ASSESSING THE CAPACITY OF RED ALGAE (*GRACILARIA VERMICULOPHYLLA)* AND AMERICAN OYSTERS (*CRASSOSTREA VIRGINICA)* TO SEQUESTER AMMONIA FROM SHRIMP (*LITOPENAEUS VANNAMEI*) IN A LAND-BASED IMTA SYSTEM.

BY

ELIZABETH MARTIN

Environmental Science: Ecosystems (B.S.), University of New Hampshire, 2020

THESIS

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Master of Science
in
Marine Biology

September 2024

This thesis was examined and approved in partial fulfillment of the requirements for the degree of Master of Science in Marine Biology by:

Thesis Director, Dr. Michael Chambers,
Research Professor of Aquaculture

Dr. Christopher Neefus, Professor of Plant Biology

Dr. David W. Fredriksson, Professor of Ocean Engineering

Dr. Michael Coogan, Postdoctoral Researcher of Aquaculture

On June 25th, 2024

Approval signatures are on file with the University of New Hampshire Graduate School.

TABLE OF CONTENTS

TABLE OF CONTENTS .... *iii*

LIST OF TABLES .... *iv*

LIST OF FIGURES .... *iv*

ABSTRACT .... *vi*

INTRODUCTION *1*

CHAPTER I. MEASURING AMMONIA UPTAKE BY GRACILARIA VERMICULOPHYLLA AND AMMONIA OUTPUT BY LITOPENAEUS VANNAMEI *8*

INTRODUCTION 8

METHODS 11

RESULTS 17

DISCUSSION 21

CHAPTER II. ASSESSING THE CAPACITY OF GRACILARIA VERMICULOPHYLLA AND CRASSOSTREA VIRGINICA TO SEQUESTER AMMONIA FROM LITOPENAEUS VANNAMEI IN A LAND-BASED IMTA SYSTEM *24*

INTRODUCTION 24

METHODS 25

RESULTS 32

DISCUSSION 40

CONCLUSION 43

CHAPTER III. COMPARING THE CAPACITY OF VARYING BIOMASS AMOUNTS OF GRACILARIA VERMICULOPHYLLA TO SEQUESTER TAN FROM LITOPENAEUS VANNAMEI IN A LAND-BASED IMTA SYSTEM *44*

INTRODUCTION 44

METHODS 45

RESULTS 48

DISCUSSION 55

CONCLUSION **58**
REFERENCES ***59***

LIST OF TABLES

TABLE 2.1: PROXIMATE ANALYSES RESULTS FOR LITOPENAEUS VANNAMEI, GRACILARIA VERMICULOPHYLLA AND CRASSOSTREA VIRGINICA BEFORE AND AFTER THE IMTA EXPERIMENT. 40

LIST OF FIGURES

FIGURE1.1: GRACILARIA VERMICULOPHYLLA IN A CONTROLLED ENVIRONMENTAL CHAMBER AT THE UNIVERSITY OF NEW HAMPSHIRE IN 500ML FLASKS CONTAINING SEA WATER WITH VARYING CONCENTRATIONS OF TAN.. 12

FIGURE 1.2: EXAMPLE OF LITOPENAEUS VANNAMEI IN A 9-LITER TANK DURING THE TAN OUTPUT EXPERIMENT AT THE UNIVERSITY OF NEW HAMPSHIRE'S COASTAL MARINE LABORATORY IN NEW CASTLE, NH. THREE TANKS EACH CONTAINED 4 GRAMS OF SHRIMP AND 1 GRAM OF FEED PELLETS. 13

FIGURE 1.3: UPTAKE RATE (μMOL/G FW/HOUR) OF GRACILARIA VERMICULOPHYLLA AS A FUNCTION OF TAN (μM). THE CURVE REPRESENTS A NONLINEAR REGRESSION FIT OF THE PARAMETERS FOR THE MICHALIS-MENTEN EQUATION. 18

FIGURE1.4: OUTPUT RATE OF TAN (μMOL/G/HOUR) PRODUCED BY SHRIMP, LITOPENAEUS VANNAMEI, FROM ZERO TO FIVE HOURS AFTER FEEDING. FEEDING OCCURRED AT TIME (HR) 0. TANKS 1, 2 AND 3 REPRESENT REPLICATES OF THE SAME TREATMENT................................. 19

