Title: Investigating transmission of white spot syndrome virus (WSSV) from invasive crayfish, *Procambarus clarkii*, to white shrimp, *Penaeus setiferus*

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Abstract:
White spot syndrome virus (WSSV) is the causative agent of the highly virulent disease, white spot disease (WSD), that is known to infect nearly 100 different decapod crustaceans. WSD has caused significant economic losses in both shrimp and crayfish aquaculture worldwide, and while WSSV is known to have rapid transmission and lethal impacts in single-species aquaculture settings, the impact of environmental factors on interspecific transmission remains unclear. Currently, live red swamp crayfish, *Procambarus clarkii,* are imported into South Carolina as both a bait and seafood product. Recent data has shown that WSSV occurs within these imported specimens. This occurrence of WSSV that can potentially be introduced into the environment, may pose a significant threat to native crustacean populations such as shrimp and blue crab, SC's largest commercial fisheries. The objective of this study is to understand transmission metrics among these crustaceans, and the impacts of environmental conditions on this transmission.

Statement of Interest

My love for the outdoors began at a young age, mapping and exploring my local woods, building a collection of leaves and rocks, and observing nature wherever I could. I always loved fishing, hiking and being outside, however I didn't realize this could be a career path until I found myself in coastal South Carolina during my undergraduate studies at the College of Charleston (CofC). It was here that I learned environmental science was a career path I could undertake, and here, where those original loves of nature developed into something more tangible that ultimately led me to my current position as a wildlife biologist with the South Carolina Department of Natural Resources (SCDNR) and a master's student in CofC's environmental and sustainability studies program (EVSS).

After graduating with a bachelor's degree in marine biology from CofC, I took an internship with the South Carolina Aquarium where I was able to see the wildlife of South Carolina in a new setting. I had previously volunteered with the estuarine finfish group at SCDNR, assisting in the sampling of organisms as part of their long-term trammel net surveys, so I knew that I wanted to pursue research. However, it was at the aquarium that I learned the value of teaching the public, particularly the youth of the community, what it meant to get outside, and learn the same lessons I did from growing up exploring the outdoors. My internship led me to a position within the crustacean research and monitoring section at SCDNR which quickly developed into my current position as a wildlife biologist with the shellfish research section, studying oysters for both their value as a keystone species as well as a method for protecting our changing shorelines. These experiences solidified my interests in conducting research within the coastal environment and in educating new generations of scientists within the greater environmental field.

After two years of working as a biologist for the state, I was ready for the next challenge, with the goal of ultimately becoming a leader and educator on the topics I am most passionate about. In order to accomplish this goal, I realized that I needed to further my education, obtaining a graduate degree in the field of environmental science. Through my experience as a biologist, I have been able to narrow my passions down to the field of marine pathology, studying diseases of marine organisms and the detrimental impacts these diseases can have within the environment. This understudied field is one of growing importance with our changing climate, and one that I ultimately hope to make a difference in throughout my career. My thesis, investigating the transmission of white spot syndrome virus (WSSV) from invasive to native crustaceans, aligns with this interest in further understanding the impacts that pathogens can have within our natural ecosystems. I hope to build off this study by continuing my research of other diseases within our coastal habitat, working with other hosts and pathogens as a wildlife biologist for the state or furthering my education as a graduate student within a PhD program. My degree in the environmental and sustainability studies program would allow me to continue my pursuit of becoming a leader within the field of marine pathology and my thesis will allow me to contribute to the greater knowledge within this area of study. We hope to publish the results of this research in leading journals for invertebrate pathology or shellfish research, and contribute to knowledge through the presentation of these results at national and regional conferences.

Teaching future generations is a passion that has never evaded me, with a goal of becoming an educator at an institute of higher education where I can mentor young scientists that share a similar passion for our changing ecosystems. I have been extremely fortunate with the opportunities that I have been given to pursue these goals, and I hope to provide others the same. I think that it is extremely important to allow students to be involved with research early in their careers, so they too can gain experience and narrow their own interests within this increasingly important field of study. My thesis and education will allow me to continue on this path and I look forward to providing these opportunities to those in the future.

