Current Velocity, Temperature, and Stress: Factors affecting movement and prevalence of White Spot Syndrome virus in *Procambarus clarkii* (Red Swamp Crayfish)

By

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Abstract

Red swamp crayfish (*Procambarus clarkii*) have invaded ecosystems worldwide and are potential carriers of white spot syndrome virus (WSSV). We monitored the prevalence of WSSV under a natural thermal regime in wild populations of *P. clarkii* and tested whether stress associated with laboratory studies could trigger WSSV virulence. Proportion of WSSV in wild populations peaked at 22.3°C but prevalence could remain low even at optimal temperatures. Stress associated with internal insertion of PIT Tags did not trigger increased WSSV count or virulence in laboratory animals. We then used PIT-tagged animals to investigate the relationship between current velocity and rheotaxis in *P. clarkii*. We found weak evidence for positive rheotaxis at low current velocities and strong evidence for negative rheotaxis at higher velocities. Results suggest that high current velocities may limit the spread of invasive *P. clarkii* in lotic systems and may be useful to increase trapping efficiency in invaded systems.

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List of Abbreviations

RSC	Red Swamp Crayfish
WSSV	White spot syndrome virus
qPCR	Quantitative PCR
STDs	Standard tube dilutions
EWSFC	E.W. Shell Fisheries Center
CL	Carapace length
SE	Standard error
PIT	Passive integrative transponders

Chapter 1: Effects of season and stress on prevalence of the White Spot Syndrome virus in *P. clarkii*

INTRODUCTION

The white spot syndrome virus (WSSV) is a pathogen in the family *Nimaviridae* that is a major concern in global crustacean aquaculture (Desrina et al., 2022) due to it's potential to cause high mortality rates in infected populations. Infection level, and subsequent mortality, vary depending on the specific isolate and amount of virus particles the host is exposed to (Marks et al., 2005; Laramore et al., 2009; Pace et al., 2016). The first discovery of WSSV was in populations of cultured shrimp, *Penaeus japonicus*, in northern Taiwan in 1992 (Chou et al., 1995). In the United States, WSSV was first detected in the shrimp farming industry in 1995 (Lightner et al., 2012) and has since spread to other crustacean aquaculture species such as crayfish (Baumgartner et al., 2009; Lee et al., 2021).

One crayfish species reported to be infected by WSSV is the red swamp crayfish (RSC; *Procambarus clarkii*). RSC is native to the southern United States and north-eastern Mexico and has been the predominant species cultured in the Louisiana crayfish aquaculture industry since the early 1900s (Hobbs, 1972; Huner & Barr, 1991). WSSV has been identified in RSC commercial aquaculture ponds in Louisiana since 2007 (Baumgartner et al., 2009). Due to its high cultural value and demand for human consumption, RSC have established populations on all continents except Antarctica and Oceania (Hobbs et al., 1989). Due to their widespread distribution and shipping, RSC have the potential to be global vectors of WSSV.

The anthropogenic movement of live organisms has been documented as a pathway for pathogens spreading in aquatic systems (Alderman, 1996). For example, the crayfish plague (*Aphanomyces astaci*), which originated in North American crayfish, has caused extreme

mortality among European crayfish species (Alderman, 1996; Holdich et al., 2009; Oidtmann 2012) via human-mediated introductions (Alderman & Polglase, 1988; Huang et al., 1994). Similarly, introductions of RSC that are carriers of WSSV may infect native crayfish, ultimately threatening crayfish biodiversity on many continents.

Increasing temperatures due to climate change have been shown to enhance disease processes (Shields, 2019) and stress as temperatures rise above the thermal optima of aquatic organisms and their immune systems. Immune response to infectious diseases can be affected by temperature with cellular immunity against the virus being more effective at lower temperatures (Korkut et al., 2018). Crustaceans rely on innate immunity to fight off infections with the crustin protein being an integral immune response to diseases such as the white spot virus (Bouallegui, 2021; Wang et al., 2017; Sun et al., 2014). In RSC, transcription of *crustin2* decreases at 25 ± 1°C but increases at 19 ± 1°C and 32 ± 1°C suggesting a more effective immune response at low and high temperatures (Jiang et al., 2019). In addition, some microbial pathogens have higher thermal optima than their hosts and can better overcome their hosts immune defenses (Shields, 2019).

The mechanism behind infection prevalence and temperature for WSSV and RSC is not likely due to WSSV having a higher thermal optima than RSC. Optimal growth for RSC occurs between 22-30°C, and thermal preference is typically between 23-27°C (Westhoff and Rosenberg, 2016). Similarly, in RSC aquaculture farms in China, WSSV viral replication was reduced at low (10-19°C) and high (30-32°C) compared to intermediate (24-25°C) temperatures (Jiang et al., 2019; Du et al., 2008) - suggesting a similar thermal optima for host and pathogen. However patterns may be driven in part by increased effectiveness of the immune system at high

and low temperatures as WSSV sometimes (but not always) peaked at intermediate temperatures where immune responses appeared to be weakest (Jiang et al. 2019).

In other crustacean species including shrimp, WSSV infectivity has been correlated to changes in temperature and seasonality. Water temperature fluctuations between 3 to 4°C during a day activated white spot disease outbreaks in cultured *Penaeus monodon* (Tendencia & Verreth 2011). Further, Piamsomboon et al. (2016) found white spot disease cases occurred 2.53 times more when monthly average temperatures fell within 24.5 to 27.2°C than months where average temperatures fell within 28.6 to 29.8°C indicating WSSV infections in crustaceans may be impacted by season and temperature.

Prevalence of WSSV in RSC populations appeared to be partly driven by a not fully understood interaction of season with temperature. Prevalence initially increased above 19 ± 1°C, peaking at 25 ± 1°C, and declined back to zero as temperatures oscillated from 30-32°C in summer. However, prevalence did not increase when fall temperatures fell to the previously observed late spring optimum of 25-26°C (Jiang et al., 2019). Little is known about the prevalence of WSSV and its relation to temperature in the wild as, to our knowledge, there are no seasonal surveys of this virus occurring in wild RSC populations in or near their native range.

In the spring of 2022, RSC held in laboratory raceways at the E.W. Shell Fisheries Center (EWSFC) in Auburn, AL, USA (N32.65247, W-85.48560) began exhibiting lethargy and high mortality rates. Crayfish from affected raceways were subsequently tested and confirmed positive for WSSV (Bruce et al., 2024). Because the majority of these crayfish had been trapped from wild populations at EWSFC, we began testing wild populations across two watersheds to determine if WSSV was widespread in station ponds and streams (Fig. 1.1) and if so, whether detection patterns varied with seasonal changes in temperature (Objective 1). Additionally, we

tested whether the stress associated with research activities resulted in the detection and onset of virulence of WSSV in previously negative animals that were suspected to be carriers of WSSV (Objective 2). We hypothesized that 1) WSSV was widespread in wild crayfish populations at the EWSFC, 2) probability of WSSV detection would increase when daily average water temperatures fell within 19-30°C, and 3) stressors associated with laboratory studies (i.e., internal PIT tagging) would trigger WSSV virulence in outwardly healthy crayfish that were suspected to be carriers.

METHODS: OBJECTIVE 1

Crayfish collection

Crayfish were collected from multiple sites at the E.W. Shell Fisheries Center (EWSFC; Auburn University) from June 2022 – December 2023 (Fig. 1.1). At each site, minnow traps were baited with a 5.5-ounce can of Friskies Pate Wet Cat Food and deployed overnight with a set time of ~18h. Traps were deployed every 2 weeks except for periods when ponds were drained or streams had dried.

Evaluation for WSSV and viral load quantification

On each collection date, a total of 5-10 crayfish were randomly chosen at each site for WSSV testing. Carapace length (mm), sex, and species were recorded for each individual. A single pleopod was removed from each crayfish and placed into a collection tube. Optimally, two tubes containing 5 pleopods each (i.e. 10 total crayfish sampled) were collected from each site. However, low catch rates in traps sometimes resulted in less than two tubes or less than 5 pleopods per tube being collected for a given sampling event. Collection tubes were placed in coolers, returned to the lab, and stored at -80°C. Tubes were removed and thawed for DNA

extraction processing within 11 months of collection. Once thawed, samples were homogenized in the collection tubes containing 2 mm ceramic beads, using a VWR 4-place bead mill (Radnor, PA). An Omega BioTek DNA Tissue Kit (Norcross, GA) was used to extract DNA.

DNA quantity and quality (4.7–71.7 ng μL^{-1}; 260/280 range of 1.66–1.87) was assessed using a NanoDrop One[c] (ThermoFisher Scientific, Waltham, MA). DNA from each sample was diluted to 5 ng µL solution for a quantitative PCR (qPCR) assay based on Durand and Lightner (2002). The 25 µL reaction was comprised of 12.5 µL of TaqMan Fast Advanced Master Mix (ThermoFisher Scientific, Waltham, MA), 5 µL of extracted DNA, 0.75 µL of forward primer (0.3 µM; WSS1011F), 0.75 µL of reverse primer (0.3 µM; WSS1079R), 0.375 µL of probe (0.15 µM) and 5.625 µL of nuclease-free water. The qPCR was performed using a Quantstudio 5 (Applied Biosystems, Waltham, MA) with the following conditions: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. A standard curve was generated using a 75-bp oligonucleotide (100 µM stock, Muhammad et al., 2020) was included with a 10-fold of standard tube dilutions (STDs; 3 through 8) in place of the DNA; crayfish tissue that previously tested positive for the virus was used as a positive control; and nuclease-free water in place of the DNA was used as a negative control.

The virus genome copies per µL were calculated according to the Ct values using the standard Ct mean for each run. Samples were at detectable levels of WSSV if Ct values were below 38. Any samples with a Ct value of >38 were considered undetected for WSSV. This was determined based on the standard curve we created that did not exceed results past 38 Ct.

Temperature collection

Temperature for each site was collected every 30 minutes using a HOBO Pendant MX Water Temperature Data Logger (Bourne, MA) and used to calculate daily average temperature at each site throughout the collection period.

Data analysis

To assess the relationship between temperature and the probability of detecting WSSV, data from all collection events was pooled across sites. The probability of detecting WSSV was calculated by dividing the number of positive samples (i.e., a sample that exhibited >38 Ct) by the number of total samples within each 4°C temperature bin (i.e. 8-12°C…32-36°C). The proportion of positive samples within each bin was then plotted against the mean temperature within each bin and fitted with a 3rd order polynomial quadratic regression using SigmaPlot 15 (Systat Software©, Inc. 2014).

To assess the relationship between temperature and intensity of WSSV in positive samples only, the copies/µL estimated for each positive sample was plotted against the average daily temperature on the collection date for a given positive sample. Copy data was log transformed to pass the Shapiro-Wilk normality test (P=0.9822) and a quadratic regression was fitted to the data using SigmaPlot 15 (Systat Software©, Inc. 2014).

RESULTS: OBJECTIVE 1

Spatial prevalence of WSSV

WSSV was detected in crayfish on at least one date from the single location sampled in watershed I and from each of the six locations sampled in watershed II (Table 1; Fig. 1.2, 1.3). Ct values ranged from a marginally positive detection of 37.8 with a count of 25,346 copies/µL, to

strong detections of 16.8 with a count of 47,651,802 copies/µL. Three ponds (pond E46, recirculation pond, and retention pond) produced at least one sample with counts exceeding 1,000,000 copies/µL whereas stream sample counts were always <1,000,000 copies/µL. At the watershed I site, four positive samples included pleopods from species other than *P. clarkii* (i.e. *Procambarus acutissimus* and *Lacunicambarus dalyae*) (Table 1). Within the watershed II sites, no species other than *P. clarkii* were collected in traps.

Relationship between temperature and WSSV

When pooled across all sites and dates, the majority of WSSV detections fell within the optimal temperature range (19-30°C) for WSSV found for *P. clarkii* in China (Jiang et al., 2019). However, some positive detections fell outside of this range and the majority of samples collected within the optimal temperature range were negative (Fig. 1.4). The probability of collecting a positive sample showed a unimodal relationship with temperature ($y=-0.4513+0.0581x-0.0013x^2$; $P=0.02$, $R^2=0.85$) peaking at 22.3°C, and declining to zero at the lower and upper mean bin temperatures of 10 and 34°C, respectively (Fig. 1.5). When considering the positive samples only, there was not a significant quadratic relationship ($P=0.51$) between viral concentration in samples (copies/µL) and temperature (Fig. 1.6).

