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ASSESSING THE UTILITY OF ENVIRONMENTAL DNA TECHNIQUES TO MONITOR WHITE SHRIMP (*LITOPENAEUS SETIFERUS*) ABUNDANCE ON THE GEORGIA COAST

by

RAVEN HURT

(Under the Direction of John Carroll)

ABSTRACT

Environmental DNA (eDNA) surveys have developed over the last decade from once being a novel tool to now acting as an effective technology often used in complement to traditional capture surveys for assessing the distribution of organisms in freshwater and marine environments. However, many uncertainties on how to properly develop, operate, and analyze eDNA based techniques still hinder this technology's effectiveness in the field. The white shrimp, *Litopenaeus setiferus*, is a common, commercially and recreationally important species in the United States, with landings exceeding $254 million in 2020. *L. setiferus* is also used as a key indicator species to changes in estuarine water quality and habitat. Given the commercial and ecological value of *L. setiferus*, they represented a useful species to explore the utility of eDNA techniques for fishery monitoring. In this study, I examined how *L. setiferus* abundance and biomass correlated to the amount of eDNA found in the environment, while investigating how factors like temperature effect eDNA detection over time. However, this study yielded mixed results bearing difficulties with assay specificity, and sample amplification, highlighting the challenges associated with using eDNA sampling on marine crustaceans. Ultimately, these findings emphasized the need for standardized assay validations and the importance of appropriate selection of target species, environment, sampling, and detection method before trying to comprehensively use eDNA technologies for fisheries management.

INDEX WORDS: White shrimp, *Litopenaeus setiferus*, Environmental DNA, eDNA, Species monitoring, Crustacean, Assay design

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by

RAVEN HURT

B.S., Illinois State University, 2019

A Thesis Submitted to the Graduate Faculty of Georgia Southern University

in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

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by

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Electronic Version Approved:
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CHAPTER 1

AN OVERVIEW OF WHITE SHRIMP (*L. SETIFERUS*)

Study Introduction

Environmental DNA (eDNA) surveys have emerged as powerful tools used in complement to traditional capture surveys for assessing the distribution of organisms in freshwater and marine environments over the last decade. Organisms continuously shed DNA into surrounding environments through sloughed off cells, hair, gametes, fecal matter, etc. This genetic material can then be collected through water samples, captured through filtration, extracted, and amplified, resulting in detection and spatial distribution data. Therefore, researchers and managers suggest eDNA based surveys may fill an important gap in broad-scale monitoring of biodiversity and resources in the future. The white shrimp, *Litopenaeus setiferus*, is a common commercially and recreationally important species in the United States with landings exceeding $254 million in 2020. *L. setiferus* is also used as a key indicator species to changes in estuarine water quality and habitat. Given the commercial and ecological value of *L. setiferus*, they may represent a useful species to explore the utility of eDNA techniques for fishery monitoring.

Litopenaeus setiferus Biology

White shrimp, *Litopenaeus setiferus* (Linnaeus, 1797), are one of three commercially important species of Penaeid shrimp found off the Atlantic and Gulf coasts of the United States (Ditty, 2011), commonly found in regions distinguished by vast inland, brackish marshes (Anderson et al., 1949). Though they inhabit coastal marshes for development and growth, spawning occurs just offshore in the spring and is triggered by increasing bottom water

temperatures (Lindner & Anderson 1956, Dall et al., 1990). Male shrimp transfer a spermatophore to the underside of female shrimp where spawning and fertilization is thought to occur immediately (Perez- Farfante, 1969). The eggs then become demersal and sink to the ocean floor (Anderson, 1966; Lindner & Cook, 1970). Throughout the next 12-24 hours the eggs hatch and become larvae that then rise and move into the water column (Klima et a1., 1982, Anderson, 1966). Developing larvae of *L. setiferus* have three distinct planktonic larval stages, i.e., nauplius, protozoea, and mysis coupled with several molts within each stage (Palomino et al., 2001). The transitioning process through larval stages can take weeks and the rate of their transition is highly dependent on water temperature (Lindner & Cook 1970). Eventually, larvae are moved inshore via wind currents and tides where they further undergo metamorphosis to benthic postlarval shrimp and settle in estuarine nursery habitats (Whitaker, 1983a; Anderson et al., 1949; Williams, 1955). They spend 7-9 months of their lives in the estuary to grow, feed, and avoid predation before returning to shelf waters as sub-adults (Renaud, 1986; Zein-Eldin, 1986; Chow et al., 1993; Misamore & Browdy, 1996; Rosas et al., 1999, 2004). When shrimp reach approximately 12.7-20.32 cm, they are considered adults and are mature enough to reproduce (Whitaker, 1983b).

Many factors affect the distribution of *L. setiferus* throughout their lifetimes. Hydrodynamic influences such as bottom currents (McKenzie, 1981), spring tides, robust tidal exchanges during storms, paired with temperature, body size, predation, and age (Shipman, 1983; White & Boudreaux, 1977; Klima et a1., 1982) all influence the migrations of white shrimp into and from estuaries. Specifically, smaller, juvenile shrimp are routinely found in higher abundances in the upper reaches of tidal creeks (Hackney & Burbanck, 1976; Webb & Kneib, 2002). It is here where they feed on a high abundance of live and dead animal and plant

matter that is commonly found along the marsh edge and in the headwaters of marsh creeks (Rozas & Odum, 1987; McTigue & Zimmerman, 1998). Juveniles also utilize the shallow upper reaches of tidal creeks as a refuge from predators (Boesch & Turner, 1984), with predation being the key source of natural mortality in juvenile shrimp populations (Minello & Zimmerman, 1991). *L. setiferus* are commonly consumed by a variety of marine species including blue crab, many species of finfish, insect larvae, and even other shrimps (Minello & Zimmerman, 1983). Risk of predation declines with increased individual size (Ruiz et al., 1993) which allows the growing shrimp to migrate their way out more successfully from the upper reaches of estuaries and slowly make their way to open waters to reproduce (Kneib, 1997; Webb & Kneib, 2002). Salinity was once considered to be a principal factor in *L. setiferus* distribution within estuaries (Zein-Eldin & Renaud, 1986; Wenner & Beatty, 1993), but it is unlikely that salinity alone drives this size and age distribution, and it is instead due to the variety of factors mentioned above (Webb & Kneib, 2002).

Growth of white shrimp is also highly dependent on an array of interacting environmental factors. Food availability and habitat conditions directly effects the growth and survival of shrimp during larval, juvenile, and adult stages (Johnson and Fielding, 1956; Perez-Velazquez, 2013). Temperature also has a large influence on the growth rate of *L. setiferus*; growth occurs during intermolt periods that have been linked to higher water temperatures (Dall et al., 1990; Criales et al., 2003).

***Litopenaeus setiferus* Population Structure**

There are a limited number of studies examining the population structure *of L. setiferus* throughout their range. However, Ball and Chapman (2003) completed a population genetic analysis on *L. setiferus* collected from North Carolina, South Carolina, Georgia, the Atlantic and

Gulf coasts of Florida, Louisiana, Texas, and Mexico. Genetic variation was assessed through the comparison of six microsatellite loci with results from all samples suggesting highly polymorphic loci and deviations in expected genotype frequency (Hardy-Weinberg proportions) indicating that some selection had occurred although the presence of null alleles makes interpretation difficult. This study distinguished broad-scale genetic similarity superimposed over random temporal and geographical variations with low FST and RST values suggesting little population structure and no considerable degree of differentiation among populations. By pooling samples from the western Atlantic and pooling samples from the Gulf of Mexico they were able to find weak but significant genetic variation that is likely due to genetic mixing from pelagic larvae and adult migrations and by the relatively recent separation of the two populations (Ball & Chapman, 2003). However, a separate study examined the population genetic history of *L. setiferus* and found a geographically structured complex haplotype phylogeny that consisted of two distinct lineages and two less well-defined sub lineages (McMillen-Jackson & Bert, 2003). Overall, genetic variation of *L. setiferus* throughout their North American range is weak, showing little to no population structure or deviations.

***Litopenaeus setiferus* Fishery**

Penaeid shrimps support highly valuable fisheries throughout the world (Velázquez & Gracia, 2000). In the United States, *L. setiferus* has been an economically important species dating back to 1709 when they were identified as the first commercially important shrimp species in the country (McKenzie, 1981; Muncy, 1984). The *L. setiferus* fishery has then been monitored since the 1930s (Burkenroad, 1939), with historic periods of high exploitation, such as the 1950s in the Gulf of Mexico (Condrey & Fuller, 1992). *L. setiferus* is now one of three commercially important penaeid species, which also includes brown shrimp, *Farfantepenaeus*

aztecus and pink shrimp *Farfantepenaeus duorarum* that both inhabit the US Atlantic and Gulf coasts (Ditty, 2011). While the other penaeid species have reduced some pressure on *L. setiferus* harvest, *L. setiferus* still dominates the shrimp fishery landings along the Atlantic coast, comprising >70% of the shrimp catch in North Carolina to Florida (NMFS) and over 85% of the penaeid harvest in the state of Georgia alone (Webb & Kneib, 2002). With most recent (2020*) L. setiferus* landings data in the U.S. totaled to be approximately 110 million pounds, and a monetary value of $254 million (NOAA Fisheries Commercial Fishing Landings Database, 2020).

