Fall 2023

Sustainability Practices in Aquaculture: Using Algae Turf Scrubber Biomass to Raise Black Soldier Flies as an Alternative Feed in Blue Tilapia, Oreochromis aureus, Culture

Michelle C. Lowery

SUSTAINABILITY PRACTICES IN AQUACULTURE:

USING ALGAE TURF SCRUBBER BIOMASS TO RAISE BLACK SOLDIER FLIES AS AN ALTERNATIVE FEED IN BLUE TILAPIA, *OREOCHROMIS AUREUS*, CULTURE

by

MICHELLLE LOWERY

(under the direction of Anthony Siccardi)

ABSTRACT

Since the 1980s, non-algal aquaculture has grown to encompass 49% of all seafood production in response to a growing human population and increased seafood demand (FAO, 2022). Hurdles exist to aquaculture sustainability, including dependence on wild sourced fishmeal (FM) and the impacts wastewater discharge. It takes 4-5 tons of wild forage fish to produce one ton of dry FM (Miles and Chapman, 2006) and as aquaculture is primarily conducted in earthen ponds and public open water bodies (FAO, 2022), finfish culture can have a high impact on the surrounding environment by discharging excess nutrients. This study used algae turf scrubber (ATS) biomass grown on a 9.14-meter ATS placed at the outflow of a Statesboro wastewater treatment facility to raise black soldier fly (BSF), *Hermetia illucens*, as a FM replacement protein in blue tilapia diets. Five diets were formulated to include a 100% FM diet, 50% FM replacement, and 100% FM replacement with commercial feeds used as controls. These were fed to 125 juvenile Blue Tilapia stocked in 60.6-liter tanks in a randomized design over six weeks. Results showed no differences amongst the diets with respect to growth, survival, hepatosomatic index, visceral index, intraperitoneal fat, or muscle mass. Excess nutrients (nitrogen and phosphorus) from discharge waste can be recycled by ATS biomass converted into feed for blue tilapia using BSF larvae. This study demonstrated a possible sustainability solution in both aquaculture and wastewater management.

Index words: Algae turf scrubber, Waste management, Aquaculture, Black soldier flies, Fish meal, Productivity, Algae, Sustainability

SUSTAINABILITY PRACTICES IN AQUACULTURE:

USING ALGAE TURF SCRUBBER BIOMASS TO RAISE BLACK SOLDIER FLIES AS AN

ALTERNATIVE FEED IN BLUE TILAPIA, *OREOCHROMIS AUREUS*, CULTURE

by

MICHELLE LOWERY

BS, Georgia Southern University, 2010

MAT, Georgia Southern University, 2013

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial

Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE IN BIOLOGY

SUSTAINABILITY PRACTICES IN AQUACULTURE:

USING ALGAE TURF SCRUBBER BIOMASS TO RAISE BLACK SOLDIER FLIES AS AN ALTERNATIVE FEED IN BLUE TILAPIA, *OREOCHROMIS AUREUS*, CULTURE

by

MICHELLE LOWERY

Major Professor: Anthony Siccardi

Committee: Michele Guidone

Heather Joesting

Electronic Version Approved:

December 2023

ACKNOWLEDGMENTS

Completion of this thesis was made possible with the help and support of my thesis committee including Dr. Anthony Siccardi, Dr. Heather Joesting, Dr. Michelle Guidone, and the SARC facility curator Brigette Brinton. I would like to express my gratitude to the undergraduate students for their invaluable assistance during this research. A special thanks to my advisor, Dr. Anthony Siccardi, for his unwavering support, mentorship, and dedicated guidance throughout the entire process of researching and authoring this thesis.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

LIST OF ABBREVIATIONS ANS ACRONYMS

ADC	Apparent digestibility coefficient
AFDW	Ash free dry weight
ANOVA	Analysis of variance
AOAC	Association of Official Agricultural Chemists
ASP	Annual surplus production
ATS	Algae turf scrubber
BOD	Biological oxygen demand
BSF	Black soldier fly
CF	Condition factor
DW	Dry weight
EU	European Union
FAO	Food and Agricultural Organization of the United Nations
FCR	Feed conversion ratio
FER	Feed efficiency ratio
FM	Fish meal
HSI	Hepatosomatic index
IPF	Intraperitoneal fat ratio
MM	Muscle mass ratio
NOAA	National Oceanic and Atmospheric Administration
PER	Protein efficiency ratio
Q1	Quarter 1
Q2	Quarter 2

Q3	Quarter 3
Q4	Quarter 4
SARC	Sustainable Aquaponic Research Center
SS	Suspended solids
TAN	Total ammonia nitrogen
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
VSI	Viscerosomatic index

CHAPTER 1

INTRODUCTION

Purpose of Study

An ever-growing human population has placed a strain on wild seafood sources and water supplies. Eighty percent of the world's population live in areas where water security and river biodiversity are threatened (Vörösmarty et al., 2010). The purpose of wastewater treatment is to sanitize and discard effluent into the environment within governmental regulations, and to ensure the water can be reused (Salgot and Folch, 2018). Novel methods of wastewater treatment have been explored to reduce the increased presence of excess nutrients, microplastics, harmful compounds, and any other toxic substances in waste. At the same time, methods of food acquisition and production are consistently researched to meet a growing demand as well.

Global fisheries and aquaculture production is estimated to have reached a record 214 million tons in 2020. This was mostly attributed to aquaculture, which produced a record 122.6 million tons (FAO, 2022). Aquaculture (not including algae culture) accounted for 49% of the total seafood production, while 51% came from capture fisheries. Since the 1980s aquaculture has grown exponentially. Its growth was spurred by declines in wild fish populations, competitive prices, and increases in human population and seafood demand (Bostock et al., 2010; Naylor et al., 2021). Over time, aquaculture has been viewed as a means to increase sustainability in seafood production because it can reduce human reliance and impact on natural ecosystem population dynamics. Unfortunately, poor aquaculture practices and increased intensification have been responsible for mangrove habitat destruction, high nutrient discharge, introduction of nonnative species, and a continued reliance on fisheries resources, including fishmeal sourced from wild fish populations (Naylor et al., 2000; Martinez-Porchas and Martinez-Cordoza, 2012).

The purpose of this study was to investigate a sustainable method of wastewater treatment and fish production through aquaculture. An algae turf scrubber (ATS) was used as a method of municipal wastewater treatment in recycling excess nutrients (nitrogen and phosphorus) through the production of ATS biomass. The ATS biomass was then used to raise BSF larvae as a fishmeal replacement in the culture of blue tilapia. The goal was to demonstrate one method of increasing the sustainability of aquaculture and wastewater treatment through nutrient recapture and fishmeal replacement under practical conditions.

CHAPTER 2

A PILOT SCALE STUDY OF NUTRIENT EXTRACTION AND BIOMASS PRODUCTION FROM AN ALGAE TURF SCRUBBER RECEIVING MUNICIPAL WASTEWATER EFFLUENT.

Wastewater treatment occurs in two or three stages depending on the facility. Primary treatment is used to remove solids by physical means though sedimentation, filtration, coagulation, or floatation (Sonune and Ghate, 2004; Gupta et al., 2012). This process can remove up to 50% of the biochemical oxygen demand (BOD), 70% of the suspended solids (SS), and 65% of the oil and grease (Sonune and Ghate, 2004). Secondary treatment involves chemical treatment or biological filtration that uses aerobic, anaerobic and/or facultative bacteria (Sonune and Ghate, 2004; Gupta et al., 2012). The goal of this step is to catch any remaining suspended solids and organic compounds not removed during primary treatment. After secondary treatment up to 65% chemical oxygen demand (COD), up to 99% of microplastics, and 95% BOD and SS are removed from wastewater (Sonune and Ghate, 2004; Gupta et al., 2012; Carr et al, 2016; Gies et al., 2018). As primary and secondary treatment strategies used to treat wastewater primarily remove BOD, organic compounds, and suspended solids, inorganic compounds, such as heavy metals and nutrients, can persist in wastewater effluent without tertiary treatment.

Tertiary treatment encompasses any other added treatment process to remove residual suspended solids, organic, inorganic compounds, and toxic substances making the water safe for reuse or even human consumption (Sonune and Ghate, 2004; Gupta et al., 2012; Salgot and Folch, 2018). This step can remove heavy metals otherwise destined for the environment (Rahman et al., 2018) and often targets nutrients such as nitrogen and phosphorus, which can lead to eutrophic conditions downstream (Abdel-Raouf et al., 2012). Examples of tertiary treatment include an extra filtration step, an added clump formation step (coagulation and flocculation), disinfection, reverse osmosis, or even desalination if high in salinity. Unfortunately, tertiary water treatment can be very costly and result in increased sludge production (Abdel-Raouf et al., 2012; Bashar et al., 2018). As a result, tertiary treatment is not required in wastewater treatment. Much research in the last decades has been geared toward effective and sustainable wastewater treatment practices, including nature inspired treatments, such as biomass formation, lagoons, or constructed wetlands (Costa et al., 2000; Körner et al., 2003; Adey et al., 2013; Fan et al., 2023). Other strategies include oxidation, nanofiltration, solids absorption, and ion exchange (Crini and Lichtfouse, 2019). The focus for this research is on the use of algal biomass in the removal of excess nitrogen and phosphorus from treated wastewater effluent as a tertiary treatment strategy.

By 2050, nutrient discharge into the environment will increase by 10%–70% (Van Puijenbroek et al., 2019). Due to the consequences of wastewater discharge and government regulation, much research has been done on nutrient extraction by a variety of means. Water hyacinth ponds have been demonstrated able to remove up to 50% of organic loads from pig farm effluent, as well as total nitrogen and total phosphorus (Costa et al, 2000). Constructed wetlands are a minimal maintenance tool for wastewater treatment that have been demonstrated to be efficient at organics and suspended solids removal but inefficient at phosphorus removal, often requiring further treatment (Vymazal, 2010). Duckweed has been used as well, but it is subject to mortality at un-ionized ammonia concentrations of 1.0 mg/l and pH concentrations greater than 7.8 (Körner et al, 2003). In this study, total ammonia nitrogen (TAN), a combination of unionized and un-unionized ammonia, reached as high as 15.90 mg/l post-secondary wastewater treatment, a level of ammonia detrimental to duckweed.

Algae have been studied as a means of nitrogen and phosphorus extraction as well. For example, benthic algae, also known as periphyton, have been demonstrated to be capable of treating dairy farm effluent. However, despite significant nutrient uptake by the algae, elevated levels of nitrate may remain in the effluent. This has been attributed to the initially elevated levels of nitrogen in the waste and preferential assimilation of ammonium by the algae when present (Mulbry and Wilkie, 2002). Cyanobacteria have demonstrated promise in phytoremediation as well as facilitating the growth of certain crops, such as rice (Tripathi et al., 2008; Saadatnia et al. 2009), and leafy vegetables (Wuang et al. 2016) due to nitrogen fixation properties in supplying nutrients to the plants. However, the high temperature and excess nutrient loads favor the growth harmful cyanobacterial blooms, producing hepatotoxins and neurotoxins harmful to humans and any aquatic and terrestrial life (Jöhnk et al., 2008; Paerl and Huisman, 2009; AVMA, 2023). Various other algae, such as Ulva (Lawton et al., 2013) and diatoms (Li et al., 2017), have been shown to be effective in nutrient extraction as well.

Much nutrient removal research with algae has been targeted toward single species cultivation. However, maintaining a monoculture is energy intensive, subject to biological contamination, susceptible to aquatic consumers, and presents certain challenges of light penetration at commercial scale production (Shurin et al., 2013; Kazamia et al., 2014). The practical application of controlling the growth of one algal species is limited by seasonal temperature fluctuations, disease, nutrient requirements, competition, and crop interaction. Periphytic algal communities in shallow water vary by seasonality and macrophyte interaction (Mulbry et al., 2010; Adey et al., 2013). Rather than attempting to manage the growth of one algal species to remove nutrient content from wastewater, this study proposes using multiple species of self-seeded algae to increase sustainability.

The method used, called an algal turf scrubber (ATS), takes advantage of the properties of benthic algae utilizing a designated substrate as a controlled anchor for algal growth (Adey, et al., 1993). Algae turf scrubber biomass is a combination of micro and macroalgae, as well as micro-invertebrates, ciliates, bacteria, and more. Kangas et al., 2017 identified over 180 species and 7 phyla of algae on a Susquehanna River ATS, diatoms and green algae being the most dominant species. Such biodiversity promotes a balance of species, where interspecific competition, top-down herbaceous predation, and bottom-up variations in light and temperature can prevent any one species from dominating this benthic ecosystem for extended periods of time (Feminella and Hawkins, 1995; Hillebrand, 2005; Yang et al., 2009; Adey et al., 2013).

This self-sustained benthic ecosystem has been extensively studied as a form of bioremediation for over thirty years. It has been used to decrease ammonia, total carbon, nitrogen, and phosphorus in nutrient rich river systems, industrial wastewater treatment, dairy farms, and aquaculture facility effluent (Mulbry et al., 2008; Mulbry et al., 2010; Adey et al., 2011; Kangas et al., 2014; Ray et al., 2015; de Souza Celente et al., 2019; Gian et al., 2023). While decreasing excess nutrients, the ATS maintains healthy dissolved oxygen levels (Ray et al., 2015). Poor nutrient management leads to algal blooms, spikes in ammonia and nitrates, decreases in dissolved oxygen, and increased bacterial presence downstream from the site of effluent output (Abdel-Raouf et al., 2012; Wang et al., 2015). All of which can be lethal to aquatic life, reduce biodiversity, and diminish our dwindling drinking water supplies (de Jonge et al., 2002; de Souza Celente et al., 2019). Algae turf scrubbers are considered one of the most efficient treatment strategies for nonpoint source pollution in a watershed (Adey et al., 2013). The research presented is a pilot scale study using an ATS used to recover nutrients from municipal wastewater effluent. Though this is not a novel research objective, the goal of this study is determining the capacity of the ATS for nutrient recovery and biomass production of municipal effluent over the course of a year as a tool for sustainable wastewater treatment.

METHODS

ATS Collection and Processing

A 9.14 m^2 ATS was set up at the municipal wastewater treatment plant in Statesboro, GA as a means of tertiary treatment. Artificial turf grass was used as a 3D substrate and set at a 2% incline to maximize surface area for algal recruitment and the capture nutrients from wastewater effluent. A partial flow of wastewater was diverted to pass over the 0.31m wide ATS flowing continuously 24hr per day at a rate of 3.15 l/s. The ATS was set up outdoors with no cover or weather barrier at the treatment plant outflow. The wastewater facility already had a primary and secondary treatment setup; where primary treatment was in the form of settlement tanks and secondary treatment was an activated sludge system.

The data in this study was collected over the course of a year from the start of the study in July 2022 to the end in June 2023. ATS biomass was sampled weekly and analyzed for dry weight (DW) and percentage ash, from which ash free dry weight (AFDW) was calculated and used to determine productivity (Sluiter et al., 2005). Weekly water quality samples were collected at the end of the ATS and analyzed for available nitrogen (Hach method 8155 using salicylate, 10th edition) and phosphorus (Hach method 8048 for reactive phosphorus determination using ascorbic acid) using the HACH 3900. Sample collection was separated into four quarters of 12 weeks and composited for quarterly percent moisture (AOAC.930.15), crude protein (AOAC 990.03), crude fat (AOAC 920.39), and total phosphorus (digestion followed by inductively coupled plasma atomic emission spectroscopy; AOAC 990.08) analyzed by the New Jersey Feed

Laboratory Inc., NJ. June samples were not collected due to a period of interrupted water flow over the ATS at the wastewater treatment facility.

Phosphorus and nitrogen removal rates were calculated by multiplying quarterly percent phosphorus and nitrogen, respectively, with harvested algal mass (Kangas and Mulbry, 2014; Kangas et al., 2017). Nitrogen content was reverse calculated from crude protein by dividing by 6.25 based on the Kjeldahl method of protein determination used by the feed lab, AOAC 990.03, which analyzes the nitrogen content in the biomass and multiplies the result by 6.25. This is done on the basis that protein is made up of 16% nitrogen. This is currently considered the standard method of protein measurement in mixed samples (Mariotti et al., 2008; Chang and Zhang, 2017; Hayes, 2020). As the size of the ATS used was not sized to a scale that allow nutrient content removal from the wastewater effluent, recovery was determined based on biomass nitrogen and phosphorus content. Phosphorus has no gaseous state, so the sole source of phosphorus for the ATS biomass is the wastewater facility effluent. Nitrogen does have a gaseous state (the atmosphere is 78% nitrogen) which can be fixed by algae such as cyanobacteria. These blue-green algae have been observed in the ATS biomass. However, high nitrogen loads can suppress nitrogen fixing species of cyanobacteria (Donald et al., 2011; Posch et al., 2012). For the purposes of this study, it was assumed all nitrogen content in the biomass came from the wastewater effluent for nutrient recovery determination.