Introduction

Viruses are the most abundant biological entity in the water column of the world's oceans, with 1mL of productive coastal seawater containing an estimated 1 billion viruses (Suttle, 2005). In addition to being highly abundant, the majority of viruses within the natural marine viral community are known to be infectious (Wilhelm *et al*., 1998). Pathogens, including viruses, tend to remain in a homeostatic relationship with their host and environment, whereas a change in any of these components drives periodic disease outbreaks (Burge & Hershberger, 2020). When these outbreaks occur, they can have profound impacts on both managed and natural systems, resulting in diminished growth and survival of affected marine organisms (Lafferty *et al*., 2015). The causative agents of infectious viral diseases can also produce negative consequences on human nutritional, economic and environmental health (Behringer & Duermit-Moreau, 2020). Due to the range of impacts resulting from marine viral diseases, it is important to gain a better understanding of the inter-relationships between host, pathogen, and the environment, particularly given the dynamic nature of these interactions.

In the United States, estuarine ecosystems support nearly half of the U.S. economic output, estimated at approximately $8.8 trillion gross domestic product in 2018, while only comprising 4% of U.S. total land use (Rouleau *et al.*, 2021). Estuaries are also among the most biologically productive ecosystems in the world (SCDNR, 2020a). In South Carolina, these estuaries are dominated by salt marsh habitat, estimated at 344,500 acres, the most of any state on the east coast of the U.S. (SCDNR, 2020b). It is therefore critical to understand host-virus-environment relationships that occur between key species that both rely on these habitats as well as contribute to the importance of these systems, particularly within coastal South Carolina, USA.

White shrimp, *Penaeus setiferus,* utilize estuaries throughout their life histories, and, according to data from 2015 to 2020, can account for upwards of $9 million annually in commercial landings in South Carolina (ACCSP, 2015). In addition, *P. setiferus* play a vital role in the structure and function of estuarine communities (Muncy, 1984). In 1999, wild-caught *P. setiferus* and *Penaeus aztecus* (brown shrimp) from South Carolina were found to test positive with white spot syndrome virus (WSSV), a highly virulent pathogen that causes white spot disease (WSD) in shrimp (Chapman *et al*., 2004), and ultimately results in total mortality of infected populations under normal culture conditions in as few as 3 to 5 days (Chou *et al.,* 1995). This virus, thought to be introduced to the U.S. via the frozen shrimp trade (Lightner *et al*., 1997), infects more than 100 arthropod species, with the vast majority being decapod crustaceans (Dey *et al*., 2020). Since its emergence in the early 1990's on cultured shrimp farms in eastern Asia (Takahashi *et al*., 1994; Chou *et al*., 1995), it has been estimated to cause negative economic impacts of 1 to 3 billion USD annually since 1994 (Lightner, 1999).

White spot syndrome virus has recently been discovered to have large impacts on other aquaculture species such as that of the red swamp crayfish, *Procambarus clarkii*. This freshwater crustacean is native to the lower Mississippi River drainage and Gulf coastal plain, but has established non-native populations in freshwater wetlands worldwide (Nagy *et al.,* 2021). *P. clarkii* are common in aquaculture as a food source as well as for the aquarium trade, and non-native populations are most likely a result of the release of individuals following the importation of live crayfish (Nagy *et al.* 2021). In 2007, WSSV was first identified in large-scale farming operations as well as wild crayfish populations of Louisiana, causing mortality rates of greater than 90% (Baumgartner *et al.,* 2009).

Non-native populations of *P. clarkii* are well established in the coastal plain of South Carolina, and can tolerate salinities up to 35 ppt, allowing for the potential invasion of estuarine and brackish water habitats (Dörr *et al.*, 2020). Horizontal transmission of WSSV has been demonstrated experimentally from the red claw crayfish, *Cherax quadricarinatus,* to the Asian tiger shrimp, *Penaeus monodon* (Soowannayan & Phanthura, 2011). Because of crayfish's tolerance to saline waters, the establishment of wild populations in South Carolina and the usage of low salinity environments during the early part of penaeid shrimp life histories, there is potential for interactions between these crayfish and native shrimp species. Due to the transmission potential between these species, understanding interactions in natural environments is of great importance.

