High protein diets can lead to loosely formed feces (Moccia and Bevan, 2010). In this experiment, rainbow trout were found to have feces that were visibly more particulate than formed strands as normally seen in an aquaculture setting. Factors likely contributing to the high solubility of nutrients expelled by the trout include dietary and physiological traits. Feed constituent makeup can affect the nutrient dissipation and fecal pellet consistency. Physiological characteristics like stress responses can also negatively affect fecal pellet formation.

Nutrient Production

RAS nutrient production is a function of control exerted on an individual system. In this type of RAS during pseudo-steady state operation, nutrients are slowly and consistently released through drum filter backwash cycles. In this study, 98% daily water recirculation rates contributed to the observed accumulation of all measured nutrients. Trout tend to assimilate many of the nutrients provided in their diet. Individual nutrient production reported in this study are only representative for systems of similar setup, operational, maintenance routines. All nutrients, excluding boron, were accumulated over the course of the 26-day study period. Daily

boron production was not accurately quantified because many samples contained less boron than the detectable limit of the ICP-OES resulting in a zero or negative value. Negative nutrient production rates indicate a net loss of nutrients from the system. These negative values combined with large standard deviations indicate that ICP-OES may not be adequate for characterizing RAS nutrient production. This observation is consistent with early RAS nutrient production research

Nutrient Fate

This study observed nutrient fate through quantifying solid versus dissolved nutrients. Nearly all the macronutrients were dissolved in the wastewater, however, only two micronutrients, chlorine and molybdenum, were mostly solubilized. The remaining micronutrients, boron, copper, iron, manganese, and zinc were all sequestered in the solid wastes. This nutrient fate is the direct result of combined effects of the water quality, chosen filters, operational procedures, feed, and fish species. Water quality parameters like temperature, pH, and electrical conductivity affect chemical speciation which influences the availability or lack thereof for nutrients to plants (Seawright et al., 1998; Schneider et al., 2005; Rakocy et al., 2006). Previous research indicates that fish species and aquafeed also play a factor in the amount and solubility of nutrients through interactions with digestive enzymes, acids, and digestive tract length (Sarker et al. 2018; Sarker et al. 2020). Rainbow trout produced soft formed feces during this study which could be a major contributor to the high solubility of macronutrients. Additional binding agents could be added to the aquafeed to encourage better formed feces. This type of study gives producers a better idea of the effluent nutrients loads and their solubility. This information gives RAS producers the necessary information to better control their effluent stream through improved filtration and adapting aquafeeds.

Study Limitations

Failures to maintain pseudo-steady state conditions throughout this experiment arose from multiple causes. Daily changes such as system water use, fish feed intake, fish stress, and equipment malfunction all lead to problematic situations that negatively affected pseudo-steady-state conditions. In this experiment, all the previously mentioned factors existed throughout the entire 183-day data collection. These factors led to the omission of many of the early study data which lacked pseudo-steady-state conditions. At the beginning of the experiment, stressed fish refused feed and produced less feces. This was also problematic because the feed became waterlogged and stuck to the bottom of the standpipe well resulting in a slow dissolution of nutrients into the water. Equipment malfunctions also occurred during the experiment when the drum filter screens developed tears in the screen allowing solids to pass through the filter and remain in the system. Another mechanical problem experienced during the experiment was the sticking of the drum screen activation float valve which was stuck in the 'ON' position allowing the system to use more than 3,000 gal of water in one night severely diluting the system nutrients.

Another shortcoming of this study was the lack of an indigestible tracer in the feed. Through the inclusion of a tracer, this research would be able to confirm the full accounting of nutrient production based on the differential nutrient production and fish assimilation due to species specific effects. Individual effects of species on nutrient production are not well known, however a concentration effect has been described in previous aquafeed studies which contributes to proportion of fish nutrient assimilation versus excretion (Timmons et al., 2018). Measurements of expulsed nutrients in this study could be skewed. The measured nutrients in the

fish excrement exist in artificially higher percentages than in the gut leading to a misrepresentative sample (Timmons et al., 2018). The concentration effect is influenced by water quality and fish stress resulting in a variation of fish nutrient assimilation and release (Wendelaar, 1997; Sommerville et al., 2014). Aquafeed researchers often adds an indigestible marker to their feeds to calculate a concentration factor in the gut (Sarker et al., 2020). Under commercial production settings, specialized feed is manufactured by companies for each species and age class (Sarker et al., 2016, 2018, 2020).