Statistics and Analysis

All calculations were performed using R-Studio (version 4.3.0; Posit Software, PBC). Descriptive statistics were used to summarize results, such as mean and standard deviation of yearly productivity, harvested mass, nutrient removal rates, and percentage ash; as well as analyzed trends in productivity per quarter and by season. As environmental conditions and nutrient removal rates were not normally distributed and smaller values were analyzed, Kendall rank correlation was used to investigate correlations between productivity, nutrient removal rates, and measured environmental conditions. All statistical analyses were checked for normality and variance to determine the correct statistical test for significance. A p-value <0.05 was considered significant (α). Seasons were used as a proxy for temperature data. Solstice seasons were used to define seasonal months as follows: March-May (Spring), June-August (Summer), September-November (Fall), and December-February (Winter).

RESULTS

On average, TAN discharge was at or above fish toxicity minimums (0.05 mg/l). The average TAN of the wastewater passing over the algae turf scrubber was 1.14 ± 3.30 mg/l (range = 0-15.90 mg/L). The average effluent phosphorus content was 0.89 ± 1.55 mg/l (range = 0.01-10.28 mg/L). Nitrogen recovery was 1.07 ± 0.44 g-N/m^2/d. Phosphorus recovery was 0.24 ± 0.09 g-P/m^2/day. There was no correlation between effluent nutrient concentrations and ATS biomass nutrient removal rates ($p>0.05$). There were also no seasonal or quarterly correlations between wastewater nutrient concentrations and nutrient removal rates (Figure 1.1).

Productivity, nutrient concentrations, nutrient recovery, and proximate composition per quarter are shown in Table 1.1. ATS biomass was high in ash throughout the 11-month trial and low in lipid concentrations. The mean productivity of the wastewater ATS was 13.83 ± 5.09g AFDW/m^2/day (Figure 1.2). Q1 had the highest productivity, 19.49 ± 4.10g AFDW/m^2/day, and Q3 had the lowest productivity, 10.27 ± 2.80 g AFDW/m^2/day. This followed seasonal variations with the highest productivity being in the summer and the lowest in the winter (Figure 1.2). There was no correlation between productivity and the effluent nutrient concentrations; TAN ($\tau = -0.15$, $p > 0.05$) or phosphorus ($\tau = -0.11$, $p > 0.05$). There was no seasonal or quarterly correlation between nutrient removal rates and effluent nutrient contents either ($p < 0.05$) (Figure 1.3). There was a positive correlation between productivity and nutrient removal rates; nitrogen ($\tau = 0.76$, $p < 0.05$) or phosphorus ($\tau = 0.78$, $p < 0.05$) (Figure 1.4).

DISCUSSION

The primary purpose of this study was to determine ATS productivity and nutrient removal from a Statesboro wastewater treatment facility. Mean productivity for the year was 13.83 g AFDW/m^2/day. By dry weight, this is 39.09 g/m^2/day. This is higher than the productivity of a pilot scale study done in central Florida, which had a productivity of 12 g/m^2/day and a similar nutrient recovery of 3% nitrogen and 0.75% phosphorus (Hydromentia, Inc., 2005) as compared to our findings of 2.67% nitrogen recovery and 0.6% phosphorus recovery. Productivity was comparable to a 22-month study performed on the Great Wicomico River in Chesapeake Bay, with a 3D substrate ATS, which had a productivity of 39.6 g/m^2/day (Adey et al., 2013) and another full-scale pilot study in Patterson, CA which had a productivity of 35 g/m^2/day with a nitrogen recovery of 3.1% and a phosphorus recovery of 2.1% (Craggs et al. 1996).

There was no correlation between effluent nutrient concentration and biomass nutrient removal rates. This may be because it has been shown that productivity varies seasonally, as it is affected by light and temperature (Hessen et al. 2002; Adey et al, 2013; Kangas et al., 2017). Removal rates and productivity had a positive correlation. Productivity tends to be higher in the summer and lower in the winter (Adey et al., 2013; Mulbry et al., 2010; Kangas et al., 2017). Seasonal analysis of productivity trends indicated productivity was highest in the summer months and lowest in the winter months for this study (fig. 1.3). This was also corroborated by quarterly productivity findings (Table 1.1). Q1, which encompassed summer months (including July and August), had the highest productivity and Q3, which mainly encompassed winter months (including January and February), had the lowest productivity. Productivity has also been attributed to nutrient load and variation in the algae composition (Mulbry et al., 2008; Mulbry et al., 2010; Wang and Lan 2011; Gan et al., 2023). This was described as seasonal species succession made possible by species adaptations, life cycles, and environmental tolerance (Kangas et al., 2017). In this study, there was no correlation between effluent nutrient concentrations and productivity. This may be a result of the small size of the ATS. At 9.14m^2, there was insufficient surface area to maximize nutrient uptake from a municipal wastewater treatment facility which may habitually receive high nutrient loads from city sewage. TAN averaged above fish toxicity minimums during this study.

This study saw a steadily high percent ash content year-round despite the wide variations in productivity seasonally and in some cases weekly. This may be due to the high diatom activity glimpsed in summer and fall samples consistent with findings by Li et al., 2017. Diatoms were found to be the most abundant algae in non-point source periphyton communities (Adey et al., 2013; Marella et al., 2019). Marella et al., 2019 were able to maintain 90% diatom activity on a wastewater ATS raceway. At 0.6%, low phosphorus recovery may be attributed to precipitation due to pH or algae composition. At a pH of 8-10, phosphorus tends to precipitate out of the water column, forming calcium hydroxyapatite (Adey et al., 2013). Variations in algae composition and species capacity for phosphorus removal can also affect percent phosphorus recovery (den Haan et al., 2016). Further analysis for the presence of this compound and algal composition would be necessary to determine if this is the case. Also, in the future, pH may need to be monitored in conjunction with phosphorus. Though the ATS biomass may not assimilate the phosphorus as nutrition in its precipitated form, it can serve as a temporary sink as it is deposited and held in the algal mat. With periodic harvest, precipitated phosphorus may be removed as well. Phosphorus waste is a critical issue because it can not only lead to eutrophic waters downstream (Conley et al, 2009), but it is a finite and expensive resource.

Algae turf scrubbers as tertiary treatment strategies present an opportunity for sustainable reuse of the absorbed excess limiting nutrients. In lieu of costly sludge management practices or less sustainable methods of disposal, such as incineration and disposal in landfills (Gude, 2015), the generated biomass could potentially be recycled as biofuel (Adey et al., 2011; Sandefur et al., 2011; Newby et al., 2016; Salama et al., 2017), compost (Mulbry et al., 2005; Biswas et al., 2017), livestock feed (Wilkie and Mulbry, 2002), or even concrete (Britt and Kangas, 2016). At an average fat content 0.51%, based on averaged quarterly composite samples, this biomass was fairly low in percent lipid concentration which is not ideal for biofuel production. High-rate algal ponds can produce algae with as high as 40% lipid content (Park and Craggs, 2011). Recycling the algae as compost or feed is an option. In aquaculture, between 30% and 85% of phosphorus is discharged as waste (Seawright et al., 1998; Shneider et al., 2005). An algae turf scrubber could be set up to capture the waste of a culture system, whether it be agricultural drainage, aquaculture effluent or farm runoff into a nearby waterway and the biomass could then be reused rather than treated as waste.

For aquaculture, one option would be to convert the ATS biomass into aquaculture feed. There have been many studies that have investigated the potential of algae as a fishmeal alternative that have shown positive results. It was shown to enhance disease immunity in pigs (Turner et al., 2002) and improve lipid metabolism in fish (Güroy et al., 2010). Mustafa et al., 1995 demonstrated that 5% fish meal replacement by macroalgae resulted in increased body weight, feed utilization, and muscle protein deposition in red sea bream fingerlings. Hussein et al., 2013 demonstrated that up to 50% corn gluten replacement by biofuel algae was beneficial to growth in Nile tilapia before adverse developmental effects were seen. There was no significant difference between 100% soybean meal and 10% replacement by defatted microalgae biomass in juvenile gilthead seabream growth (Pereira et al., 2020). Biswas et al., 2017 showed supplementing feed with periphyton resulted in a higher fish growth and percent survival of the striped, gray mullet, an omnivorous euryhaline species.

Through least-cost feed formulation, we were able to determine the percent fishmeal replacement of this wastewater produced ATS biomass in tilapia, *Oreochromis sp.*, feed using the proximate composition data presented in Table 1.2. Diet formulations suggest that this ATS biomass can replace up to 5% fishmeal, as a protein alternative, in a practical tilapia diet. Full replacement was hindered by the low protein concentrations and especially high ash content of the ATS biomass (Table 1.2). Single species cultivated algal colonies have been used to create feeds with up to 100% fishmeal replacement as a means of minimizing human impact on ocean resources (Olvera-Novoa et al., 1998; García-Ortega et al, 2016). There is still little research on the use of ATS biomass as feed, but this may be due to the reasons previously discussed for variation in composition and inconsistent productivity.

Common organic wastewater contaminants may be another reason for the lack of research, as they have been found in wastewater effluent, streams, and groundwater (Lapworth et al., 2012). These contaminants include flame retardants, plasticizers, nonprescription drugs, pesticides, insecticides, and antibiotics (Lapworth et al., 2012; Reddy and Lee, 2012). These are potential contaminants to fish feed with the use of municipal wastewater in generating ATS biomass. Heavy metals contamination is a concern because of the nonpoint source nature of municipal waste and the possibility of biomagnification. Heavy metals, such as mercury, copper, chromium, and cadmium can affect growth, development, cause cancer, and have a wide range of other health effects (Martin and Griswold, 2009; Mahurpawar, 2015). Algae has demonstrated the potential to bioaccumulate these metals in the short-term and long-term depending on the species removing them from the effluent discharged in the environment (Dwivedi, 2012). In this study, a composite sample from Q1 was analyzed for the presence of heavy metals (Table 1.3). Though none were found to accurately determine whether heavy metals have been present in the biomass for the duration of the study, all four quarterly composite samples would need to have been analyzed for heavy metal contamination.

Conclusion and Future Directions

The algae turf scrubber set up at the Statesboro wastewater treatment facility was efficient in the removal of nitrogen and phosphorus from the effluent water column. It had a yearly mean productivity and nutrient recovery comparable to previous pilot scale and full scale ATS studies in Maryland, Florida, and California with maintenance restricted to setup and weekly harvest. There has been exhaustive research on the merit of algae turf scrubbers as a form of bioremediation, but there are still roadblocks to commercial use and studies have focused on its potential for nutrient extraction. This study only used a partial flow of wastewater effluent over a small scale 9.14 m^2 ATS. Assessing the environmental impacts of the ATS would be an important step in assessing its sustainability. Data on the efficacy of a multilane or full-scale system receiving 100% of the wastewater effluent with environmental assessments before and after a designated period, including species impacts, is necessary.

Algae turf scrubbers have exhibited success as a secondary or tertiary treatment strategy, but it generates biomass, in addition to the sludge generated by the wastewater treatment process, that needs disposal. Currently, sludge management accounts for up to 65% of the total operating costs of a wastewater treatment plant in the United States (Gude, 2015). Continued research into successful ways of recycling the ATS is necessary if an ATS is to be used for sustainability purposes. The recycled nutrients must be reused or disposed of in a way that will not put them back into the environment. Understanding the impact of seasonal variation on productivity is important in understanding ATS limits in nutrient recovery and the amount of biomass that can be generated per square meter at any given time. The biomass generated can then be used as a desired product, rather than a byproduct requiring disposal. The next chapter will discuss a means of recycling this excess biowaste, in a way that may be more sustainable than some current sludge management practices, such as incineration, dumping in landfills (Gude, 2015), or even biofuel which results in up to 70% residual biomass post lipid extraction (Ward et al., 2014).

CHAPTER 3

FISHMEAL REPLACEMENT IN BLUE TILAPIA, OREOCHROMIS AUREUS, DIETS BY BLACK SOLDIER FLY LARVAE, HERMETIA ILLUCENS, RAISED USING ALGAE TURF SCRUBBER BIOMASS

In 2020, most fisheries catch was meant for human consumption, but 22 million tons of fish were designated for non-food uses, such as fish meal, fish oil, medical models, and aquariums (FAO, 2022). Fishmeal, a flour made from dried and ground fish, is used as an ingredient in feed because it is rich in polyunsaturated fatty acids and high in protein, ranging between 25% and 72% (Pike, 1999). It contains all the essential amino acids a cultured fish needs (Miles and Chapman, 2006), is highly digestible, and palatable making it a prime choice for fish feed in aquaculture. From an economic standpoint, fishmeal is currently priced at $1600 per ton (FAO, 2020). Though prices have fluctuated over time, they have remained between $1000-$2000 over the last decade, spending much of that time above $1500 per ton. By contrast, soybean meals fluctuate between $200 and $600 per ton. Because resources used to produce fishmeal still rely on traditional fisheries methods, it tends to be an expensive option despite being nutritionally replete.

Fishmeal is derived from small, pelagic wild-caught industrial grade fish, fisheries bycatch, and fish processing byproducts. Industrial grade fish refers to fish strictly used for non-human consumption purposes. However, 90% of fish considered industrial grade fish are food grade as well (Cashion et al., 2017). Bycatch refers to any undesired organism caught while targeting a specific species and fish processing byproducts are the entrails, bones, and any parts not used for consumption. In 2021, global fishmeal trade reached 3.67 million tons. This is a 12% increase from the previous year, 2020 (FAO, 2022). According to Miles and Chapman, 2006, it takes about 4-5 tons of fish to make 1 ton of dry fishmeal. If this is true, this means about 18.35 million tons of fish were used to make fishmeal in 2021. In addition to increased demand, industrial grade fish used in fishmeal, such as anchovies, may no longer be a consistent resource. Peru, the leading producer in fishmeal, generates 1/3 of the global production. In 2022, they had a 3.28 million ton catch quota, a 9% increase from the previous year but they did not meet that quota due to an increased presence of juveniles and severe weather (FAO, 2022).

When it comes to fisheries, it is important that demand for fish populations does not surpass the annual surplus production (ASP). This is the excess population size, when no fishing occurs, needed to maintain the same population size at the beginning of the next fishing season (Ricker, 1975). In other words, a negative ASP means individuals have been harvested from a population above the maximum sustainable yield. There would be insufficient stock for harvest and therefore a possible population decline in the long term. This can result from overfishing or other environmental conditions. Not only is future fishery production affected but this competes with environmental predators. Anchovies, herring, sardines, shad, menhaden, capelin, ocean perch, and whiting are all examples of fish intended for use in fish meal. Most of these fish have stable populations dedicated to fishmeal production, but some see increased demand due to competing human consumption. The Atlantic herring, *Clupea harengus*, is one such fish considered overfished and significantly below target population level (NOAA, 2022). These fish populations are a food source for ocean predators such as bass, cod, salmon, sharks, skates, seabirds, and marine mammals. Negative impacts to fish stock populations can have cascading effects in the marine biome (Pinnegar et al., 2000; Daskaloz, 2002; Möllmann et al., 2008; Baum and Worm, 2009).

Direct competition is not the only threat for predators. They are susceptible to bycatch during fishing hauls, as is the case with seabirds, dogfish, sharks, and marine mammals. In 2015, nearly 838 million pounds of bycatch was captured out of over 6.5 billion pounds of fish caught in the U.S. alone (Benaka et al., 2019). Though effort is made to return these organisms to the ocean, stress due to physical injury, air exposure, and temperature and pressure changes can lead to death onsite or post release (Davis and Olla, 2001; Ryer, 2002; Baker et al. 2013). Increased ocean traffic also leads to increased vessel strikes (Guzman et al., 2013; Thomas et al., 2016). These are especially an issue for endangered whale species and other endangered marine mammals (Elvira et al., 2021). According to Van der Hoop et al., 2013, 45% of the North Atlantic right whale mortalities from 1970-2010 were due to vessel strikes. Finding alternatives to fishmeal with equivalent or better benefits can help decrease marine traffic, reduce fishing efforts, and loosen human strain on the marine ecosystem.

Since 1990, fishmeal production has been declining, from nearly 8 million tons produced in 1990 to about 5 million tons in 2020 (FAO, 2022). Fishmeal is considered both environmentally and ecologically unsustainable. There are societal and economic pressures on the aquaculture industry to find alternative proteins (Egerton, et.al., 2020). For example, NOAA and the USDA put together an initiative in 2011 to reduce reliance on fishmeal and pursue alternative feeds in aquaculture. One result is that fishmeal use in salmon diets has dropped from an estimated 70% of salmon diets in 1980, to 25% in 2017 (Rust et al., 2011).