METHODS: OBJECTIVE 2

Crayfish collection and husbandry

Procambarus clarkii were collected in August 2022 from an outdoor pond (E46; Figure 1.1) at the E.W. Shell Fisheries Station in Auburn, AL (N32.65247, W-85.48560) using baited (Friskies wet cat food) minnow traps. Pleopods from 10 crayfish (5 crayfish/sample vial) at the collection site were sampled and frozen at -80°C for later WSSV analysis. All experimental

systems were disinfected prior to adding study animals using Virkon® Aquatic. Crayfish were held communally in 3.0 m x 0.67 m flow through raceways that were filled with water from an ~8 ha reservoir (Farm Pond 11) at ambient temperatures for one week, at which time pleopods were collected from 10 crayfish (5 crayfish/sample vial) and frozen at -80°C for later WSSV analysis. Thirty-one crayfish (16 females, 15 males) with carapace lengths between 28 mm - 36 mm (mean CL = 31.3 ± 0.35 (SE) mm) were then transferred to 1.5 L individual tanks (Pentair AHAB units) within a recirculation system equipped with a mechanical filter and a biofilter. Each tank contained a shelter made from a 3.81cm dia PVC pipe. Water temperature of the recirculation system was raised to 30°C at a rate of 2°C/day under a 12 h light:12 h dark photoperiod, and crayfish acclimated to these conditions for one week before being pit-tagged. The 30°C temperature was chosen because it was high enough to cause a reduction in WSSV replication (Jiang et al., 2019) but too low to cause lethal thermal stress (Huner & Barr, 1991; Westhoff & Rosenberger, 2016). Crayfish were fed three shrimp pellets/day (Ziegler Shrimp Grower SI-35-EX) three days a week and monitored daily for molting.

Pit tag laboratory study

Individuals were randomly assigned to control (n = 16) or internally tagged treatment (n = 15). Crayfish in the tagged treatment were internally tagged using 12 mm (HDX ISO 11784/11785 Oregon RFID) PIT tags (0.1 g) > one week after their last observed molt to allow ample time for carapace hardening and initial carapace length was measured to the nearest mm. Following the central insertion methodology, crayfish were held ventral side up, and a 3 mm incision was made on the left lateral side directly below the left fifth pereiopod using a 12-gauge hypodermic needle (Hamilton et al., 2022). The PIT tag was inserted through the incision and

pushed anteriorly using forceps until the tag was no longer visible. Growth and survival were monitored for 86 days after the initial tagging.

On day 86 two pleopods were removed from each of 31 crayfish from the control and tagged treatments and placed into collection tubes containing 2 mm ceramic beads (2 pleopods/tube). Final carapace length was measured to the nearest mm and was used to calculate final growth (initial carapace length – final carapace length (mm)). Crayfish were euthanized following pleopod collection. Vials were stored at -80ºC until qPCR analysis was conducted. DNA was extracted and quantified for these and the initial WSSV samples using the same methodology as described in objective 1.

RESULTS: OBJECTIVE 2

Growth

There was no significant difference (Mann-Whitney Rank Sum Test; U=113; P=0.777) in final growth (carapace length (mm)) between control (n=16; mean = 1.4 ± 0.4 (SE) mm) and tagged (n=15; mean = 1.8 ± 0.6 (SE) mm) crayfish. Both groups exhibited 100% survival throughout the three-month study period. Tag retention was 100% for all tagged crayfish.

White spot virus samples

Of the two samples (5 crayfish/sample vial) analyzed from the source pond, one sample tested negative (Ct = undetermined) and one tested positive (Ct = 25.4, copies/µL = 13,595,757). Both of the samples (5 crayfish/sample vial) analyzed from the flow-through holding tank prior to initiating the experiment tested negative (Ct = undetermined). Of the 16 control crayfish tested individually (1 crayfish/sample vial) at the end of the three-month study period, 14 tested negative (Ct > 38 or "undetermined") while 2 tested marginally positive (Ct = 37.5 and 37.8,

copies/µL = 1,281, 1,082 respectively). Of the 15 tagged crayfish tested individually (1 crayfish/sample vial), all tested negative (Ct > 38 or "undetermined") at the end of the three-month study period.

DISCUSSION

White spot syndrome virus is a pathogen of major concern for shrimp and crayfish aquaculture and has the potential to also affect wild crustacean populations (Sasson et al., 2024). Assessing risk of WSSV to wild and cultured crayfish populations requires an understanding of spatial and temporal patterns of WSSV infections across the landscape of interest. In North America, WSSV was first detected in Louisiana crawfish farms in 2007 (Baumgartner et al., 2009). WSSV has been detected in commercially available RSC (Sasson et al. 2020), which are shipped throughout the world, often live, for research and human consumption (Oficialdegui et al., 2020). WSSV may be more prevalent across wild crayfish populations in North America and other parts of the world than is currently recognized. WSSV was first detected in Alabama at the E.W. Shell Fisheries Research Center in April, 2022 (Bruce et al., 2024). Subsequent sampling (June 2022 – December 2023) of sites across the two watersheds located at EWSFRC revealed that WSSV was widespread in wild crayfish from pond and stream habitats and detected at every site sampled. Our results support the conclusion of Bruce et al. (2024) that WSSV was likely already present in wild populations on the station prior to the detection in laboratory animals in April, 2022. Additional studies are needed to determine whether WSSV is also widespread across wild populations in other parts of Alabama.

Because Alabama hosts the highest diversity of crayfish taxa (100 species currently recognized; Bearden et al., 2023) of any southeastern state, it is important to determine whether

WSSV is confined to RSC or if it is also spreading to other crayfish species. Samples in our survey contained pleopods from multiple animals, and sometimes multiple species, so we were not able to determine which individuals within a given sample were infected. However WSSV was detected in one sample that only contained pleopods from *P. acutissimus* and in another sample that contained only pleopods from *P. acutissimus* and *L. dalyae*. Thus WSSV occurred in populations of at least one (*P. acutissimus*) and possibly two (*L. dalyae*) crayfish species on the research station in addition to RSC. The southeastern United States contains the highest diversity of freshwater crayfish in the world. Crayfish are one of the most threatened taxonomic groups globally (Ricciardi & Rasmussen, 1999; Taylor et al., 2007; Richman et al., 2015) with infectious diseases introduced by invasive species being a large threat to native populations (Holdich et al., 2009). Based on our study, WSSV is not isolated to RSC. Its prevalence in wild crayfish populations should be explored further to determine if it is threatening the health and survival of native species and whether some species are more susceptible to infection than others.

Although WSSV was spatially widespread and detected at least once at every site sampled, it was not consistently detected across time at any site. The majority of samples collected over the survey period were negative for WSSV. Multiple factors may trigger outbreaks of WSSV in crayfish, but temperature has been indicated as an important environmental factor (Lutz, 2022; Jiang et al. 2019). In our survey, the proportion of positive samples increased as average daily water temperatures rose above 10°C, peaked at ~22°C, and subsequently decreased to zero as temperatures rose to 34°C. This pattern is similar to that observed in RSC populations in China where WSSV infectivity began to increase at 19°C, peaked at 25°C and decreased at 30°C (Jiang et al., 2019), and helps explain the seasonality of WSSV outbreaks in Louisiana crayfish ponds that typically exhibit outbreaks in March and April (Lutz, 2022). Additional

studies looking at the correlation between WSSV and temperature in other crustaceans including shrimp have found infection rates caused mortality at 27 to 30°C compared to no mortality occurring at 33°C in *Litopenaeus vannamei* (Rahman et al., 2006), with optimum WSSV proliferation occurring at 25°C in *Fenneropenaeus chinensis* (Gao et al., 2011). Although prevalence of infection may be affected by temperature in crayfish, we found no evidence that severity of infection was correlated with temperature. There was no significant relationship between copies/µL in positive samples, and average daily temperature at the time of collection.

The prevalence of samples with undetectable levels of WSSV may also be related to the use of baited minnow traps as our collection method. Baited traps typically select for larger, more aggressive individuals that may reduce the number of smaller, less aggressive crayfish from entering the traps (Hein et al., 2007; Gherardi et al., 2011). WSSV has been shown to cause lethargic behavior in infected crayfish (Pace et al., 2016; Qian & Zhu, 2019). Therefore, our collection method may have favored individuals with minimal to no infection (i.e., no signs of lethargy).

Prevalence of infection may also be related to population density. The ingestion of WSSV-infected tissue successfully inoculates crayfish (Davidson et al., 2010). Cannibalism in crayfish populations increases as density increases (Houghton et al., 2017). Hence, the prevalence of WSSV may increase in populations of crayfish that occur at higher densities and should be explored in future studies.

In addition to environmental factors, an important question is whether stress associated with research activities can trigger an outbreak in otherwise healthy individuals that may be carrying WSSV at undetectable levels. Crayfish research typically requires individuals to be removed and transported from their natural habitats, placed into artificial holding systems, and

subjected to potentially stressful research procedures. If WSSV is known to be present in a given population, a valid concern is whether or not research activities will trigger an outbreak in the laboratory. In our experiment, we used crayfish that were likely carriers of WSSV. The virus was detected in the population from which crayfish were collected, but WSSV was at undetectable levels in experimental animals just prior to the study. Crayfish were subjected to an internal tagging procedure known to be stressful (McFarlane et al., 2019). We found no evidence that the combined stressors of laboratory housing and experimental procedures triggered WSSV to increase to detectable levels or become pathogenic in laboratory animals. One important caveat is that we conducted this study at 30°C, a temperature that is at the upper end of the optimal range for RSC growth (Huner, 1988) and likely to inhibit WSSV (Jiang et al., 2019). Additional studies are needed to determine effects of laboratory stress at optimal temperatures for the virus (i.e. 22-25°C; this study, Jiang et al., 2019). Future studies should also consider testing effects of additional water quality parameters such as ammonia, pH, and low dissolved oxygen on pathogenicity of the white spot virus in crayfish held in research labs or in aquaculture ponds.

In conclusion, temperature appears to have a strong impact on prevalence of WSSV in wild crayfish populations but prevalence can still remain low, even at optimal temperatures. WSSV is not limited to RSC but likely spreading among other crayfish species in the wild. The suspected presence of WSSV in a source population does not necessarily preclude research activities. At warm temperatures (i.e., 30°C), stress associated with artificial laboratory conditions and tag insertion did not trigger an outbreak of WSSV in the experimental population. Due to the complexity of virus pathogenicity and interactions with environmental variables, we provide several suggestions for future research to increase our understanding of interactions between WSSV, crayfish, and the environment.

References

Alderman, D. J., 1996. Geographical spread of bacterial and fungal diseases of crustaceans. Revue Scientifique et Technique 15: 603–632.

Alderman, D. J., & J. Polglase, 1988. Pathogens, parasites and commensals. In: Holditch, D. M., & R. S. Lowery (eds), Freshwater Crayfish: Biology, Management and Exploitation 167–212.

Baumgartner, W., J. Hawke, K. Bowles, P. Varner, & K. Hasson, 2009. Primary diagnosis and surveillance of white spot syndrome virus in wild and farmed crawfish (*Procambarus clarkii, P. zonangulus*) in Louisiana, USA. Diseases of Aquatic Organisms 85: 15–22.

Bearden, R. A., A. C. Grider, B. D. Holt, & S. W. McGregor, 2023. Alabama's Crayfish Species Count Reaches Century Mark: Confirmation of *Procambarus barbiger* Occurring in Alabama. Southeastern Naturalist Eagle Hill Institute 22: N13–N16.

Bouallegui, Y., 2021. A Comprehensive Review on Crustaceans' Immune System With a Focus on Freshwater Crayfish in Relation to Crayfish Plague Disease. Frontiers in Immunology 12: 667787.

Bruce, T. J., J. W. Abernathy, N. Tripp, N. Barnes, C. E. Harrison, A. A. Oladipupo, J. D. Krol, A. L. Wise, J. V. Warg, & J. A. Stoeckel, 2024. White spot syndrome virus in Alabama red swamp crayfish (*Procambarus clarkii*). Journal of Fish Diseases 47: e13873.

Chou, H., C. Huang, C. Wang, H. Chiang, & C. Lo, 1995. Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. Diseases of Aquatic Organisms 23: 165–173.

Davidson, E. W., J. Snyder, D. Lightner, G. Ruthig, J. Lucas, & J. Gilley, 2010. Exploration of potential microbial control agents for the invasive crayfish, *Orconectes virilis*. Biocontrol Science and Technology 20: 297–310.

Desrina, S. B. Prayitno, M. C. J. Verdegem, J. A. J. Verreth, & J. M. Vlak, 2022. White spot syndrome virus host range and impact on transmission. Reviews in Aquaculture 14: 1843–1860.

Du, H., W. Dai, X. Han, W. Li, Y. Xu, & Z. Xu, 2008. Effect of low water temperature on viral replication of white spot syndrome virus in *Procambarus clarkii*. Aquaculture 277: 149–151.