FIGURE1.5: AVERAGE RATE OF TAN EXCRETION EVERY HOUR AFTER SHRIMP, LITOPENAEUS VANNAMEI, FEEDING. MEAN VALUES WITH A LETTER IN COMMON ARE NOT SIGNIFICANTLY DIFFERENT ($P > 0.05$). 20

FIGURE1.6: CALCULATED TAN (μM) AT VARIOUS RATIOS OF SEAWEED:SHRIMP RATIOS BASED ON EQUATION (4) AND EXPERIMENTALLY DETERMINED TAN UPTAKE RATES BY SEAWEED (GRACILARIA VERMICULOPHYLLA) AND TAN PRODUCTION RATES BY SHRIMP (LITOPENAEUS VANNAMEI). 21

FIGURE 2.1: ILLUSTRATION OF THE LARGE TANK HOLDING SYSTEM WHERE POST LARVAL SHRIMP LITOPENAEUS VANNAMEI WERE GROWN PRIOR TO BEING TRANSFERRED TO THE EXPERIMENTAL AQUATIC HABITAT SYSTEM. 26

FIGURE 2.2: ILLUSTRATION OF THE EXPERIMENTAL AHAB SYSTEM USED FOR THE LAND-BASED IMTA EXPERIMENT CONTAINING LITOPENAEUS VANNAMEI, GRACILARIA VERMICULOPHYLLA AND CRASSOSTREA VIRGINICA. 27

FIGURE 2.3: ILLUSTRATION OF A SINGULAR IMTA AHAB SYSTEM INCLUDING VOLUME AND HEIGHT MEASUREMENTS. 29

FIGURE 2.4: TAN, COMBINED NITRITE + NITRATE AND TOTAL NITROGEN COLLECTED DAILY FROM THE IMTA SYSTEM CONTAINING LITOPENAEUS VANNAMEI, GRACILARIA VERMICULOPHYLLA AND CRASSOSTREA VIRGINICA BEFORE FEEDING. DATA IS SEPARATED BY TREATMENT TYPE.33

FIGURE 2.5: LOG TOTAL NITROGEN MEASURED EVERY DAY FROM EACH SYSTEM AS A FUNCTION OF TIME (DAYS) DURING THE IMTA EXPERIMENT CONTAINING LITOPENAEUS VANNAMEI, GRACILARIA VERMICULOPHYLLA AND CRASSOSTREA VIRGINICA. THERE IS A SIGNIFICANT DIFFERENCE BETWEEN THE SLOPE OF TREATMENTS AIR AND OYSTERS ($P \leq 0.032$) AND NO SIGNIFICANT DIFFERENCE BETWEEN THE SLOPE OF AIR AND SETTLE TANK TREATMENTS. 34

FIGURE 2.6: MEAN TAN CONCENTRATIONS IN WATER SAMPLES COLLECTED BEFORE FEEDING (T0) AND UP TO FIVE HOURS AFTER FEEDING ONCE A WEEK FOR THREE WEEKS DURING THE IMTA EXPERIMENT CONTAINING LITOPENAEUS VANNAMEI, GRACILARIA VERMICULOPHYLLA AND CRASSOSTREA VIRGINICA. ERROR BARS ARE STANDARD ERROR. GRAPHS ARE SEPARATED BY WEEK. WITHIN EACH GRAPH, BARS WITH A LETTER IN COMMON ARE NOT SIGNIFICANTLY DIFFERENT. 36

FIGURE 2.7: LITOPENAEUS VANNAMEI AVERAGE BIOMASS (G) COMPARED ACROSS EACH WEEK OF THE EXPERIMENT. ERROR BARS ARE STANDARD ERROR. DIFFERENT LETTERS REPRESENT SIGNIFICANT DIFFERENCES BETWEEN WEEKS. 37

FIGURE 2.8: CRASSOSTREA VIRGINICA AVERAGE BIOMASS MEASURED ONCE A WEEK DURING THE 4-WEEK EXPERIMENT. ERROR BARS ARE STANDARD ERROR. NO SIGNIFICANT DIFFERENCES WERE FOUND BETWEEN WEEKS. 38