To reduce cost and increase sustainability, animal and plant-based alternatives to fishmeal have been investigated for aquaculture (New and Wijkström, 2002; Hodar et al., 2020). A few examples include poultry byproduct meal, blood meal, meat and bone meal, krill meal, insect protein, artemia biomass, soybean meal, corn gluten meal, seed meals, and algae (Kaushik et al., 2004; Henry et al., 2015; García-Ortega et al., 2016; Montoya-Camacho et al., 2018; Hodar et al., 2020; Sarker et al., 2020). There are basic criteria when considering commercially viable alternatives to fishmeal (Hodar et al., 2020). The first is that the replacement protein source needs to be cheaper to produce than fish meal and readily available. Secondly, the replacement protein source needs to have similar nutritional qualities as fish meal. This is to ensure that the organisms get their nutritional needs without affecting health and/or growth. The replacement protein also needs to be palatable and digestible. This is so the constructed feed gets ingested by the organism and the nutrients are absorbed by the digestive system, and not simply excreted.

This study proposes to use black soldier fly (BSF) larvae as a fishmeal alternative in blue tilapia feed and as a method of sustainably processing ATS biomass, thereby recycling wastewater nutrients. BSF have been extensively studied as livestock feed since the 1970s, a means of recycling organic waste since the 1990s, and as an alternative protein to fishmeal since the early 2000s (Newton et al., 2008). They are native to North America but can be found worldwide. They are a cheaper source of protein than fishmeal as they can be locally grown using waste, such as from a brewery, food remains (Adebayo et al., 2020), manure (Sheppard et al., 1994), as well as grains, chicken feed, and fruits (Adebayo et al., 2020). However, the feed source chosen for the BSF larvae has been shown to affect their body composition; including protein, fat, and ash content (St. Hilaire et al., 2007; Zheng et al., 2012). Adebayo et al., 2020 demonstrated that BSF larvae fed chicken feed had higher protein content than those fed fruit waste. Despite a variable protein range, it is possible to create a 100% fishmeal replacement feed with BSF larvae, making them nutritionally comparable to fishmeal (Belghit et al., 2019; Priyadarshana et al., 2021; Tippayadara et al., 2021). BSF larvae are also capable of a higher apparent digestibility coefficient than fishmeal in Nile Tilapia, *Oreochromis niloticus* (Tippayadara et al., 2021) and red hybrid tilapia (Muin and Taufek, 2024).

In addition to satisfying the basic criteria for fishmeal replacement in commercial feed, BSF must be able to process nutrients and survive in unfavorable conditions, which may result from using the ATS biomass grown in municipal wastewater as feed. Experiments with livestock have demonstrated that BSF larvae are able to remove up to 80.5% of total nitrogen and 75.7% of phosphorus from swine and cow manure (Williams et al., 2006; Myers et al., 2008; Newton et al., 2008). Seaweed has been used to replace up to 50% plant-based feeds in BSF larvae before growth, nutrient retention and lipid levels were affected (Liland et al., 2017). Zhang et al., 2022 demonstrated that BSF larvae are capable of breaking down 62% of mycrocystins toxins unaided and 82% with supplemented antioxidants. They exhibit a high tolerance for aflatoxin B1, a fungal mycotoxin, able process 0.5 mg/kg of feed and stay below detection limits by European Union (EU) standards of 0.02 mg/kg (Bosch et al., 2017). BSF are considered a sustainable, cost-effective food staple in raising livestock due to their ability to convert organic waste into high quality protein. Though there have been several studies on growth optimization of BSF larvae and fishmeal replacement, no study has used wastewater treatment algae turf scrubber (ATS) biomass to raise the BSF larvae as feed for blue tilapia, *Oreochromis aureus*. This study evaluated the effects of using BSF larvae, raised on sustainably sourced ATS biomass, to replace fishmeal in blue tilapia feed. The study assessed effects on growth, health, survival, body composition, and feed protein digestibility.

METHODS

Black Soldier Fly Larvae Collection and Processing

Forty thousand BSF larvae, *Hermetia illucens*, were purchased at 0.32 cm in size and stored in two mesh ventilated clear bins, measuring 49.53 cm wide, 104.14 cm in length, and 14 cm in depth. The container was partially filled with hay with a depth of 5-8 cm. 20,000 BSF larvae were placed in each container. BSF larvae were maintained in a climate-controlled shed, bin lid closed, at a room temperature of 76°F. ATS biomass from an experimental algae turf scrubber set up at the Statesboro wastewater treatment facility (Chapter 1) was used to feed the BSF larvae weekly. ATS biomass was scraped once a week and directly fed to the BSF larvae the next day without further processing (Figure 2.1). Due to the distance between the ATS site in Statesboro, GA and the larva habitat in Savannah, GA, ATS biomass samples were stored in an open bucket on ice overnight prior to BSF feeding the next day.

BSF larvae have multiple larval stages prior to metamorphosis. The stage with the highest protein content is the prepupae stage (Larouche, 2019). The larvae were grown in their food source, the ATS biomass, in addition to the hay which was used to prevent the larvae from drowning. As feed was consumed and defecated by the BSF larvae, substrate humidity increased. The larvae have a behavioral adaptation, whereby they migrate to dry areas in preparation for metamorphosis during the prepupae stage (Diener et al., 2011; Larouche, 2019). Relying on this behavioral migration is known as self-collection or self-harvesting (Sheppard et al., 1994; Diener et al., 2011; Rana et al., 2015). This stage can be identified by its brown coloration (Figure 2.1). In addition to migration, the larvae formed their pupae within the created habitat by surrounding themselves in frass. For this reason, the prepupae BSF were separated from the substrate manually as well by weekly sifting for prepupae after 8 weeks. BSF larvae were fully harvested within 5 months by a combination of self- harvesting and periodic sifting. Effort was made to collect prepupae larvae for use in the feed trial as determined by behavior, color change, and size (Larouche, 2019). Larvae collected from any other life stage were returned to the habitat.

The BSF larvae were rinsed upon collection, washed, weighed, and frozen for future processing. Upon thawing, larvae were washed again, bleached, and all debris removed. The larvae were placed on an aluminum tray and photographed with a ruler for future analysis length and width using image J. A sample size of 100 larvae were used to estimate BSF population length and width. After photographs were taken, BSF larvae were oven dried at 50°C for 48hrs. They were then ground-up in preparation for cold extrusion into feed pellets. A 60g sample of the feed was ground and homogenized for proximate composition analysis; including percent moisture (AOAC.930.15), crude protein (AOAC 990.03), crude fat (AOAC 920.39), ash (AOAC 942.05), and crude fiber (AOAC 978.10) by the New Jersey Feed Laboratory Inc., NJ (Table 2.1). The amino acid profile of BSF larvae was determined by high performance liquid chromatography(AOAC 994.12/985.28) by the New Jersey Feed Laboratory Inc., NJ. The commercial control BSF used as a control was analyzed for proximate composition as well. Results of this analysis were used to formulate Tilapia feed using Agri-Data Systems Pro 5 (version 2.41, Agri-Data Systems).

Feed Preparation

Four tilapia diets were cold extruded using a commercially optimized diet adopted from Nguyen et al., 2009 (Table 2.2). The diets were 100% fishmeal (FM), 50% FM replacement (50% BSF), 100% FM replacement (100% BSF), and 100% commercial BSF, which used commercially raised BSF larvae from Grubbly Farms. The purpose of the commercial BSF was to compare

BSF raised on ATS biomass to commercially available oven dried BSF larvae. One commercial control feed (Purina Aquamax) using fishmeal as its protein source was purchased as a control for the extruded FM diet, to ensure proper preparation of the extruded FM feed.

The feed ingredients were ground, weighed, and then homogenized in a Hobart mixer (Hobart Inc.) for 10 min. Water and alginate were added to produce a paste viable for extrusion. All diets were cold extruded using a KitchenAid® food grinder and mixer with the 3mm die. Feed was then air dried at room temperature with the aid of a fan for 72 hrs and stored in zip lock bags until fed to the tilapia. Feed percent moisture ranged from 9.75% - 9.91%. Experimental diets were analyzed for percent moisture (AOAC.930.15), crude protein (AOAC 990.03), crude fat (AOAC 920.39), ash (AOAC 942.05), and crude fiber (AOAC 978.10) by the New Jersey Feed Lab Inc., NJ. Diets were formulated to include 34% protein and 5.41% fat. The commercial control feed (Purina Aquamax) was pond fish feed designed for catfish and tilapia with 32% protein and 3% fat. 0.55% methionine was added to all extruded diets except the 100% fishmeal diet. The menhaden fish meal already contained 0.52% methionine. Proximate analyses for all diets used in the feed trial are presented in Table 2.3.

Feed Trial

An *in vivo* six-week feed trial was performed to assess the effects of using BSF larvae raised on ATS biomass to replace fish meal in tilapia diets. The trial was conducted at the Georgia Southern Sustainable Aquaponic Research Center (SARC) using blue tilapia. The study was performed in a randomized design with five treatments, five replicate tanks per treatment and six juvenile blue tilapia per tank.

The juvenile blue tilapia were stocked in a system of 32 individual 60.6-liter polyethylene tanks connected by a recirculating water filtration system. The system had a water recirculation rate of

1.9 lpm per tank and a 651 L sump. The water temperature was maintained at 78° F ± 2° using a chiller and heater (Aqua Logic) with a room light/dark cycle of 12:12 hrs. The tanks were aerated by forcing a high-water flow rate through a narrow water dispensing nozzle, creating localized water turbulence. The system had a 40-watt UV sterilizer (Pentair Aquatic Ecosystems) and sand filter filled with 0.085 cubic meters of Perma Beads (Advanced Aquaculture Systems Inc.) for water treatment by disinfection and filtration.

The initial combined fish weight per tank averaged 35.92 ± 1.78 g with no significant differences among tanks (χ^2_4=5.38, $p > 0.05$). Tilapia were fed twice a day at 3% body weight. Feed was adjusted weekly based on the monitored weekly growth rate of randomly selected tanks. All fish, from three randomly chosen tanks, were captured with a nylon net, and transferred to a tared, water-filled container for weight determination. After the weight was recorded, the fish were returned to their respective tanks. No tank was selected twice for the duration of the study to minimize fish stress. The average weight of all captured fish was used to represent the average weight of all fish in the system. During the second and third weeks, there was no growth observed based on the randomly chosen tanks. Since all tanks consumed their diets within 15 mins of feeding, an assumed 1% growth was used to minimize the chance of insufficient feed affecting growth. Water quality parameters, including ammonia (Nitrogen, Ammonia; Method 8155), nitrite (USEPA Diazotization Method; Method 8507), and nitrate (Cadmium reduction method, Method 8039) were sampled weekly and analyzed the same day as sampled (Hach 3900). Samples were taken from randomly selected tanks within the recirculating system. Temperature, pH, dissolved oxygen, and turbidity were measured weekly as well (U-50 Series, Horriba multimeter).

At the termination of the feeding trial, all fish were weighed, measured for total body length, and photographed. Total weight gain, estimated protein efficiency ratio (PER), feed conversion ratio (FCR), feed efficiency ratio (FER), and percent survival were calculated.

Calculations were as follows:

$$PER = \frac{g\ weight\ gained}{g\ total\ protein\ ingested}$$

$$FCR = \frac{g\ feed\ given}{g\ animal\ weight\ gained}$$

$$FER = \frac{g\ weight\ gained}{g\ dry\ feed\ offered}$$

One fish per tank (five per diet) was randomly selected for analysis of proximate body composition, including percent moisture (AOAC.930.15), crude protein (AOAC 990.03), crude fat (AOAC 920.39)(Wendt Thiex, 2023), and ash (AOAC 942.05) by the New Jersey Feed Lab Inc., NJ. Due to insufficient dry weight (DW) for analysis, fish samples were composited by diet (two tanks combined and homogenized), so that two samples per diet were analyzed for proximate body composition. One fish per tank (five per diet) was dissected and analyzed for the following biometric parameters: hepatosomatic index (HSI), intraperitoneal fat ratio (IPF), visceral somatic index (VSI), muscle mass ratio (MM), and condition factor (CF).

Calculation were as follows:

$$HSI = 100 \times \left(\frac{g\ liver\ weight}{g\ body\ weight}\right)$$

$$IPF = 100 \times \left(\frac{g\ IPF\ weight}{g\ body\ weight}\right)$$

$$VSI = 100 \times \left(\frac{g\ viscera\ weight}{g\ body\ weight}\right)$$

$$MM = 100 \times \left(\frac{g\ fillet\ weight}{g\ body\ weight}\right)$$

$$CF = 100 \times \left(\frac{g\ body\ weight}{cm^3\ total\ length}\right)$$

Digestibility

An in vivo digestibility trial was performed to assess the apparent digestibility of crude protein in blue tilapia for two feed types and a control. Treatment feeds were comprised of 70% the control diet and 30% the test ingredients (fish meal and BSF meal) with 1% chromium oxide added as an inert marker. The trial was conducted in the 32-tank recirculating system at the SARC facility. Three days were allotted between the feed trial and the digestibility trial for digest feed acclimation and fish de-stress. After feed trial measurements, fish were returned to their respective tanks and tank selection for each digestibility feed was randomized with no further movement or manipulation to the fish. A randomized design was used to determine feed and protein digestibility, including three dietary treatments (one control, one fish meal, and one BSF meal) and eight replicates (each containing from 3-5 juvenile blue tilapia). As with the feed trial, temperature was monitored daily, but all water quality parameters continued to be recorded weekly.

The fish were fed based on 4% body weight twice daily for 7 days. This was increased from 3% during the feed trial in an effort to maximize fecal output to acquire sufficient fecal samples dry weight for analyses. Fecal samples were collected by siphoning the system 1 hour after every feed. Any unconsumed feed was removed by siphoning prior to siphoning the feces. If separation could not be performed, the siphoned material was not used as part of the pooled sample. Fecal samples were immediately oven dried at 50°C for 24hrs and stored at room temperature in zip lock bags. At the end of the trial, three samples per diet were analyzed for protein (AOAC 990.03) and chromium (digestion followed by inductively coupled plasma atomic emission spectroscopy; AOAC 990.08) by the New Jersey Feed Lab Inc., NJ. The apparent digestibility coefficient (ADC) for the diets was calculated using chromium oxide as the indicator and protein as the nutrient (Pond et al., 1995). The ADC for protein was calculated using 70:30 ratio of reference diet to treatment ingredient (Cho et al., 1982). The equations are provided below:

$$\text{Diet ADC (\%)} = 100 - \left[100 \times \left\{\left(\frac{\text{\% diet indicator}}{\text{\% feces indicator}}\right) \times \left(\frac{\text{\% nutrient in feces}}{\text{\%}\ nutrient\ in\ diet}\right)\right\}\right]$$

$$\text{Protein ADC (\%)} = \left(\frac{100}{30}\right) \times \left[treatment\ ADC\ - \left\{\left(\frac{70}{100}\right) \times \text{reference ADC}\right\}\right]$$

Statistics and Analysis

Statistical analyses were performed using R-Studio (version 4.3.0; Posit Software, PBC). Descriptive statistics were used to summarize trial results, such as mean and standard deviation of growth table summaries, health indices, BSF lengths, and environmental parameters. All variables were analyzed based on the mean value per tank. Parametric data were analyzed using one-way ANOVA to determine if there were significant differences ($p < 0.05$) among diets. Tukey-Kramer was used to investigate the differences among treatment means. Kruskal-Wallis was used to determine significant differences in nonparametric data. No significant differences needed to be investigated for nonparametric data. All statistical analyses were checked for normality using the Shapiro-Wilk test and confirmed visually using a normal quantile plot.

Levene's test was used for variance homogeneity determination. Significance was determined based on a p-value < 0.05. A Mann Whitney U test was used to compare ATS biomass raised BSF larvae to commercial larvae length and width.

RESULTS

Black Soldier Flies

In this study, the BSF larvae were harvested within 5 months. The BSF began showing signs of maturing after 8 weeks with few prepupae stage larvae being harvested. A total of 11g DW was collected over the first month of harvest. After that, in 2 weeks, 62.93 g DW of larvae was collected and this trend continued until the end of the study. It was observed that the greatest batches of maturing larvae occurred between 3 and 4 months of the habitat set up. Out of 100 prepupae larvae measured, the ATS biomass raised BSF averaged 1.68 ± 0.22 cm in length and 0.39 ± 0.07 cm in width. The commercial BSF larvae averaged 1.81 ± 0.35 cm in length and 0.36 ± 0.11 cm in width. The commercial BSF larvae had larger prepupae than the BSF raised on ATS biomass in both length ($W = 3969$, $p < 0.05$) and width ($W=5953$, $p < 0.05$). However, the heteroscedasticity of the samples suggests this could be a random occurrence.