Durand, S. V., & D. V. Lightner, 2002. Quantitative real time PCR for the measurement of white spot syndrome virus in shrimp. Journal of Fish Diseases 25: 381–389.

Gao H, J. Kong, Z. Li, G. Xiao, X. Meng, 2011. Quantitative analysis of temperature, salinity and pH on WSSV proliferation in Chinese shrimp *Fenneropenaeus chinensis* by real-time PCR. Aquaculture 312: 26−31.

Gherardi, F., L. Aquiloni, J. Diéguez-Uribeondo, & E. Tricarico, 2011. Managing invasive crayfish: is there a hope? Aquatic Sciences 73: 185–200.

Hamilton, G. L., T. L. Cooper, & T. F. Turner, 2022. Field estimates of crayfish mortality associated with passive integrated transponder tagging. Aquatic Conservation: Marine and Freshwater Ecosystems 32: 1809–1816.

Hein, C. L., M. J. Vander Zanden, & J. J. Magnuson, 2007. Intensive trapping and increased fish predation cause massive population decline of an invasive crayfish. Freshwater Biology 52: 1134–1146.

Hobbs, H. H. 1972. Crayfishes (Astacidae) of North and Middle America. Biota of freshwater ecosystems. US Environmental Protection Agency, Water Pollution Control Research Service Identification Manual 9. US Environmental Protection Agency, Washington, DC.

Hobbs, H. H., J. P. Jass, & J. V. Huner, 1989. A Review of Global Crayfish Introductions with Particular Emphasis on Two North American Species (Decapoda, Cambaridae). Crustaceana Brill 56: 299–316.

Holdich, D. M., J. D. Reynolds, C. Souty-Grosset, & P. J. Sibley, 2009. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. Knowledge and Management of Aquatic Ecosystems 394-395.

Houghton, R. J., C. Wood, & X. Lambin, 2017. Size-mediated, density-dependent cannibalism in the signal crayfish *Pacifastacus leniusculus* (Dana, 1852) (Decapoda, Astacidea), an invasive crayfish in Britain. Crustaceana 90:417-435.

Huang, T., L. Cerenius, & K. Söderhäll, 1994. Analysis of genetic diversity in the crayfish plague fungus, Aphanomyces astaci, by random amplification of polymorphic DNA. Aquaculture 126: 1–9.

Huner, J.V., 1988. *Procambarus* in North America and elsewhere. In: Holdich DM, Lowery RS (eds) Freshwater crayfish: 346 Rev Fish Biol Fisheries (2016) 26:329–349 123 biology, management and exploitation. Croom Helm, London.

Huner, J. V., & J. E. Barr, 1991. Red Swamp Crawfish: Biology And Exploitation. https://repository.library.noaa.gov/view/noaa/46389.

Jiang, N., X. Pan, Z. Gu, W. Liu, K. Si, Y. Zhou, Y. Zhou, L. Zhai, Y. Fan, & L. Zeng, 2019. Proliferation dynamics of WSSV in crayfish, *Procambarus clarkii*, and the host responses at different temperatures. Journal of Fish Diseases 42: 497–510.

Korkut, G. G., C. Noonin, & K. Söderhäll, 2018. The effect of temperature on white spot disease progression in a crustacean, *Pacifastacus leniusculus*. Developmental & Comparative Immunology 89: 7–13.

Laramore, S. E., J. Scarpa, C. R. Laramore, & J. Lin, 2009. Virulence Variation of White Spot Syndrome Virus in Pacific White Shrimp *Litopenaeus vannamei*. Journal of Aquatic Animal Health 21: 82–90.

Lee, C., J. H. Kim, S.-K. Choi, H. J. Jeon, S. H. Lee, B. K. Kim, Y. K. Kim, K.-J. Lee, & J. E. Han, 2021. Detection of infectious white spot syndrome virus in red claw crayfish (*Cherax quadricarinatus*) and red swamp crayfish (*Procambarus clarkii*) imported into Korea. Aquaculture 544: 737117.

Lightner, D. V., R. M. Redman, C. R. Pantoja, K. F. J. Tang, B. L. Noble, P. Schofield, L. L. Mohney, L. M. Nunan, & S. A. Navarro, 2012. Historic emergence, impact and current status of shrimp pathogens in the Americas. Journal of Invertebrate Pathology 110: 174–183.

Lutz, G. 2022. White Spot Syndrome Virus in Louisiana Crawfish. Louisiana Agriculture 65:19.

Marks, H., J. J. A. van Duijse, D. Zuidema, M. C. W. van Hulten, & J. M. Vlak, 2005. Fitness and virulence of an ancestral White Spot Syndrome Virus isolate from shrimp. Virus Research 110: 9–20.

McFarlane, A., J. O'Brien, B. Nelson, & M. Gammell, 2019. The behavioural response of the white-clawed crayfish (*Austropotamobius pallipes*) to five standard marking techniques over a 14-day period. Marine and Freshwater Behaviour and Physiology 52: 181–197.

Muhammad, M., J. M. Lotz, R. B. Blaylock, & S. S. Curran, 2020. White spot syndrome virus in decapods from Mississippi Sound, USA, and susceptibility of *Palaemonetes pugio* and *Uca panacea* to a Chinese isolate. Diseases of Aquatic Organisms 138: 121–131.

Oficialdegui, F. J., M. I. Sánchez, & M. Clavero, 2020. One century away from home: how the red swamp crayfish took over the world. Reviews in Fish Biology and Fisheries 30: 121–135.

Oidtmann, B, 2012. Crayfish plague (*Aphanomyces astaci*) Manual of Diagnostic for Aquatic Animals 2012. World Organization for Animal Health, Paris, pp 101-118.

Pace, B., J. Hawke, R. Subramanian, & C. Green, 2016. Experimental inoculation of Louisiana red swamp crayfish *Procambarus clarkii* with white spot syndrome virus (WSSV). Diseases of Aquatic Organisms 120: 143–150.

Piamsomboon, P., C. Inchaisri, J. Wongtavatchai, 2016. Climate factors influence the occurrence of white spot disease in cultured penaeid shrimp in Chanthaburi province, Thailand. Aquatic Environmental Interactions 8: 331-337.

Qian, X., & F. Zhu, 2019. Hesperetin protects crayfish *Procambarus clarkii* against white spot syndrome virus infection. Fish & Shellfish Immunology 93: 116–123.

Rahman M, C. Escobedo-Bonilla, M. Corteel, J.J. Dantas-Lima, M. Wille, V. Alday Sanz, M.B. Pensaert, P. Sorgeloos, & H.J. Nauwynck, 2006. Effect of high water temperature (33°C) on the clinical and virological outcome of experimental infections with white spot syndrome virus (WSSV) in specific pathogen-free (SPF) *Litopenaeus vannamei*. Aquaculture 261: 842−849.

Ricciardi, A., & J. B. Rasmussen, 1999. Extinction Rates of North American Freshwater Fauna. Conservation Biology 13: 1220–1222.

Richman, N. I., M. Böhm, S. B. Adams, F. Alvarez, E. A. Bergey, J. J. S. Bunn, Q. Burnham, J. Cordeiro, J. Coughran, K. A. Crandall, K. L. Dawkins, R. J. DiStefano, N. E. Doran, L. Edsman, A. G. Eversole, L. Füreder, J. M. Furse, F. Gherardi, P. Hamr, D. M. Holdich, P. Horwitz, K. Johnston, C. M. Jones, J. P. G. Jones, R. L. Jones, T. G. Jones, T. Kawai, S. Lawler, M. López-Mejía, R. M. Miller, C. Pedraza-Lara, J. D. Reynolds, A. M. M. Richardson, M. B. Schultz, G. A. Schuster, P. J. Sibley, C. Souty-Grosset, C. A. Taylor, R. F. Thoma, J. Walls, T. S. Walsh, & B. Collen, 2015. Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). Philosophical Transactions of the Royal Society B: Biological Sciences Royal Society 370: 1-11.

Sasson, D. A., J. M. Allen, M. J. Walker, J. H. Huber, G. K. Rothman, P. R. Kingsley-Smith, T. L. Darden, & M. R. Kendrick, 2024. Prevalence of white spot syndrome virus (WSSV) in wild-caught and commodity decapod crustaceans in coastal South Carolina, USA. Journal of Crustacean Biology 44: 1-6.

Shields, J. D., 2019. Climate change enhances disease processes in crustaceans: case studies in lobsters, crabs, and shrimps. Journal of Crustacean Biology 39: 673-683.

Sun, Y., F. Li, Z. Sun, X. Zhang, S. Li, C. Zhang, & J. Xiang, 2014. Transcriptome Analysis of the Initial Stage of Acute WSSV Infection Caused by Temperature Change. PLOS ONE Public Library of Science 9: e90732.

Taylor, C. A., G. A. Schuster, J. E. Cooper, R. J. DiStefano, A. G. Eversole, P. Hamr, H. H. Hobbs III, H. W. Robison, C. E. Skelton, & R. F. Thoma, 2007. A Reassessment of the Conservation Status of Crayfishes of the United States and Canada after 10+ Years of Increased Awareness. Fisheries 32: 372–389.

Tendencia, E.A., J. Verreth, 2011. Temperature fluctuation, low salinity, water microflora: risk factors for WSSV outbreaks in *Penaeus monodon*. Israeli Journal of Aquaculture-Bamidgeh 63:1−7.

Wang, Z., B. Sun, & F. Zhu, 2017. Epigallocatechin-3-gallate inhibit replication of white spot syndrome virus in *Scylla paramamosain*. Fish & Shellfish Immunology 67: 612–619.

Westhoff, J. T., & A. E. Rosenberger, 2016. A global review of freshwater crayfish temperature tolerance, preference, and optimal growth. Reviews in Fish Biology and Fisheries 26: 329–349.

Table 1.1: Ct values and viral concentration information associated with samples that tested positive for WSSV. Each sample consisted of a sampling tube containing pleopods from up to 5 individual crayfish. A given sample tube could contain pleopods from more than one species.

Watershed	Site	Date	Ct value	Copies/µL	Species
I	Stream: Upper Station	6/23/2022	37.8	25,346	*Procambarus clarkii, Procambarus acutissimus*
		9/14/2022	34.3	230,631	*Procambarus clarkii*
		9/27/22	33.1	401,344	*Procambarus clarkii, Procambarus acutissimus*
		9/27/22	32.9	440,342	*Procambarus acutissimus*
		3/29/2023	30.8	242,340	*Procambarus acutissimus, Lacunicambarus dalyae*
		3/29/2023	32.1	10,174	*Procambarus clarkii*
II	Pond: E46	6/9/2022	16.8	47,651,802	*Procambarus clarkii*
		6/9/2022	31.4	63,495	*Procambarus clarkii*
		8/3/2022	25.4	13,595,757	*Procambarus clarkii*
	Pond: recirc.	6/9/2022	21.1	33,236,988	*Procambarus clarkii*
		6/9/2022	34.6	73,421	*Procambarus clarkii*
		9/14/2022	30.5	1,409,519	*Procambarus clarkii*
		3/21/2023	29.1	721,672	*Procambarus clarkii*
	Stream: Lower Station	3/29/2023	28.8	880,593	*Procambarus clarkii*
		3/29/2023	32.7	65,892	*Procambarus clarkii*
	Pond: F14	6/9/2022	34.8	65,946	*Procambarus clarkii*
		6/9/2022	33.8	102,160	*Procambarus clarkii*
		6/23/2022	38.2	21,708	*Procambarus clarkii*
		3/29/2023	30.3	329,484	*Procambarus clarkii*
		3/29/2023	34.0	28,156	*Procambarus clarkii*
		9/6/2023	34.1	79,974	*Procambarus clarkii*
		9/19/2023	36.7	15,982	*Procambarus clarkii*
	Pond: Retention	6/9/2022	35.9	38,937	*Procambarus clarkii*
		6/9/2022	28.5	1,164,315	*Procambarus clarkii*
		4/26/2023	33.8	32,699	*Procambarus clarkii*
	Pond: H-series	6/9/2022	34.5	74,363	*Procambarus clarkii*
		6/9/2022	36.4	31,326	*Procambarus clarkii*