Many studies have been conducted to evaluate the impacts and transmission dynamics of WSSV for major shrimp farming species such as the whiteleg shrimp, *Penaeus vannamei*, and the Asian tiger shrimp, *Penaeus monodon* (Chou *et al*., 1995; Durand *et al*., 1996; Soto *et al*., 2001; Tuyen *et al*., 2014). Furthermore, there are growing concerns surrounding the impacts of WSD on crayfish aquaculture (Huang *et al.,* 2001; Shi *et al.,* 2005; Du *et al.,* 2008). While extensive research has been conducted on WSSV in controlled environments, significant knowledge gaps exist related the potential for outbreaks in natural systems through interspecific transmission. Previous research has addressed the susceptibility of the host to WSD under salinity-stressed conditions (Joseph & Philip, 2007; Carbajal-Sánchez *et al.,* 2008; Raj *et al.,* 2012; Vaseeharan *et al.,* 2013; Ramos-Carreño *et al.,* 2014) however, varying transmission rates and responses are still not well understood under these conditions. In addition, variation among viral strains could lead to differences in these parameters (Gao *et. al.,* 2014). This study intends to investigate the effects of environmentally relevant salinities and temperatures of transmission potential of WSSV from the invasive crayfish, *P. clarkii,* to the native white shrimp, *P. setiferus*, that can commonly be found in South Carolina estuarine environments.

Experimental Design and Objectives

Experimental Design

Two separate experiments will be conducted to identify transmission metrics from WSSV-positive *P. clarkii* to native *P. setiferus* across a range of salinities. Transmission metrics observed will include transmission rate, mortality rate, and infection intensity at mortality. Each parameter will provide insight into the effects of salinity on the responses associated with interspecific transmission of WSSV. The specific strain of WSSV used for this experiment will be a strain known to infect *P. clarkii* that originates from aquaculture facilities in Louisiana. Since farm-raised crayfish from Louisiana are imported to South Carolina, this strain likely represents a threat to crustaceans native to South Carolina. Each trial will incorporate an environmentally relevant salinity gradient, similar to those experienced by both species in transitional estuarine areas. The first trial will utilize a broad range of salinities, as well as two variations in temperature, to identify how environmental factors relate to transmission between the experimental organisms. The second trial will look at a finer-scale salinity gradient, to identify variation across this range. The salinities used in the second trial will be determined by observed differences in mortality shown in the first trial. Each trial will utilize a total of 30 aquaria, whereas each aquaria represents a replicate of a specific treatment. The first trial will incorporate 3 salinities (5, 12 and 19 ppt) at 2 temperatures (25°C and 28°C), with 5 replicates at each salinity-temperature combination. The second trial will use either 5 or 6 replicates at 5 or 6 salinities based on findings from the first trial. (Figure 1). Each 3-gallon polycarbonate aquarium

will house one experimentally infected crayfish and one shrimp specimen, separated by a perforated PVC barrier to allow for water exchange but to avoid direct contact.