Feed Trial

All descriptive variables are presented as mean concentration over the six-week period ± standard deviation. The weekly randomized water temperature checks averaged 25.4°C ± 1.15, dissolved oxygen averaged 11 ± 0.73 mg/l, pH averaged 8.15 ± 0.19, and turbidity averaged 2.04 ± 1.04 NTU. Average ammonia was 0.03 ± 0.02 mg/l, nitrite was 0.001 ± 0.001 mg/l, and nitrate was 1.55 ± 0.50 mg/l. Environmental parameters for tilapia growth were within optimum growth conditions (Bolorundoro and Abdullah, 1996; Riche and Garling, 2003; Abd El-Hack et al., 2022).

A summary of growth performance and feed utilization are presented in Table 2.4, including initial weight, final weight, growth, survival, FCR, PER, FER, and CF. All diets had a 100% survival rate. For all diets, irrespective of tank grouping, final weights ranged between 1.94g and 56.09g and averaged 19.11 ± 11.33g. Growth averaged 13.24 ± 3.20 g. There were no significant differences in feed utilization among diets; FCR ($F_{4,20} = 2.42$, $p > 0.05$), FER ($F_{4,20} = 0.52$, $p > 0.05$), or PER ($F_{4,20} = 2.43$, $p > 0.05$). The CF, a measure of the well-being of the tilapia, showed no major differences across the diets either ($F_{4,20} = 0.85$, $p > 0.05$). There was no observable difference in the consumption of feed across the diets and no diet significantly affected tilapia weight gain; final weight ($F_{4,20} = 2.76$, $p > 0.05$) or growth ($F_{4,20} = 1.32$, $p > 0.05$).

Health indices are provided in Table 2.5. There were no significant differences in the viscera index ($F_{4,20} = 1.48$, $p > 0.05$), hepatosomatic index ($F_{4,17} = 0.39$, $p > 0.05$), intraperitoneal fat ratio ($\chi^2_4 = 2.76$, $p > 0.05$)(n=20), or muscle mass to body weight ratio ($F_{4,20} = 0.47$, $p > 0.05$). The tilapia were tested for proximate body composition, including fat, ash, and protein (Table 2.6). There was fairly low variability in tilapia ash content (averaged 16.96 ± 0.57%) as compared to protein (66.75 ± 1.86%) and fat (15.82 ± 2.55%) across all diets. However, a higher sample size was necessary to determine if there were significant differences among the diets. The commercial control diets had the lowest fat content.

Digestibility

Apparent digestibility coefficients are shown in Table 2.7. There was no significant difference in digestibility between BSF feed and fishmeal feed ($F_{1,4} = 1.34$, $p > 0.05$). There was also no significant difference in protein digestibility ($F_{1,4} = 1.34$,$p > 0.05$).

DISCUSSION

This study investigated the health and growth effects of replacing fish meal in tilapia feed with BSF larvae raised on ATS biomass. The ATS biomass was generated from wastewater effluent used as a means of tertiary treatment to recycle excess nutrients, such as nitrogen and phosphorus. The ATS demonstrated success at nutrient uptake and productivity (Chapter 1) but can only be considered nutrient recycling if the biomass generated is productively utilized. The feed trial (Chapter 2) showed how ATS biomass could be productively recycled as feed for BSF larvae, converting nutrients from waste into tilapia diets without adverse effects on growth, health, or feed utilization by the fish. The BSF larvae were able to transform a high ash, low protein biomass waste into a high protein, low ash feedstock (Table 2.1). Since BSF larvae body composition is affected by what they eat, food source choice can be critical in producing a high protein BSF larvae (StHilaire et al., 2007; Zheng et al., 2012; Adebayo et al., 2020). The ATS biomass was able to produce BSF larvae high enough in protein to replace fishmeal as a source of protein in a practical tilapia diet.

The significant difference between the length and width of the commercial BSF larvae and the treated BSF, as well as the prolonged larval stage suggest there may have been confounding variables in the BSF rearing process. The BSF commercial control larvae were larger in width and length. Where our BSF were raised on ATS biomass produced from treated sewage, the BSF commercial control larvae were reportedly fed a wholesome diet of fruits, grains, and vegetables. Feed source quality (organic waste versus ATS biomass grown from treated wastewater) could be a factor for the BSF disparity in size, but there was no significant difference in the effect of the BSF as a diet on tilapia, including growth, feed utilization, or health indices. One explanation for the difference is larvae processing. Some larvae may have been collected too early in their life cycle, as was observed in some of the photos. Additionally, photos for image analysis were completed on dried fully extended and crisp commercial larvae, whereas the BSF larvae were thawed and previously frozen. Photos could not be taken of the dried larvae due to their shriveled morphology post oven drying. This was attributed to the Maillard reaction cause by the oven drying process, which can induce a browning coloration in addition to the shrinkage of BSF larvae (Melis et al., 2018; Purschke et al., 2018).

Feed source and environmental conditions can affect larval weight, percent protein, and development time (Newton et al., 2005; Gobbi et al., 2013; Adebayo et al., 2020; Lalander et al., 2020). Under ideal conditions, a larvae can pupate in less than 3 weeks (Larouche, 2009). The BSF larvae were delayed in development, lasting 2-4 months in the larval stages prior to becoming adults. This may have been caused by overpopulation in the habitat leading to competition for resources. Food shortages and low temperatures can extend the larval stage up to four months (Furman et al., 1959). The biomass generated by the ATS weekly was observed to be insufficient for the amount of larvae present. They processed the feed provided within a couple of days but were fed once a week. This is not necessarily due to the productivity, given that it was comparable to other units of full-scale size, but is likely due to the size of the ATS. 9.14m^2 was too small to impact the treated wastewater and too low a surface area to produce enough biomass to feed 40,000 BSF larvae.

It was also found that there were no significant differences between the 100% BSF diet and the extruded FM control diet. The FM diet exhibited no significant differences in final weight, growth, feed utilization, health effects, fat, or ash to the commercial control. This suggests the FM diet was an acceptable control for the BSF diets. The lack of significant differences between the BSF diets and FM indicates BSF can replace FM in a tilapia diet. Final weights within diets were highly variable as seen by the wide range in weights 1.98g and 56.09g and high overall standard deviation in mean final bodyweight. This may have been due to the high sexual dimorphism exhibited by blue tilapia with respect to weight (Lind et al., 2015). Gender was not taken into consideration when stocking for this experiment. This may explain the wide range of the final weights, but no significant differences among the diets for final weights.

Assuming blue tilapia have a similar amino acid requirement to Nile tilapia, *Oreochromis niloticus*, and/or Mozambique tilapia, *Oreochromis mossambicus*, the BSF meal amino acid profile did not meet the amino acid requirement for either Tilapia species as shown in Table 2.8 (Mjoun et al., 2010). In spite of that, there were no growth effects or significant deficits caused by the replacement of fishmeal with BSF meal. The BSF larvae diet and BSF protein in the diet were comparable to fishmeal in digestibility. Though not significant, the BSF diet actually had a higher protein digestibility and greater final bodyweight and growth. Though tilapia body composition comparisons were inconclusive due to low sample size, there were no significant differences in the muscle mass to body weight ratio or any of the health indices. There was also no difference in the CF, indicating comparable well-being across the diets.

There was no significant difference between the 100% BSF diet and any other diet (controls or treatments) for any measured parameters. These results are supported by Nairuti et al., 2021, who showed no differences between BSF larvae and fishmeal feed in terms of growth or survival on Nile tilapia. Overall, the 100% survival rate, lack of adverse health effects, palatability, digestibility, and comparable growth to the control diets indicates BSF raised with ATS biomass could be used either to supplement commercial feed or serve as a fishmeal alternative in freshwater aquaculture. Based on this study, BSF larvae can replace up to 100% fish meal in a commercially optimized dual protein diet.

One of the concerns with using ATS biomass grown from treated municipal waste was nonpoint source pollution. Heavy metals as one of the possible contaminants is a concern due to the possibility of biomagnification. Heavy metals, such as mercury, copper, chromium, and cadmium can affect growth, development, cause cancer, and have a wide range of other health effects (Martin and Griswold, 2009; Mahurpawar, 2015). In organic waste, BSF larvae have proven an ability to reduce heavy metal contamination (Attiogbe et al., 2019). The greater the quantity of larvae present, the less mercury metal contamination was found in the BSF larvae, demonstrating the potential to meet EU standards for animal feed, with a maximum limit of 0.5mg/kg (Attiogbe et al., 2019). This is a useful trait for the dissipation of trace metals that may be found in waste but are below the legal limit. BSF larvae have the potential to not only replace fishmeal but may also be used as a means of reducing trace pollutants, especially for those contaminants that are not known to be capable of biomagnification, such as bacterial pathogens.

Several pathogens are able to survive secondary wastewater treatment. Human fecal bacteria, such as *Bacteroidales*, *Clostridiaceae*, and *Bifidobacterium* have been found to persist in effluent post-secondary treatment (Wéry et al., 2010). Rains, 2016 showed that an algae turf scrubber is capable of removing e. coli from the water column. This means using the biomass could infect the BSF. However, BSF larvae have been shown to possess antimicrobial properties, including suppression of methicillin resistant *Staphylococcus aureus*, *E. coli*, and other bacteria (Liu et al., 2008; Park et al., 2014; Kinny et al., 2022). In high densities, they are capable of inhibiting egg deposition by the common house fly, *Musca domestica* (Sheppard, 1983). BSF have a broad spectrum of antimicrobial peptides that trigger immune responses that are diet dependent (Vogel et al., 2018). Additionally, BSF lack digestive organs as adults making them a non-vector species, reducing the chances of disease transmission (Sheppard et al., 2002; Newton, 2005).

Though municipal waste may not be ideal due to the dangers of nonpoint source pollutants, ATS biomass can be grown on other waste streams, such as aquaculture, dairy farms, or agricultural runoff. An ATS set up as part of an aquaculture system or farming effluent would serve a dual purpose. It would reduce system nutrients and serve as feed for BSF larvae, which in turn feed the tilapia. The BSF larvae would supply a fishmeal comparable protein and reduce the likelihood of any contamination from the reuse of system discharged waste. In this scenario, BSF larvae potentially serve as an added wastewater treatment step.

Due to increasing demand, intensification practices have increased in lieu of facility expansion leading to higher waste production in aquaculture (Henriksson et al., 2018; Dauda et al., 2019). Finfish aquaculture is primarily conducted in earthen ponds by middle income countries, and to a lesser extent cages and pens in public open waterbodies (FAO, 2022). Under these conditions, disease transmission is at an increased risk depending on intensification practices. An algae turf scrubber could be set up to capture discharged waste, recycling nitrogen and phosphorus from the nutrient rich effluent. The BSF could recycle nutrients from the biomass and sustainably replace fishmeal in tilapia feed. More has been done to optimize BSF husbandry, but this study was accomplished under practical conditions to facilitate replication. The process used to grow the ATS biomass and raise the BSF required minimal maintenance, theoretically making this a low-cost system. This is ideal for aquaculture, which produces nutrient rich waste. The results of this experiment offer sustainability solutions and a means to reduce nutrient pollution in aquaculture and wastewater treatment.

Future Directions

Though results showed no significant difference in growth, health indices, or feed utilization, the amino acid profile of BSF meal and FM vary. Muin et al., 2017 showed that at different diet inclusion the amino acid profile of BSF diets will significantly vary. Further investigation of blue tilapia amino acid requirements and ATS biomass effects on the BSF amino acid profile may be warranted. The question to investigate then is whether this variability extends to the tilapia being fed these diets. Also, if further sustainability is to be reached, research into BSF frass and residue biomass would take this system closer to zero-waste. The waste left behind by larvae processing holds nutrients which could potentially supplement feed or be used as fertilizer, bringing the process closer to a zero-waste system.

REFERENCES

Adey, W. H., Kangas, P. C., & Mulbry, W. (2011). Algal Turf Scrubbing: Cleaning Surface Waters with Solar Energy while Producing a Biofuel. *BioScience*, *61*(6), 434–441. https://doi.org/10.1525/bio.2011.61.6.5

Adey, W., Luckett, C., & Jensen, K. (1993). Phosphorus Removal from Natural Waters Using Controlled Natural Production. *Society for Ecological Restoration*, *1*(1), 29–39. https://doi.org/10.1111/j.1526-100X.1993.tb00006.x

Adey, W. H., Laughinghouse IV, H. D., Miller, J. B., Hayek, L.-A. C., Thompson, J. G., Bertman, S., Hampel, K., & Puvanendran, S. (2013). Algal turf scrubber (ATS) floways on the Great Wicomico River, Chesapeake Bay: Productivity, algal community structure, substrate and chemistry1. *Journal of Phycology*, *49*(3), 489–501. https://doi.org/10.1111/jpy.12056

Abdel-Raouf, N., Al-Homaidan, A. A., & Ibraheem, I. B. M. (2012). Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences*, *19*(3), 257–275. https://doi.org/10.1016/j.sjbs.2012.04.005

Abd El-Hack, M. E., El-Saadony, M. T., lNader, M. M., Salem, H. M., El-Tahan, A. M., Soliman, S. M., & Khafaga, A. F. (2022). Effect of environmental factors on growth performance of Nile tilapia (Oreochromis niloticus). *International Journal of Biometeorology*, *66*(11), 2183–2194. https://doi.org/10.1007/s00484-022-02347-6

Adebayo, H., Kemabonta, K., Ogbogu, S., Elechi, M., & Obe, M. (2021). Comparative assessment of developmental parameters, proximate analysis, and mineral compositions of black soldier fly (*Hermetia illucens*) prepupae reared on organic waste substrates. *International Journal of Tropical Insect Science*, *41*. https://doi.org/10.1007/s42690-020-00404-4

AOAC Official Methods of Analysis (2012) 19th edition, AOAC INTERNATIONAL, Gaithersburg, MD, Method 930.15

AOAC Official Method of Analysis (1995). 16th edition, AOAC INTERNATIONAL, Gaithersburg, MD, Method 990.08

AOAC Official Methods of Analysis (2005). 18th edition, AOAC INTERNATIONAL, Gaithersburg, MD, Method 942.05

AOAC Official Methods of Analysis (1995). 16th edition, AOAC INTERNATIONAL, Gaithersburg, MD, Method 920.38

AOAC Official Methods of Analysis (2005). 18th edition, AOAC INTERNATIONAL, Gaithersburg, MD, Method 978.10

AOAC Official Methods of Analysis (2005). 18th edition, AOAC INTERNATIONAL, Gaithersburg, MD, Method 990.03

AOAC Official Methods of Analysis (2016). 20th edition, AOAC INTERNATIONAL, Gaithersburg, MD, Method 994.12

AOAC Official Methods of Analysis (1995). 16th edition, AOAC INTERNATIONAL, Gaithersburg, MD, Method 985.28

Attiogbe, F. K., Ayim, N. Y. K., & Martey, J. (2019). Effectiveness of black soldier fly larvae in composting mercury contaminated organic waste. *Scientific African*, *6*, e00205. https://doi.org/10.1016/j.sciaf.2019.e00205

AVMA. *Harmful algal blooms (HABs)*. (2023). American Veterinary Medical Association. https://www.avma.org/resources-tools/animal-health-and-welfare/animal-health/harmful-algal-blooms-habs

Baker, M. R., Gobush, K. S., & Vynne, C. H. (2013). Review of factors influencing stress hormones in fish and wildlife. *Journal for Nature Conservation*, *21*(5), 309–318. https://doi.org/10.1016/j.jnc.2013.03.003

Bashar, R., Gungor, K., Karthikeyan, K. G., & Barak, P. (2018). Cost effectiveness of phosphorus removal processes in municipal wastewater treatment. *Chemosphere*, *197*, 280–290. https://doi.org/10.1016/j.chemosphere.2017.12.169

Baum, J. K., & Worm, B. (2009). Cascading top-down effects of changing oceanic predator abundances. *Journal of Animal Ecology*, *78*(4), 699–714. https://doi.org/10.1111/j.1365-2656.2009.01531.x

Belghit, I., Liland, N. S., Gjesdal, P., Biancarosa, I., Menchetti, E., Li, Y., Waagbø, R., Krogdahl, Å., & Lock, E.-J. (2019). Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (Salmo salar). *Aquaculture*, *503*, 609–619. https://doi.org/10.1016/j.aquaculture.2018.12.032

Benaka, L.R., D. Bullock, A.L. Hoover, and N.A. Olsen (editors). U.S. National Bycatch Report First Edition Update 3. 2019. U.S. Dept. of Commerce, NOAA. NOAA Technical Memorandum NMFS-F/SPO-190, 95 p.