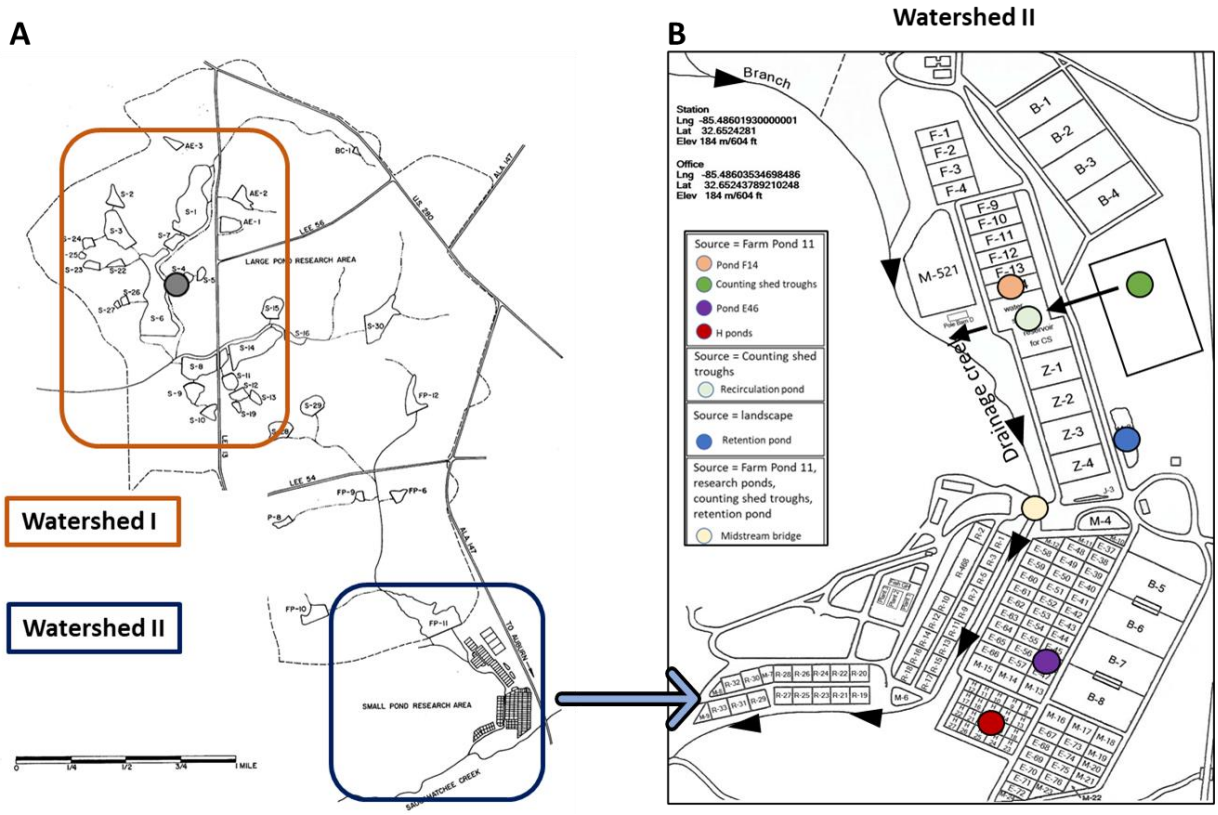

Figure 1.1: A) Map of the E.W. Shell Fisheries Center in Auburn, AL showing the locations of watershed I and II, and the single site sampled in watershed I. B) Location of six sites sampled within watershed II. Arrows show direction of flow in pond drainage canal.

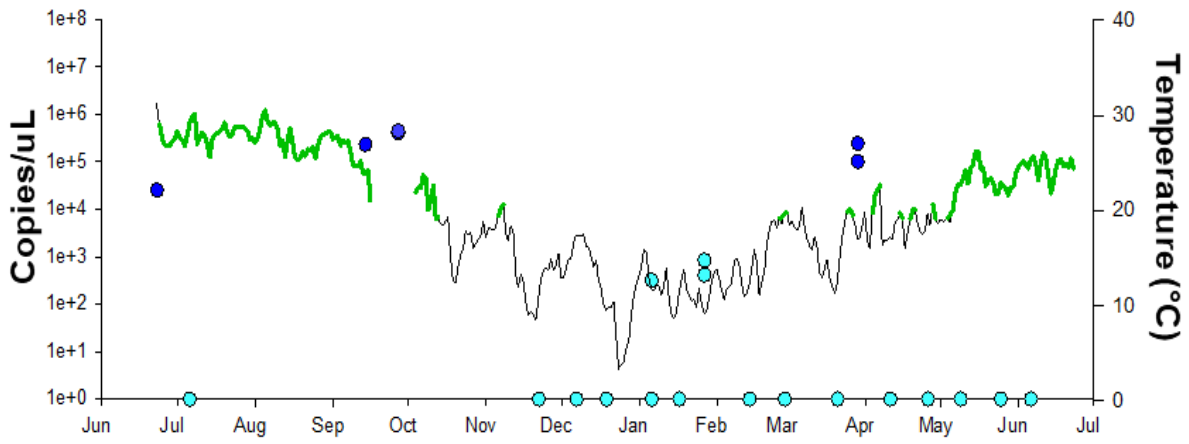

Figure 1.2: Copies/µL detected in WSSV samples and mean daily water temperature recorded at the Watershed I sampling site from June 2022 – June 2023. Green line represents the hypothesized optimal temperature range for WSSV based on Jiang et al. (2019). Dark blue samples represent samples where WSSV was detected and light blue represent samples where it was not detected (Ct value >38, or "undetermined").

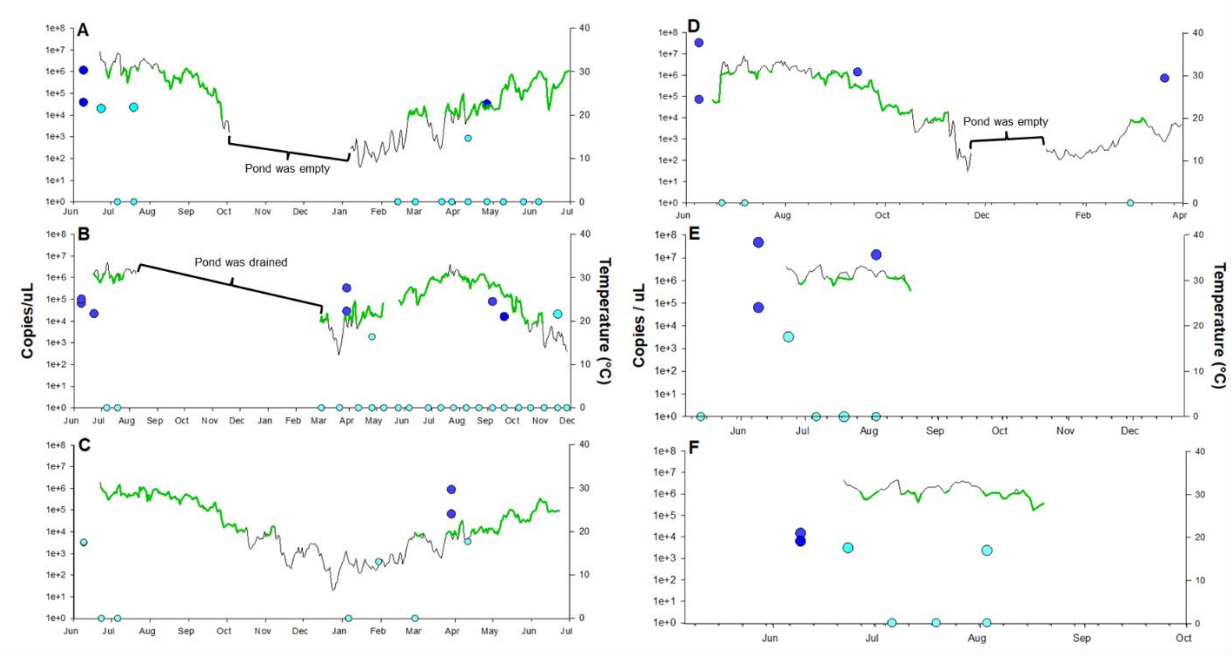

Figure 1.3: Copies/µL detected in WSSV samples and mean daily water temperature recorded at the A) retention pond, B) F14 pond, C) lower station stream, D) recirculation pond, E) E46 pond, and F) H pond sampling sites in Watershed II from June 2022 – June 2023. Green line represents the hypothesized optimal temperature range for WSSV based on Jiang et al. (2019). Dark blue samples represent samples where WSSV was detected and light blue represent samples where it was not detected (Ct value >38, or "undetermined").

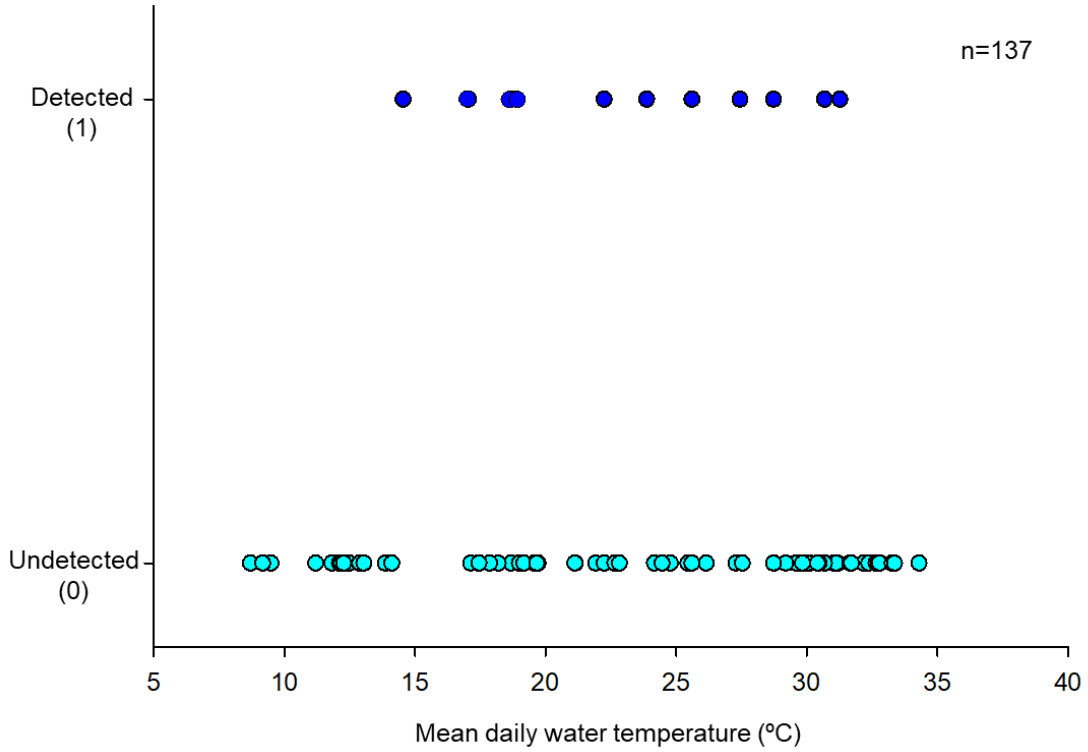

Figure 1.4: Binomial plot of WSSV detection versus mean daily water temperature at the sampling site on the day of collection.

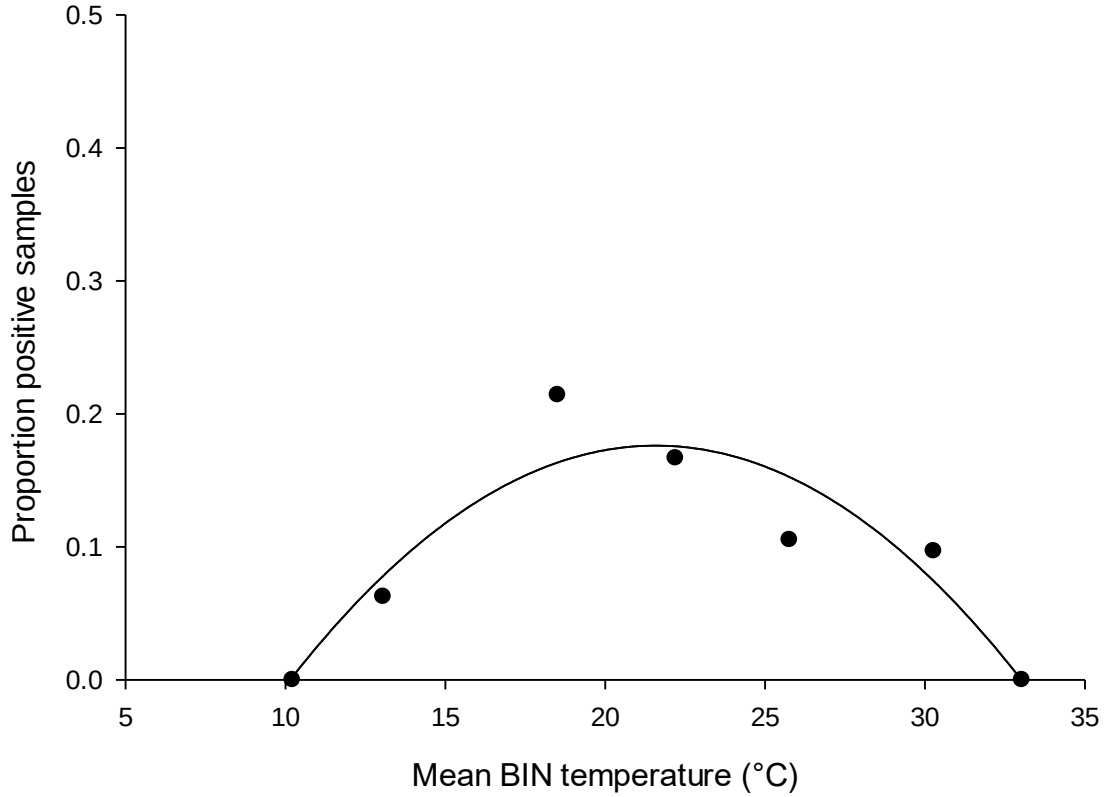

Figure 1.5: Relationship between proportion of positive samples and water temperature for all samples collected in watersheds I and II. Each dot represents the proportion of positive samples collected within a 4°C temperature bin (i.e. 8-12°C…34-36°C) plotted against the average temperature within that bin.