Conducting nutrient production research under actual production conditions requires significant infrastructure. This experimental design was ultimately limited because of the space and resource requirements. The limitation of replicate systems reduced the scope of the experiment. Initial plans to test three aquafeeds would require a 3x3 randomized complete block design to adequately compare nutrient production for three feeds. Experimental designs were limited to testing a single variable with three replications because the research facility can only hold three small commercial scale systems. In this regard, future research should use a minimum 3x3 randomized complete block experimental design to improve testing of multiple variables.

CONCLUSIONS

Evaluating rainbow trout RAS nutrient production reveals how commercial farms can analyze their effluent streams and tailor water treatment for more effective remediation. In some instances, effluent can be treated for reintegration as a fertilizer for plants in a hydroponic system. The value of RAS effluent has not been realized or effectively utilized (Seawright et al., 1996; Mugg et al., 2007). The methods developed in this study to characterize nutrient production can offer RAS operations valuable insight on their own effluent streams. This would allow RAS producers an opportunity to exert more control on their systems and potentially profit

from their waste stream. Due to the dynamic nature of these systems, water reuse in the system is the single most important critical control point in nutrient retention.

From this research, macro-nutrients, Ca, K, Mg, P, S, and N, and micro-nutrients, Cl and Mo are soluble in the liquid waste. Alternately, micro-nutrients, B, Cu, Fe, Mn, and Zn retained in the solid portion of the waste require liberation before they may be utilized. This reveals an opportunity for applying further waste treatment practices, to liberate and reintroduce reclaimed nutrients to a nutrient solution before reintegration in a hydroponic system. RAS already captures and treats nutrients, simply adapting waste treatment processes would allow for the storage and reuse of waste nutrients and therefore an economically and environmentally friendly model of agricultural RAS production.

Overall, RAS shows the capacity to capture, treat, store, and reuse nutrients simultaneously replacing expensive economic and environmental costs with more profitable and environmentally sustainable options. Understanding the distribution of wasted nutrients between wastewater versus waste-solids allows treatment processes to become targeted for efficient treatment. This study lays the foundation of a nutrient mass balance strategy for RAS producers by identifying key variables that influence nutrient production and retention in a system. Future research should include a feed tracer for better nutrient assimilation accounting. Solid nutrient reclamation should also be addressed to better assess the value of reusing these nutrients. Finally, studying the nutrient production of multiple commercial scale farms would contribute a better understanding of general RAS nutrient production.

GRAND CONCLUSIONS

by

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INTRODUCTION

Recirculating Aquaculture Systems (RAS) technology can reduce environmental impacts by capturing and reusing nutrients from aquaculture wastewater. Nutrients produced in RAS can be integrated with hydroponics, water-based plant production. This integration would offset environmental impacts of wastewater discharge with a potentially profitable fertilizer asset. Reclaimed RAS nutrients could also be applied to terrestrial agriculture if new strategies are developed to dewater the nutrients before injection into soil. Understanding how nutrients are produced and move in RAS is necessary for determining the best treatment processes for nutrient reuse.

Aquafeed Nutrient Characterization

Aquafeed nutrient profiles act as inputs into the mass balance study and contribute to the differences in nutrient production between species. Average tilapia and trout feed nutrient concentrations are reported in Table 13 ; nutrient profiles were very similar for the feed used in the tilapia and rainbow trout studies. Boron, molybdenum, sulfur, calcium, potassium, magnesium, phosphorus, and total nitrogen concentrations were similar, while iron, sulfur, and manganese differed significantly between the two feeds. While the trout feed had higher protein content based on percent weight, total nitrogen was only slightly higher than the tilapia feed on a

dry weight basis (73.50±3.51 g kg^{-1} versus 64.74±2.81 g kg^{-1} respectively). Because trout don't use carbohydrates efficiently, their feeds are typically formulated with higher levels of protein and fat. One drawback of high protein diets is that up to 65% of the protein can be lost to the environment, with most of the nitrogen being excreted as ammonia (Craig et al., 2017).