Biswas, G., Sundaray, J. K., Bhattacharyya, S. B., Shyne Anand, P. S., Ghoshal, T. K., De, D., Kumar, P., Sukumaran, K., Bera, A., Mandal, B., & Kailasam, M. (2017). Influence of feeding, periphyton and compost application on the performances of striped, grey mullet (Mugil cephalus L.) fingerlings in fertilized brackish water ponds. *Aquaculture*, *481*, 64–71. https://doi.org/10.1016/j.aquaculture.2017.08.026

Bolorunduro, P.I., Abdullah, A.Y. Water quality management in fish culture, National Agricultural Extension and Research Liaison Services, Zaria, Extension Bulletin No. 98, 1996.

Bosch, G., van der Fels-Klerx, H. J., de Rijk, T. C., & Oonincx, D. G. A. B. (2017). Aflatoxin B1 Tolerance and Accumulation in Black Soldier Fly Larvae (Hermetia illucens) and Yellow Mealworms (Tenebrio molitor). *Toxins*, *9*(185), 1–10. https://doi.org/doi:10.3390/toxins9060185

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I., & Corner, R. (2010). Aquaculture: Global status and trends. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1554), 2897–2912. https://doi.org/10.1098/rstb.2010.0170

Britt, K. & Kangas, Patrick. (2016). A Preliminary Assessment of Dried Algal Biomass as a Filler Material in Concrete. *Journal of Algal Biomass Utilization*, *7*(2), 147–152.

Carr, S., Liu, J., & Tesoro, A. (2016). Transport and Fate of Microplastic Particles in Wastewater Treatment Plants. *Water Resear ch*, *91*, 1–9. http://dx.doi.org/10.1016/j.watres.2016.01.002

Chang, S.K.C., Zhang, Y. (2017). Protein Analysis. In: Nielsen, S.S. (eds) Food Analysis. Food Science Text Series. Springer, Cham. https://doi.org/10.1007/978-3-319-45776-5_18

Cho, C. Y., Slinger, S. J., & Bayley, H. S. (1982). Bioenergetics of salmonid fishes: energy intake, expenditure, and productivity. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *73*(1), 25-41.

Conley, D. J., Paerl, H. W., Howarth, R. W., Boesch, D. F., Seitzinger, S. P., Havens, K. E., Lancelot, C., & Likens, G. E. (2009). Controlling Eutrophication: Nitrogen and Phosphorus. *Science*, *323*(5917), 1014–1015. https://doi.org/10.1126/science.1167755

Costa, R.H.R, Bavaresco, A.S.L, Medri, W., Pholippi, L.S. (2000) Tertiary treatment of piggery wastes in water hyacinth ponds. *Water Science and Technology*, 42 (10-11), 211-214. Doi:10.2166/wst.2000.0645

Craggs, R. J., Adey, W. H., Jessup, B. K., & Oswald, W. J. (1996). A controlled stream mesocosm for tertiary treatment of sewage. *Ecological Engineering*, *6*(1–3), 149–169. https://doi.org/10.1016/0925-8574(95)00056-9

Crini, G., & Lichtfouse, E. (2019). Advantages and disadvantages of techniques used for wastewater treatment. *Environmental Chemistry Letters*, *17*(1), 145–155. https://doi.org/10.1007/s10311-018-0785-9

Daskalov, G. (2002). Overfishing drives a trophic cascade in the Black Sea. *Marine Ecology Progress Series*, *225*, 53–63. https://doi.org/10.3354/meps225053

Dauda, A. B., Ajadi, A., Tola-Fabunmi, A. S., & Akinwole, A. O. (2019). Waste production in aquaculture: Sources, components, and managements in different culture systems. *Aquaculture and Fisheries*, *4*(3), 81–88. https://doi.org/10.1016/j.aaf.2018.10.002

Davis, M. W., & Olla, B. L. (2001). Stress and Delayed Mortality Induced in Pacific Halibut by Exposure to Hooking, Net Towing, Elevated Seawater Temperature and Air: Implications for Management of Bycatch. *North American Journal of Fisheries Management*, *21*(4), 725–732. https://doi.org/10.1577/1548-8675(2001)021<0725:SADMII>2.0.CO;2

de Jonge, V. N., Elliott, M., & Orive, E. (2002). Causes, historical development, effects, and future challenges of a common environmental problem: eutrophication. *In Nutrients and Eutrophication in Estuaries and Coastal Waters: Proceedings of the 31st Symposium of the Estuarine and Coastal Sciences Association (ECSA), held in Bilbao, Spain, 3–7 July 2000* (pp. 1-19). Springer Netherlands. https://doi.org/10.1007/978-94-017-2464-7_1

de Souza Celente, G., Colares, G. S., Machado, Ê. L., & Lobo, E. A. (2019). Algae turf scrubber and vertical constructed wetlands combined system for decentralized secondary wastewater treatment. *Environmental Science & Pollution Research*, *26*(10), 9931–9937. https://doi.org/10.1007/s11356-019-04425-6

den Haan, J., Huisman, J., Brocke, H. J., Goehlich, H., Latijnhouwers, K. R. W., van Heeringen, S., Honcoop, S. A. S., Bleyenberg, T. E., Schouten, S., Cerli, C., Hoitinga, L., Vermeij, M. J. A., & Visser, P. M. (2016). Nitrogen and phosphorus uptake rates of different species from a coral reef community after a nutrient pulse. *Scientific Reports*, *6*(1), Article 1. https://doi.org/10.1038/srep28821

Diener, S., Studt Solano, N. M., Roa Gutiérrez, F., Zurbrügg, C., & Tockner, K. (2011). Biological Treatment of Municipal Organic Waste using Black Soldier Fly Larvae. *Waste and Biomass Valorization*, *2*(4), 357–363. https://doi.org/10.1007/s12649-011-9079-1

Donald, D. B., Bogard, M. J., Finlay, K., & Leavitt, P. R. (2011). Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. *Limnology and Oceanography*, *56*(6), 2161–2175. https://doi.org/10.4319/lo.2011.56.6.2161

Dwivedi, S. (2012). Bioremediation of heavy metal by algae: current and future perspective. *J Adv Lab Res Biol*, *3*(3), 195-199.

Egerton, S., Wan, A., Murphy, K., Collins, F., Ahern, G., Sugrue, I., Busca, K., Egan, F., Muller, N., Whooley, J., McGinnity, P., Culloty, S., Ross, R. P., & Stanton, C. (2020). Replacing fishmeal with plant protein in Atlantic salmon (Salmo salar) diets by supplementation with fish protein hydrolysate. *Scientific Reports*, *10*(1), Article 1. https://doi.org/10.1038/s41598-020-60325-7

Elvira, B., Nicola, G. G., Ayllón, D., & Almodóvar, A. (2021). Determinants of large-scale spatial distribution and seasonal microhabitat selection patterns of the endangered freshwater blenny

Salaria fluviatilis in the Ebro River basin, Spain. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *31*(11), 3261–3275. https://doi.org/10.1002/aqc.3671

Fan, L., Mohseni, A., Schmidt, J., Evans, B., Murdoch, B., & Gao, L. (2023). Efficiency of lagoon-based municipal wastewater treatment in removing microplastics. *Science of The Total Environment*, *876*, 162714. https://doi.org/10.1016/j.scitotenv.2023.162714

FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en

FAO. 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. https://doi.org/10.4060/cc0461en

Feminella, J. W., & Hawkins, C. P. (1995). Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. *Journal of the North American Benthological Society*, *14*(4), 465-509.

Furman, D. P., Young, R. D., & Catts, P. E. (1959). Hermetia illucens (Linnaeus) as a factor in the natural control of Musca domestica Linnaeus. *Journal of Economic Entomology*, *52*(5), 917-921.

Gan, X., Klose, H., & Reinecke, D. (2023). Stable year-round nutrients removal and recovery from wastewater by technical-scale algal Turf Scrubber (ATS). *Separation and Purification Technology*, *307*, 122693. https://doi.org/10.1016/j.seppur.2022.122693

García-Ortega, A., Kissinger, K. R., & Trushenski, J. T. (2016). Evaluation of fish meal and fish oil replacement by soybean protein and algal meal from *Schizochytrium limacinum* in diets for giant grouper, *Epinephelus lanceolatus*. *Aquaculture*, *452*, 1–8. https://doi.org/10.1016/j.aquaculture.2015.10.020

Gies, E., LeNoble, J., Noel, M., Etemadifar, A., Bishay, F., Hall, E., & Ross, P. (2018). Retention of microplastics in a major secondary wastewater treatment plant in Vancouver, Canada. *Marine Pollution Bulletin*, *133*, 553–561. https://doi.org/10.1016/j.marpolbul.2018.06.006

Gobbi, P., Martínez-Sánchez, A., & Rojo, S. (2013). The effects of larval diet on adult life-history traits of the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). *European Journal of Entomology*, *110*(3), 461–468. https://doi.org/10.14411/eje.2013.061

Gude, Veera Gnaneswar. (2015). Energy positive wastewater treatment and sludge management. *Edorium Journal of Waste Management*, *1*, 10–15.

Gupta, V., Ali, Prof. I., Saleh, T., Nayak, A., & Agarwal, S. (2012). Chemical Treatment Technologies for Waste-water Recycling–an Overview. *RSC Adv.*, *2*, 6380–6388. https://doi.org/10.1039/C2RA20340E

Güroy, D., Güroy, B., Merrifield, D. L., Ergün, S., Tekinay, A. A., & Yiğit, M. (2010). Effect of dietary ulva and spirulina on weight loss and body composition of rainbow trout, Oncorhynchus mykiss (Walbaum), during a starvation period. *Journal of Animal Physiology and Animal Nutrition*, *95*(3), 320–327. https://doi.org/10.1111/j.1439-0396.2010.01057.x

Guzman, H. M., Gomez, C. G., Guevara, C. A., & Kleivane, L. (2013). Potential vessel collisions with Southern Hemisphere humpback whales wintering off Pacific Panama. *Marine Mammal Science*, *29*(4), 629–642. https://doi.org/10.1111/j.1748-7692.2012.00605.x

Hach. (2018). Nitrate: Cadmium Reduction Method (2nd ed.). Hach Company/Hach Lange GmbH. DOC316.53.01465

Hach. (2019). Nitrite: USEPA Diazotization Method (11th ed.). Hach Company/Hach Lange GmbH. DOC316.53.01074

Hach. (2015). Nitrogen, Ammonia: Salicylate Method (10th ed.). Hach Company/Hach Lange GmbH. DOC316.53.01077

Hach. (2018). Phosphorous, Reactive (Orthophosphate): USEPA PhosVer 3 (Ascorbic) Method (10th ed.). Hach Company/Hach Lange GmbH. DOC316.53.01119

Hayes, M. (2020). Measuring Protein Content in Food: An Overview of Methods. *Foods*, *9*(10), Article 10. https://doi.org/10.3390/foods9101340

Henriksson, P. J. G., Belton, B., Jahan, K. M.-, & Rico, A. (2018). Measuring the potential for sustainable intensification of aquaculture in Bangladesh using life cycle assessment. *Proceedings of the National Academy of Sciences*, *115*(12), 2958–2963. https://doi.org/10.1073/pnas.1716530115

Henry, M., Gasco, L., Piccolo, G., & Fountoulaki, E. (2015). Review of the use of insects in the diet of farmed fish: Past and future. *Animal Feed Science and Technology*, *203*, 1–22. https://doi.org/10.1016/j.anifeedsci.2015.03.001

Hessen, D. O., Færøvig, Per J., & Andersen, Tom. (2002). Light, nutrients, and P:C ratios in algae: grazer performance related to food quality and quantity. *Ecological Society of America*, *83*(7), 1886–1898. https://doi.org/10.1890/00129658(2002)083[1886:LNAPCR]2.0.CO;2

Hillebrand, H. (2005). Light regime and consumer control of autotrophic biomass. *Journal of Ecology*, *93*(4), 758–769. https://doi.org/10.1111/j.1365-2745.2005.00978.x

Hodar, A., Vasava, R., Mahavadiya, D., & Joshi, N. (2020). Fish meal and fish oil replacement for aquafeed formulation by using alternative sources: a review. *J.Exp.Zool.India*, *23*(1), 13–21.

Hussein, E. E.-S., Dabrowski, K., El-Saidy, D. M. S. D., & Lee, B.-J. (2013). Enhancing the growth of Nile tilapia larvae/juveniles by replacing plant (gluten) protein with algae protein. *Aquaculture Research*, *44*(6), 937–949. https://doi.org/10.1111/j.1365-2109.2012.03100.x

Hydromentia, Inc. (2005). *S-154 Pilot Single Stage Algal Turf Scrubber® (ATS^{TM}) Final Report* (pp. 1–90) [Final Report]. South Florida Water Management District Contract No. C-13933. https://hydromentia.com/wp-content/uploads/2015/06/2005_HMI_S1540-Single-Stage-ATS-Final-Report.pdf

Jöhnk, K. D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P. M., & Stroom, J. M. (2008). Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biology*, *14*(3), 495–512. https://doi.org/10.1111/j.1365-2486.2007.01510.x

Kangas, P., & Mulbry, W. (2014). Nutrient removal from agricultural drainage water using algal turf scrubbers and solar power. *Bioresource Technology*, *152*, 484-489. doi: 10.1016/j.biortech.2013.11.027

Kangas, Patrick, Mulbry, Walter, Klavon, Philip, Laughinghouse, H. Dail, & Adey, Walter. (2017). High diversity within the periphyton community of an algal turf scrubber on the Susquehanna River. *Ecological Engineering*, *108*, 564–572. http://dx.doi.org/10.1016/j.ecoleng.2017.05.010

Kaushik, S. J., Coves, D., Dutto, G., & Blanc, D. (2004). Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, Dicentrarchus labrax | Elsevier Enhanced Reader. *Aquaculture*, *230*, 391–404. https://doi.org/doi:10.1016/S0044-8486(03)00422-8

Kazamia, E., Riseley, A. S., Howe, C. J., & Smith, A. G. (2014). An Engineered Community Approach for Industrial Cultivation of Microalgae. *Industrial Biotechnology (New Rochelle, N.Y.)*, *10*(3), 184–190. https://doi.org/10.1089/ind.2013.0041

Kinney, M., Moyet, M., Bernard, E., & Alyokhin, A. (2022). Suppression of Methicillin-Resistant Staphylococcus aureus and Reduction of Other Bacteria by Black Soldier Fly Larvae Reared on Potato Substrate. *Microbiology Spectrum*, *10*(5), e02321-22. https://doi.org/10.1128/spectrum.02321-22

Körner, S., Vermaat, J. E., & Veenstra, S. (2003). The Capacity of Duckweed to Treat Wastewater. *Journal of Environmental Quality*, *32*(5), 1583-1590. doi:10.2134/jeq2003.1583

Lalander, C., Ermolaev, E., Wiklicky, V., & Vinnerås, B. (2020). Process efficiency and ventilation requirement in black soldier fly larvae composting of substrates with high water content. *Science of the Total Environment*, *729*, 138968. https://doi.org/10.1016/j.scitotenv.2020.138968

Lapworth, D. J., Baran, N., Stuart, M. E., & Ward, R. S. (2012). Emerging organic contaminants in groundwater: A review of sources, fate, and occurrence. *Environmental Pollution*, *163*, 287–303. https://doi.org/10.1016/j.envpol.2011.12.034

Larouche, J. (2019). *Processing methods for the black soldier fly (Hermetia illucens) larvae: From feed withdrawal periods to killing methods*. Universite Laval.

Lawton, R. J., Mata, L., Nys, R. D., & Paul, N. A. (2013). Algal Bioremediation of Waste Waters from Land-Based Aquaculture Using Ulva: Selecting Target Species and Strains. *PLoS ONE*, *8*(10). doi: 10.1371/journal.pone.0077344

Li, X., Marella, T. K., Tao, L., Peng, L., Song, C., Dai, L., Tiwari, A., & Li, G. (2017). A novel growth method for diatom algae in aquaculture waste water for natural food development and nutrient removal. *Water Science and Technology*, *75*(12), 2777–2783. https://doi.org/10.2166/wst.2017.156

Liland, N. S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C. G., Waagbø, R., Torstensen, B. E., & Lock, E.-J. (2017). Modulation of nutrient composition of black soldier fly (Hermetia illucens) larvae by feeding seaweed-enriched media. *PLOS ONE*, *12*(8), e0183188. https://doi.org/10.1371/journal.pone.0183188

Lind, C. E., Safari, A., Agyakwah, S. K., Attipoe, F. Y. K., El-Naggar, G. O., Hamzah, A., Hulata, G., Ibrahim, N. A., Khaw, H. L., Nguyen, N. H., Maluwa, A. O., Zaid, M., Zak, T., & Ponzoni, R. W. (2015). Differences in sexual size dimorphism among farmed tilapia species and strains undergoing genetic improvement for body weight. *Aquaculture Reports*, *1*, 20–27. https://doi.org/10.1016/j.aqrep.2015.03.003

Liu, Q., Tomberlin, J. K., Brady, J. A., Sanford, M. R., & Yu, Z. (2008). Black Soldier Fly (Diptera: Stratiomyidae) Larvae Reduce <I>Escherichia coli</I> in Dairy Manure. *Environmental Entomology*, *37*(6), 1525–1530. https://doi.org/10.1603/0046-225X-37.6.1525

Mahurpawar, M. (2015). Effects of heavy metals on human health. *Int J Res Granthaalayah*, *530*(516), 1-7.