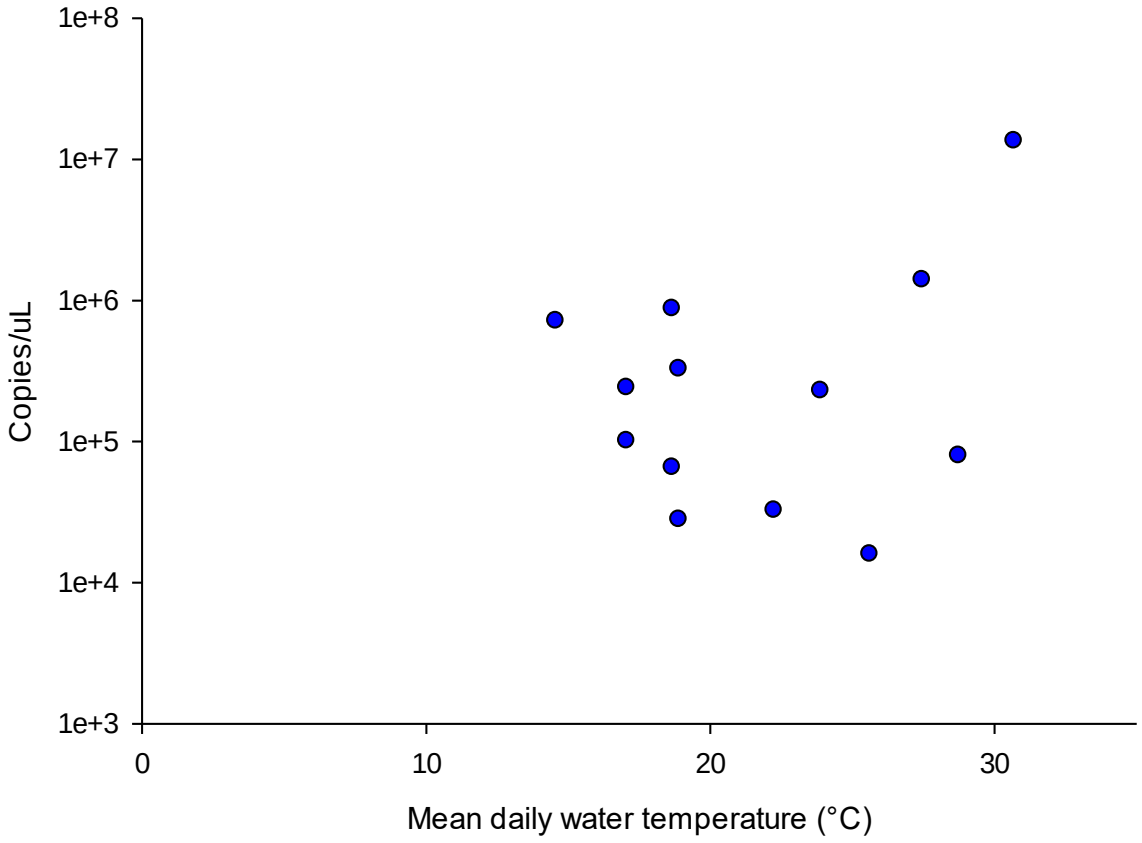

Figure 1.6: Relationship between copies/µL and mean daily water temperature at the sampling site on the day of collection for all samples containing detectable levels of WSSV.

Chapter 2: Effects of current velocity on rheotaxis and exploratory behavior in *P. clarkii*

INTRODUCTION

According to the World Wide Fund for Nature Living Planet Index, the greatest population declines across the globe are occurring in freshwater ecosystems with freshwater species experiencing an 83% population decrease from 1970 to 2014 (WWF, 2018). Amongst the freshwater taxa, freshwater crayfish and mussels have the highest proportion of species that are either extinct or under some level of threat (vulnerable to critically imperiled) (Strayer and Dudgeon, 2010). Freshwater invertebrates face threats including, but not limited to, habitat degradation, overexploitation, diseases, and invasive species (Reid et al., 2019).

A major threat to freshwater ecosystems can occur from invasive species introductions, especially in relatively isolated environments where new species introductions can have disproportionately large effects. Introductions contribute to declines in native species biodiversity and can cause food web alteration at local scales (Vander Zanden et al., 1999; Havel et al., 2015; Gallardo et al., 2016). Species introductions are a persistent problem in terrestrial and aquatic systems throughout the world. They can often be attributed to anthropogenic movement of organisms that result in purposeful release or accidental escapement of organisms into novel habitats. Introductions can be facilitated by habitat alterations, especially those that increase the connectedness of previously isolated systems (Moyle & Light, 1996; Crook et al., 2015).

Establishment and spread of invasive crayfish are particularly problematic in freshwater systems (Strayer, 2010). Some, like the red swamp crayfish (*Procambarus clarkii*), can have direct ecosystem impacts such as declines in macrophyte abundance and increased predation on

native animal species (Souty-Grosset et al., 2016). Red swamp crayfish (RSC) are only native to the Southcentral United States and Northeastern Mexico but now have established invasive populations on every continent except Antarctica and Australia – an issue that warrants global attention (Huner, 1977; Huner & Avault, 1979; Souty-Grosset et al., 2016). Red Swamp Crayfish not only impact native biodiversity and resource availability but can directly modify invaded environments through construction of terrestrial burrows. Burrows can damage shoreline stability, cause banks to collapse, and increase water turbidity (Correia & Ferreira, 1995; Angeler et al., 2001).

Development of effective control methods for RSC has become a major focus of invasive crayfish research. Previous removal strategies to control invasive RSC populations have included biological, physical, biocidal, and chemical techniques. Biological techniques include the sterilization of male crayfish (Manfrin et al., 2021), the introduction of predators (i.e., freshwater European eels (Aquiloni et al., 2010)), and the release of "neo-female" RSC that produce all-male progeny (Savaya et al., 2020). Biocidal and chemical techniques include pyrethrum and carbon dioxide (Cecchinelli et al., 2012; Fredricks et al., 2020; Abdelrahman et al., 2021). Because no single technique is effective on its own, biological and biocidal/chemical techniques are often combined with physical removal via baited funnel and minnow traps. However, trapping can be time and resource-expensive as well as size and sex-biased by targeting large, male individuals (Freeman et al., 2010; Gherardi et al., 2011).

Recent invasive crayfish research has focused on developing methodology to repel crayfish and thus "push" them, or attract them and thus "pull" them, into specific areas for easier collection and removal. For example, carbon dioxide can "push" crayfish towards the water surface or pond edges for easier collection (Abdelrahman et al. 2021; Elliott, 2022). Water flow

may also serve as a useful, non-chemical, repellent or attractant. Some crayfish, such as the Australian Redclaw (*Cherax quadricarinatus)* are positively attracted to flow. “Box-n-ramp” traps utilize flowing water down a ramp to induce redclaw crayfish to self-harvest from a pond. The crayfish will crawl from an occupied pond, up a flowing ramp and into a collecting bin (Masser & Rouse, 1997). Red Swamp Crayfish are a lentic species whose response to flow is not fully understood. High flow velocities (>0.3 m/s) prevented their upstream movement in a previous flume study (Kerby et al., 2005). Conversely, in another laboratory study, they were attracted to the inflow end of raceways suggesting an attraction to flow (Elliott, 2022). However velocity was not measured in that study so the velocity range to which they were attracted is unknown. In a subsequent pond study, airlifts were placed in ponds to generate subsurface and bottom flows of <0.06 m/s. Catch rates of baited traps placed within a flow zone did not differ from baited traps in control ponds with no flow, suggesting no effect of flow (Elliott, 2022).

To improve our understanding of the relationship between RSC rheotaxis and water velocity, we conducted flume studies in the laboratory utilizing a range of velocities that remained constant, or increased in upstream zones. We hypothesized that 1) RSC are attracted to low flows but repelled by higher flows, and 2) Exploratory behavior is highest at intermediate flow rates that represent neutral attraction and repulsion behaviors. In particular, we predicted that:

1. As velocity increases from zero m/s, RSC will initially exhibit positive rheotaxis and spend less time downstream as they are attracted to the lower, upstream current velocities. As velocity continues to increase, RSC will switch to negative rheotaxis and spend more time downstream as they are being repulsed by the higher current velocities.

2. Random exploratory behavior (the number of transitions between adjacent zones) will decrease with increasing velocity if crayfish are either strongly attracted or repelled by increasing velocity. Number of transitions will remain relatively constant if crayfish movement is not strongly affected by velocity (neutral rheotaxis).
3. Effects of current velocity will be size-dependent due to increased drag of high velocities on larger-sized crayfish.

METHODS

Animal collection and husbandry

Procambarus clarkii were collected from March to July in 2023 from outdoor ponds at the E.W. Shell Fisheries Center (EWSFC) in Auburn, AL (N32.65247, W-85.48560) using baited (Friskies wet cat food) minnow traps. Only male crayfish fitting into one of three size-classes (small, 27-31 mm CL; medium, 35-39 mm CL; and large, 41-44 mm CL) were retained and used for experiments. Crayfish were immediately transported to the lab and placed in plastic containers (1 crayfish per container) that had a volume of 0.009 m^3 (0.1651 x 0.1143 x 0.0508 m). Multiple ~6 mm diameter holes were drilled into the sides to facilitate water exchange, and each container was labeled with a unique identification number associated with its crayfish. A thin layer of gravel (River Rock ~6 mm size; American Countryside, Alabama) was added to each container to provide surface area for the establishment of biofilter bacteria. Holding containers were distributed among 3 (3.0 x 0.7 m) rectangular troughs (maximum of 39 housing containers per trough) located within indoor laboratory facilities at the EWSFC. To further promote the establishment of biofilter bacteria, shredded PVC ribbon (Pentair Bio-Fill BF250) was placed in each trough.

Troughs were filled with water from an ~8 ha reservoir (Farm Pond 11) located a short distance away and were operated as recirculation systems. During the holding period, water quality was monitored twice weekly (Ammonia, pH, Alkalinity, Hardness, Nitrite). Partial water changes were initiated if water quality parameters fell outside the ranges presented in Table 2.1. The water temperature of each trough was controlled with an 800-watt heater (Finnex Titanium Model TH-800) and a temperature controller (Inkbird Model ITC-306T). Following initial collection, crayfish were acclimated for one week to water temperatures that matched their collection site. Water temperature was then increased by 2°C/day until trough temperatures reached 30°C. This temperature was chosen because white spot syndrome virus (WSSV) had recently been detected at EWSFC (Bruce et al. 2024) and high (≥30°C) temperatures may inhibit WSSV viral loads in crayfish (Jiang et al. 2019). Crayfish were acclimated to 30°C for a minimum of 1 week and then internally PIT tagged (as described in chapter 1) ≥1 week prior to initiation of movement experiments (McFarlane et al., 2019).

Flow study design: flume system

Experiments were conducted in flumes that were 2.36 meters long and 0.69 meters wide. Each flume was constructed using vinyl gutter material and was held within a larger trough (3 x 0.67 meter) (Fig. 2.2). The trough was filled with water from the same source as the acclimation tanks such that the water level in the trough was just below the top of the experimental flume. A submersible pump (120v, 3,096 L/h) was placed at the inflow end of the flume. Both ends of the flume were covered with 12.7 mm mesh to allow water to flow through the system while preventing the escape of the crayfish. Weighted coverings covered the top of the flume to prevent escapement and to keep the flume interior uniformly dark and minimize the effects of light on movement. A layer of gravel (River Rock ~6 mm size; American Countryside, Alabama) was

added such that a thin layer covered the flume bottom to provide a natural gripping surface for crayfish as they moved through the flume. This layer was replaced with fresh gravel between trials.