Litopenaeus setiferus as a Useful Indicator/Model/Study Species

Consumer demand for shrimp has increased dramatically over the past 20 years, with 4.4 pounds being consumed per person annually (Webb & Kneib, 2002, NOAA), that coupled with high economic value of the fishery product has led to increased intensity of fishing efforts and destructive aquaculture practices which threaten wild shrimp populations and coastal habitats (Nance et al., 2007; Baker & Minello, 2010; Rozas & Minello, 2011). In addition to commercial value, white shrimp play a vital role in helping maintain healthy and balanced ecosystems as both an important predator and prey species in coastal habitats. *L. setiferus* is commonly used as a key indicator species and model organism for examining ecosystem changes since their abundance is highly dependent upon healthy available saltmarsh habitat (Belcher & Jennings, 2004; Webb & Kneib, 2002). Given the economic and ecological importance of *L. setiferus*, it is essential to be able to regularly monitor the population of this important species, suggesting they are a useful model organism to examine developing biomonitoring techniques.

CHAPTER 2

DEVELOPMENT OF A SPECIES-SPECIFIC ASSAY TO DETECT THE PRESENCE OF ONLY WHITE SHRIMP (*L. SETIFERUS*) eDNA

Introduction

The white shrimp, *Litopenaeus setiferus*, is an economically and socially important fisheries species in the United States. As the largest penaeid along the US eastern seaboard, *L. setiferus* is highly sought after for human consumption, with landings valued at $254 million in 2020, making it a highly valued fishery in the southeastern US (NOAA Fisheries Commercial Fishing Landings Database, 2020). Additionally, they are important for coastal ecosystems since they are essential links in estuarine food webs by eating small estuarine organisms while also serving as prey for a variety of recreationally and commercially important finfish species (Belcher & Jennings, 2004; Webb & Kneib, 2002). *L. setiferus* may also be a useful indicator species for examining ecosystem changes since their abundance is highly dependent upon healthy available saltmarsh habitat (Belcher & Jennings, 2004; Webb & Kneib, 2002). Given the commercial and ecological importance of *L. setiferus*, it is essential to have effective and efficient monitoring programs to manage populations.

Fisheries management programs rely on predictive models coupled with biological monitoring to track the status of fishery species while also relating environmental factors to production (Kelly et al., 2014). For *L. setiferus*, successful monitoring programs can be hampered by multiple factors. First, faunal datasets for *L. setiferus*, although extensive, lack consistency due to missing timepoints and varied methodologies across sampling years and locations. Second, commercial species monitoring is heavily reliant on methods such as bottom trawling and drop ring sampling, which are destructive (Collie et al., 2000) and can only be

performed in areas when and where conditions are favorable (Thomsen et al., 2012). Finally, these methodologies are often financially expensive to conduct. After factoring in for the number of workers needed, personnel hours required to achieve sampling events, cost of equipment upkeep, fuel, and more, traditional monitoring methods can burden limited resources within any conservation department.

Given the importance of monitoring programs to fisheries management, and current issues associated with these efforts, future management may rely on the development of sustainable cost-effective monitoring techniques. Environmental DNA (eDNA) sampling offers a unique survey technique that could remedy the current limitations of traditional monitoring. Organisms continuously shed DNA into surrounding environments through sloughed off cells, hair, gametes, fecal matter, etc. (Poinar et al., 1998; Bunce et al., 2005; Lydolph et al., 2005). This genetic material can then be collected through water samples, captured through filtration, extracted, and amplified, resulting in detection and spatial distribution data (Itakura et al., 2019). Using DNA from environmental samples has demonstrated great potential to provide information on species, populations, and communities (Baird & Hajibabaei, 2012; Kelly et al., 2014). Depending on the eDNA technology used and the intensity effort of the traditional surveys being compared, the per-survey costs associated with eDNA are usually significantly lower than traditional field surveys (Biggs et al., 2015; Sigsgaard et al., 2015). Recent studies have also revealed that eDNA methodologies may increase the power of species detection, area able to be sampled, and frequency of sampling when compared to traditional methods (Cantera et al., 2019; Wood et al., 2020). Since eDNA sampling only requires a small water sample to identify species presence (typically 500ml-2L; Rees et al., 2014), it is also far less invasive and destructive than traditional techniques.

A number of eDNA studies rely on next generation sequencing technologies to identify all taxa present in a sample (shotgun metagenomics). Unfortunately, this approach has some disadvantages, as it relies on well-curated sequence reference libraries, is expensive, and does not allow for accurate abundance calculations. An alternative to next generation-based methods is the use of species-specific quantitative real-time polymerase chain reaction (qPCR) that allow for the detection of specific taxa based on single nucleotide differences (Lacoursiere-Roussel et al., 2016a; Horiuchi et al., 2019; Thomas et al., 2020). These probes potentially allow for estimations of species abundance or density based on relative standard curves, demonstrating that eDNA can be a useful tool for species monitoring providing more than just presence absence data (Klymus et. al, 2020; Sassoubre et. al, 2016). Therefore, the objective of this study was to develop a species-specific TaqMan Minor Groove Binder (MGB) probe based on single nucleotide polymorphisms (SNP) to detect the presence of only *L. setiferus* eDNA from water samples.

Methods

Both nuclear and mitochondrial DNA can be detected through eDNA techniques, however, I targeted the mitochondrial cytochrome C oxidase subunit 1 gene (COI) for this study since highly abundant mitochondria within each cell increases the chances of amplifying this gene (Taanman, 1999). In addition, COI is the most expansively sequenced gene region of the animal kingdom due to its high resolution at the species level (Pentinsaari, et al., 2016; Thomsen & Willerslev, 2015), making it exceedingly accessible via universal sequence databases (e.g., GenBank and BOLDSYSTEMS). Finally, the protective circular structure of mitochondrial DNA keeps it from degrading as quickly when compared to the linear chromatin structure of nuclear DNA and is therefore thought to be better at calculating and detecting the fate of eDNA over

longer periods of times (Foran et al., 2006; Alaeddini et al., 2010; Moushomi et al., 2019). This can be especially important in marine environments where eDNA has been found to more quickly degrade then when compared to freshwater environments (Collins et al., 2018).

To develop species-specific primers and probes, the COI region of *Litopenaeus setiferus* collected from North Carolina, South Carolina, Georgia, the Atlantic and Gulf coasts of Florida, Louisiana, Texas and Mexico (shrimp collected from 1995 to 1999; Ball & Chapman, 2003) were aligned using Geneious software. The goal of this first alignment was to identify a highly conserved region among the North American *L. setiferus* populations. Once the first alignment was complete, the highly conserved COI sequences of *L. setiferus* and multiple crustacean organisms that commonly inhabit the waters of coastal Georgia, including both shrimp and crabs, were aligned. The goal of the second alignment was to identify areas with single nucleotide polymorphisms (SNPs) (Figure2.1). TaqMan® Probes design protocols where then followed to construct multiple sets of forward and reverse primers and *L. setiferus* specific TaqMan probes (Table 2.1).

I designed three primer-probe assays and tested the sensitivity and specificity of each. I used Qiagen DNeasy Blood & Tissue Kits (QIAGEN®, Hilden, Germany) to extract tissue samples of *L. setiferus*, *Farfantepenaeus aztecus* (brown shrimp), *Callinectes sapidus* (blue crab), and *Palaemonetes* spp. (grass shrimp). In addition, I performed filter extractions that were collected from one 37.8 L glass aquaria containing only *Palaemonetes* spp. in artificial seawater (salinity = 29), and one 151.4 L plastic holding tank containing only *L. setiferus* filled with artificial seawater (salinity = 29). One 500 mL water sample was collected from both tanks and taken to a separate clean laboratory to be filtered to avoid contamination. Samples were filtered through 47 mm diameter 1.2 µm glass microfiber filters (Whatman®) (GMF) (Moushomi et al.,

2019; Foran, 2006). A modified protocol of the manufacturer's recommendations of Qiagen DNeasy Blood & Tissue Kits (QIAGEN®, Hilden, Germany) was used to extract DNA from filter samples (Renshaw et al., 2015). A quarter cut was removed from each filter and immersed in a 2-mL tube containing 567 µL buffer ATL and 63 µL Proteinase-K (recommended 180 and 20 µL) and incubated on a heating block at 65 °C for 2 hours, being vortexed every 30 minutes. Following the two-hour incubation period, filters were manually removed using forceps that were sterilized in a 30% bleach bath and then rinsed in DI water between each filter removal. After filter removal, 630 µL buffer AL and 630 µL 100% ethanol were added to the 2-mL tube, (recommended 200 µL of each solution) and vortexed. Contents were then aliquoted into spin columns with a total of three centrifugation repetitions required to unload entire contents of the 2-mL tube, (single centrifuge is normally required). The remainder of the protocol followed the manufacturer's recommendations except for a final elution step of 100 µL of AE buffer (instead of recommended 200 µL).

Tissue and filter extractions were then subject to qPCR. Individual reactions were 25 µL each, consisting of 2.25 µL of both forward and reverse primers at 50 µM concentration, 1 µL of probe at 50 µM concentration, 5 µL DNA free water, 12.5 µL of TaqPath™ ProAmp™ Master Mix (Applied Biosystems), and 2 µL of DNA extract. The qPCR reaction consisted of an initial activation step at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95 °C for 15 s and an annealing and extension step at 60 °C for 1 min.