Marella, T. K., Datta, A., Patil, M. D., Dixit, S., & Tiwari, A. (2019). Biodiesel production through algal cultivation in urban wastewater using algal floway. *Bioresource Technology*, *280*, 222–228. https://doi.org/10.1016/j.biortech.2019.02.031

Mariotti, F., Tomé, D. D., & Mirand, P. P. (2008). Converting Nitrogen into Protein – Beyond 6.25 and Jones' Factors. *Critical Reviews in Food Science and Nutrition*, *48*(2), 177–184. https://doi.org/10.1080/10408390701279749

Martinez-Porchas, M., & Martinez-Cordova, L. R. (2012). World Aquaculture: Environmental Impacts and Troubleshooting Alternatives. *The Scientific World Journal*, *2012*, 1–9. https://doi.org/10.1100/2012/389623

Martin, S., & Griswold, W. (2009). Human Health Effects of Heavy Metals. *Environmental Science and Technology Briefs for Citizens*, *15*, 1–6.

Melis, R., Braca, A., Mulas, G., Sanna, R., Spada, S., Serra, G., Fadda, M. L., Roggio, T., Uzzau, S., & Anedda, R. (2018). Effect of freezing and drying processes on the molecular traits of edible yellow mealworm. *Innovative Food Science & Emerging Technologies*, *48*, 138–149. https://doi.org/10.1016/j.ifset.2018.06.003

Miles, R. D. & Chapman, F. A. (2006). *FA122/FA122: The Benefits of Fish Meal in Aquaculture Diets*. 1–7.

Mjoun, K., Rosentrater, K., & Brown, M. L. (2010). *TILAPIA: Environmental Biology and Nutritional Requirements*. South Dakota Cooperative Extension Service. *Factsheets*. Paper 164.

Möllmann, C., Müller-Karulis, B., Kornilovs, G., & St John, M. A. (2008). Effects of climate and overfishing on zooplankton dynamics and ecosystem structure: Regime shifts, trophic cascade, and feedback loops in a simple ecosystem. *ICES Journal of Marine Science*, *65*(3), 302–310. https://doi.org/10.1093/icesjms/fsm197

Montoya-Camacho, N., Marquez Rios, E., Castillo-Yáñez, F., Cárdenas-López, J., López-Elías, J., Ruiz-Cruz, S., Jimenez-Ruiz, E., Rivas-Vega, M., & Ocaño-Higuera, V. (2018). Advances in the use of alternative protein sources for tilapia feeding. *Reviews in Aquaculture*, *11*, 1–12. https://doi.org/10.1111/raq.12243

Muin, H., Taufek, N. M., Kamarudin, M. S., & Razak, S. A. (2017). Growth performance, feed utilization and body composition of Nile tilapia, Oreochromis niloticus (Linnaeus, 1758) fed with different levels of black soldier fly, *Hermetia illucens* (Linnaeus, 1758) maggot meal diet. *Iranian Journal of Fisheries Sciences*, *16*(2), 567–577.

Mulbry, W., Kangas, P., & Kondrad, S. (2010). Toward scrubbing the bay: Nutrient removal using small algal turf scrubbers on Chesapeake Bay tributaries. *Ecological Engineering*, *36*(4), 536-541. doi: 10.1016/j.ecoleng.2009.11.026

Mulbry, W., Kebede-Westhead, E., Pizarro, C., & Sikora, L. (2005). Recycling of manure nutrients: Use of algal biomass from dairy manure treatment as a slow-release fertilizer. *Bioresource Technology*, *96*, 451–458. https://doi.org/10.1016/j.biortech.2004.05.026

Mulbry, W., Kondrad, S., Pizarro, C., & Kebede-Westhead, E. (2008). Treatment of dairy manure effluent using freshwater algae: Algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers. *Bioresource Technology*, *99*(17), 8137-8142. doi: 10.1016/j.biortech.2008.03.073

Mustafa, G., Wakamatsu, S., Takeda, T.-aki, Umino, T., & Nakagawa, H. (1995). Effects of algae meal as feed additive on growth, feed efficiency, and body composition in Red Sea Bream. *Fisheries Science*, *61*(1), 25–28. https://doi.org/10.2331/fishsci.61.25

Myers, H., Tomberlin, J., Lambert, B., & Kattes, D. (2008). Development of Black Soldier Fly (Diptera: Stratiomyidae) Larvae Fed Dairy Manure. *Environmental Entomology*, *37*(1), 11–15. https://doi.org/10.1603/0046-225X(2008)37[11:DOBSFD]2.0.CO;2

Nairuti, R. N., Munguti, J. M., Waidbacher, H., & Zollitsch, W. (2021). Growth performance and survival rates of Nile tilapia (L.) reared on diets containing Black soldier fly (L.) larvae meal. *Die Bodenkultur: Journal of Land Management, Food and Environment*, *72*(1), 9–19. https://doi.org/10.2478/boku-2021-0002

Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Folke, C., Beveridge, Malcolm C. M., Clay, Jason, Folke, Carl, Lubchenco, Jane, Mooney, Harold, & Troell, Max. (2000). Effect of Aquaculture on World Fish Supplies. *Nature*, *405*, 1017–1024.

Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., Little, D. C., Lubchenco, J., Shumway, S. E., & Troell, M. (2021). A 20-year retrospective review of global aquaculture. *Nature*, *591*(7851), Article 7851. https://doi.org/10.1038/s41586-021-03308-6

New, M.B. & Wijkström U. N. (2002). *Use of fishmeal and fish oil in aquafeeds: further thoughts on the fishmeal trap*. FAO Fisheries Circular. https://www.fao.org/3/y3781e/y3781e.pdf

Newby, D. T., Mathews, T. J., Pate, R. C., Huesemann, M. H., Lane, T. W., Wahlen, B. D., Mandal, S., Engler, R. K., Feris, K. P., & Shurin, J. B. (2016). Assessing the potential of polyculture to accelerate algal biofuel production. *Algal Research*, *19*, 264–277. https://doi.org/10.1016/j.algal.2016.09.004

Newton, G. L., Sheppard, D. C., & Burtle, G. J. (2008). Research Briefs: Black Soldier Fly Prepupae—A Compelling Alternative to Fish Meal and Fish Oil. *Aquaculture*, *24*, 103-109.

Newton, L., Sheppard, Craig, Watson, D Wes, Burtle, Gary, & Dove, Robert. (2005). *Using the black soldier fly, Hermetia illucens, as a value-added tool for the management of swine manure.* (pp. 1–17).

Nguyen, T. N., Davis, D. A., & Saoud, I. P. (2009). Evaluation of Alternative Protein Sources to Replace Fish Meal in Practical Diets for Juvenile Tilapia, Oreochromis spp. *Journal of the World Aquaculture Society*, *40*(1), 113–121. https://doi.org/10.1111/j.1749-7345.2008.00230.x

NOAA Fisheries. (2023, September 7). *Atlantic Herring | NOAA Fisheries* (New England/Mid-Atlantic,Southeast). NOAA. https://www.fisheries.noaa.gov/species/atlantic-herring

Olvera-Novoa, M. A., Domínguez-Cen, L. J., Olivera-Castillo, L., & Martínez-Palacios, C. A. (1998). Effect of the use of the microalga Spirulina maxima as fish meal replacement in diets for tilapia, Oreochromis mossambicus (Peters), fry. *Aquaculture Research*, *29*(10), 709–715. https://doi.org/10.1046/j.1365-2109.1998.29100709.x

Paerl, H., & Huisman, J. (2009). Climate Change: A Catalyst for Global Expansion of Harmful Cyanobacterial Blooms. *Environmental Microbiology Reports*, *1*(1), 27–37. https://doi.org/10.1111/j.1758-2229.2008.00004.x

Park, J. B. K., & Craggs, R. J. (2011). Algal production in wastewater treatment high-rate algal ponds for potential biofuel use. *Water Science and Technology*, *63*(10), 2403–2410. https://doi.org/10.2166/wst.2011.200

Park, S.-I., Chang, B. S., & Yoe, S. M. (2014). Detection of antimicrobial substances from larvae of the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). *Entomological Research*, *44*(2), 58–64. https://doi.org/10.1111/1748-5967.12050

Pereira, H., Sardinha, M., Santos, T., Gouveia, L., Barreira, L., Dias, J., & Varela, J. (2020). Incorporation of defatted microalgal biomass (Tetraselmis sp. CTP4) at the expense of soybean meal as a feed ingredient for juvenile gilthead seabream (*Sparus aurata*). *Algal Research*, *47*, 101869. https://doi.org/10.1016/j.algal.2020.101869

Pike, I. H. (1999). The role of long chain omega-3 polyunsaturated fatty acids in animal feeding. *Ifoma Tec. Bull*, *3*, 1-40.

Pinnegar, J. K., Polunin, N. V. C., Francour, P., Badalamenti, F., Chemello, R., Harmelin-Vivien, M.-L., Hereu, B., Milazzo, M., Zabala, M., D'Anna, G., & Pipitone, C. (2000). Trophic cascades in benthic marine ecosystems: Lessons for fisheries and protected-area management. *Environmental Conservation*, *27*(2), 179–200. https://doi.org/10.1017/S0376892900000205

Pond, W. G., Church, D. C. , Pond, K. R. 1995. Basic animal nutrition and feeding. 4th edition, John Wiley, and Sons Inc. New York 615 pp.

Posch, T., Köster, O., Salcher, M. M., & Pernthaler, J. (2012). Harmful filamentous cyanobacteria favoured by reduced water turnover with lake warming. *Nature Climate Change*, *2*(11), 809–813. https://doi.org/10.1038/nclimate1581

Priyadarshana, K., Walpita, C., NAVEENAN, MANOHARAN, Magamage, M., & Ruwandeepika, D. (2021). Substitution of Fishmeal with Black Soldier Fly Hermetia illucens Linnaeus, 1758 Larvae in Finfish Aquaculture -A Review. *Asian Fisheries Science*, *34*. https://doi.org/10.33997/j.afs.2021.34.2.001

Purschke, B., Brüggen, H., Scheibelberger, R., & Jäger, H. (2018). Effect of pre-treatment and drying method on physico-chemical properties and dry fractionation behaviour of mealworm larvae (Tenebrio molitor L.). *European Food Research and Technology*, *244*(2), 269–280. https://doi.org/10.1007/s00217-017-2953-8

Rana, K. M. S., Salam, M. A., Hashem, S., & Islam, A. (2015). Original Article Development of Black Soldier Fly Larvae Production Technique as an Alternate Fish Feed. *International Journal of Research in FIsheries and Aquaculture*, *5*, 41–47.

Rahman, M. T., Kameda, T., Kumagai, S., & Yoshioka, T. (2018). Effectiveness of Mg-Al-layered double hydroxide for heavy metal removal from mine wastewater and sludge volume reduction. *International Journal of Environmental Science & Technology (IJEST)*, *15*(2), 263–272. https://doi.org/10.1007/s13762-017-1385-0

Ray, N. E., Terlizzi, D. E., & Kangas, P. C. (2015). Nitrogen and phosphorus removal by the Algal Turf Scrubber at an oyster aquaculture facility. *Ecological Engineering, 78*, 27-32. doi: 10.1016/j.ecoleng.2014.04.028

Reddy, D. H., & Lee, S.-M. (2012). Water Pollution and Treatment Technologies. *Journal of Environmental & Analytical Toxicology*, *02*(4). https://doi.org/10.4172/2161-0525.1000e103

Riche, M., & Garling, D. (2003). Fish: Feed and Nutrition. *Feeding Tilapia in intensive Recirculating systems*.

Ricker, W. E. (1975). Computation and interpretation of biological statistics of fish populations. *Fish. Res. Board Can. Bull.*, *191*, 1-382.

Rust, M., Barrows, F., Hardy, R., Lazur, A., Naughten, K., & Silverstein, J. (2021). *The Future of Aquafeeds* (National; Technical Memorandum NMFS F/SPO-124; Alternative Feeds Initiative). NOAA/USDA. https://www.fisheries.noaa.gov/noaa-usda-alternative-feeds-initiative

Ryer, C. (2002). Trawl stress and escapee vulnerability to predation in juvenile walleye pollock: Is there an unobserved bycatch of behaviorally impaired escapees? *Marine Ecology Progress Series*, *232*, 269–279. https://doi.org/10.3354/meps232269

Saadatnia, H., & Riahi, H. (2009). Cyanobacteria from paddy fields in Iran as a biofertilizer in rice plants. *Plant, Soil and Environment*, *55*(5), 207–212. https://doi.org/10.17221/384-PSE

Salama, E.-S., Kurade, M. B., Abou-Shanab, R. A. I., El-Dalatony, M. M., Yang, I.-S., Min, B., & Jeon, B.-H. (2017). Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation. *Renewable and Sustainable Energy Reviews*, *79*, 1189–1211. https://doi.org/10.1016/j.rser.2017.05.091

Salgot, M., & Folch, M. (2018). Wastewater treatment and water reuse. *Current Opinion in Environmental Science & Health*, *2*, 64–74. https://doi.org/10.1016/j.coesh.2018.03.005

Sandefur, H. N., Matlock, M. D., & Costello, T. A. (2011). Seasonal productivity of a periphytic algal community for biofuel feedstock generation and nutrient treatment. *Ecological Engineering*, *37*(10), 1476–1480. https://doi.org/10.1016/j.ecoleng.2011.04.002

Sarker, P. K., Kapuscinski, A. R., McKuin, B., Fitzgerald, D. S., Nash, H. M., & Greenwood, C. (2020). Microalgae-blend tilapia feed eliminates fishmeal and fish oil, improves growth, and is cost viable. *Scientific Reports*, *10*(1), 19328. https://doi.org/10.1038/s41598-020-75289-x

Schneider, O., Sereti, V., Eding, E. H., & Verreth, J. A. J. (2005). Analysis of nutrient flows in integrated intensive aquaculture systems. *Aquacultural Engineering*, *32*(3–4), 379–401. https://doi.org/10.1016/j.aquaeng.2004.09.001

Seawright, D. E., Stickney, R. R., & Walker, R. B. (1998). Nutrient dynamics in integrated aquaculture–hydroponics systems. *Aquaculture*, *160*(3–4), 215–237. https://doi.org/10.1016/S0044-8486(97)00168-3

Sheppard, C. D., Newton, L. G., Thompson, S. A., & Savage, S. (1994). A value-added manure management system using the black soldier fly. *Bioresource Technology*, *50*(3), 275–279. https://doi.org/10.1016/0960-8524(94)90102-3

Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., & Sumner, S. M. (2002). Rearing Methods for the Black Soldier Fly (Diptera: Stratiomyidae). *Journal of Medical Entomology*, *39*(4), 695–698.