PIT tag antenna system

PIT tag antennas were constructed for use with an ORMR multiple antenna reader (Oregon RFID, Portland, Oregon). Each antennae was constructed using 20-gauge AWG wire inside of a rectangular PVC frame (0.66 m (L) x 0.24 m (H)). The number of times the wire was looped through the inside of the pvc pipe frame was adjusted such that the read range of each antenna was 0.18-0.2 meters. Antennas were placed at 1 and 1.37 meters along the length of the flume systems such that they detected crayfish moving between Zones 1 and 2 and between Zones 2 and 3 (Fig. 2.2).

Flume trials

Two trials were run per day – one trial per each of two flumes. The medium size-class was tested in the first 20 trials, the small size-class in the next 18 trials, and the large size-class in the final 16 trials. A minimum of 16 crayfish were tested within each size-class. Experimental set up began the day before trials were conducted. On day 0, both experimental troughs (1 flume per trough) were filled as described previously (see flume system), and water heated to 30°C using an 800-watt heater (Finnex Titanium Model TH-800) connected to a temperature controller (Inkbird Model ITC-306T). Two experimental crayfish (one per flume) were randomly selected from the appropriate size-class and checked for any signs of molting such as a soft carapace or separation between the cephalothorax and abdomen (Penn, 1943). Crayfish showing signs of molting were not used for the trial and replaced with another randomly chosen crayfish showing no signs of molting. Crayfish that showed no signs of molting were placed back in their holding

containers and used the following day for an experimental trial. Each crayfish was only used once. Crayfish that showed signs of molting when initially selected were not used later for subsequent trials.

On the morning of the trials, the flume pump was turned on to generate and calibrate current through the flume prior to adding a crayfish. Target velocity for a given trial was randomly selected from a pre-determined set of target velocities ranging from 0 to 0.55 m/s. The pump was then adjusted such that the velocity of Zone 2 (mean of FM_2, FM_3; Fig. 2.2) matched the target velocity for that trial. The zero velocity treatment was used for two individuals per size-class.

After adjustment to match the target velocity in Zone 2, flume velocities were also measured and recorded at FM_1 and FM_4 (Fig. 2.2). The flume pump was then turned off at 09:00 and a single crayfish placed into the acclimation zone ("A"; Fig. 2.2) that was separated from the rest of the flume with a removable, plastic divider. The top of the acclimation zone was then covered to prevent the crayfish from escaping or being disturbed by any surrounding movements. During the 30 m acclimation period, dissolved oxygen and temperature were measured at the center of the flume and in the ambient water within the outer trough. The entire flume was then covered to prevent escapement and to minimize light within the flume.

At 09:30, the plastic divider was removed, and the pump turned back on. The Oregon RFID reader antennae started reading at this time. The crayfish was allowed to wander freely through the flume for 2 h (09:30-11:30). During this time, each antennae recorded the presence/absence of the crayfish within its detection range every .01 s. The crayfish was then removed from the flume system and carapace length measured. The final dissolved oxygen and

temperature were then remeasured in the flume and ambient trough water with the pump turned off to replicate the same conditions that were measured during the crayfish acclimation period.

Data analysis

A paired T test was used to test for differences in current velocity between zones 1 and 2, and between zones 2 and 3, for trials within each size class. Datasets that did not pass the normality assumptions were rank transformed and analyzed using a Wilcoxon signed rank test (Table 2.2).

To test for positive or negative rheotaxis where velocity did not differ between the initial (zone 1) and upstream (zone 2) zones, the number of times the crayfish was recorded in Zone 1 was divided by the total number of location recordings for each trial. The proportion time in Zone 1 was then plotted against current velocity of zones 1-2 for all trials within a given size-class. The resulting plot was then fit with two regression types: linear and quadratic using SigmaPlot 15 (Systat Software©, Inc. 2014). Outliers were identified (SigmaPlot 15) and removed if detected. The best fit regression was chosen as the one that passed the Shapiro-Wilk normality test, the equal variance assumption, and had the lowest (i.e. >5% difference from the next best performing regression) Akaki information criterion (AICc) score (Table 2.3). If all of the AICc scores were within 5% of each other, we chose the regression with the highest (i.e. >5% difference) R^2 value. If none of the three regressions passed normality and/or equal variance assumptions, we transformed the data using a natural log transformation to meet the assumptions and reran each model. Declines, increases, or no change in time spent in zone 1 with increasing current velocity were considered indicators of positive, negative, or neutral rheotaxis, respectively. If low current velocities stimulated positive rheotaxis but high current velocities stimulated negative rheotaxis, we expected a pattern similar to Fig. 2.1A.

To test for effects of current velocity on random exploratory behavior when velocity was held constant between the initial (zone 1) and upstream (zone 2) zones, we plotted the number of transitions from zone 1 to zone 2 against current velocity in zones 1-2 for all trials within a given size class and used the previously described procedures to pick the best fit among a linear, 3 segment piecewise, and quadratic. We considered ranges where the number of transitions declined with increasing current velocity to be evidence of decreased exploratory behavior driven by increasing strength of either positive or negative rheotaxis. Velocity ranges where number of transitions increased with increasing current velocity were considered evidence of declining strength of either positive or negative rheotaxis. A lack of change in number of transitions was considered evidence of neutral rheotaxis. If low current velocities stimulated positive rheotaxis but high current velocities stimulated negative rheotaxis, we expected a pattern similar to Fig. 2.1B.

To test for effects of current velocity on random exploratory behavior when velocity was higher in upstream (zone 3) than downstream (zone 2) zones, we plotted the number of transitions from zone 2 to zone 3 against current velocity in zone 3 for all trials within a given size class and used the previously described procedures to pick the best fit among a linear, 3 segment piecewise, and quadratic. Interpretation of results was also the same as described previously. To determine whether a decline in random exploratory behavior was driven by positive or negative rheotaxis, we plotted the proportion time spent in zone 3 against average current velocity in zone 3 and chose the best fit between a linear and quadratic regression using the previously described procedures and interpretation.

RESULTS

Water quality and flow

Current velocity in zones 1 and 2 were nearly identical across all velocities tested for small, medium, and large size-classes (Fig. 2.3 A, B, C). After rank transformation to pass normality assumptions, zone 2 velocities did not significantly differ from those of zone 1 (Table 2.2). Conversely, zone 3 velocities were consistently higher than zone 2 velocities with the magnitude of the difference increasing at higher velocities (Fig. 2.3 D, E, F). Current velocities in zone 3 were significantly higher than zone 2 for the small, medium and large size class trials (Table 2.2).

Dissolved oxygen concentrations in flumes (mean of initial and final measurements) were similar among trials for the small, medium, and large size classes (mean ± 1 SE; 6.93 ± 0.11 mg/L, 7.61 ± 0.06 mg/L, and 6.67 ± 0.16 mg/L, respectively). Water temperature was similar between troughs and flumes and varied by <1°C among size-class trials. The average temperatures (mean ± 1 SE) in the flume water for the small, medium, and large size classes were 29.7 ± 0.09 °C, 29.4 ± 0.0.14 °C, and 29.7 ± 0.12 °C, respectively.

Size class data

The average carapace lengths (mean ± 1 SE) of crayfish within the small, medium, and large size classes were 29.6 ± 0.30 mm, 36.6 ± 0.32 mm, and 41.4 ± 0.36 mm, respectively.

Constant current velocity between downstream and upstream zones

The proportion of time spent in Zone 1 increased with increasing mean velocity in zones 1-2 for all three size classes. The relationship was best fit by a quadratic regression for small and large crayfish and equally well-fit by a linear or quadratic regression for medium crayfish. We

used a quadratic regression for consistency among all three size-classes (Table 2.3, Fig. 2.4 D, E, F).

None of the relationships between the number of transitions from zone 1 to 2 and mean current velocity of zones 1-2 was best fit by the hypothesized 3 segment piecewise regression (Fig. 2.1B). The best fit for the small size-class was a polynomial quadratic regression after data had been log transformed to meet normality and variance assumptions. The best fit for the medium size-class was a quadratic for non-transformed data. The best fit for the large size-class was a quadratic regression on log-transformed data (Fig. 2.4 A, B, C; Table 2.3). Current velocity explained a greater proportion of variance in the transitions from Zone 1 to Zone 2 for the small size-class (R^2=0.85) than the large (R^2=0.65) or medium (R^2=0.43) size classes.

Increasing current velocity in upstream zone

None of the relationships between the number of transitions from zone 2 to 3 and current velocity in zone 3 was best fit by the hypothesized 3 segment piecewise regression (Fig. 2.1B). For small and large crayfish, the relationship was best fit by a linear regression through non-transformed data, and by a quadratic regression for medium crayfish (Fig. 2.5A, B, C; Table 2.5). Current velocity explained a greater proportion of variance in the transitions from Zone 2 to Zone 3 for the small size-class (R^2=0.81) and large (R^2=0.75) than the medium (R^2=0.54) size classes.

The proportion of time spent in Zone 3 decreased with increasing mean velocity in zone 3 for all three size classes. The relationship was best fit by a linear regression for large crayfish, a quadratic regression for medium crayfish, and equally well-fit by a linear or quadratic regression for small crayfish (Fig. 2.5 D, E, F, Table 2.6). Current velocity explained a greater proportion of

the variance in time spent in zone 3 for the small (R^2 = 0.85) and medium (R^2 = 0.81) than the large (R^2 = 0.39) size-classes.

DISCUSSION

Red Swamp Crayfish are one of the most invasive species worldwide. Although considered primarily a lentic species, they can also invade lotic systems (Cruz & Rebelo, 2007; Moreira et al., 2015). Knowledge of the effects of current velocity on RSC movement patterns may greatly aid in developing control techniques, dispersal barriers, and predicting patterns of spread (Kerby et al., 2005).

Some crayfish species such as the Australian Redclaw Crayfish (*Cherax quadricarinatus*) are attracted to flow. This attraction has led to the development of self-harvesting flow traps whereby flow is directed down a ramp. The crayfish crawl up the ramp and then fall into a collection bin for easy harvest (Masser & Rouse, 1997). Similarly, RSC showed some evidence for positive rheotaxis in a previous study, moving towards the inflow end of laboratory raceways (Elliot, 2022). However, current velocities were not measured in that study. Our results provide some evidence that RSC may exhibit positive rheotaxis at low current velocities for small crayfish. When velocity was held constant between zones (i.e. zone 1 and zone 2), small crayfish spent ~45% of time downstream (zone 1) when current velocity was zero, and <45% of time when current velocities were <0.15 m/s. Future studies should test more individuals from the smaller size class at current velocities <0.15 m/s to observe if positive rheotaxis clearly occurs. However, medium crayfish and large crayfish never showed convincing evidence of positive rheotaxis, and rather showed neutral rheotaxis, as there were no velocities where they spent less time downstream (zone 1) than when velocity was zero. Therefore, evidence of positive rheotaxis

as a method for attracting invasive populations of RSC, specifically smaller sized individuals, may not be useful when developing future control efforts based on our weak findings.

The lack of strong positive rheotaxis in all size classes in our study is somewhat supported by previous flow and energetic studies in other species of crayfish, but studies focused on RSC are limited. Crayfish with larger body sizes exhibit increased drag in higher flow systems (Clark et al., 2008). Smaller crayfish may be more successful in invading upstream systems since they have a better chance of migrating and using objects as shields from the increasing current velocities, which has been indicated in *Orcontectus obscurus* (Clark et al., 2008). However, the evidence found in our study only provides weak support for positive rheotaxis by smaller RSC. Red Swamp crayfish >6 cm total body length operate close to their maximum aerobic capacity when current velocities exceed 0.25 m/s, potentially leaving less energetic capacity for growth, reproduction and upstream dispersal (Rubio-Gracia et al. 2022). Our study supports the conclusions of Rubio-Gracia et al. (2022) in that proportion of time spent downstream (zone 1) by larger crayfish increased strongly as current velocities exceeded 0.25 m/s.

Studies exploring the impact of current velocity on RSC behavior are limited but generally point to negative rheotaxis. For instance, high flow velocities (>0.3 m/s) prevented invasive RSC collected from California streams from moving in upstream directions when tested in flume systems (Kerby et al., 2005). In a field setting, Gavioli et al. (2018) found that RSC did not inhabit waterways that were fast-flowing (maximum values of 1.3 m/s). Our results further support these findings by providing similar evidence across multiple size classes of RSC. In our study, crayfish rarely, if ever, transitioned to, or spent time in, the far upstream end of the flume when velocities reached or exceeded ~0.8 m/s.