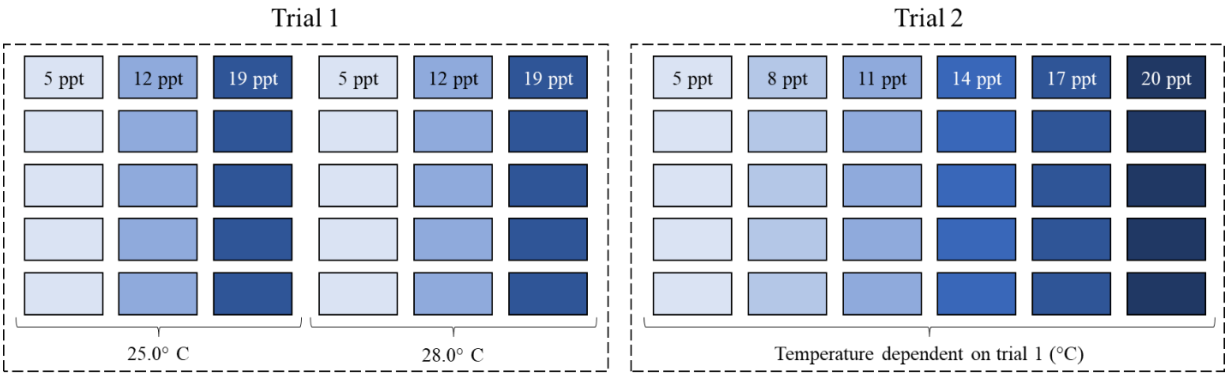

Figure 1: Schematic of the experimental design. Each box represents 3-gallon aquarium with experimentally infected *Procambarus clarkii* (red dots, n=30) and *Penaeus setiferus* (yellow dots, n=30) physically separated via perforated PVC wall. Trial 2 salinities and temperature dependent on Trial 1.

Experimental Aquaria System

Experimental aquaria will be set-up in Room F-126 in the Hollings Marine Laboratory (HML) at the Fort Johnson Marine Resources Center in Charleston, South Carolina. Each trial will utilize 30, 3-gal polycarbonate aquaria filled with a mixture of polished seawater (filtered and sterilized via ultraviolet light) and dechlorinated tap water to reach desired salinities. Temperature of the challenge room will be controlled by a one-way HVAC zoned air system and temperatures of tanks for the first trial will be controlled by small aquarium heaters. Lighting within challenge room will remain consistent with a 14-hour light to 10-hour dark photoperiod, with 30-minute transition to avoid disturbance of test organisms and maintain environmentally relevant conditions. Water quality parameters including salinity and temperature will be recorded and monitored daily to avoid fluctuations throughout the trials, and high levels of dissolved oxygen will be maintained throughout via constant aeration by an air stone. Other water quality parameters will be tested prior to trials including pH and alkalinity to avoid any covariance with salinity. Prior to trials being conducted, areas within the challenge room will be identified for any changes in temperature or light, and, during experiments, aquaria will use a randomized block design, with blocks corresponding to different light and/or temperature areas to avoid room effects on treatments.

Each aquarium will be divided with a small section of opaque perforated PVC. This will keep experimentally infected *P. clarkii* physically separated from *P. setiferus* specimens while allowing for indirect transmission through the water column. All specimens will be held in acclimation tanks until trials begin. Trails will begin once infected crayfish specimens are placed in experimental tanks. After a pre-determined time, crayfish will be removed from tank as to establish a consistent exposure for each replicate. Preliminary trials will indicate adequate time needed for confirmed exposure. Between replicate trials, all equipment will be sterilized, and complete water changes conducted.

Collection and Holding of Organisms

Collection of *P. clarkii* (ranging from 30-40 mm orbital carapace length) will be conducted by a combination of baited minnow traps and dip nets in Bear Swamp at Bulow County Park, SC. All *P. clarkii* specimens used in experimental trials will be female to limit any potential sex-linked bias. *P. setiferus* (ranging from 50 mm to 70 mm total length) will be collected from the Ashley River by the R/V *Silver Crescent* as a part of the SCDNR's Estuarine Trawl survey using a 6.1-meter otter trawl with 1.27 cm bar mesh or by a smaller 4.3-meter otter trawl as part of the SCDNR Crustacean Research and Monitoring Section's creek trawl survey (Figure 2). All collected animals will be subjected to an acclimation and quarantine period, where organisms will undergo changes in salinity to reach experimental treatment concentrations at a rate of no more than 2 ppt per day and gradual acclimations to experimental temperatures. During this acclimation and quarantine period, specimens will be subjected to a health and condition screening, where rejection of the test batch will occur if a greater than 50% mortality of individuals is observed to avoid the introduction of collection-based trauma and consequent mortality as a confounding effect on the experimental trials. Experimental animals will be fed antibiotic and hormone free commercial pellets at a rate of 5% body weight during the acclimation period to ensure they are not weakened due to starvation. Any uneaten pellets will be immediately removed from aquaria to avoid disturbances in water quality.