Sheppard, C. (1983). House fly and lesser fly control utilizing the black soldier fly in manure management systems for caged laying hens. *Environmental entomology*, *12*(5), 1439-1442.Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., & Templeton, D. (2005). Determination of ash in biomass (NREL/TP-510-42622). *National Renewable Energy*

Shurin, J. B., Abbott, R. L., Deal, M. S., Kwan, G. T., Litchman, E., McBride, R. C., Mandal, S., & Smith, V. H. (2013). Industrial-strength ecology: Trade-offs and opportunities in algal biofuel production. *Ecology Letters*, *16*(11), 1393–1404. https://doi.org/10.1111/ele.12176

Sluiter, A., Scarlata, C., Sluiter, J., & Templeton, D. (2005). *Determination of Extractives in Biomass: Laboratory Analytical Procedure (LAP)* (Technical Report NREL/TP-510-42619; pp. 1–12). National Renewable Energy Laboratory. http://www.nrel.gov/biomass/analytical_procedures.html

Sonune, A., & Ghate, R. (2004). Developments in Wastewater Treatment Methods. *Desalination*, *167*, 55–63. https://doi.org/10.1016/j.desal.2004.06.113

St-Hilaire, S., Cranfill, K., McGuire, M. A., Mosley, E. E., Tomberlin, J. K., Newton, L., Sealey, W., Sheppard, C., & Irving, S. (2007). Fish Offal Recycling by the Black Soldier Fly Produces a Foodstuff High in Omega-3 Fatty Acids. *Journal of the World Aquaculture Society*, *38*(2), 309–313. https://doi.org/10.1111/j.1749-7345.2007.00101.x

Thomas, P. O., Reeves, R. R., & Brownell Jr., R. L. (2016). Status of the world's baleen whales. *Marine Mammal Science*, *32*(2), 682–734. https://doi.org/10.1111/mms.12281

Tippayadara, N., Dawood, M., Krutmuang, P., Hoseinifar, S. H., Van Doan, H., & Paolucci, M. (2021). Replacement of Fish Meal by Black Soldier Fly (Hermetia illucens) Larvae Meal: Effects on Growth, Haematology, and Skin Mucus Immunity of Nile Tilapia, Oreochromis niloticus. *Animals*, *11*(193), 1–19. https://doi.org/10.3390/ani11010193

Tripathi, R. D., Dwivedi, S., Shukla, M. K., Mishra, S., Srivastava, S., Singh, R., Rai, U. N., & Gupta, D. K. (2008). Role of blue green algae biofertilizer in ameliorating the nitrogen demand and fly-ash stress to the growth and yield of rice (Oryza sativa L.) plants. *Chemosphere*, *70*(10), 1919–1929. https://doi.org/10.1016/j.chemosphere.2007.07.038

Turner, J. L., Dritz, S. S., Higgins, J. J., & Minton, J. E. (2002). Effects of ascophyllum nodosum extract on growth performance and immune function of young pigs challenged with salmonella typhimurium1. *Journal of Animal Science*, *80*(7), 1947–1953. https://doi.org/10.2527/2002.8071947x

Van Der Hoop, J. M., Moore, M. J., Barco, S. G., Cole, T. V. n., Daoust, P.-Y., Henry, A. G., Mcalpine, D. F., Mclellan, W. A., Wimmer, T., & Solow, A. R. (2013). Assessment of Management to Mitigate Anthropogenic Effects on Large Whales. *Conservation Biology*, *27*(1), 121–133. https://doi.org/10.1111/j.1523-1739.2012.01934.x

Van Puijenbroek, P. J. T. M., Beusen, A. H. W., & Bouwman, A. F. (2019). Global nitrogen and phosphorus in urban waste water based on the Shared Socio-economic pathways. *Journal of Environmental Management*, *231*, 446–456. https://doi.org/10.1016/j.jenvman.2018.10.048

Vogel, H., Müller, A., Heckel, D. G., Gutzeit, H., & Vilcinskas, A. (2018). Nutritional immunology: Diversification and diet-dependent expression of antimicrobial peptides in the black soldier fly Hermetia illucens. *Developmental & Comparative Immunology*, *78*, 141–148. https://doi.org/10.1016/j.dci.2017.09.008

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S. E., Sullivan, C. A., Liermann, C. R., & Davies, P. M. (2010). Global threats to human water security and river biodiversity. *Nature*, *467*(7315), Article 7315. https://doi.org/10.1038/nature09440

Vymazal, J. (2010). Constructed Wetlands for Wastewater Treatment. *Water*, *2*(3), Article 3. https://doi.org/10.3390/w2030530

Wang, B., & Lan, C. Q. (2011). Biomass production and nitrogen and phosphorus removal by the green alga Neochloris oleoabundans in simulated wastewater and secondary municipal wastewater effluent. *Bioresource Technology*, *102*(10), 5639–5644. https://doi.org/10.1016/j.biortech.2011.02.054

Wang, X., Wang, X., Huppes, G., Heijungs, R., & Ren, N.-Q. (2015). Environmental implications of increasingly stringent sewage discharge standards in municipal wastewater treatment plants: Case study of a cool area of China. *Journal of Cleaner Production*, *94*. https://doi.org/10.1016/j.jclepro.2015.02.007

Ward, A. J., Lewis, D. M., & Green, F. B. (2014). Anaerobic digestion of algae biomass: A review. *Algal Research*, *5*, 204–214. https://doi.org/10.1016/j.algal.2014.02.001

Wéry, N., Monteil, C., Pourcher, A.-M., & Godon, J.-J. (2010). Human-specific fecal bacteria in wastewater treatment plant effluents. *Water Research*, *44*(6), 1873–1883. https://doi.org/10.1016/j.watres.2009.11.027

Wuang, S. C., Khin, M. C., Chua, P. Q., & Luo, Y. D. (2016). Use of Spirulina biomass produced from treatment of aquaculture wastewater as agricultural fertilizers. *Algal Research*, *15*, 59-64. doi: 10.1016/j.algal.2016.02.009

Wilkie, A., & Mulbry, W. (2002). Recovery of dairy manure nutrients by benthic freshwater algae. *Bioresour* Younis, E.-S. M., Al-Quffail, A. S., Al-Asgah, N. A., Abdel-Warith, A.-W. A., & Al-Hafedh, Y. S. (2018). Effect of dietary fish meal replacement by red algae, *Gracilaria arcuata* , on growth performance and body composition of Nile Tilapia Oreochromis Niloticus. *Saudi Journal of Biological Sciences*, *25*(2), 198–203. https://doi.org/10.1016/j.sjbs.2017.06.012

Williams, C. M., Zering, K., & Wohlgenant, M. (2006). Technology Report: Black Soldier Fly (SF).

Yang, G. Y., Tang, T., & Dudgeon, D. (2009). Spatial and seasonal variations in benthic algal assemblages in streams in monsoonal Hong Kong. *Hydrobiologia*, *632*(1), 189–200. https://doi.org/10.1007/s10750-009-9838-1

Zhang, Z.-C., Gu, P., Yang, K.-L., Zhao, M.-X., Huang, Z.-X., & Miao, H.-F. (2022). Bioconversion of cyanobacteria by black soldier fly larvae (Hermetia illucens L.): Enhancement by antioxidants. *Science of The Total Environment*, *822*, 153524. https://doi.org/10.1016/j.scitotenv.2022.153524

Zheng, L., Hou, Y., Li, W., Yang, S., Li, Q., & Yu, Z. (2012). Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. *Energy*, *47*(1), 225–229. https://doi.org/10.1016/j.energy.2012.09.006

STATEMENT OF INDEPENDENT WORK

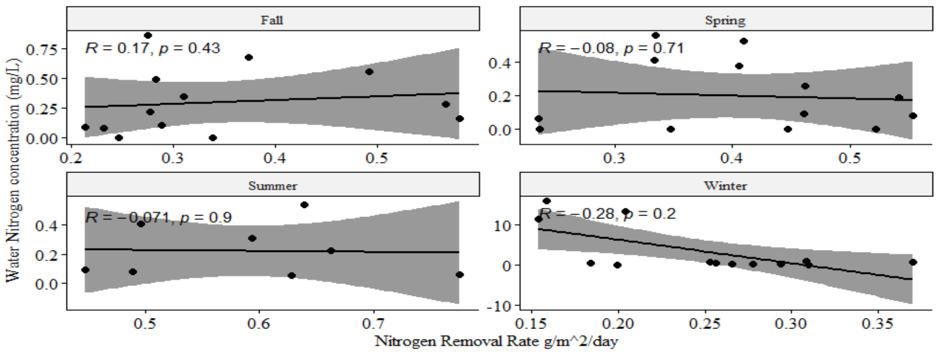
The following thesis has been composed solely by myself, Michelle Lowery. Except where stated otherwise by reference or acknowledgment, the work presented is entirely my own. I am responsible for all black soldier fly husbandry, feed trial data collection, digestibility data collection, feed preparation, ATS biomass processing, water quality analysis, statistical analysis, and written work. Undergraduate student aid was received for BSF harvest, daily tilapia feeding, and water quality checks. The ATS biomass used came from an ATS system setup as part of a larger USDA research project for which I collaborated with my advisor, Anthony Siccardi, to collect ATS water quality data and samples. This research was accomplished as a partial fulfillment of my biology master's degree. This work has not been submitted for any other degree or professional qualification except as specified.

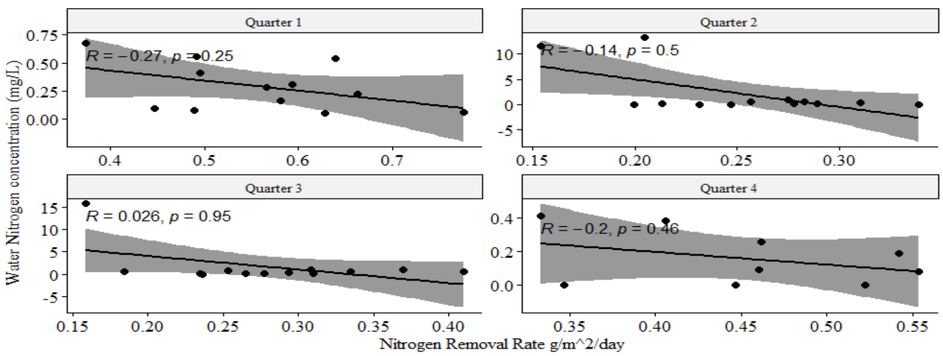
Funding: This work was supported by funding from Sigma Xi, the Scientific Research Honor Society and Georgia Southern University.

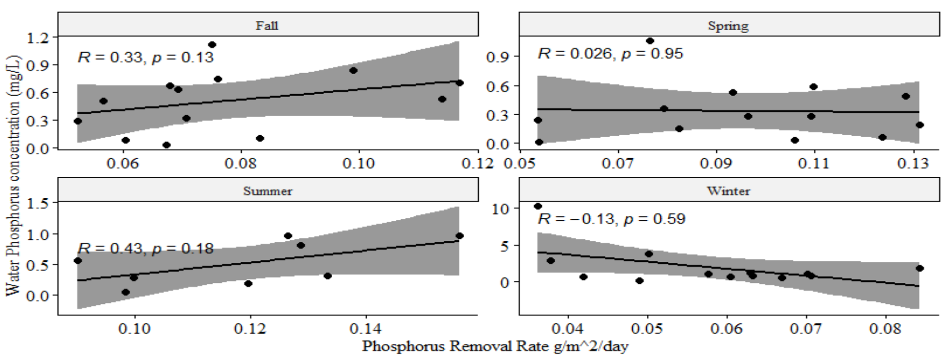
Conflicts of Interest: The author declares no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

APPENDIX A

Figures







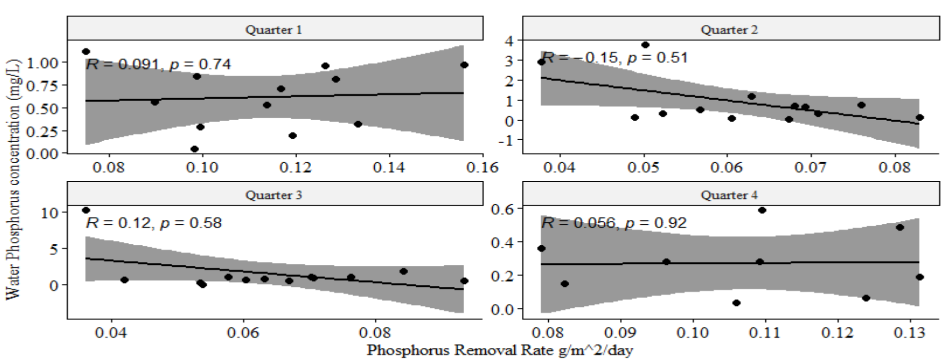


Figure 1.1. This is a scatterplot depicting correlations between effluent nutrient concentrations and ATS biomass nutrient removal rates by quarter and season for 11 months. The correlation coefficient and p-value are displayed.

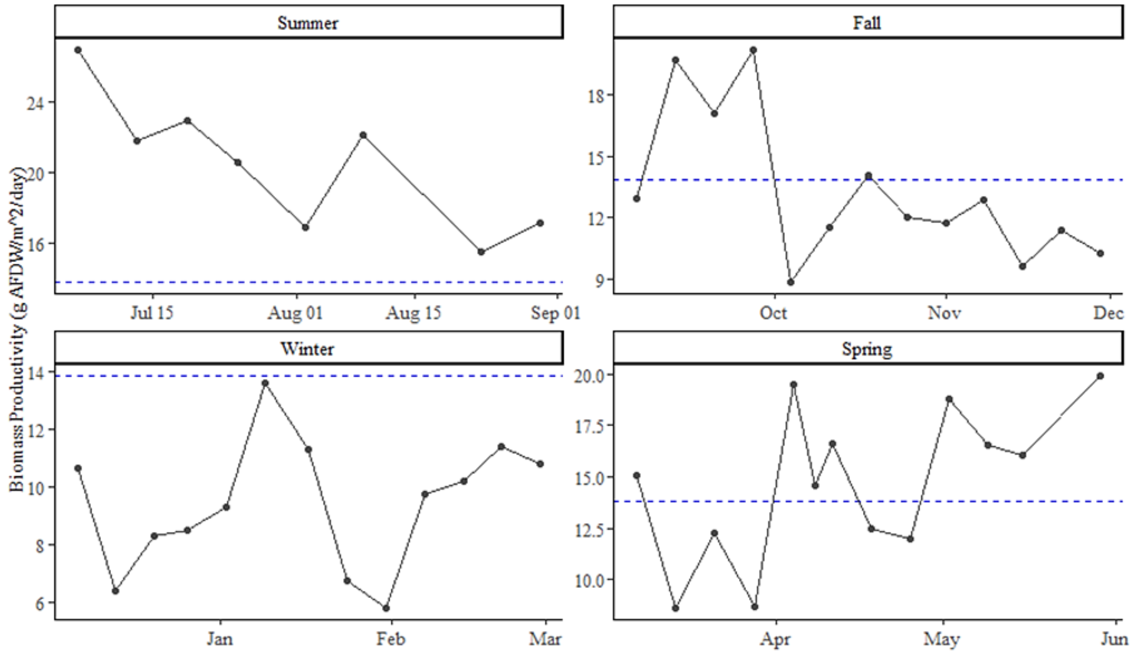
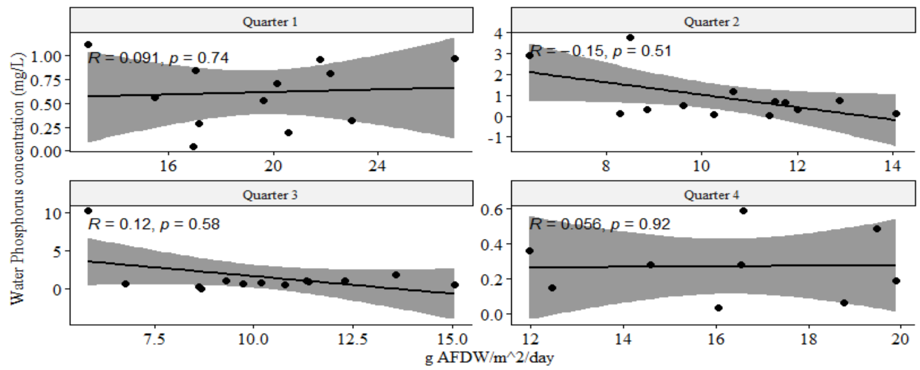
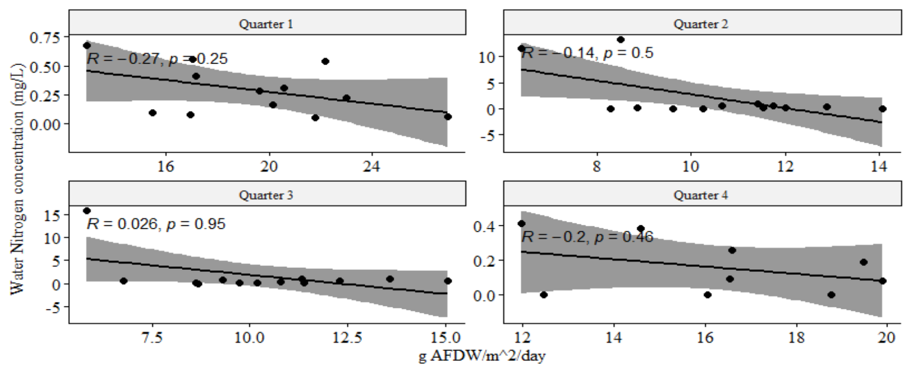
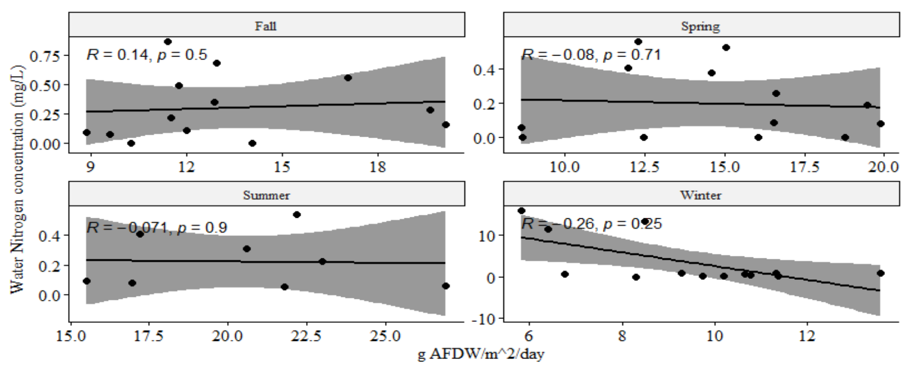


Figure 1.2 The solid line is daily productivity over 11 months. The dashed line is the mean productivity (13.8g AFDW/m^2/day) for the year.