Although exploratory behavior decreased with increasing current velocity and was primarily driven by negative, rather than positive rheotaxis, there were some differences in negative rheotaxis associated with size. When velocity was constant between upstream and downstream zones (zones 1, 2), all three size classes spent increasing time downstream as mean velocity increased. The large crayfish showed the weakest evidence for negative rheotaxis as time spent downstream (zone 1) did not exceed 33% until current velocities exceeded ~0.35 m/s whereas the small and medium crayfish spent >33% downstream (zone 1) as current velocities exceeded ~0.1 m/s. When velocity was higher in the upstream zone (i.e. zone 3), all three size-classes spent less than 33% of time in the upstream zone as velocities increased above ~0.2 m/s. The large crayfish again showed the weakest evidence for negative rheotaxis as less than 40% of the variability in time spent upstream was explained by velocity whereas velocity explained >80% of time spent upstream by the small and medium size classes.

One of the most common control techniques for invasive crayfish involves baited traps. Mechanical removal of invasive crayfish like RSC can be time and resource-heavy (Gherardi et al., 2011) but are typically more effective and require fewer resources when conducted in systems when barriers may be present and influencing migration capabilities (Reisinger et al., 2024). Another drawback of traps is that they typically favor larger crayfish (Bills & Marking, 1988; Green et al., 2018; Budnick et al., 2022). Because negative rheotaxis was stronger in small and medium, compared to large crayfish, manipulation of flow may be useful in "pushing" smaller sizes of RSC to funnel or baited minnow traps via avoidance behavior of high flow. Future studies looking at the ability of flow manipulation to increase catch rates of small and medium size-classes may be particularly useful.

High flow in natural systems can serve as passive dispersal barriers and may be useful in association with artificial barriers to aid in the downstream concentration and collection of invasive RSC (Kerby et al. 2005). Use of artificially-induced high flow for control may be feasible but would need to be scaled to larger systems. Use of pumps could be problematic in remote areas with limited access to electricity. Engineered changes in elevation could be used to increase gravity-driven flow but might interfere with dispersal of non-target species (Moreira et al., 2014). If higher current velocities can be used to concentrate crayfish in a relatively small, low-flow refuge area, and thus increase the catch per unit effort then this method is worth further exploration. Higher current velocities may harm non-target species by causing increased sedimentation or displacing organisms. Nevertheless, these impacts could be less harmful compared to other methods like pyrethrin (Peay et al. 2006) that have previously been used to control invasive populations of crayfish making the introduction of flow a potential tool for RSC removal in invaded systems.

References

Abdelrahman, H., R. Gibson, K. Fogelman, A. Cupp, A. Allert, & J. Stoeckel, 2021. Evaluation of dissolved carbon dioxide to stimulate emergence of red swamp crayfish *Procambarus clarkii* (Decapoda: Cambaridae) from infested ponds. Management of Biological Invasions 12: 952–974.

Angeler, D. G., S. Sánchez-Carrillo, G. García, & M. Alvarez-Cobelas, 2001. The influence of *Procambarus clarkii* (Cambaridae, Decapoda) on water quality and sediment characteristics in a Spanish floodplain wetland. Hydrobiologia 464: 89–98.

Aquiloni, L., S. Brusconi, E. Cecchinelli, E. Tricarico, G. Mazza, A. Paglianti, & F. Gherardi, 2010. Biological control of invasive populations of crayfish: the European eel (*Anguilla anguilla*) as a predator of *Procambarus clarkii*. Biological Invasions 12: 3817–3824.

Bills, T. D., & L. L. Marking, 1988. Control of Nuisance Populations of Crayfish with Traps and Toxicants. The Progressive Fish-Culturist Taylor & Francis 50: 103–106.

Bruce, T. J., J. W. Abernathy, N. Tripp, N. Barnes, C. E. Harrison, A. A. Oladipupo, J. D. Krol, A. L. Wise, J. V. Warg, & J. A. Stoeckel, 2024. White spot syndrome virus in Alabama red swamp crayfish (*Procambarus clarkii*). Journal of Fish Diseases 47: e13873.

Budnick, W., B. Roth, L. Nathan, S. Thomas, K. Smith, S. Walker, & S. Herbst, 2022. Evaluation of five trap designs for removal of invasive red swamp crayfish (*Procambarus clarkii* Girard, 1852) in Southern Michigan: catch per unit effort, body size, and sex biases. Management of Biological Invasions 13: 369–390.

Cecchinelli, E., L. Aquiloni, G. Maltagliati, G. Orioli, E. Tricarico, & F. Gherardi, 2012. Use of natural pyrethrum to control the red swamp crayfish *Procambarus clarkii* in a rural district of Italy. Pest Management Science 68: 839–844.

Clark, J. M., M. W. Kershner, & J. R. Holomuzki, 2008. Grain size and sorting effects on size-dependent responses by lotic crayfish to high flows. Hydrobiologia 610: 55–66.

Correia, A. M., & Ó. Ferreira, 1995. Burrowing Behavior of the Introduced Red Swamp Crayfish *Procambarus Clarkii* (Decapoda: Cambaridae) in Portugal. Journal of Crustacean Biology 15: 248–257.

Crook, D. A., W. H. Lowe, F. W. Allendorf, T. Erős, D. S. Finn, B. M. Gillanders, W. L. Hadwen, C. Harrod, V. Hermoso, S. Jennings, R. W. Kilada, I. Nagelkerken, M. M. Hansen, T. J. Page, C. Riginos, B. Fry, & J. M. Hughes, 2015. Human effects on ecological connectivity in aquatic ecosystems: Integrating scientific approaches to support management and mitigation. Science of The Total Environment 534: 52–64.

Cruz, M. J., & R. Rebelo, 2007. Colonization of freshwater habitats by an introduced crayfish, *Procambarus clarkii*, in Southwest Iberian Peninsula. Hydrobiologia 575: 191–201.

Elliott, G., 2022. An Evaluation of Carbon Dioxide and Water Flow as Control Techniques for the Invasive Red Swamp Crayfish. https://etd.auburn.edu//handle/10415/8147.

Fredricks, K., J. Tix, J. Smerud, & A. Cupp, 2020. Laboratory trials to evaluate carbon dioxide as a potential behavioral control method for invasive red swamp (*Procambarus clarkii*) and rusty crayfish (*Faxonius rusticus*). Management of Biological Invasions 11: 259–278.

Freeman, M. A., J. F. Turnbull, W. E. Yeomans, & C. W. Bean, 2010. Prospects for management strategies of invasive crayfish populations with an emphasis on biological control. Aquatic Conservation: Marine and Freshwater Ecosystems 20: 211–223.

Gallardo, B., M. Clavero, M. I. Sánchez, & M. Vilà, 2016. Global ecological impacts of invasive species in aquatic ecosystems. Global Change Biology 22: 151–163.

Gavioli, A., M. Milardi, M. Lanzoni, S. Mantovani, V. Aschonitis, E. Soana, E. A. Fano, & G. Castaldelli, 2018. Managing the environment in a pinch: red swamp crayfish tells a cautionary tale of ecosystem based management in northeastern Italy. Ecological Engineering 120: 546–553.

Gherardi, F., L. Aquiloni, J. Diéguez-Uribeondo, & E. Tricarico, 2011. Managing invasive crayfish: is there a hope? Aquatic Sciences 73: 185–200.

Green, N., M. Bentley, P. Stebbing, D. Andreou, & R. Britton, 2018. Trapping for invasive crayfish: comparisons of efficacy and selectivity of baited traps versus novel artificial refuge traps. Knowledge & Management of Aquatic Ecosystems 419:15.

Havel, J. E., K. E. Kovalenko, S. M. Thomaz, S. Amalfitano, & L. B. Kats, 2015. Aquatic invasive species: challenges for the future. Hydrobiologia 750: 147–170.

Huner, Jay V., 1977. Introductions of the Louisiana red swamp crayfish, *Procambarus clarkii* (Girard): an update. Freshwater Crayfish, 3: 193-202.

Huner, Jay V. & J. W. Avault, Jr., 1979. Introductions of Procambarus spp. Freshwater Crayfish, 4: 191-194.

Jiang, N., X. Pan, Z. Gu, W. Liu, K. Si, Y. Zhou, Y. Zhou, L. Zhai, Y. Fan, & L. Zeng, 2019. Proliferation dynamics of WSSV in crayfish, *Procambarus clarkii*, and the host responses at different temperatures. Journal of Fish Diseases 42: 497–510.

Kerby, J. L., S. P. D. Riley, L. B. Kats, & P. Wilson, 2005. Barriers and flow as limiting factors in the spread of an invasive crayfish (*Procambarus clarkii*) in southern California streams. Biological Conservation 126: 402–409.

Manfrin, C., A. Giglio, L. Pallavicini, L. Zampa, L. Vecchiet, A. Caputi, C. Chiandetti, A. Beorchia, R. Vidimari, & P. G. Giulianini, 2021. Medium-term feasibility of the management of the invasive crayfish *Procambarus clarkii* with the sterile males release technique. Pest Management Science 77: 2494–2501.

Masser M, Rouse D, 1997. Australian Red Claw Crayfish. Southern Regional Aquaculture Centre Publication. 244: 1-8. Stoneville, Mississippi, USA.

McClain R, Romaire R, Lutz CG, Shirley M, 2007. Louisiana crawfish production manual. Louisiana State University Agricultural Center, Baton Rouge, LA.

McFarlane, A., J. O'Brien, B. Nelson, & M. Gammell, 2019. The behavioural response of the white-clawed crayfish (*Austropotamobius pallipes*) to five standard marking techniques over a 14-day period. Marine and Freshwater Behaviour and Physiology 52: 181–197.

Moreira, F. D., F. Ascensão, C. Capinha, D. Rodrigues, P. Segurado, M. Santos-Reis, & R. Rebelo, 2015. Modelling the risk of invasion by the red-swamp crayfish (*Procambarus clarkii*): incorporating local variables to better inform management decisions. Biological Invasions 17: 273–285.

Moyle, P. B., & T. Light, 1996. Biological invasions of fresh water: Empirical rules and assembly theory. Biological Conservation 78: 149–161.

Peay, S., P. D. Hiley, P. Collen, & I. Martin, 2006. Biocide Treatment of Ponds in Scotland to eradicate signal crayfish. Bulletin Français de la Pêche et de la Pisciculture EDP Sciences 380-391:1363–1379.

Penn, G. H., 1943. A study of the life history of the Louisiana Red-Crawfish *Cambarus Clarkii* Girard. Ecology 24: 1–18.

Reid, A. J., A. K. Carlson, I. F. Creed, E. J. Eliason, P. A. Gell, P. T. J. Johnson, K. A. Kidd, T. J. MacCormack, J. D. Olden, S. J. Ormerod, J. P. Smol, W. W. Taylor, K. Tockner, J. C. Vermaire, D. Dudgeon, & S. J. Cooke, 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biological Reviews 94: 849–873.

Reisinger, L., N. Tripp, A. Atchia, S. Bolds, D. Cook, K. Fralick, D. Ganas, & B. Hayman, 2024. Can intensive trapping to reduce the abundance of an invasive crayfish benefit an imperiled congener? Aquatic Conservation: Marine and Freshwater Ecosystems 34: e4123.

Rubio-Gracia, F., C. Galobart, L. Benejam, B. Martí, A. Rubio, & A. Vila-Gispert, 2022. Oxygen consumption in relation to current velocity and morphology in the highly invasive crayfish *Procambarus clarkii*. Hydrobiologia 849: 2845–2857.

Savaya, A., G. De Leo, E. Aalto, T. Levy, O. Rosen, R. Manor, E. Aflalo, E. Tricarico, & A. Sagi, 2020. The IAG gene in the invasive crayfish *Procambarus clarkii* – towards sex manipulations for biocontrol and aquaculture. Management of Biological Invasions 11: 237–258.

Souty-Grosset, C., P. M. Anastácio, L. Aquiloni, F. Banha, J. Choquer, C. Chucholl, & E. Tricarico, 2016. The red swamp crayfish *Procambarus clarkii* in Europe: Impacts on aquatic ecosystems and human well-being. Limnologica 58: 78–93.

Strayer, D. L., 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biology 55: 152–174.

Strayer, D. L., & D. Dudgeon, 2010. Freshwater biodiversity conservation: recent progress and future challenges. Journal of the North American Benthological Society 29: 344–358.

Vander Zanden, M. J., J. M. Casselman, & J. B. Rasmussen, 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature Publishing Group 401: 464–467.

WWF. 2018. Living planet report—2018: Aiming higher. In: M. Grooten, R. E. A. Almond (Eds.). Gland: WWF.

Table 2.1: Acceptable ranges of AHAB water quality parameters (McClain et al., 2007).