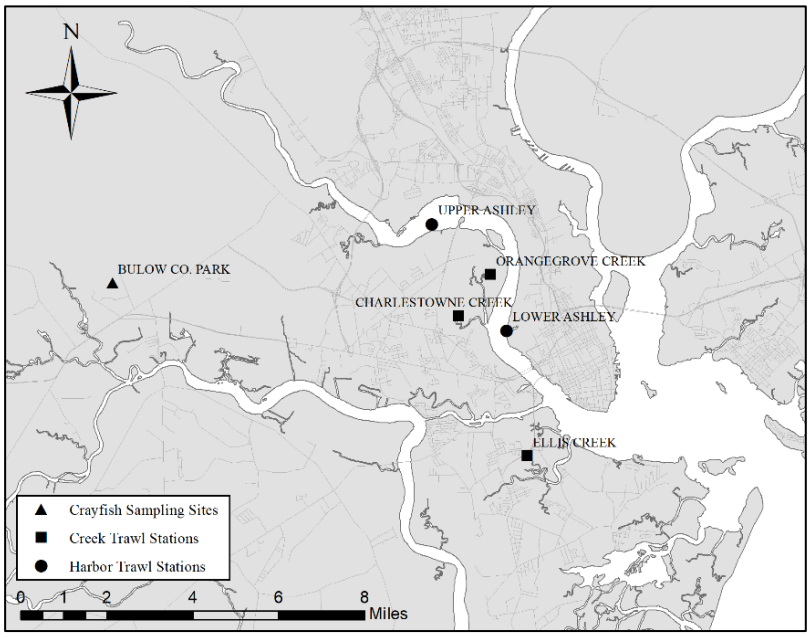

Figure 2: Map of the greater Charleston, South Carolina area, displaying potential collection sites for *P. clarkii* specimens in Bulow County Park, and *P. setiferus* specimens along the Ashley River.

Shrimp specimens will undergo initial acclimation in a 110-gallon holding tank, with salinity and temperature pre-conditioned to those recorded at capture. Water temperature in the holding tank will be allowed to rise or fall naturally to that within the challenge room, prior to separating individuals for different salinity treatments. Secondary acclimation will occur in 20-gal polycarbonate aquaria, with water quality being maintained using pre-conditioned airlift-driven sponge filters. Individuals will be physically separated from one another using 16 oz. floating polypropylene containers with 15, ¼ in. diameter holes drilled out of the sides to allow for water exchange. Specimens of *P. clarkii* will undergo a similar acclimation process, however initial acclimation for these organisms will occur in 20-gal polycarbonate aquaria similar to those used for secondary acclimation of shrimp. These individuals will also be physically separated using the floating polypropylene container setup.

Viral Inoculant Preparation

In order to produce the viral inoculant needed for these trials, gill tissue will be removed from experimentally infected organisms of known viral isolates obtained from the Louisiana State University (LSU) School of Veterinary Medicine and processed via a modified technique derived from Du *et al.* (2007) and Pace *et al.* (2016). In summary, gill tissue will be homogenized in TNE buffer (0.05M Tris-HCl, 0.1M NaCl, 0.001M EDTA, pH 7.4) prior to being centrifuged at 8000 × g (20 min at 4°C) followed by the supernatant being passed through a 0.45 μm filter. The filtrate will then be layered onto a 30% sucrose solution and centrifuged at 30 000 × g (2 hours at 4°C) before resuspension of the viral pellet in 100 μl of TNE buffer. This final pure viral inoculant will be stored in a -80°C freezer until use. Approximately 3mL of pure viral inoculant will be needed to experimentally infect a total of 60 crayfish specimens for both trials (n =30 per trial). Viral inoculant will be prepared at a dilution of 1 part inoculant to 9 parts sterile saline followed by filtration through a 0.45 μm filter to keep consistent viral quantities across all individuals.

WSSV inoculations of experimental animals

To ensure positive infection of *P. clarkii,* inoculant will be directly injected into experimental organisms. Prior to injection, wet weights of individuals will be recorded in order to calculate the amount of inoculant needed for each animal at a 20 μL viral solution per gram body weight dosage. Using a 1 mL syringe with a 26-gauge needle, each animal will be injected intramuscularly with proportional volume of viral solution in the fourth abdominal segment. Injection sites of crayfish will be rinsed with distilled (DI) water prior to placement in treatment tanks to ensure no excess inoculant is added to the systems.