Quarter 1
R = 0.091, p = 0.74
Quarter 2
R = −0.15, p = 0.51
Quarter 3
R = 0.12, p = 0.58
Quarter 4
R = 0.056, p = 0.92
Water Phosphorus concentration (mg/L)
g AFDW/m^2/day


Quarter 1
R = −0.27, p = 0.25
Quarter 2
R = −0.14, p = 0.5
Quarter 3
R = 0.026, p = 0.95
Quarter 4
R = −0.2, p = 0.46
Water Nitrogen concentration (mg/L)
g AFDW/m^2/day


Fall
R = 0.14, p = 0.5
Spring
R = −0.08, p = 0.71
Summer
R = −0.071, p = 0.9
Winter
R = −0.26, p = 0.25
Water Nitrogen concentration (mg/L)
g AFDW/m^2/day

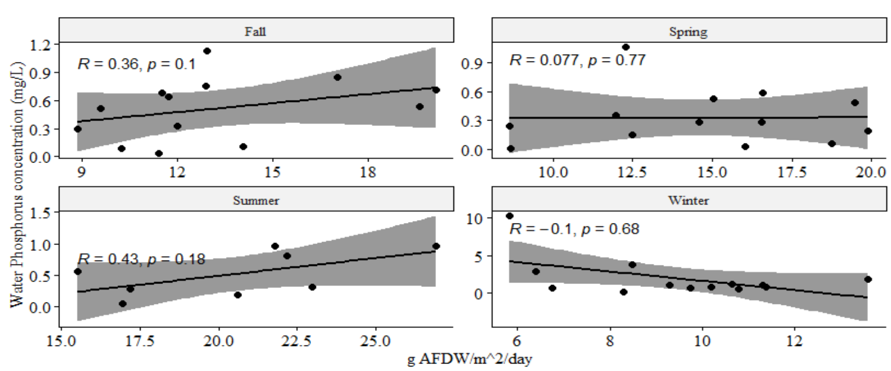

Fall
R = 0.36, p = 0.1
Spring
R = 0.077, p = 0.77
Summer
R = 0.43, p = 0.18
Winter
R = −0.1, p = 0.68
Water Phosphorus concentration (mg/L)
g AFDW/m^2/day

Figure 1.3. This is a scatterplot depicting correlations between effluent nutrient concentrations and productivity by quarter and season for 11 months. The correlation coefficient and p-value are displayed.

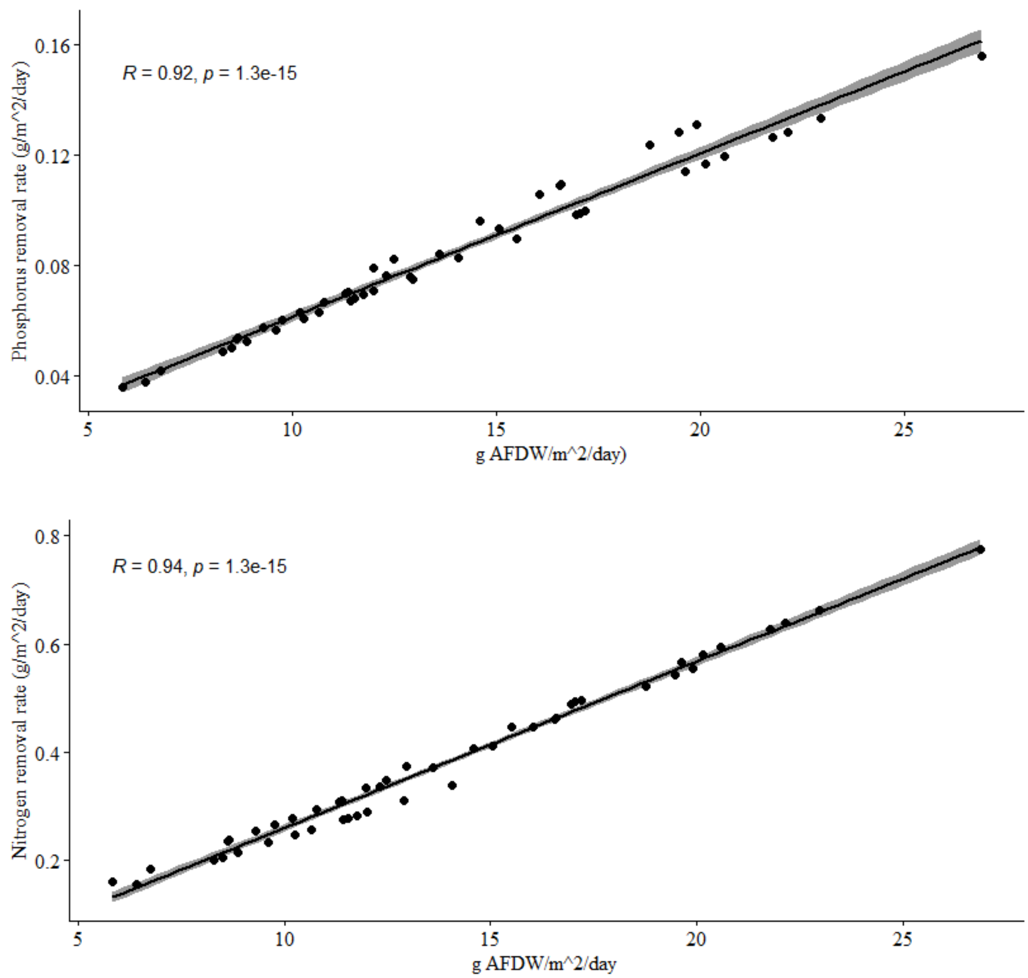


Figure 1.4. This is a scatterplot depicting correlations between Productivity and ATS biomass nutrient removal rates for 11 months. The correlation coefficient and p-value are displayed.

Fig. 2.1. This figure is a picture representation of different stages in larvae processing. A is a photo of the initial larvae size. B is a photo of the larvae added to the grow bin and ATS biomass , which served as their sole diet. C is a photo of the larvae sifting process for manual harvest of BSF larvae. D is a photo of the prepupae larval stage collected for tilapia feed.

Tables:

Table 1.1 ATS biomass quarterly production, nutrient content and proximate composition.

Variable	Quarter 1	Quarter 2	Quarter 3	Quarter 4	Year
Harvest (g) [4]	13,274.54	7,057.71	7,460.94	8,053.09	35,846.28
Productivity [1,2]	19.49 ± 3.78	10.48 ± 2.11	10.27 ± 2.57	16.26 ± 2.86	13.83 ± 4.90
Total Phosphorus (g)	230.98	124.92	138.77	159.45	162.44
Total Nitrogen (g)	1,148.83	509.85	609.53	672.21	731.65
% Ash [2]	65.41 ± 6.08	61.91 ± 3.36	64.86 ± 3.15	64.57 ± 3.55	64.13 ± 4.31
% Fat	1.08	0.00	0.08	1.12	0.51
% Protein	18.03	15.05	17.02	17.39	16.80
% Nitrogen	2.88	2.41	2.72	2.78	2.69
% Phosphorus	0.58	0.59	0.62	0.66	0.61
Phosphorus Removal Rate [3]	0.33	0.16	0.18	0.30	0.24
Nitrogen Removal Rate [3]	1.64	0.67	0.80	1.28	1.07

[1] Productivity was measured in g-AFDW/m2/day. [2] Mean ± standard deviation. [3] Removal rates were calculated as g/m2/day. [4] Harvest (g) is the sum of all weekly harvested ATS biomass. All variables except productivity, calculated on a dry weight basis. Q1=Jul-Sept; Q2=Oct-Dec; Q3=Jan-Mar; Q4= Apr-May.

Table 1.2 Fish meal and ATS proximate compositions

Proximates	FM	WW_ATS
Protein_Crude	63.2	16.50
Fat_Crude	9.0	1.07
Fiber_Crude	0.9	4.40
Ash	19.0	66.04

The following table describes the proximate composition of fish meal (FM) and ATS biomass generated from municipal wastewater effluent (WW_ATS).

Table 1.3. Metals analysis of ATS biomass

Metal	Analysis_µg/l
Tungsten	0
Tin	0
Tellerium	0
Titanium	0
Scandium	0
Silver	0
Selenium	0
Copper	0
Gallium	0
Indium	0
Bismuth	0
Lead	0
Cadmium	0
Aluminum	0
Antimony	0
Arsenic	0

An Q1 ATS biomass composite sample was tested for metals contamination.

Table 2.1 Meal proximate compositions

Proximates	FM	BSF	WW_ATS
Protein_Crude	63.2	40.45	16.50
Fat_Crude	9.0	40.65	1.07
Fiber_Crude	0.9	6.22	4.40
Ash	19.0	5.63	66.04

FM= Fish meal. BSF= Black Soldier Flies. WW_ATS= wastewater treatment algae turf scrubber.

Results are presented on a dry weight basis.

Table 2.2 Ingredient composition of formulated diets

Ingredients_per kg	FM Control	100% BSF	50% BSF	Commercial BSF
Alginate [1]	10.00	10.00	10.00	10.00
ATS BSF [2]	NA	96.36	47.70	NA
Commercial BSF [3]	NA	NA	NA	86.43
Fish meal [4]	60.00	NA	30.00	NA
Methionine [1]	NA	0.88	0.63	0.94
Soybean oil [5]	36.50	3.81	19.91	6.67
Calcium phosphate, dibasic [6]	26.00	26.00	26.00	26.00
Vitamin mix [7]	20.00	20.00	20.00	20.00
Mineral mix [8]	5.00	5.00	5.00	5.00
Soybean meal [9]	559.92	559.92	559.92	559.92
Vitamin C [1]	1.00	1.00	1.00	1.00
Whole wheat [10]	200.00	200.00	200.00	200.00
Wheat starch [11]	82.44	77.04	79.84	84.04

[1] Source: Sigma-Aldrich, St. Louis, MO. [2] Source: Black Soldier Flies raised at Georgia Southern University. [3] Source: Grubbly Farms, Atlanta, GA. [4] Source: Omega Protein Corporation, Houston, TX. [5] Source: IGA, Inc., Chicago, IL. [6] Source: Research Products International Corporation, Mount Prospect, IL. [7] Contents (mg/g premix): cobalt chloride, 0.004; cupric sulfate pentahydrate, 0.250; ferrous sulfate, 4.000; magnesium sulfate anhydrous, 13.862; manganous sulfate monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate hepahydrate, 13.193; alpha-cellulose, 67.964. [8] Contents (g/kg premix): thiamin HCl, 0.44; riboflavin, 0.63; pyridoxine HCl, 0.91; dl pantothenic acid, 1.72; nicotinic acid, 4.58; biotin, 0.21; folic acid, 0.55; inositol, 21.05; menadione sodium bisulfite, 0.89; vitamin A acetate, 0.68; vitamin D3, 0.12; dl-alpha-tocoperol acetate, 12.63; alpha-cellulose, 955.59. [9]

Source: Flint River Mills, Inc., Bainbridge, GA. [10] Source: King Arthur Baking, Norwich, VT.

[11] Source: Sadaf Foods, Los Angeles, CA.

Table 2.3 Proximate composition of Tilapia diets

	FM	100% BSF	50% BSF	Commercial BSF	Commercial Control
Protein	37.05	37.14	37.60	37.51	36.01
Fat	5.48	5.33	5.34	5.24	3.12
Ash	8.34	7.96	7.95	8.02	7.71
Fiber	3.29	3.24	3.36	3.56	4.19

Results are presented on a percent dry weight basis.

Table 2.4 Growth Summary

Variable	100% BSF[1]	50% BSF[1]	Commercial BSF[1]	Commercial FM[1]	FM[1]	p-value	PSE
Initial Weight	5.87 ± 0.30	6.00 ± 0.33	6.13 ± 0.30	5.80 ± 0.30	6.13 ± 0.18	0.25[2]	0.05
Final Weight	21.60 ± 1.76	20.00 ± 3.13	19.14 ± 3.29	15.93 ± 1.21	19.46 ± 4.06	0.07[3]	0.52
Growth	15.73 ± 1.69	14.00 ± 3.26	13.01 ± 3.09	10.13 ± 1.04	13.33 ± 4.10	0.07[3]	0.51
survival							
100	5 (100%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)		
FER	1.02 ± 0.14	0.83 ± 0.16	0.86 ± 0.22	0.66 ± 0.13	0.91 ± 0.27	0.08[3]	0.03
FCR	0.99 ± 0.15	1.25 ± 0.29	1.22 ± 0.30	1.60 ± 0.44	1.17 ± 0.33	0.08[3]	0.06
PER	0.11 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.07 ± 0.01	0.10 ± 0.03	0.08[3]	0.00
CF	2.47 ± 1.70	3.04 ± 4.04	2.11 ± 0.25	1.90 ± 1.24	2.46 ± 2.85	0.45[3]	0.20

[1] Mean ± SD. [2] Kruskal-Wallis rank sum test. [3] One-way ANOVA. Weights are presented in grams. FER= Feed Efficiency Ratio (weight gain/ dry feed offered). FCR= feed conversion ratio (feed given/weight gain). PER= protein efficiency ratio (weight gain/protein ingested). CF= condition factor (100 x {g body weight / total length in cm3}).

Table 2.5 Health Parameters

Variable	100% BSF[1]	50% BSF[1]	Commercial BSF[1]	Commercial Control[1]	FM[1]	p-value	PSE
VSI	7.49 ± 1.15	8.50 ± 1.17	8.14 ± 1.27	7.04 ± 0.49	8.24 ± 1.24	0.24[2]	0.20
HSI	0.12 ± 0.04	0.21 ± 0.09	0.19 ± 0.09	0.18 ± 0.10	0.07 ± 0.05	0.09[2]	0.02
IPF	0.02 ± 0.05	0.14 ± 0.23	0.07 ± 0.15	0.07 ± 0.08	0.03 ± 0.06	0.60[3]	0.02
MM	24.06 ± 4.75	26.07 ± 3.35	22.87 ± 1.61	24.36 ± 2.97	25.53 ± 6.29	0.76[2]	0.74

[1] Data is presented as mean ± standard deviation. [2] Analyzed using one-way ANOVA. [3] Analyzed using Kruskal-Wallis rank sum test. Values with different letters are statistically significant ($p < 0.05$). VSI= viscerosomatic index; HSI= hepatosomatic index; IPF= intraperitoneal fat; MM= muscle mass index; CF= condition factor

Table 2.6 Tilapia body composition

	100% BSF	50% BSF	Commercial BSF	Commercial Control	FM
Fat	16.76	16.85	12.35	14.49	18.67
Protein	66.63	66.59	68.21	68.47	63.85
Ash	17.66	16.79	16.68	16.70	16.95

All results are presented on a percent dry weight basis.

Table 2.7 Apparent Digestibility Coefficients (ADC)

Variable	BSF[1]	FM[1]	p-value[2]	PSE
Diet_ADC	91.19 ± 1.55	89.67 ± 1.65	0.31	0.53
Protein_ADC	76.5 ± 5.2	71.4 ± 5.5	0.31	1.78

[1] Data is presented as mean ± standard deviation (n=3). [2]Analyzed by one-way ANOVA.

Table 2.8 Meal amino acid compositions

Amino Acids	Fish Meal	BSF Meal	ATS Meal
Methionine	1.78	0.59	2.78
Cystine	NA	0.30	NA
Lysine	4.80	1.63	4.94
Phenylalanine	2.50	1.10	6.64
Leucine	4.55	1.70	7.82
Isoleucine	2.93	0.96	5.68
Threonine	2.54	1.12	4.49
Valine	3.27	1.73	2.75
Histidine	1.47	0.85	1.92
Arginine	3.81	1.55	7.40
Glycine	NA	1.52	NA
Asparatic Acid	NA	2.77	NA
Serine	NA	1.26	NA
Glutamic Acid	NA	3.65	NA
Proline	NA	1.69	NA
Hydroxyproline	NA	0.33	NA
Alanine	NA	1.81	NA
Tyrosine	NA	1.88	NA

Amino acid composition is presented in g/100g dry weight.