Table 13. Summary statistics for aquafeed nutrient characterization for each the tilapia and rainbow trout feeds for a side-by-side comparison of basic nutrient inputs into a recirculating aquaculture system. Statistics based on the average nutrient concentrations of aquafeeds throughout each experiment.

	Tilapia Feed Zeigler Finfish Silver (40% PC, 10%F)	Rainbow Trout Feed Bio-Oregon Bio-Trout (45% PC, 24%F)
Micronutrients (mg/kg)		
B	5.63 ± 1.38	3.51 ± 1.56
Cu	13.83 ± 4.35	13.09 ± 1.71
Fe	62.35 ± 3.37	169.90 ± 18.78
Mn	3.04 ± 0.81	34.19 ± 6.13
Mo	0.33 ± 0.47	0.89 ± 0.11
Zn	46.12 ± 10.25	151.55 ± 15.81
Macronutrients (g/kg)		
Ca	17.06 ± 1.08	13.92 ± 3.33
K	8.66 ± 0.62	5.23 ± 0.28
Mg	1.86 ± 0.30	1.30 ± 0.07
P	13.54 ± 0.51	9.55 ± 1.69
S	514.00 ± 726.91	624.09 ± 95.27
TN	64.74 ± 2.81	73.50 3.51

Tilapia vs. Rainbow Trout Nutrient Production

Species effects can be observed when comparing daily nutrient production for tilapia and rainbow trout. Average daily nutrient production for each species is presented in Table 14. Rainbow trout and tilapia nutrient production were similar in mass for micro-nutrients and similar in proportion for macro-nutrients. Rainbow trout produced more nutrients on average even though higher system water usage likely diluted nutrients in that study. The maximum recorded total nitrogen (TN)production for tilapia, 6361.82 g per 100kg feed, was similar to the max TN production rate for rainbow trout, 6325.50 g per 100kg feed. This is an interesting

finding because the trout feed contained 45% protein while the tilapia feed contained only 40% protein. Aquafeed nutritional labels list protein contents as a minimum percent indicating that the 40% protein tilapia feed could at times contain a higher protein percentage than listed. The feed constituent analysis indicated that the trout feed was higher in nitrogen on a dry mass basis. However, the nutrient mass balance comparison indicates double the production of nitrogen for trout, 5729.49±540.33g per 100kg feed, as for tilapia, 2896.13 ± 4133.70 g per 100kg feed. The high variability seen in the tilapia nitrogen production data is due to excess water removal from equipment malfunctions during the study. Negative nutrient production rates indicate a net loss of nutrients from the system. These negative values combined with large standard deviations indicate that ICP-OES may not be adequate for characterizing RAS nutrient production.

Table 14. Summary statistics of average daily nutrient production by species. Statistics based on the pseudo-study state periods for each experiment, 81 days and 26 days for tilapia and rainbow trout respectively.

	Mean ± Standard Deviation; (minimum, maximum)	
Micronutrients (g per 100kg feed)	Tilapia	Rainbow Trout
B	-4.36±4.78 (-9.21, 2.15)	-0.48±0.08 (-0.56, -0.37)
Cl	-76.72±350.20 (-571.82, 181.32)	706.29±49.58 (665.65, 776.09)
Cu	3.39±0.55 (2.87, 4.16)	1.01±0.51 (12.77, 14.03)
Fe	10.78±1.90 (8.14, 12.56)	13.41±0.51 (12.77, 14.03)
Mn	5.61±1.78 (3.20, 7.43)	7.08±0.71 (6.25, 7.97)
Mo	0.23±0.08 (0.13, 0.32)	3.11±0.57 (2.39, 3.77)
Zn	7.26±0.89 (6.33, 8.47)	11.95±0.58 (11.17, 12.55)
Macronutrients (g per 100kg feed)		
Ca	1172.44±706.72 (235.33, 1941.91)	2043.37±29.18 (2011.42, 2081.97)
K	405.27±740.68 (-635.99, 1024.57)	659.48±51.15 (605.17, 728.01)
Mg	181.72±196.13 (-94.55, 341.17)	445.58±7.61 (436.97, 455.48)
P	704.34±582.04 (-97.64, 1265.91)	690.10±42.57 (631.12, 730.05)
S	-19.97±163.61 (-24.51, 140.66)	312.95±45.59 (271.50, 376.45)
Total Nitrogen (* Constituents)	2896.13±4133.70 (-2913.84, 6361.82)	5729.49±540.33 (5017.34, 6325.50)
*NH_4-N	10.06±29.54 (-23.50, 48.39)	38.27±1.91 (36.06, 40.73)
*NO_3-N	-1205.84±1286.35 (-2963.57, 79.03)	-190.27±388.79 (-565.50, 345.39)
*Urea	-5.55±0.59 (-6.02, -4.71)	0.36±0.64 (-0.29, 1.23)