Transmission

Initial transmission rates of WSSV from *P. clarkii* to *P. setiferus* will be observed by testing specimens using quantitative polymerase chain reaction (qPCR). At 24 and 48 hours post challenge, left pleopod samples will be removed from each shrimp, rinsed in DI water, and stored in 95% ethanol. These samples will be provided to the Genetics Section of the Marine Resources Research Institute of the SCDNR for processing. Although pleopods are not the primary target site for initial replication, this tissue will be used to avoid unnecessary stress on the organisms during the trials. If mortality occurs prior to the 24- or 48-hour marks, tissue will be removed upon initial observation of moribund specimen and processed for additional post-mortality analyses, including removal of gill tissue for confirmation of diagnosis via qPCR as well as fixation of tissue for histopathological analysis.

Mortality Rates

Mortality rates will be recorded as cumulative mortality per hours post challenge. Cumulative mortality will be calculated for each salinity when total mortality occurs across all replicate treatments. Each replicate, n =5, for the first trial, or n =5/6 for the second, will represent a given percentage of mortality for that salinity treatment. Trials will run for up to 10 days, whereas, if total mortality does not occur within the allotted time, remaining individuals will be euthanized and processed for additional post mortality analyses.

Upon observation of moribund shrimp, specimens will be immediately fixed using 10% seawater buffered formalin for a minimum of 48 hours and then transferred to 70% ethanol. Techniques for routine histological diagnosis of Penaeid shrimps as presented by Bell &

Lightner (A Handbook of Normal Penaeid Shrimp Histology, The World Aquaculture Society, Baton Rouge, 1988) will be used for fixation, paraffin embedding and hematoxylin and eosin (H&E) staining of target tissues of moribund shrimp. Target tissues for WSSV replication occur across all body systems, therefore, the entirety of organs held within the cephalothorax of each individual will be fixed for this analysis.

Infection Intensity

Using histopathological techniques, infection intensity will be categorized based on a scale from 0-4, where 0 represents no identifiable infection and 4 represents a severe infection (Lightner, 1996). This diagnosis for WSSV infections will include the observation of eosinophilic to pale basophilic intranuclear inclusion bodies in hypertrophied nuclei of epithelial cells and connective tissue cells, primarily those of cuticular origin (Lightner, 1996) (Figure 3). Scaling of infection intensity will be defined by total presence of these infected cells in a given field of view by increments of 25% (1-25% = 1, 25-50% = 2, 50-75% = 3 and 75-100% = 4). In addition to intensity, areas of infection will be recorded to identify means of pathogenesis within the organism. Areas of differing intensities will be recorded as such if variation across samples is observed.

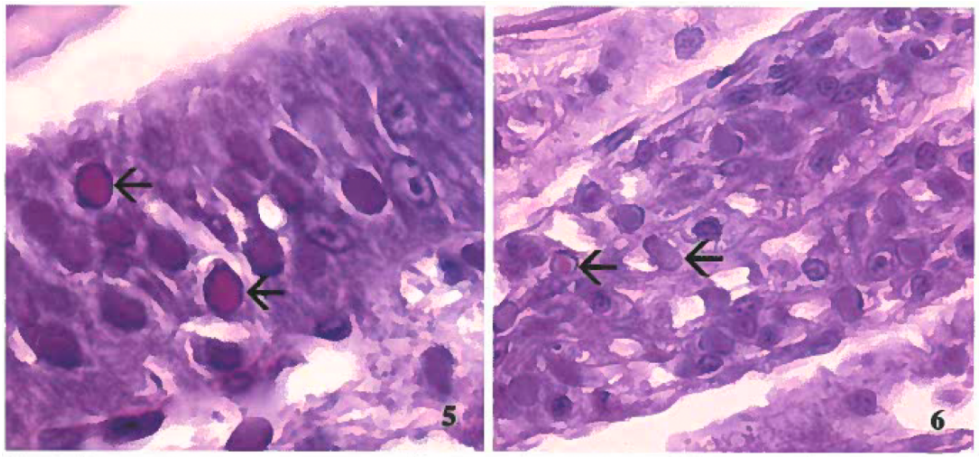

Figure 3: Histological sections processed with Meyer-Bennett H&E stain from juvenile Chinese white shrimp, *Penaeus chinensis* displaying advanced (*i.e.,* stage 3) WSSV infection in stomach tissue (left) and gill tissue (right), characterized by fully developed and developing intranuclear inclusion bodies filling hypertrophied nuclei (arrows). Magnification 1300X (left) & 900X (right). (Lightner, 1996).