FIGURE 2.9: GRACILARIA VERMICULOPHYLLA AVERAGE BIOMASS (G) COMPARED ACROSS EACH WEEK OF THE EXPERIMENT. ERROR BARS ARE STANDARD ERROR. DIFFERENT LETTERS REPRESENT SIGNIFICANT DIFFERENCES BETWEEN WEEKS. BARS WITH A SHARED LETTER ARE NOT SIGNIFICANTLY DIFFERENT FROM EACH OTHER. 39

FIGURE 3.1: RESEARCH DESIGN DIAGRAM SHOWING THE AQUATIC HABITAT SETUP CONTAINING LITOPENAEUS VANNAMEI AND GRACILARIA VERMICULOPHYLLA AND TREATMENT LEVELS.. 45

FIGURE 3.2: TAN, COMBINED NITRITE + NITRATE AND TOTAL NITROGEN COLLECTED EVERY DAY FROM THE IMTA SYSTEM CONTAINING LITOPENAEUS VANNAMEI AND GRACILARIA VERMICULOPHYLLA BEFORE FEEDING DURING THE 8-DAY EXPERIMENT. DATA IS SEPARATED BY TREATMENT TYPE. 49

FIGURE 3.3: LOG TRANSFORMED NITRITE + NITRATE MEASURED EVERY DAY FROM EACH SYSTEM COMPARING A RATIO OF 1:2, 1:3, AND 1:4 OF LITOPENAEUS VANNAMEI TO GRACILARIA VERMICULOPHYLLA GRAPHED AGAINST TIME IN DAYS. THERE IS A SIGNIFICANT DIFFERENCE BETWEEN THE SLOPE OF TREATMENT 1:3 AND 1:4 ($P \leq 0.008$), 1:2 AND 1:3 ($P \leq 0.030$) AND NO SIGNIFICANT DIFFERENCE BETWEEN 1:2 AND 1:4 ($P \leq 0.8786$). 50

FIGURE 3.4: LOG TOTAL NITROGEN MEASURED EVERY DAY FROM EACH SYSTEM GRAPHED AGAINST TIME IN DAYS DURING THE IMTA EXPERIMENT CONTAINING LITOPENAEUS VANNAMEI AND GRACILARIA VERMICULOPHYLLA. THERE IS A SIGNIFICANT DIFFERENCE BETWEEN THE SLOPE OF TREATMENTS 1:3 AND 1:4 ($P \leq 0.0339$) AND NO SIGNIFICANT DIFFERENCE BETWEEN THE SLOPE OF 1:2 AND 1:4.. 51

FIGURE 3.5: AVERAGE TAN(μM) IN THE AHAB SYSTEM BEFORE FEEDING AND UP TO FIVE HOURS AFTER FEEDING DURING THE 8 DAY EXPERIMENT COMPARING A RATIO OF 1:2, 1:3, AND 1:4 OF *LITOPENAEUS VANNAMEI* TO *GRACILARIA VERMICULOPHYLLA*. AN ANOVA FOUND SIGNIFICANT DIFFERENCES IN TAN CONCENTRATION AT DIFFERENT TIMES AFTER SHRIMP WERE FED, REPRESENTED BY DIFFERENCES IN LETTERS ON EACH BAR. ERROR BARS REPRESENT STANDARD ERROR. NO SIGNIFICANT DIFFERENCES ON TREATMENTS WERE FOUND ($P < 0.05$). 52

FIGURE 3.6: AVERAGE % GRACILARIA VERMICULOPHYLLA GROWTH PER TREATMENT BETWEEN DAY 1 AND 8 OF THE 8 DAY IMTA EXPERIMENT. DIFFERENCES WERE NOT SIGNIFICANT. ERROR BARS REPRESENT STANDARD ERROR. 53

FIGURE 3.7: AVERAGE INITIAL AND AVERAGE FINAL GRACILARIA VERMICULOPHYLLA BIOMASS (G) DURING THE 8 DAY IMTA EXPERIMENT. DIFFERENT LETTERS REPRESENT SIGNIFICANT DIFFERENCES BETWEEN WEEKS ($P > 0.05$). ERROR BARS REPRESENT STANDARD ERROR. 53

FIGURE 3.8: CHANGE IN LITOPENAEUS VANNAMEI BIOMASS BETWEEN INITIAL AND FINAL MEASUREMENTS COMPARED ACROSS TREATMENTS DURING THE 8-DAY IMTA EXPERIMENT. ERROR BARS REPRESENT STANDARD ERROR. THERE WAS NO SIGNIFICANT DIFFERENCE BETWEEN TREATMENTS.. 54

FIGURE 3.9: AVERAGE INITIAL AND AVERAGE FINAL LITOPENAEUS VANNAMEI BIOMASS (G). THERE WAS NO SIGNIFICANT DIFFERENCE BETWEEN INITIAL AND FINAL BIOMASS. ERROR BARS REPRESENT STANDARD ERROR. 55

.