Results

All three designed assays (Table 2.1) had a positive amplification of *L. setiferus* tissue around the same average cycle threshold (Ct value). Assay 1 at 18.53, assay 2 at 19.04, and assay 3 at 19.92, indicating strong positive reactions and powerful affinity to *L. setiferus* DNA.

However, two of the other crustaceans tested, blue crab *Callinectes sapidus* tissue, grass shrimp *Palaemonetes* spp. tissue, and grass shrimp *Palaemonetes* spp. filter extractions, all amplified as well with a CT values of 32 ± 2. Brown shrimp *Farfantepenaeus aztecus* tissue and the *L. setiferus* holding tank filter did not amplify for any of the three assays (Table 2.2, Figure 2.2). Specificity of all three assays were moderately good but sensitivity was not as strong for the eDNA filters compared to tissue extractions. This qPCR reaction was run twice and generated similar findings for each reaction.

Though all three assays amplified other crustacean species, CT value was notably different for *C. sapidus* and *Palaemonetes* spp. when compared to *L. setiferus*, indicating a less powerful affinity to the other two crustacean species tested. Because these assays were primarily used in controlled laboratory experiments where *L. setiferus* was the only species present, I identified assay 3, forward primer 5′-TATAAGCTTCTGACTTCTACCTC-3′, reverse primer 5′-TATACTGTTCATCCAGTTCCAAC-3′, and TaqMan™ MGB probe (Applied Biosystems®) 6FAM- TTCCCTTACTCTTCTTCTATCTAGAGGAATMGB as the best primer-probe set, at 100 bp long.

Discussion

The results of this study showed that all three assays designed had low average Ct values showing a powerful affinity to *Litopenaeus setiferus* DNA. However, all three assays amplified two out of the other three crustaceans tested in the laboratory. *Callinectes sapidus* and *Palaemonetes* spp. tissue extractions amplified around the same CT value of 30, and the *Palaemonetes* spp. filter extraction amplified at a slightly higher CT value of 34. The use of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene for assay design has been used in eDNA studies targeting marine crustacean species (Forsström & Vasemägi, 2016; Roux et al.,

2020; Crane et al., 2021) due to ample variability across the gene for species-level detection and accessibility of reference sequences (Roux et al., 2020). However, results from my study highlight that variability of this gene across geographically close marine crustaceans may not vary enough to develop highly sensitive species-specific assays.

Few other eDNA studies targeting marine crustaceans have designed their own assay for species-specific detection (Roux et al., 2020; Forsström & Vasemägi, 2016). To verify assay specificity for the invasive European green crab *Carcinus maenas*, Roux et. al (2020) tested 26 nontarget species, including ten other crab species in the laboratory, none of which amplified. Field validations also successfully detected *C. maenas* at all five sampled sites (Roux et al., 2020). However, a second study that designed a species-specific assay for the detection of mud crabs *Rhithropanopeus harrisii*, only tested the specificity of their primers in silico by comparing the sequences to other crab species (Forsström & Vasemägi, 2016). A third eDNA study targeting *C. maenas* used an unpublished COI assay (Neigel and Germane, unpub.) that has not yet been validated with local species and its specificity was only tested in silico as well (Crane et al., 2021).

The previous studies on marine crustacean eDNA have varied in their methodological approaches. Water sample volume ranges from 15 mL maintained in sodium acetate and ethanol (Forsström & Vasemägi, 2016), to 50 mL filtered through 1.5 µm GMF (VWR International, LLC., Radnor, PA) (Crane et al., 2021) to 1 L water samples filtered through 1 µm cellulose filters (Whatman®) (Roux et al., 2020). In addition, the studies varied in their extraction techniques, using Qiagen® DNeasy Blood & Tissue kits (QIAGEN®, Hilden, Germany) (Crane et al., 2021; Roux et al., 2020) and QIAcube Connect system (QIAGEN®, Hilden, Germany) following the QIAamp DNA Mini Kit protocol for filter extractions (Crane et al., 2021), or the

Macherey-Nagel® NucleoSpin Tissue kit (Forsström & Vasemägi, 2016). The studies also utilize different quantification techniques. Therefore, the variation in success of the different eDNA approaches between the previous studies and my study are likely due to different methods used among studies and lack of overall standardization for sampling.

My study attempted to test the specificity of the three assays both in silico via alignments, and in vitro via both tissue and filter extractions of local crustaceans. I did not achieve the same level of in vitro specificity as Roux et. al (2019), although they tested their primers against 26 other crustacean species, while I tested my primers and probes against only 3 species. This variation in assay specificity testing, and or, the magnitude of COI similarities in the crustaceans tested in this study, could have caused the variation in success between our two studies. In addition, I used glass microfiber filters while Roux et al. (2019) used cellulose membrane filters and DNA binding affinity may differ between the two filter materials (Hinlo et al., 2017; Liang & Keeley, 2013). As above, there is no agreed upon, standardized approach to quantifying eDNA for marine crustaceans, which make comparisons difficult and limits the technology's usefulness in fisheries monitoring.

It has also been proposed that low DNA shedding rates from shelled invertebrate likely hinders eDNA detectability (Geerts et al., 2018; Roux et al., 2020; Crane et al., 2021). Mächler et al., (2014) noted mixed results from eDNA based studies on macroinvertebrates and suggested that the low levels of eDNA found and released from these animals is a significant challenge. Forsström and Vasemägi (2016) found that DNA release rate for *R. harrisii* was much higher during times of greater stress and activity level. While Dunn et al. (2017) and Crane et al. (2021) suggested that reproductive biology has the greatest influence on invertebrate eDNA detection. One approach might be to target the planktonic larval life stages for detection of most shelled

invertebrate species (Roux et al., 2020). Overall, better insights and a call for standardizations for assay specificity and sensitivity testing, sampling methods, and optimal sampling season is crucial for interpreting eDNA results of any marine crustacean species.

Conclusion

Environmental DNA sampling has swiftly shifted over the last decade from being a novel technology used primarily for the detection of microbial taxa to currently a well-explored biomonitoring tool commonly used to identify macro-organisms in an array of environments (Thomsen & Willerslev, 2015; Roux et al., 2020). However, there are still many hurdles and uncertain in the implementation and interpretation of this technology for many species. It has now been observed in many field based eDNA studies that environmental sample results commonly push qPCR-based detection limits, especially when trying to quantify hard shelled invertebrates (Roux et al., 2020). These findings therefore put a large emphasis on the need for standardized assay validation and highly sensitive assays to properly detect any species as eDNA methodologies continue to become a fundamental management tool (Goldberg et al., 2016; Roux et al., 2020).

Table 2.1: Table of the three designed assays specific to the COI gene of *Litopenaeus setiferus*.

Sequence Name	Seq 5' to 3'	% GC Content
Probe 1	[6FAM]CCACGCTGGRGCCTCAGTAGA[BHQ1aQ]	64.3
Forward Primer 1	ACCCTCCTTTATCTGCTAGTATCG	45.8
Reverse Primer 1	ACTGCTCCTAGAATAGAGGATACAC	44
Probe 2	[6FAM]TCTAGGAGCAGTAAACTTCATAACAACCGT[BHQ1aQ]	40
Forward Primer 2	TCTCCACTTAGCTGGTGTATCC	50
Reverse Primer 2	AGGTATTCGGTCTATAGTTATTCCTGT	37
Probe 3	[6FAM]TTCCCTTACTCTTCTTCTATCTAGAGGAAT[BHQ1aQ]	36.7
Forward Primer 3	TATAAGCTTCTGACTTCTACCTC	39.1
Reverse Primer 3	TATACTGTTCATCCAGTTCCAAC	39.1

Table 2.2: Table of the averaged CT values for *Litopenaeus setiferus* tissue, *Palaemonetes* spp. tissue, *Callinectes sapidus* tissue, *Farfantepenaeus aztecus* tissue, *Litopenaeus setiferus* holding tank filter, and *Palaemonetes* spp. filter extractions.

	Litopenaeus setiferus	***Palaemonetes* spp.**	***Callinectes sapidus***	***Farfantepenaeus aztecus***	***Litopenaeus setiferus* Filter**	***Palaemonetes* spp. Filter**
Assay 1	18.395	30.065	30.775	No CT	No CT	33.235
Assay 2	19.051	31.422	31.552	No CT	No CT	34.670
Assay 3	19.845	31.261	30.393	No CT	No CT	33.742

Figure 2.1: Condensed alignment of *Litopenaeus setiferus* COI gene with other relevant Georgia crustacean species.