Interpretation of Results

Effect of salinity on initial transmission

Transmission of WSSV from experimentally infected *P. clarkii* to *P. setiferus* is expected to happen quickly (*i.e.,* within 48 hours post challenge) during both trials as observed by Soowannayan & Phanthura (2011); however, it is unknown how or if this rate changes under these given environmental conditions. Initial transmission will be determined by taking tissue samples and testing for positivity using qPCR at 24 hours and 48 hours post challenge. These binary data will display either a positive or negative result, indicating a percent positive among individuals at specific salinities within a known amount of time post exposure. Due to restricted capabilities on running multiple assays as well as minimizing stress to experimental animals, a narrower time approach is not possible. For the first trial, salinity and temperature will be handled as categorical variables and an ANOVA will be used to identify any significance in transmission across treatments. The second trial will utilize logistic regression to test for significant effects of the larger salinity gradient on transmission.

Effect of salinity on mortality rates

Due to the freshwater nature of viral strain used in this study, lower salinities may display higher levels of mortality than that of higher salinities. For the first trial, effects of salinity and temperature on mortality will be compared using ANOVA and a mixed effects approach will be used to identify the effects of salinity and temperature on mortality. For the second trial, both a Kaplan-Meier survival curve as well as a regression approach will be used to look for effects of salinity on mortality. Total mortality at hours post challenge could change across salinities displaying either; (i) a positive relationship among mortality and increasing salinity; (ii) a negative relationship between mortality and increasing salinity; (iii) an intermediate salinity showing higher mortality than extreme salinities; (iv) extreme salinities showing higher mortality than intermediate salinities; or (v) no relationship between salinity and mortality.

Previous findings have shown that survival of experimentally infected *P. vannamei* increases at intermediate salinities (15 and 28 ppt) while decreasing at extreme salinities (5 and 54 ppt) (Ramos-Carreño et al., 2014). These findings bolster the predicted results, based upon the experimental design, where a negative linear relationship could be observed among mortalities with increasing salinity due to selected salinities being lower for environmental relevance. This could, however, suggest that extreme salinities show higher mortality than those at intermediate. An ANOVA may be performed to determine differences between individual salinities.

Effect of salinity on infection intensity

Infection intensity will be determined through histopathological assessments, measuring overall tissue damage resulting from WSSV infections. Individual shrimp will be considered replicates across their respective environmental challenges. Infection intensity across the gradient in the second trial will be analyzed using a regression-based approach to observe relationships between intensity and increasing salinity. Because this viral strain is infectious primarily in freshwater, there could be a trend of higher intensities at lower salinities compared to higher salinities, due to the replicability of viral particles in preferred low salinity conditions.

Other variations in infection intensity could also occur, as observed by Carbajal-Sánchez et al. (2008) who reported more severe infection of both the gills and gastric epithelia in *P. vannamei* held at an intermediate treatment of 15 ppt than at other salinity treatments. This finding suggests the possibility of contrasting trends between infection and mortality in relation to salinity. In this proposed study, ANOVA may be used to determine if infection intensity is significantly different among treatment salinities. In addition to looking at overall intensity, areas of infection will be recorded throughout and specific areas within tissues will be scaled based on intensity. These results could suggest differences in pathogenesis across different conditions, and therefore if variation is present, results will be compared for significance.

Conclusion

This proposed investigation of the effects of salinity on indirect transmission from non-native *P. clarkii* to native penaeid shrimp ultimately aims to provide better insight on the potential for WSD outbreaks in wild populations. With little literature investigating environmental effects on transmission of WSSV in relation to natural environments, this potential vector of transmission from a non-native to native species represents a gap in knowledge of estuarine ecosystems of South Carolina. Understanding the transmission metrics among these crustaceans, and the impacts of environmental conditions, can inform management decisions related to minimizing disease outbreaks that impact population status.