ABSTRACT

ASSESSING THE CAPACITY OF RED ALGAE (*GRACILARIA VERMICULOPYLLA*) AND AMERICAN OYSTERS (*CRASSOSTREA VIRGINICA)* TO SEQUESTER AMMONIA FROM SHRIMP (*LITOPENAEUS VANNAMEI*) IN A LAND-BASED IMTA SYSTEM.

By

Elizabeth Martin

University of New Hampshire, September 2024

Growing shrimp with extractive species is gaining momentum to reduce effluent waste in aquaculture systems. Preliminary experiments were conducted at the University of New Hampshire's Spaulding laboratory to calculate total ammonia nitrogen uptake rates by *Gracilaria vermiculophylla* and output rates by whiteleg shrimp, *Litopenaeus vannamei*. Based on these results, oysters and red algae were incorporated into a shrimp recirculating aquaculture system at University of New Hampshire's Coastal Marine Laboratory to determine their effectiveness in reducing dissolved nitrogen. This is a critical step in mitigating negative impacts to ecosystems surrounding shrimp aquaculture operations, while producing valuable biproducts.

An aquatic habitat (AHAB) system was used, which consisted of 36 individual 9L tanks vertically stacked into 9 columns and 4 rows, each column making up an isolated system. A 4-week trial was conducted comparing three treatment groups: 1) shrimp grown with oysters and red algae, 2) shrimp grown with only red algae, and 3) shrimp grown with red algae as well as

aeration to re-suspend waste particles. No additional biofilters or mechanical filters were used. Oysters were found to have a significant impact on maintaining low nitrogen levels in the system over time compared to treatments with no oysters ($p \leq 0.032$).

A second one-week trial was conducted to compare systems with different ratios of shrimp to seaweed biomass. Shrimp were grown with the same ratio as the first trial; a shrimp:seaweed ratio of 1:2, and an increased ratio of 1:3 and 1:4. Water samples for both trials were collected once a day before feeding, and once a week before feeding and at regular intervals up to five hours after feeding. Biomass data was also collected once a week throughout the experiment. Results suggest a higher growth rate for *G. vermiculophylla* in a ratio of 1:3 shrimp:seaweed, as well as a significantly higher combined nitrite + nitrate concentration maintained in the 1:3 ratio treatment compared to the other two treatments.

INTRODUCTION

Since the 1960s, wild fish stocks have declined to the point where they can no longer meet human demand (FAO, 2022). Seafood consumption has increased on a global scale, outpacing the rate that wild fish and fishery products can be sustainably harvested. Aquaculture production has increased, filling the gap left between the supply and demand for fishery products. In the coming years, aquaculture production is projected to become increasingly important to sustainably feed a growing world population (FAO, 2022).

Global seafood consumption has increased by 3% per year since 1960, and is projected to continue growing, resulting in an additional 15% increase by 2030. Simultaneously, fisheries continue to decline due to pollution, climate change and habitat loss. These factors have led to the increase of world aquaculture production, which, led by Asia, generated 87.5 million tons of finfish, mollusks and crustaceans and 35.1 million tons of algae in 2020. Comparatively, 90.26 million tons of fishery products were produced from capture fisheries from marine and inland waters in the same year (FAO, 2022).

Current aquaculture practices typically involve high-intensity monoculture and large-scale production for maximum revenue. This leads to densely populated growing areas and subsequently high concentrations of nitrogen produced by animal waste (Aubin et al., 2009). High concentrations of nitrogen are considered a pollutant (US EPA, 2013) because it can disrupt ecosystem function and decrease biodiversity (Kalansooriya & Wijesinghe, 2012). As seafood products will increasingly be sourced from aquaculture, the sustainable and equitable development of aquaculture is essential to preserve ecosystem health, mitigate pollution and protect biodiversity and social equality (FAO, 2022). By following these priorities, aquaculture can help alleviate pressure on wild fisheries, improve ecosystem function, supply healthy protein and generate jobs and livelihoods worldwide.