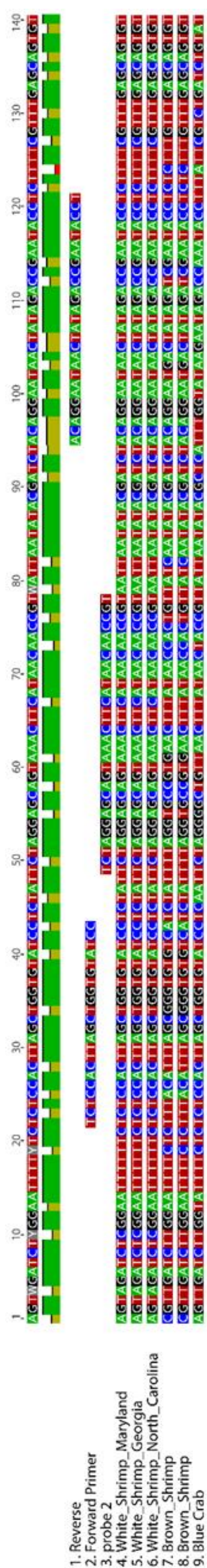
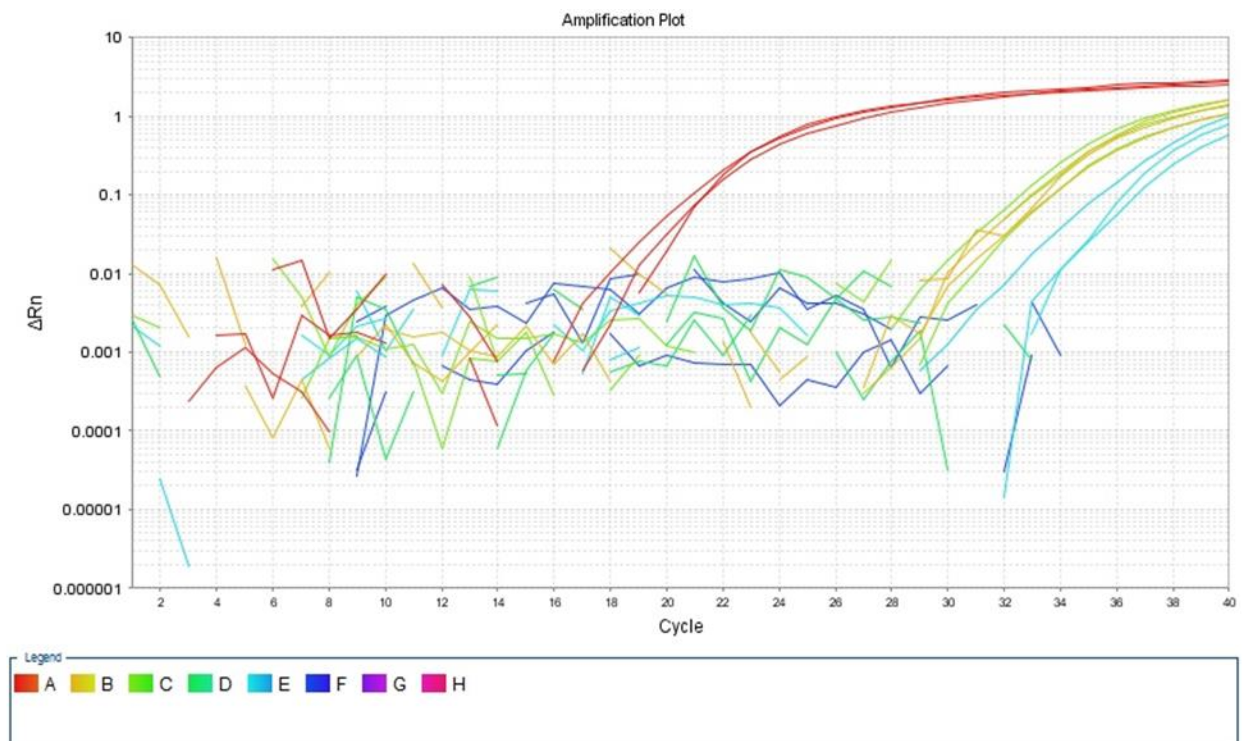


Figure 2.2: Amplification plot of the three designed assays. The red lines represent assays 1-3 for *L. setiferus* tissue with CT values 18-19, and the yellow, green, and blue lines represent assays 1-3 for *Palaemonetes* spp. and *Callinectes sapidus* tissue and *Palaemonetes* spp. filter extraction with CT values ranging from 30-34.



CHAPTER 3

RELATIONSHIPS BETWEEN ENVIROMENTAL DNA PRESENCE AND CONCENTRATION AND SHRIMP ABUNDANCE AND BIOMASS IN LABORATORY AND FIELD ENVIRONMENTS

Introduction

Environmental DNA (eDNA) surveys have emerged as powerful tools used in complement to traditional capture surveys for assessing the distribution of organisms in freshwater and marine environments over the last decade. Organisms continuously shed DNA into surrounding environments through sloughed off cells, hair, gametes, fecal matter, etc. (Poinar et al., 1998, Bunce et al., 2005, Lydolph et al., 2005). This genetic material can then be collected through water samples, captured through filtration, extracted, and then amplified, resulting in detection and spatial distribution data (Itakura et al. 2019). Therefore, researchers and managers suggest eDNA based surveys may fill an important gap in broad-scale monitoring of biodiversity and resources in the future (Lodge et al. 2012; Bohmann et al. 2014). eDNA techniques have become especially useful for determining presence/absence of species (Pochardt et al. 2020; Rees et al. 2014; Lacoursière-Roussel et al. 2016b; Wilcox et al. 2016) as well as detecting rare species that might be missed by traditional methods (Stoeck et al., 2010; Jerde et al., 2011; Goldberg et al., 2016). However, the ability of these methods to quantify abundance or biomass of organisms is currently limited due to the complexities associated with this sampling method and mixed field results. For eDNA to be a useful replacement to traditional monitoring methods, especially for fisheries management, estimation of population size and trends in addition to assessing presence or absence is also needed (Pochardt et al. 2020). Therefore, the ability to estimate species abundance and biomass from eDNA is vital in increasing its utility.

The potential application for eDNA monitoring beyond species presence has received attention over the past 10 years. Current findings suggests that eDNA quantified with Real-time PCR can offer an approximation for actual abundance in controlled experiments (Rees et al. 2014), ponds (Takahara et al., 2012), streams (Doi et al. 2015; Levi et al. 2019; Lodge et al. 2012; Tillotson et al. 2018; Wilcox et al. 2016), and in marine bays (Plough et al. 2018; Pochardt et al. 2020). In addition, aquatic organisms may release DNA into the water in proportion to their biomass (Takahara et al. 2012), and some studies have confirmed a relationship between eDNA and biomass in the lab (Thomsen et al. 2012) and field (Takahara et al. 2012, Takahara et al. 2013). However, the effectiveness of eDNA based indices of abundance and biomass in natural settings has been mixed (Kelly et al. 2014; Yates et al. 2019) likely due to the complex relationships between biomass, organism size, and abundance, and has not yet been thoroughly assessed in a management context (Pochardt et al. 2020).

The concentration of eDNA in the field, and the subsequent effectiveness of eDNA based tools, are highly dependent on the turnover rate of the molecule itself (i.e., release and degradation rate; Takahara et al., 2012). eDNA can come in many different forms from extracellular, intracellular, free, or adsorbed and the fates of each have numerous possibilities and potential interactions with environmental factors to degrade or preserve eDNA (Singh et al., 2006; Levy-Booth et al., 2007; Nielsen et al., 2007; Pietramellara et al., 2009). Factors that likely effect eDNA can fit into three main categories: characteristics of the DNA molecule itself as well as abiotic and biotic variables (Barnes et al., 2014). Length, shape, and containment by membranes directly effects how DNA degrades in a given environment over time (Romanowski et al., 1993; Ogram et al., 1994; Gallori et al., 1994). High temperatures increase the degradation of eDNA by increasing enzyme kinetics and microbial metabolism (Okabe & Shimazu, 2007;

Corinaldesi et al., 2008), and high salinity environments have been found to influence eDNA shape and stability, restricting exonuclease activity limiting eDNA degradation (Hofreiter et al., 2001; Borin et al., 2008). Extracellular enzymes may also contribute to eDNA degradation (Dell'Anno & Corinaldesi, 2004; Flemming & Wingender, 2010; Corinaldesi et al., 2011). The utility of eDNA as a proxy for species presence or abundance is then highly dependent on understanding how these factors function in each environment.

The white shrimp, *Litopenaeus setiferus*, is a common commercially and recreationally important species in the United States. *L. setiferus* is the largest of the commercially important penaeid shrimp along the US Atlantic coast, with landings exceeding $254 million. (NOAA Fisheries Commercial Fishing Landings Database, 2020). In Georgia, the *L. setiferus* is the top commercial fishery comprising over 85% of the penaeid harvest (Webb & Kneib 2002) and has averaged over $9 million in landings over the last decade. The presence and abundance of shrimp is highly dependent upon available healthy shrimp habitat, salt marshes (Turner 1977, Webb & Kneib 2002), and shrimp distribution and abundance in estuaries is linked to environmental characteristics such as temperature and salinity gradients (Wenner & Beatty, 1993). Therefore, *L. setiferus* can also be a key indicator species to changes in estuarine water quality and habitat. Due to the commercial and ecological value of *L. setiferus*, the Georgia Department of Natural Resources (GA DNR) monitors *L. setiferus* populations along the coast using traditional methods including monthly trawl surveys and drop ring sampling. Given the costs associated with traditional monitoring and issues surrounding consistency of sampling, it is critical to explore other cost-effective tools to monitor commercially important fisheries populations.