Aquaculture Effluent Nutrient Fate

Nutrient production rates only resolve part of the problem; understanding where nutrients are concentrated informs whether they are directly available for reuse. Nutrient fate is important because treating water is easier and cheaper than treating solids (Mugg et al., 2007). Nutrients can be in either liquid or solid form. Table 15 details RAS effluent nutrient fate by species for solid and liquid portions. For both species, a minimum of 95% of macro-nutrients, (Ca, K, Mg, P, S, and N) and the micro-nutrient, Cl, were dissolved in the water. Most micro-nutrients (B, Cu, Fe, Mn, and Zn) were observed in the solid fraction of the effluent stream.

Table 15. Summary of effluent nutrient mass fate for both tilapia and trout. Wastewater was filtered to 1.5-microns; solids were defined as anything captured on the 1.5-micron glass fiber filters.

	Mean ± Standard Deviation; (minimum, maximum)			
	Tilapia		Trout	
	Wastewater	Waste-solid	Wastewater	Waste-solid
Micronutrients				
B	3%	97%	8%	92%
Cl	100%	0%	100%	0%
Cu	46%	54%	18%	82%
Fe	8%	92%	22%	78%
Mn	9%	91%	7%	93%
Mo	-	-	100%	0%
Zn	8%	92%	4%	96%
Macronutrients				
Ca	99%	1%	98%	2%
K	100%	0%	100%	0%
Mg	100%	0%	100%	0%
P	99%	1%	95%	5%
S	98%	2%	100%	0%
N	100%	0%	99%	1%

Water quality parameters such as pH and temperature can affect the speciation of chemicals in the culture water therefore influencing nutrient solubility (Seawright et al., 1998; Schneider et al., 2005; Rakocy et al., 2006). Sarker et al., (2018) found that fish also influence the quantity and physical state of a chemical through their digestive processes. Nutrients that are

most effectively utilized from diets are expected to become limiting nutrients in a naturally derived fertilizer solution. This shows how feeds can be adapted to fit not only the nutritional requirement for fish, but also any limited nutrients which would otherwise make aquaculture effluent an incomplete fertilizer for hydroponics.

Supplementing extra nutrients in aquafeeds are one viable option to improve the usability of effluent, but simply liberating the nutrients from the solid waste could be enough to produce a nutritionally complete plant fertilizer. Liberating nutrients from RAS solid waste could be achieved through the addition of further waste treatment processes like digestion. Every RAS facility and individual system can add or modify water treatment processes to improve nutrient reclamation. Simply adding the digestion process to the effluent stream produces a nutrient rich fertilizer that can be utilized in hydroponics rather than dispersed to natural bodies of water. The inherent value of the nutrient rich aquaculture effluent has been overlooked and continues to cause problems with nutrient loading in the environment and costly treatment for aquaculture producers (Seawright et al., 1996; Mugg et al., 2007).

CONCLUSIONS

This thesis demonstrates how to characterize RAS nutrient production, species effects on it, and critical points of control for RAS nutrient retention. The research supports the development of a new strategy for the remediation and reuse of RAS effluent by examining nutrient production at a small farm scale. Evaluating RAS nutrient production under small-scale farm conditions informs strategies to reduce environmental impacts and increase revenue simultaneously. These small farm-scale analyses of nutrient production reveal the impacts of fluctuations on small systems. This work can contribute to understanding what nutrients are produced and at what capacity while the larger implications of this research include the

improvement of effluent treatment standards and the potential to convert a costly waste stream into revenue. This research supports the assumption that nutrient production and availability are dependent on operational standards and specific water quality conditions. Characterizing and quantifying aquaculture effluent as a potential nutrient source is critical to the development of a nutrient reuse strategy.