This study focuses on reducing nitrogenous waste from aquaculture systems containing fed species through the addition of shellfish and macroalgae. This solution not only has the ability to reduce waste but to diversify marketable products (NOAA, 2016). The key to creating a successful system with multiple aquaculture species is understanding the role that each species plays in the nitrogen cycle. Fed species, which include most finfish and shrimp, contribute to the nitrogen cycle by releasing ammonium as a waste product. Shrimp aquaculture generally occurs in large-scale artificial ponds where water is exchanged with surrounding ecosystems to prevent ammonium build-up in the pond. This exchange leads to nitrogen overloading in the surrounding ecosystem resulting in algal blooms. Algae is then decomposed by bacteria, using up all the available oxygen in the water column, creating what is known as zero oxygen "dead" zones. This process, called eutrophication, can severely impact the species that rely on oxygen where dead zones occur (Burford & Williams, 2001; Kalansooriya & Wijesinghe, 2012). In addition, shrimp farms are frequently constructed in mangrove forests and responsible for an estimated 20% decline of these habitats worldwide. Because of the ecosystem services that mangroves provide, this also negatively impacts biodiversity, coastal erosion and emits high levels of carbon dioxide (Herbeck et al., 2013).

Bivalves are filter feeders that consume particulate matter and plankton in the water column. In this way, they can act as a nitrogen sink or extractive species in estuaries, storing excess nitrogen in tissue (Humphries et al., 2016). Oysters remove large amounts of nitrogen through filter feeding. Their bio deposits additionally contribute to ammonium transformation and removal (Labrie et al., 2023). Although bio deposits contain ammonium (Buzin et al., 2015), the ammonium on surface sediment becomes available for sediment nitrifying bacteria. These bacteria convert ammonium into nitrite and nitrate, converting it into a less toxic form that can be removed by macroalgae. Additionally, in deeper anoxic sediment, ammonium in oyster bio deposits goes through the process of denitrification, where $NO3^-$ is converted into dinitrogen gas and released into the atmosphere (Bricker et al., 2020). This last form of nitrogen removal, which completely removes nitrogen from the ecosystem, is found to occur at a higher rate in areas that contain oysters (Labrie et al., 2023).

Macroalgae take up nutrients from the water column, capturing dissolved carbon, phosphorus and nitrogen and storing it in their tissue (Duan et al., 2019). An increase in growth in multiple species of macroalgae has been observed to be correlated to an increase in nitrogen concentration (Peckol et al., 1994). The nitrogen removal capacity of these algal species is greater than $5g\ N\ m^{-1}d^{-1}$, suggesting that they can effectively reduce nitrogen concentrations (Peckol et al., 1994). In a natural ecosystem, fed species, bivalves and macroalgae are found growing together. They provide a nutrient balance, with fish and shrimp releasing ammonium into the water column, stimulating the growth of phytoplankton. The phytoplankton are then filtered out of the water by bivalves, and the remaining nitrogen in the form ammonium, nitrite and nitrate are taken up by extractive macroalgae species (Hadley et al., 2016). Aquaculture practices have begun to incorporate this natural ecosystem model to mitigate the discharge of nitrogen into surrounding ecosystems. This form of aquaculture is referred to as IMTA, or Integrated Multi-Trophic Aquaculture (NOAA, 2016).

Nitrogen removal from shrimp wastewater was quantified by *Mawi et al* (2020) in an IMTA system containing two species of *Gracilaria*. The study found that *Gracilaria* took up nitrogen more readily in the form of ammonium than nitrate and acted as an improved biofilter for shrimp excretion. The study concluded that *Gracilaria* can decrease nutrient output from shrimp farming (Mawi et al., 2020).