L. setiferus may represent a useful species to explore the utility of eDNA techniques for fishery monitoring. Previous cost comparisons of targeted eDNA approaches (specific taxa

using real-time PCR) suggest that eDNA can be more cost effective than traditional sampling methods, especially for fishes, turtles, and invertebrates (Davy et al. 2015; Huver et al. 2015; Sigsgaard et al. 2015). For example, per-site eDNA survey costs were 10-50%, 37-110%, and 17% the cost of traditional survey methods for aquatic turtles (Davy et al. 2015), Brook trout (Evans et al. 2017), and aquatic invertebrates (McInerney & Rees, 2018), respectively. As such, many state and federal agencies have adapted eDNA monitoring techniques to map the occurrence of threatened/rare species (Coble et al., 2019; Doğdu & Turan, 2016; Jerde et al., 2011) and identify presence of invasive species (Klymus et al. 2017). Additionally, several studies have successfully quantified species abundance using eDNA, including for the invasive mud snails (Ponce, 2019), jack mackerel (Horiuchi et al. 2019), and lake trout (Lacoursiere-Roussel et al. 2016a), suggesting the potential for biomass estimates in species management. To date, no studies have applied these techniques to marine crustacean fishery species like *L. setiferus*.

Although it is possible that marine invertebrates like *L. setiferus* might be useful model organisms to link eDNA concentrations to abundance and biomass, critical information on biomass-eDNA concentration relationships, molecule turnover rates, and field methods need to be validated before this methodology can be used for comprehensive management. Therefore, the main objectives for this study were to link eDNA concentration to *L. setiferus* abundance and biomass using a series of lab experiments and field surveys. Specifically, I sought to (1) examine the relationship between eDNA concentration and shrimp abundance/biomass in controlled lab settings, (2) determine the eDNA degradation rate under varying temperatures pertinent to *L. setiferus* abundance and distribution, and (3) attempt to link eDNA prevalence and abundance in the field with paired traditional surveys.

Methods

Biomass Gradient Experiment

Although eDNA methodologies may be used to quantify species abundance and biomass (Thomsen et al., 2012), there is still considerable uncertainty in the field (Pochardt et al., 2020). To resolve these ambiguities, I conducted a series of in lab experiments aimed at determining a shrimp-qPCR amplification relationship for *L. setiferus*. *L. setiferus* were either harvested locally using a cast net or purchased from local marinas, returned to the lab, and maintained in 151.4 L plastic tanks filled with artificial seawater. Tanks were filtered with cannister filters and aerated.

During experiments, shrimp were distributed among eight 37.8 L aquaria comprising of a biomass gradient, created by using different numbers of shrimp, including a no shrimp control tank. Shrimp abundance ranged from 1 to 15 shrimp and biomass from 1.2 g to 97.3g (Figure 3.1). Experimental aquaria were set up to mimic natural estuarine conditions, being kept at 22°C and a salinity of 29 ppt (median for Ga DNR water quality data 2017-2018) with a 12-hour daylight cycle and equipped with air stones ensuring full admixture and oxygenation (Thomsen et al., 2012). At hour 24, prepared sampling kits (individually bagged sterilized bottles and gloves) were used to collect triplicate 500 ml water samples from each of the 8 aquaria, taken to a separate clean laboratory to be filtered away from experimental tanks and avoid contamination. Samples were filtered through 1.2 μm pore Whatman glass microfiber filters (GMF), folded and then stored in individually labeled dry centrifuge tubes and frozen at -20 °C.

Temperature Degradation Experiment

Given that temperature degrades DNA molecules, I sought to examine how different temperatures affected the rate of eDNA degradation using a series of lab experiments. Shrimp were collected and maintained as described above prior to experiments. During experiments, 6

shrimp (biomass range 13.5-44.5g) were placed in each of 12 sterilized replicate 37.8 L aquaria, with an additional 3 aquaria as no shrimp controls. Aquaria were assigned to one of three levels of biologically relevant temperatures representing seasonal variability along the Georgia coast (19°C, 24°C, 29°C) (Figure 3.2), and a salinity of 29 ppt (median for Ga DNR water quality data 2017-2018) with a 12-hour daylight cycle and equipped with air stones ensuring full admixture and oxygenation (Thomsen et al 2012). Shrimp were maintained in experimental aquaria for 24 hours, after which they were removed, and prepared sampling kits were used to collect an initial 500ml water sample that was immediately taken to determine the starting concentrating of eDNA. Water samples were then collected at 3, 6, 12, 24, 48, and 72-hour intervals to determine degradation of eDNA over time. Samples were filtered and stored as stated above.

Field Study

An important step toward applicability for management is to ensure that similar results are obtained in both laboratory and field settings. Therefore, to establish detection limits and the ability to properly interpret field results we leveraged *L. setiferus* surveys conducted by Savannah State University. Briefly, water samples were collected monthly in June, July, and August of 2021 from 9 different tidal creeks in the Savannah area (Figure 3.3, Table 3.1). Field samples were collected with sterilized prepared sampling kits in each creek by tossing and rinsing a collection bucket over the side of the boat three times before collecting water to fill a sterilized 500ml sample bottle while wearing nitrile gloves. At the same time, a 500ml sample bottle filled with DI was opened for 10 seconds (roughly the time it takes to collect an actual sample) holding the cap in one hand in the bottle in the other to serve as a field blank. Once samples were taken, they were then individually bagged to avoid contamination, placed in a cooler on ice and transported back to the lab (approximately 3-4hrs) where they were either filter

immediately or frozen until later filtration. Samples were filtered and stored as stated above. At each location, a 5-minute trawl was paired with 10 cast net throws to collect and enumerate shrimp abundance.

DNA Extraction, qPCR, and Fluorometer

Filters were thawed at room temperature, unfolded, and a ¼ section cut (except for the biomass experiment, which utilized whole filters) was taken from the filter and placed into a microcentrifuge tube for DNA extraction. We used a modified protocol of the manufacturer's recommendations of Qiagen DNeasy Blood & Tissue Kit (QIAGEN®, Hilden, Germany) (Renshaw et. al, 2015; see Chapter 2). After extractions were completed, they were frozen immediately at -20°C. The assay targets a 100 bp fragment of the cytochrome c oxidase 1 (COI) region and uses forward primer 5′-TATAAGCTTCTGACTTCTACCTC-3′, reverse primer 5′-TATACTGTTCATCCAGTTCCAAC-3′, and TaqMan™ MGB probe (Applied Biosystems®) 6FAM- TTCCCTTACTCTTCTTCTATCTAGAGGAAT-MGB. These extractions were then analyzed via qPCR. Individual reactions were 25 µL, consisting of 2.25 µL of both forward and reverse primers at 50 µM concentration, 1 µL of probe at 50 µM concentration, 5 µL of DNA free water, 12.5 µL of TaqPath™ ProAmp™ Master Mix (Applied Biosystems), and 2 µL of DNA extract. The qPCR reaction consisted of an initial activation step at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95 °C for 15 seconds and an annealing and extension step at 60 °C for 1 minute. DNA concentrations were also quantified via fluorometer Invitrogen Qubit 3.0 (Thermo Fisher®, Waltham, MA), following the manufacturer's protocol for Qubit dsDNA HS Assay kit using 2 µL of sample.

Data Analysis

Linear regression were used to test for a relationship between *L. setiferus* abundance or biomass and CT value from qPCR runs. All analyses were performed using JMP® Pro 16. Due to inconsistencies in amplification that affected the sample size at different timepoints of the eDNA degradation experiment, no analyses were performed. Likewise, field filters did not amplify white shrimp eDNA so samples could not be compared to densities via trawling.

Results

Biomass Gradient Experiment

Across the 7 total control tanks (21 filters), 5 total filters from 4 different tanks amplified during qPCR, all with an average weak CT value of 35.3, showing small amounts of *L. setiferus* cross contamination during either experimentation, filtration, or extraction. From the 49 experimental tanks (147 filters), a total of 68 filters from 30 tanks amplified with a sharp decline in amplification coming from experimental tanks with more than 32g of *L. setiferus* and no amplification from experimental tanks with more than 56g of *L. setiferus*. Results from Qubit 3.0 Fluorometric Quantification indicated that DNA concentration increased from 1-7 shrimp per tank (1.2g to 32g), but a sharp decline in DNA concentration from 9-15 shrimp per tank (>32g). Overall, there was no relationship between eDNA concentration and white shrimp biomass (linear regression p = 0.159, r2 = 0.067; Figure 3.4) or abundance (linear regression p = 0.0517, r2 = 0.124295; Figure 3.5).

Temperature Degradation Experiment

Although no filters from control tanks (6 tanks, 42 filters) amplified DNA, suggesting that cross-contamination did not occur during experimentation, filtration, or extraction, only 99 out of 168 filters from experimental tanks amplified DNA. Inconsistencies in amplification across tanks and time periods made statistical analysis impossible. Therefore, filters that did

amplify at each temperature and time point were pooled together to determine and average CT value to observe any trends. The highest concentrations occurred at the initial sampling point and decreased over time at similar rates across temperature treatments (Figure 3.6)

Field Study

Trawl and cast net catch identified the presence of *L. setiferus* at all 9 sampling locations during each month. However, white shrimp eDNA from field samples from all 9 sites across the 3-month sampling period (24 total filters) did not amplify, despite even high abundance and biomass at some sample points. Since no filters amplified white shrimp eDNA, comparative analysis between sampling techniques could not be performed.