From this research, macro-nutrients (Ca, K, Mg, P, S, and N), and micro-nutrients (Cl and Mo) were found in the liquid waste with an immediate availability to plants. Micro-nutrients (B, Cu, Fe, Mn, and Zn) were retained in the solid portion of the waste and would require liberation before they may be utilized. This reveals an opportunity for applying further waste treatment practices to liberate and reintroduce reclaimed nutrients to a nutrient solution before reintegration in a hydroponic system. RAS already captures and treats nutrients, simply adapting waste treatment processes would allow for the storage and reuse of waste nutrients and therefore an economically and environmentally friendly model of agricultural RAS production.

Overall, RAS shows the capacity to capture, treat, store, and reuse nutrients simultaneously replacing expensive economic and environmental costs with more profitable and environmentally sustainable options. Understanding the distribution of wasted nutrients between wastewater versus waste-solids allows treatment processes to become targeted for efficient remediation. This study lays the foundation of a nutrient mass balance strategy for RAS producers by identifying key variables that influence nutrient production and retention in a system. Future research should include a feed tracer for better nutrient assimilation accounting. Solid nutrient reclamation should be addressed to better assess the value of reusing these nutrients. Finally, studying the nutrient production of multiple commercial scale farms would contribute a better understanding of general RAS nutrient production.

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APPENDIX

Appendix 1. Tilapia IACUC Approval Letter

IACUC **IACUC**

University *of* New Hampshire
Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

29-Nov-2016

Guerdat, Todd C
Biological Sciences
Rudman Hall
Durham, NH 03824

IACUC #: 151207
Project: Applying Engineering Principles to Recirculating Aquaponic Systems (RAqs)
Approval Expiration Date: 26-Jan-2017
Protocol Three-Year Expiration Date: 26-Jan-2019

Institutional Animal Care and Use Committee (IACUC) Annual Review Form

Federal regulations require annual review of all approved projects involving vertebrate animal care/use. Accordingly, please supply the information requested below in questions 1-5, and return to the IACUC at the above address **at least 4 weeks prior to the study expiration date.** If you have any questions, please contact Dean Elder at 603/862-4629 or Julie Simpson at 603/862-2003. Thank you.

1. Is this project still active? If NO, please sign and return. Yes_X_ No___

2. Will there be modifications of procedures described and approved in the original application? Yes___ No_X_

   If YES, please specify, using additional paper if necessary. Modifications must be submitted to the IACUC for review and approval prior to implementation.

3. Animal Numbers (If your project involves non-target species/by-catch, please provide the information requested about these animals separately as an attachment):

SPECIES	CURRENT INVENTORY (live animals on-hand)	USED TO DATE (since start of project)	TOTAL # APPROVED (3-year total)
Tilapia (*Oreochromis spp.*)	900	950	4500

4. The IACUC is required to ensure that animal care personnel regularly engage in continuing education activities. Please identify the methods you used to provide continuing education to animal care personnel (including students) on this project during the past year (as applicable to the project).

___ Not applicable to this project.
___ No project personnel other than PI.
_X__ Direct supervision: PI works closely with personnel during procedures/activities involving animals.
_X__ Discuss and address issues relating to animal care and use during lab/staff meetings or regular

Appendix 2. Tilapia and Rainbow Trout IACUC Approval Letter

University *of* New Hampshire

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

17-Jan-2018

Guerdat, Todd C
Biological Sciences
Rudman Hall
Durham, NH 03824

IACUC #: 171201
Project: Applying Engineering Principles to Recirculating Aquaponic Systems (RAqS)
Approval Date: 14-Dec-2017

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under pain or distress category C - *the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics or other assessments.*

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

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

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

For the IACUC,

Jessica A. Bolker, Ph.D.
Chair

cc: File