An ecological model was built in another study to integrate oysters (*Crassostrea sp.*) and macroalgae (*Gracilaria sp.)* into an existing large-scale shrimp pond in Indonesia (Astriana, 2012). The model quantified the flow of nitrogen in a land based IMTA system using three separate ponds for shrimp, oysters and macroalgae. This study identified some key factors that impact model output, such as a shrimp consumption of feed ratio of 24%, reflecting that shrimp typically only consume 24% of feed in large-scale ponds. This consumption of feed ratio must be considered when designing and operating a shrimp production system (Burford & Williams, 2001). In this model, the growth of phytoplankton played an important role by utilizing dissolved nitrogen, making it bioavailable to oysters. *Gracilaria* was used for its ability to take up nitrogen in the form of ammonium, directly removing dissolved waste products from shrimp and oysters. The study identified a need for future studies to implement this model into an existing system to quantify results and increase its accuracy. The current study quantifies nitrogen concentrations in a land-based IMTA system containing shrimp, oysters and red macroalgae. These species were chosen for their nitrogen cycling capacity, market value, and aquaculture compatibility.

Shrimp is the highest valued import market in the United States, at US$6.4 billion in 2020 (Fisheries, 2022b). It is estimated that over 50% of the imported seafood in the United States is from aquaculture (Fisheries, 2022a), including a large portion of shrimp from ponds in Asia and Ecuador (NOAA, 2016). The demand for shrimp in the United States creates potential for local production and provides an opportunity to change traditional shrimp production to smaller, more sustainable systems, a need that has been identified nationally (Fisheries, 2022). The shrimp used in this study are known as Pacific white shrimp or whiteleg shrimp, *Litopenaeus vannamei.* White shrimp are native to the Pacific Coast of Latin America with a range that extends from Peru to Mexico (Liao & Chien, 2011). They prefer tropical marine habitats in waters above 20°C. They are a semi-pelagic species, living as adults in the open ocean and spending their juvenile life stage in coastal estuaries, lagoons and mangrove habitats. *L. vannamei* were first produced in Asia in the 1990s (Perry, 2023) for their favorable traits in aquaculture. They have an increased resilience to pathogens, high larval survival, fast growth rate, tolerance to high stocking density, tolerance to increased ammonium and nitrite concentrations, and are adaptable to low salinities (Liao & Chien, 2011). Shrimp are grazers, causing the release of particulate organic matter during feeding. This makes them ideal species to pair with bivalves, which filter the leftover feed particles out of the water column.

The East Coast of the U.S. has a growing oyster industry, which contributed, along with clams and mussels, to a revenue of US$340 million in 2016 (Fisheries, 2022a). This study used American oysters, *Crassostrea virginica,* which are native to the East Coast of the U.S., inhabiting waters from the Gulf of the St. Lawrence to the Gulf of Mexico. They are more tolerant to warmer and lower salinity water than other shellfish such as mussels, which corresponds to the habitat preferences of *L. vannamei. C. virginica* prefer brackish and salty waters in estuaries and tidal habitat ranging from 8 to 35ft deep. Oysters are historically known for creating large reefs similar in shape to sand bars, consisting of the accumulation of large numbers of oysters. These reefs, as well as modern oyster farms provide habitat for fish, invertebrates, macrofauna and birds (Fisheries, 2023). Wild populations that make up these reefs have declined due to disease, overharvesting, habitat loss and poor water quality. In the Northeastern U.S., there has been a growing industry of oyster farming, as well as conservation efforts to restore native oyster populations (Humphries et al., 2016). Oyster restoration is driven by research highlighting important ecosystem services that oysters provide, including nitrogen cycling through filter feeding and bio deposits, habitat for marine life, and storm surge mitigation (Fisheries, 2023). Oysters have the ability to filter particulate matter out of the water column, thereby removing particulate nitrogen. However, like shrimp, they produce ammonia as a waste product. For this reason, paring shrimp and oysters with seaweed is crucial to the success of this IMTA system.

Production of macroalgae has also increased in recent years on U.S coastlines from the Northeast to Alaska. Seaweed is sold for use as food, feed ingredients and fertilizer. It also has potential for biofuel and new materials. *Gracilaria vermiculophylla* is a genus of macroalgae in the Phylum Rhodophyta (the red seaweeds), and the seaweed chosen for this study. There are over 100 different species of *Gracilaria* found worldwide, and multiple species in the Northeastern United States, although not all are native (USDA, 2019). It is important to note that because of the