Discussion

The results of my study highlight difficulties in using sampling techniques such as eDNA as a replacement for traditional fisheries monitoring, particularly for invertebrate fisheries. Using a quantitative PCR approach to not just identify the presence of white shrimp, *Litopenaeus setiferus*, but to quantify the abundance remains elusive. Although I was able to identify a species-specific assay for *L. setiferus* using pure tissue samples (Chapter 2), issues in experimental set-ups with diluted DNA, clogged filters, and amplification failures impacted my ability to detect any patterns associated with eDNA concentration and shrimp biomass, calculate degradation rates, or try to pair field abundance to eDNA samples. Thus, while this technology may have potential, numerous issues remain before it might be used as a viable management tool for white shrimp.

In my biomass experiment, only 46% of the samples in this experiment amplified. Low levels of amplification of *L. setiferus* eDNA in these laboratory studies is most likely due to small amounts of total DNA being expelled from the shrimp over the duration of the experiment.

In comparison, a study on the invasive European green crab (*Carcinus maenas*) found very small concentrations of eDNA (<10 copies/µL) in laboratory trials from aquaria containing male and female non-ovigerous hard- and soft-shelled crabs, regardless of the crab's abundance (Crane at al., 2021). Another study on crayfish (*Procambarus clarkii*) also experiences relatively low concentrations of eDNA in aquaria containing individual crayfish (Geerts et al., 2018). The existence of the exoskeleton likely hinders the amount of DNA released into the environment via sources like epidermal cells, mucus, sloughed off tissues, or extracellular DNA (Deiner & Altermatt, 2014; Dougherty et al., 2016; Tr´eguier et al., 2014,). In contrast, aquaria studies performed on fish with comparable biomass values result in much higher eDNA concentrations (Mizumoto et al., 2018). Differences in activity levels could play a role, although I did not make activity observations over the course of my trials. My results coupled with those in the literature suggest that more research is needed to understand taxon-specific effects on eDNA detection and determine whether activity level can affect concentrations.

In addition, while the lack of amplification occurred across all biomass levels, I observed a large decline in amplification in samples taken from tanks with more than 7 individuals or 32g of shrimp, and no amplification in samples taken from tanks with more than 56g of shrimp. Total DNA concentration was quantified on a fluorometer (Qubit, Invitrogen) and yielded similar results to the qPCR outputs. Samples showed an increasing gradient of total DNA per sample up until approximately 7-9 shrimp per tank or a biomass of ~30g. Samples from higher levels of shrimp biomass per tank had a large decline in total DNA per sample from a few ng/µl to below the detectable limit. The exact causation of these outcomes is not completely understood, though it was recorded during experimentation that tanks with 7 or more shrimp were notably cloudier than tanks with 1-5 shrimp, and filters from these higher biomass tanks were visibly much darker

than lower biomass tanks. One possible explanation could be that shrimp excrete urea and ammonia via their feces and gills (Burford & Williams, 2001), and high concentrations of urea significantly lower the activity of hydrogen ions and increases the measured pH of aqueous solutions (Bull et al, 1964). DNA extractions via Qiagen DNeasy Blood & Tissue Kit (QIAGEN®, Hilden, Germany) are highly sensitive to pH and it is therefore likely that the tanks containing more shrimp had much higher levels of urea, therefore effecting the kit's ability to effectively extract *L. setiferus* DNA.

Issues with amplification also impacted my ability to conduct analyses on the degradation experiments, because only 59% of the 168 samples amplified DNA during the 72-hour experiment. Interestingly, I could not amplify initial samples from some tanks but was later able to detect DNA (i.e., after 12 hours). However, to visualize the samples that did amplify, all CT values from the same temperature treatment and hour sampled were pooled together and averaged. From this pooled data, there does appear to be eDNA degradation occurring from hour 0, immediately after shrimp were removed, to hour 72. Even though analyses could not be conducted, my results look similar to many other eDNA degradation studies in that a large amount of eDNA degradation occurs within the first few days after species removal (Thomsen et al., 2012; Barnes et al., 2014; Strickler et al., 2015; Forsström & Vasemägi, 2016). However, in natural environments there are many other factors effecting eDNA degradation than just time alone, such as temperature (Strickler et al., 2015).

In my experiment, I observed degradation across three different temperatures, and based on the data I could collect, it appears that degradation rate does not change meaningfully amongst the three temperature treatments tested (Figure 3.6). Overall, many studies have reported that eDNA degradation rate is significantly affected by water temperature over time,

with higher temperatures degrading eDNA faster (mean ± standard deviation 10 ± 0.6°C, 20 ± 0.1°C, and 30 ± 0.2°C, Tsuji et al., 2017; 5 °C, 15°C, 25°C, and 35 °C, Eichmiller et al., 2016; 5°C, 20 °C, and 35°C.Strickler et al., 2015). Water temperature is thought to indirectly affect eDNA degradation through enzymatic hydrolysis from microbes and extracellular nucleases (Barnes and Turner, 2016). Higher temperatures increase extracellular enzyme and microorganism activity, resulting in faster degradation of eDNA (Barnes and Turner, 2016). Degradation studies utilizing bullfrog tadpoles, *Lithobates catesbeianus*, (Strickler et. al, 2015), common carp, *Cyprinus carpio*, (Eichmiller et al., 2016), Japanese jack mackerels, *Trachurus japonicus,* (Jo et al., 2019), and the Japanese eel *Anguilla japonica*, (Kasai et al., 2020) all demonstrated that temperature significantly affected eDNA degradation. However, attempts at examining eDNA degradation rates in another crustacean, *Rhithropanopeus harrisii*, also experienced low initial eDNA concentrations and finding variation in the detectability of crab eDNA between aquaria (Forsström & Vasemägi, 2016). Therefore, the lack of differences in observed degradation rates across the three temperatures examined could be due to a small range of temperatures examined (10ºC) and to the overall low detectability and amplification issues during the experiment, or both.

Given the issues in the controlled laboratory experiments, it is perhaps not surprising that *L. setiferus* eDNA did not amplify from a single water sample across the 9 sites and 3 months of sampling, despite variable but at times high abundances of shrimp. The cast net and trawl sampling found that all 9 sites for each month had *L. setiferus* in the vicinity of where water samples were collected. To date, only one study performed on marine crustaceans has experienced high levels of success in field sample detection of the European green crab *C. maenas* at all five sampled sites (Roux et al., 2020). However, a different recent field

experiment with *C. maenas* failed to detect crab eDNA in or around all experimental traps containing hard-shelled green crabs in an estuary environment (Crane et al., 2021). Likewise, eDNA was only detected at 1 of 10 sites where *Rhithropanopeus harrisii* crabs were known to be abundant (Forsström & Vasemägi, 2016).

Other environmental factors could also impact the utility of eDNA techniques on species monitoring for crustaceans, especially when those crustaceans inhabit coastal waters. In a study looking at the effects of salinity and pH on eDNA degradation, they found that eDNA degrades 1.6 times faster in inshore environments when compared to offshore environments (Collins et al., 2018). High salinity is also believed to increase inhibition during PCR due to either direct interactions with DNA or interferences with DNA polymerases, further hindering eDNA technologies in marine environments when compared to freshwater systems (Díaz-Ferguson & Moyer, 2014). The combined results of these studies suggest that the concentrations of coastal crustacean eDNA in field settings is most likely below qPCR detection limits (Crane et al., 2021; Forsström & Vasemägi, 2016). Mixing and dilution through hydrodynamics (tides and currents) makes accurate eDNA detection for any species in this environment type difficult, then combining those effects along with the low levels of DNA that's expelled from crustaceans results in the likelihood of having little to no detection in natural environments (Foote et al., 2012; Schmelzle & Kinziger, 2016; Thomsen et al., 2012; Crane et al., 2021).

Since estimation of initial eDNA concentration is essential to improving estimations of a species biomass via eDNA techniques (Barnes & Turner, 2016), coastal crustacean species may not be an applicable group for this type of monitoring (Collins et. al, 2018). Despite the difficulties in my study, use of eDNA for species monitoring could still be an important tool. Many field studies have focused on soft bodied organisms that may shed DNA at higher rates

and were performed in freshwater environments (Mahon et al. 2013; Dejean et al. 2012; Ficetola et al. 2008). Marine environments differ from freshwater environments in chemical composition, hydrodynamics, and the immense volume of sea water in relation to species biomass yields higher eDNA dispersal, dilution, and degradation (Thomsen, 2012; Sassoubre et. al, 2016; Díaz-Ferguson & Moyer, 2014). Thus, while eDNA detection has been demonstrated as possible in marine environments, it is still thought to be much less reliable (Foote et al. 2012). For eDNA to be useful for marine fisheries monitoring in the future, standardized protocols should be established for all species.