Future research

Future research directions (time and resources allowing) could include further experimental trials with other important native crustacean species of South Carolina, including species such as blue crab, *Callinectes sapidus,* or brown shrimp, *Penaeus aztecus.* In 2004, WSSV in wild SC populations was confirmed by a positive individual caught in the Charleston Harbor during a screening of the wild population (n=300) (Powell *et al.,* 2015).

Other directions for future research would include investigating the physiological effects of WSD on *P. setiferus* at various salinities. This could be done through the screening of hemocyte counts to better understand immune responses to infection or through the activity of immune related enzymes such as phenol oxidase (PO) and peroxidase (POD) (Gao *et al.,* 2014). Salinity and WSSV-infection have been shown to impact immune response in *Penaeus indicus* (Vaseeharan *et al.,* 2013), and understanding this response could help explain variation in mortalities and transmission. In addition, testing different strains of WSSV that may be present in South Carolina waters would be of interest moving forward. This study looks to investigate a strain that primarily infects crayfish, however other strains have been observed in wild populations of other invasive species, such as the Asian tiger shrimp, *Penaeus monodon*, (Kendrick, pers comm.), a non-native species now established in the South Atlantic Bight.

Projected timeline

	2022									2023		
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Preliminary Trials												
Collection of Organisms												
Salinity & Temp (trial 1)												
Salinity (trial 2)												
Tissue Processing												
Data Analysis												
Writing												
Defend Thesis												
Graduation												

	2023									2024		
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Preliminary Trials												
Collection of Organisms												
Salinity & Temp (trial 1)												
Salinity (trial 2)												
Tissue Processing												
Data Analysis												
Writing												
Defend Thesis												
Graduation												

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South Carolina Department of
Natural Resources

Robert H. Boyles, Jr.
Director
Blaik Keppler
Deputy Director for
Marine Resources

Dear proposal review committee,

I am writing this letter in strong support of this application to the Graduate Program in Environmental and Sustainability Studies for the 2022-2023 academic year research awards. This award would support Greg Rothman's thesis project on the role of invasive species as vectors of disease. I first met Greg in the Spring of 2019, when I hired him as an hourly employee with the South Carolina Department of Natural Resources to assist with projects in the Shellfish Research Section. Greg quickly showed strong initiative and problem-solving skills and was soon offered a full-time position as a Wildlife Biologist. As a biologist Greg has proven to be a valuable team member, working effectively on multiple concurrent projects, and always with a positive attitude and thoughtful, pragmatic perspectives.

Greg started the master's program at the College of Charleston in 2021, choosing to work on the project described in this proposal, seeking to better understand the role of invasive species as vectors of disease to native crustaceans in South Carolina. This project aims to refine our understanding of how environmental conditions such as salinity and temperature influence the transmission and impact of diseases in aquatic organisms. By bridging the freshwater and saltwater habitats of the South Carolina coast, this project highlights the interconnectedness of aquatic habitats in the Lowcountry and how environmental factors can mediate disease transmission dynamics in the wild. Greg has already made substantial progress in developing the methodology for these lab-based trials, providing a keen and critical view to experimental design options, and regularly engaging with his committee. Greg's thoroughness in both lab-based and field-based work, his question-oriented view of experimental design, and his commitment to conserving natural resources will help ensure a successful thesis project. The funds provided by program will support the acquisition of supplies to ensure detailed monitoring of relevant environmental conditions, sample processing costs, travel to a regional or national conference to present this work, and costs associated with publishing the findings in the peer-reviewed literature. As demonstrated through his experiences and skillsets, I believe that Greg brings a wealth of experiences to this project that will help ensure its success.

The transportation of aquatic animals across the globe is leading to a homogenization of aquatic communities that threatens the structure, function, and biodiversity of environments. The impacts of non-native species in introduced environments can be vast, but research is only beginning to understand the role of non-native species as vectors of non-native diseases and parasites. The development of climate-conscious models is needed for evaluating threats posed by non-native species. An in-depth knowledge of how environmental conditions may mediate the impacts of non-native species, for instance, can help resource managers prioritize threats to natural environments.

Please let me know if there is any additional information I can provide in support of this application.

Sincerely,

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