Conclusion

Environmental DNA technologies are projected to become a powerful tool for biomonitoring. However, because detection rate is highly dependent on multiple biological (target species, density, age and life-stage), environmental (salinity, pH, temperature, hydrodynamics), and technical factors (amount and method of environmental sample, extraction method, detection approach) there is still a large amount of uncertainty that comes with eDNA results (Deiner et al., 2014; Goldberg et al., 2016; Kelly et al., 2014; Mächler et al., 2014; Strickler et al., 2015). As more studies are being completed on a wider range of species and environment types, these uncertainties are becoming clearer which will in turn allow eDNA management efforts to be better constructed. eDNA methodologies have been shown to work well in freshwater environments and with species that are known to shed high amounts of DNA (Goldberg et al., 2016; Mahon et al., 2013; Dejean et al., 2012; Ficetola et al., 2008). On the other hand, marine or brackish water environments and species with exoskeletons have yielded more mixed results (Forsström & Vasemägi, 2016; Crane et al., 2021). Therefore, while there is considerable potential for eDNA as a quantification tool, the selection of appropriate target

species, environment, sampling, and detection method paired with preliminary controlled experiment results will be essential before trying to comprehensively use eDNA technology for fisheries management (Kelly et al., 2014).

Table 3.1: Environmental DNA water sampling locations. Nine different creek locations in the Savannah area were visited once each month through June – August of 2021.

General Location	Creek	Latitude	Longitude
SSU Marine Science Research Center	Country Club Creek	32.020555	-81.056853
SSU Marine Science Research Center	Pearl Creek	32.00054	-81.062793
SSU Marine Science Research Center	Herb Creek	31.987847	-81.068095
McQueen/Oatland Island	McQueen Creek	32.057492	-80.972081
McQueen/Oatland Island	Mud Creek	32.056084	-80.98961
McQueen/Oatland Island	Richardson Creek	32.057429	-81.003697
Halfmoon River	Sheepshead Creek	31.967285	-80.997371
Halfmoon River	Tom Creek	31.993879	-80.969011
Halfmoon River	Long Creek	31.980991	-80.943903

Figure 3.1: Experimental set up to establish a shrimp-qPCR amplification relationship based on biomass. Shrimp abundance ranged from 1 to 15 shrimp and biomass from 1.18g to 97.32g across the 7 total trials. At hour 24, triplicate 500ml water samples from each of the 8 aquaria.

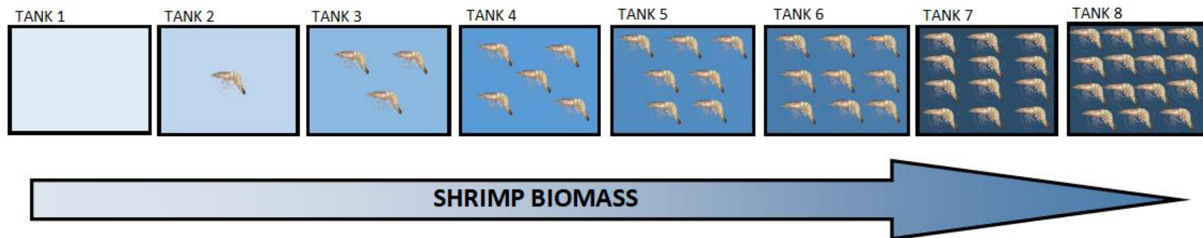


Figure 3.2: Experimental set up for the degradation experiment. Abundance of shrimp was kept constant across all aquaria with 6 shrimp per tank. Aquaria were assigned to one of three levels of biologically relevant temperatures representing seasonal variability along the Georgia coast (19°C, 24°C, 29°C). Shrimp were maintained and removed from experimental aquaria after 24 hours. 500ml water samples were collected from each aquaria at hour 0, 3, 6, 12, 24, 48, and 72- to determine degradation of eDNA over time.

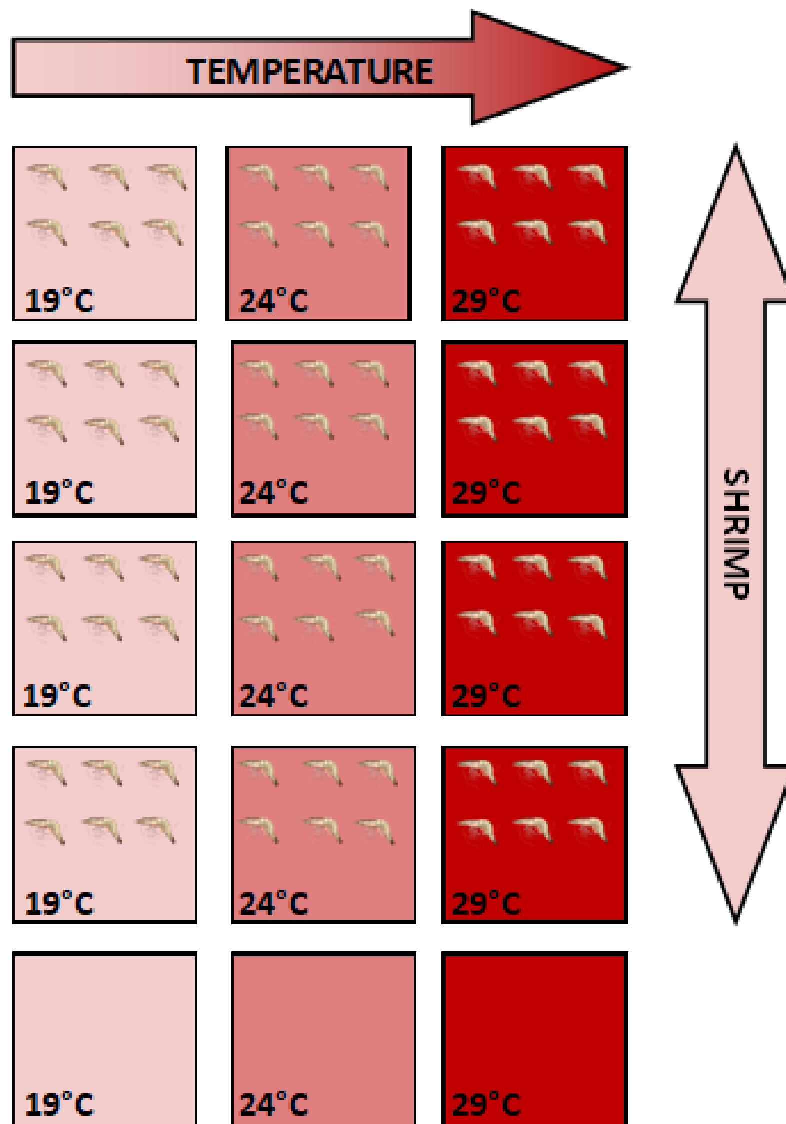


Figure 3.3: Environmental DNA water sampling locations. Nine different creek locations in the Savannah area were visited once each month through June – August of 2021. SSU Marine Science Research Center (Country Clun creek, Pearl Creek, Herb Creek), McQueen/Oatland Island (Richardson Creek, Mud Creek, McQueen Creek), Halfmoon River (Tom Creek, Long Creek, Sheepshead Creek).

Figure 3.4: Graph of the relationship between *L. setiferus* biomass and CT Value. Linear regression $p = 0.159$, $r^2 = 0.067$; All analyses were performed using JMP® Pro 16

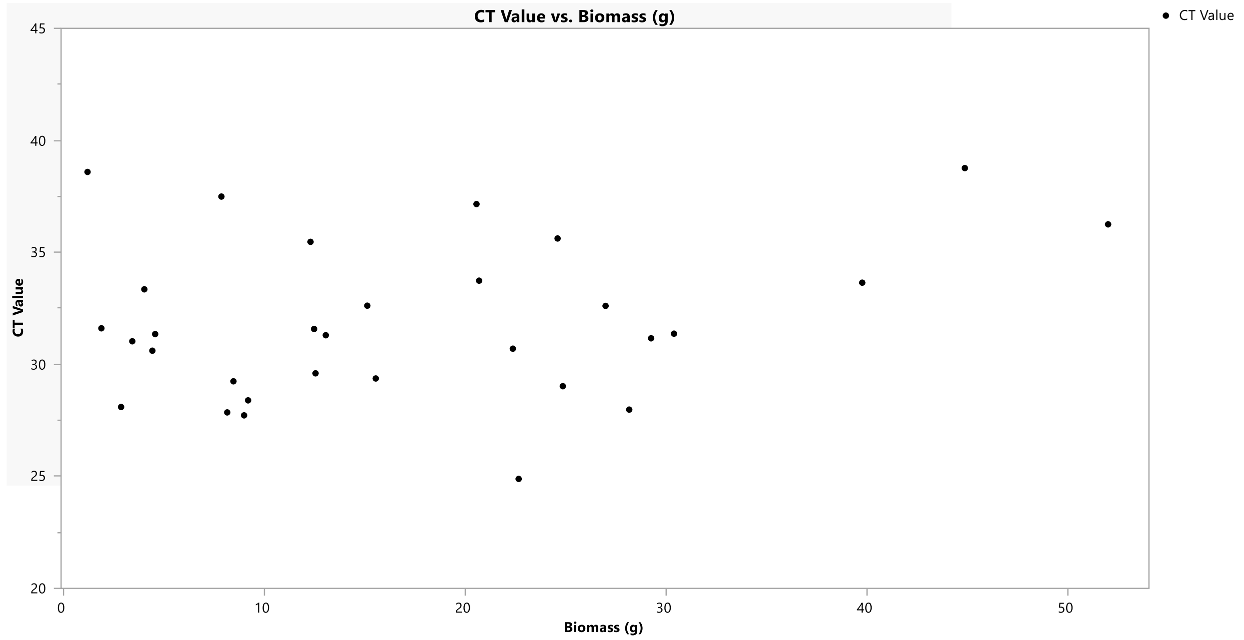


Figure 3.5: Graph of the relationship between *L. setiferus* Abundance and CT Value. Linear regression $p = 0.052$, $r^2 = 0.124$; All analyses were performed using JMP® Pro 16.

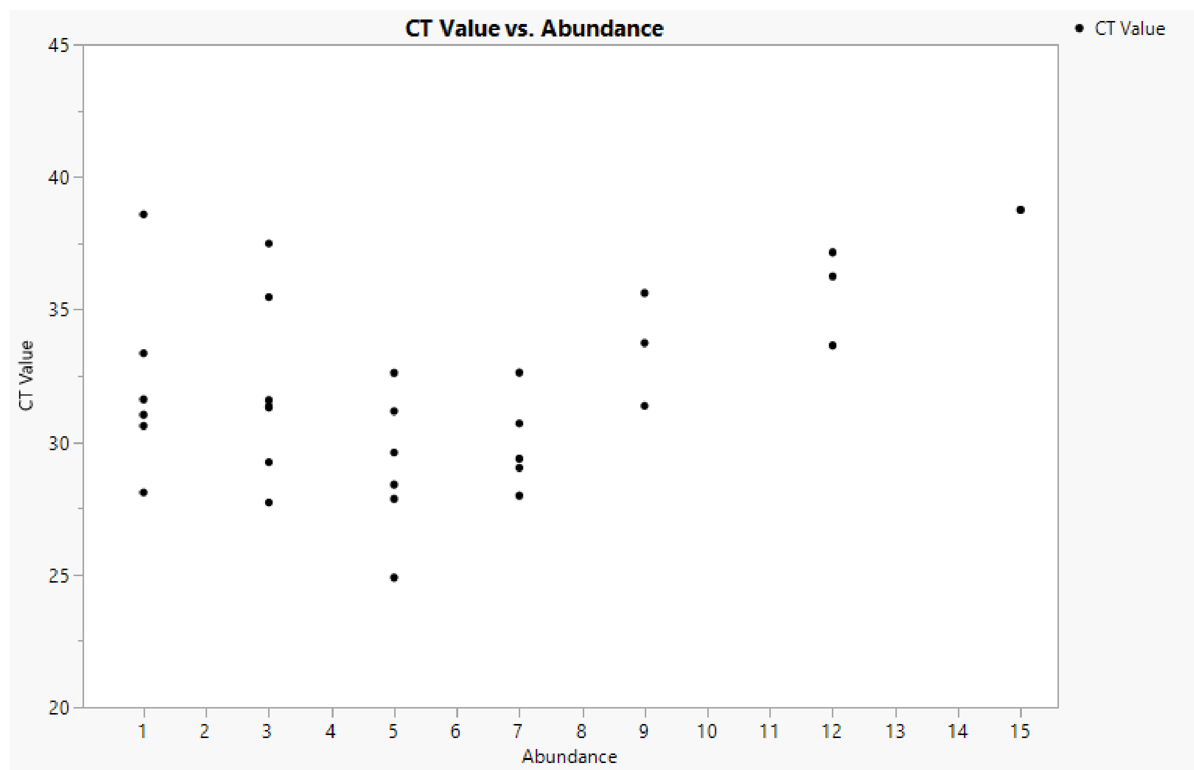
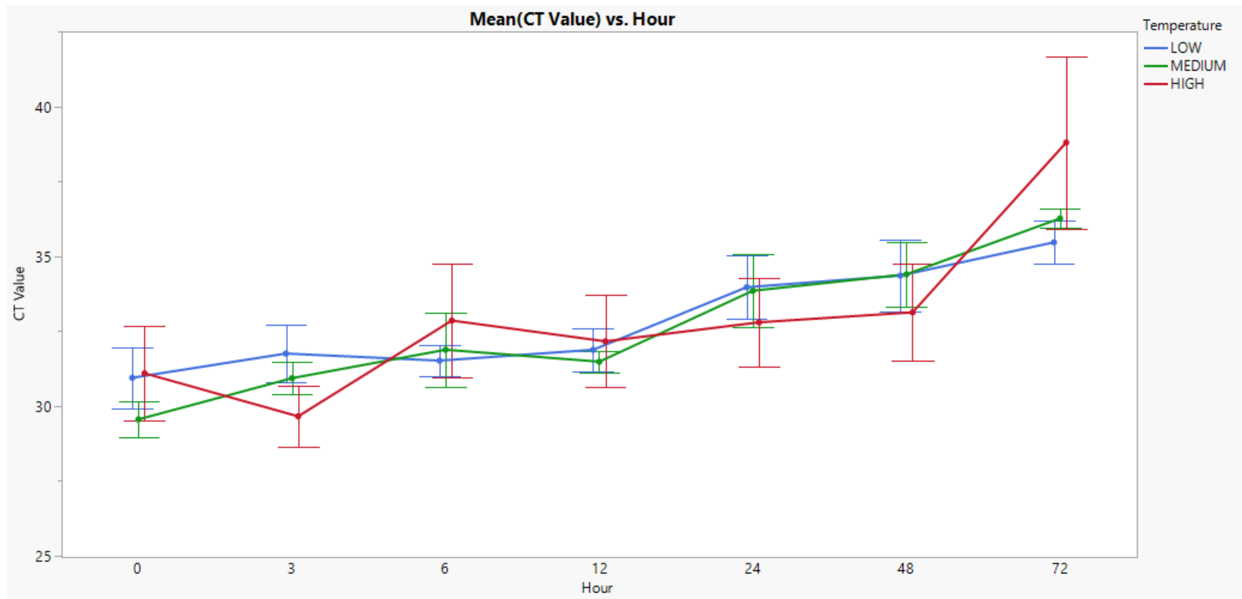


Figure 3.6: Graph of the pooled together filters at each temperature and time point that amplified to determine and average CT value to observe possible trends paired with Standard error bars. The lowest CT value occurred at the initial sampling point and increased over time at similar rates across temperature treatments.



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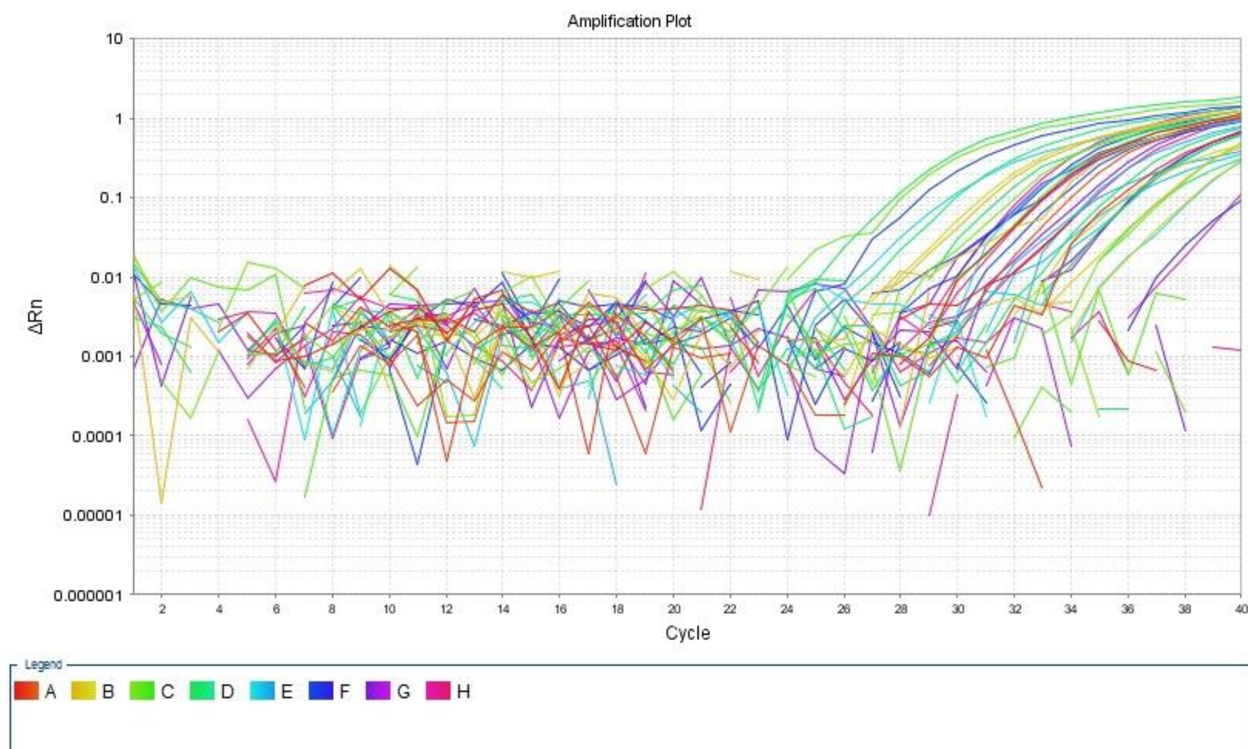
APPENDIX A

AMPLIFICATION PLOT OF THE DEGRADATION EXPERIMEMNT.



APPENDIX B

AMPLIFICATION PLOT OF FIELD SAMPLE. BLUE LINES REPRESENT THE POSITIVE CONTROLS OF *L. SETEFERUS* TISSUE