Water quality parameter	**pH**	**Alkalinity (mg/L $CaCO_3$)**	**Hardness (mg/L $CaCO_3$)**	**Ammonia (mg/L TAN)**	**Nitrite (mg/L N)**
Lower limit	6.5	20	50	-	-
Upper limit	8.5	-	-	0.3	0.6

Table 2.2: Results of statistical tests for differences in current velocity between downstream (lower zone #) and upstream (higher zone #) zones for trials involving each of three size classes. If a significant difference was found, the zone with the higher velocity is in bold.

Zones	Size-Class	Test Used	Sample Size	Z-Statistic	Probability
1 vs 2	Small	Wilcoxon signed rank	18	0.0517	P=0.980
1 vs 2	Medium	Wilcoxon signed rank	20	-0.457	P=0.671
1 vs 2	Large	Wilcoxon signed rank	16	0.891	P=0.413
2 vs **3**	Small	Paired t-test	18	-	P<0.001
2 vs **3**	Medium	Wilcoxon signed rank	20	3.724	P<0.001
2 vs **3**	Large	Paired t-test	16	-	P<0.001

Table 2.3. Statistical information for the two regressions applied to the relationship between percent time spent in zone 1 versus mean velocity in zones 1-2 for each size class. The best regression was chosen based on it passing normality and equal variance assumptions and having a >5% lower AICc or >5% greater R^2 than the other regression. Best regressions for each size-class are indicated in bold. If they were considered equally valid then both are bolded.

Size-Class	Regression type	Log Transformed?	Normality	Variance	AICc	R^2	Probability
Small	linear	No	Yes	Yes	-66.961	0.78	$P<0.0001$
	polynomial quadratic	No	Yes	Yes	-67.733	0.83	$P<0.0001$
Medium	**linear**	No	Yes	Yes	-74.603	0.84	$P<0.0001$
	polynomial quadratic	No	Yes	Yes	-72.597	0.86	$P<0.0001$
Large	linear	No	No	No	-41.306	0.39	$P=0.0092$
	polynomial quadratic	No	Yes	Yes	-42.297	0.55	$P=0.0058$

Table 2.4. Statistical information for the three regressions applied to the relationship between the number of transitions between zone 1 to zone 2 versus mean current velocity of zones 1-2 for each three size classes. The best regression was chosen based on it passing normality and equal variance assumptions and having a >5% lower AICc or >5% greater R^2 than the other regression. Best regressions for each size-class are indicated in bold. If two were considered equally valid based on AICc scores, then both are bolded.

Size-Class	Regression type	Log Transformed?	Normality	Variance	AICc	R^2	Probability
Small	linear	Yes	Yes	Yes	-48.1749	0.77	$P<0.0001$
	3 segment piecewise	Yes	Yes	Yes	-39.6109	0.86	$P<0.0001$
	polynomial quadratic	Yes	Yes	Yes	-52.9061	0.85	$P<0.0001$
Medium	linear	No	Yes	Yes	74.757	0.28	$P=0.0160$
	3 segment piecewise	No	Yes	Yes	83.756	0.49	$P=0.0663$
	polynomial quadratic	No	Yes	Yes	73.312	0.43	$P=0.0085$
Large	linear	Yes	No	Yes	-32.5685	0.58	$P=0.0007$
	3 segment piecewise	Yes	Yes	Yes	-16.8925	0.68	$P<0.0261$
	polynomial quadratic	Yes	Yes	Yes	-32.0983	0.65	$P=0.0011$

Table 2.5. Statistical information for the three regressions applied to the relationship between the number of transitions between zone 2 to zone 3 versus upstream current velocity (zone 3) for each three size classes. The best regression was chosen based on it passing normality and equal variance assumptions and having a >5% lower AICc or >5% greater R^2 than the other regression. Best regressions for each size-class are indicated in bold.

Size-Class	Regression type	Log Transformed?	Normality	Variance	AICc	R^2	Probability
Small	**linear**	No	Yes	Yes	51.0486	0.81	$P<0.0001$
	3 segment piecewise	No	Yes	Yes	65.3887	0.84	$P=0.0002$
	polynomial quadratic	No	Yes	Yes	54.3207	0.82	$P<0.0001$
Medium	linear	No	Yes	Yes	74.8229	0.50	$P=0.0005$
	3 segment piecewise	No	Yes	No	87.3748	0.57	$P=0.0225$
	polynomial quadratic	No	Yes	Yes	76.0896	0.54	$P=0.0013$
Large	**linear**	No	Yes	Yes	49.1579	0.75	$P<0.0001$
	3 segment piecewise	No	Yes	Yes	68.7641	0.75	$P=0.0074$
	polynomial quadratic	No	Yes	Yes	52.7869	0.75	$P=0.0001$

Table 2.6. Statistical information for the three regressions applied to the relationship between percent time spent in zone 3 versus mean velocity in that zone for each three size classes. The best regression was chosen based on it passing normality and equal variance assumptions and having a >5% lower AICc or >5% greater R^2 than the other regression. Best regressions for each size-class are indicated in bold.

Size-Class	Regression type	Log Transformed?	Normality	Variance	AICc	R^2	Probability
Small	**linear**	No	Yes	Yes	-85.2750	0.85	$P<0.0001$
	polynomial quadratic	No	Yes	Yes	-81.9151	0.85	$P<0.0001$
Medium	linear	No	Yes	Yes	-80.3049	0.74	$P<0.0001$
	polynomial quadratic	No	Yes	Yes	-83.3618	0.81	$P<0.0001$
Large	**linear**	No	Yes	Yes	-50.3361	0.39	$P=0.0093$
	polynomial quadratic	No	Yes	Yes	-46.7694	0.40	$P=0.0375$

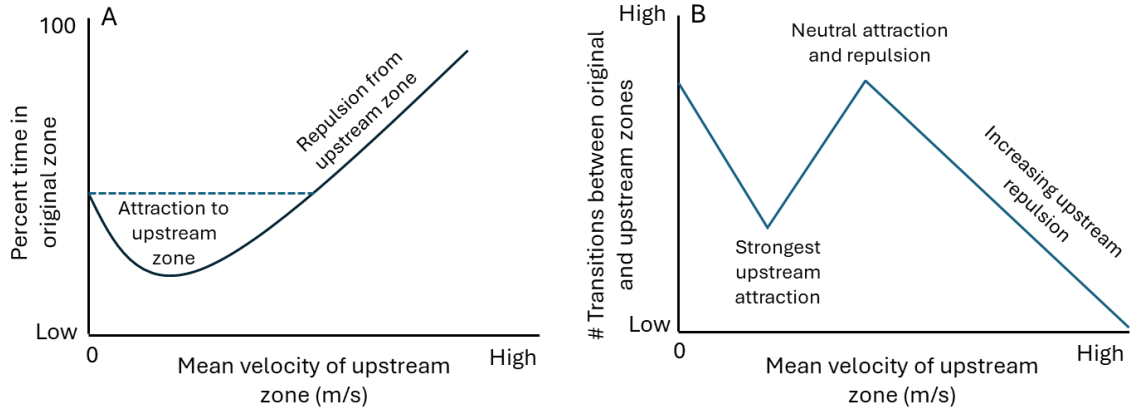

Figure 2.1: A) Hypothesized relationship between percent time spent in the initially occupied flume zone and mean velocity of the upstream zone. As the attraction to upstream velocity increases, time spent in the initial zone decreases. As the repulsion to the upstream velocity increases, time spent in the initial zone increases. The blue dotted line represents neutral attraction and repulsion B) Hypothesized relationship between # transitions between initial and upstream zones and mean velocity of the upstream zone. The number of transitions between zones decreases as crayfish are either strongly attracted to the upstream zone and spend less time in the initial zone, or are strongly repulsed by the upstream zone and spend more time in the initial zone.

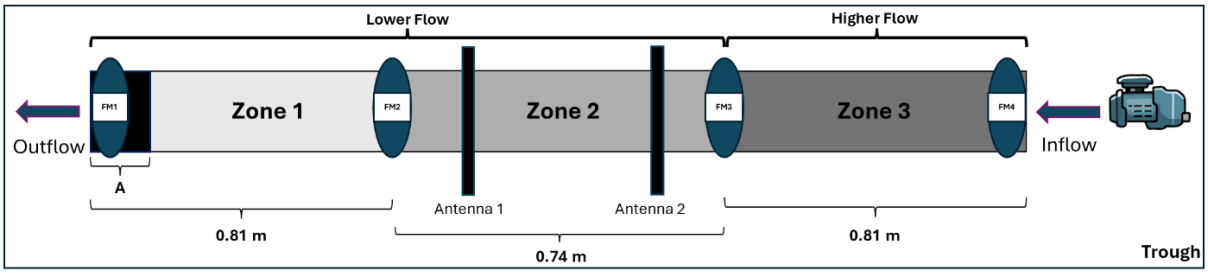

Figure 2.2: Flume system used in movement experiments. Flow measurements were taken at four locations (FM). Crayfish were acclimated before experiments in a 0.15 m zone (A). The inflow, where the highest flow area was located, occurred at the front of zone 3. Two antennae were placed in zone 2.

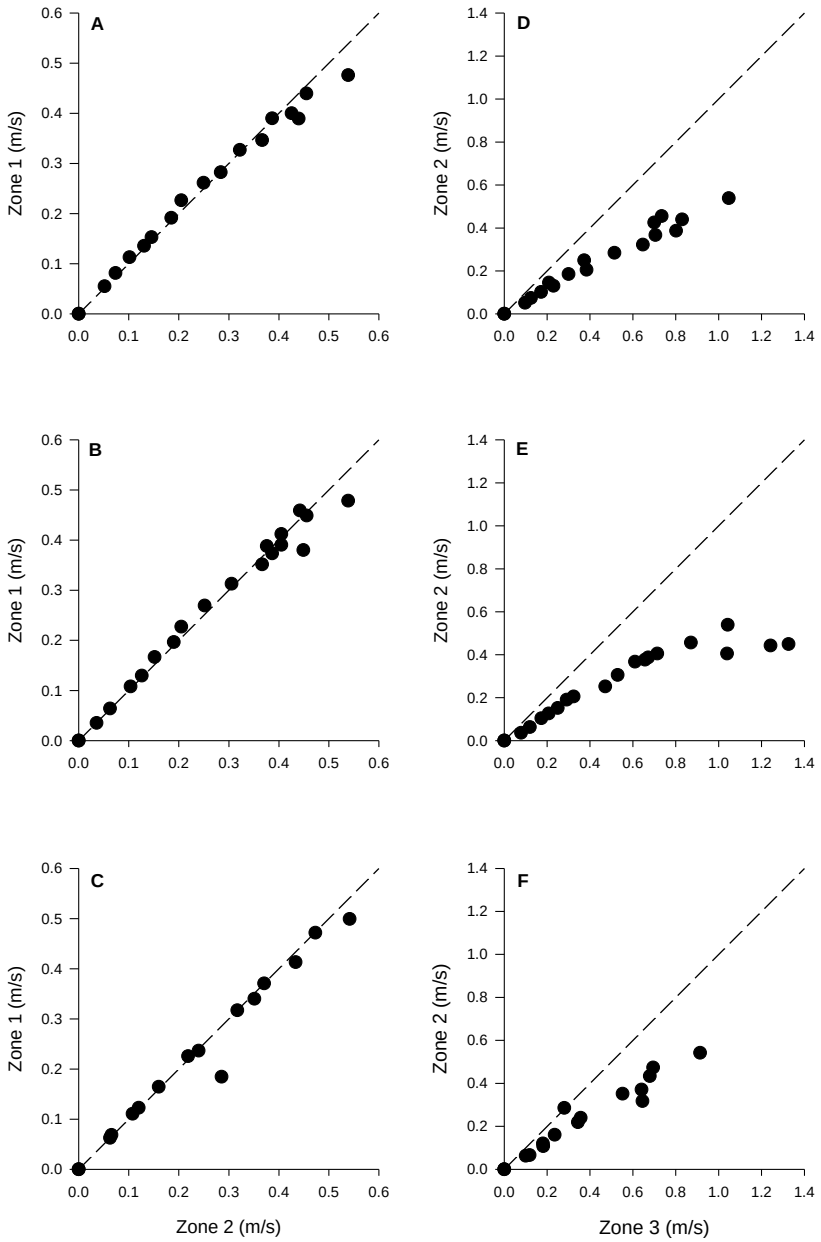

Figure 2.3: The relationship between the current velocity in Zone 2 and current velocity in Zone 1 for each of the three size classes in the following order: small (A), medium (B) and large (C). The relationship between the current velocity in Zone 3 and the current velocity in Zone 2 for each of the three size classes in the following order: small (D), medium (E) and large (F). Dotted lines represent a hypothetical 1:1 relationship between current velocity of each zones for reference purposes.

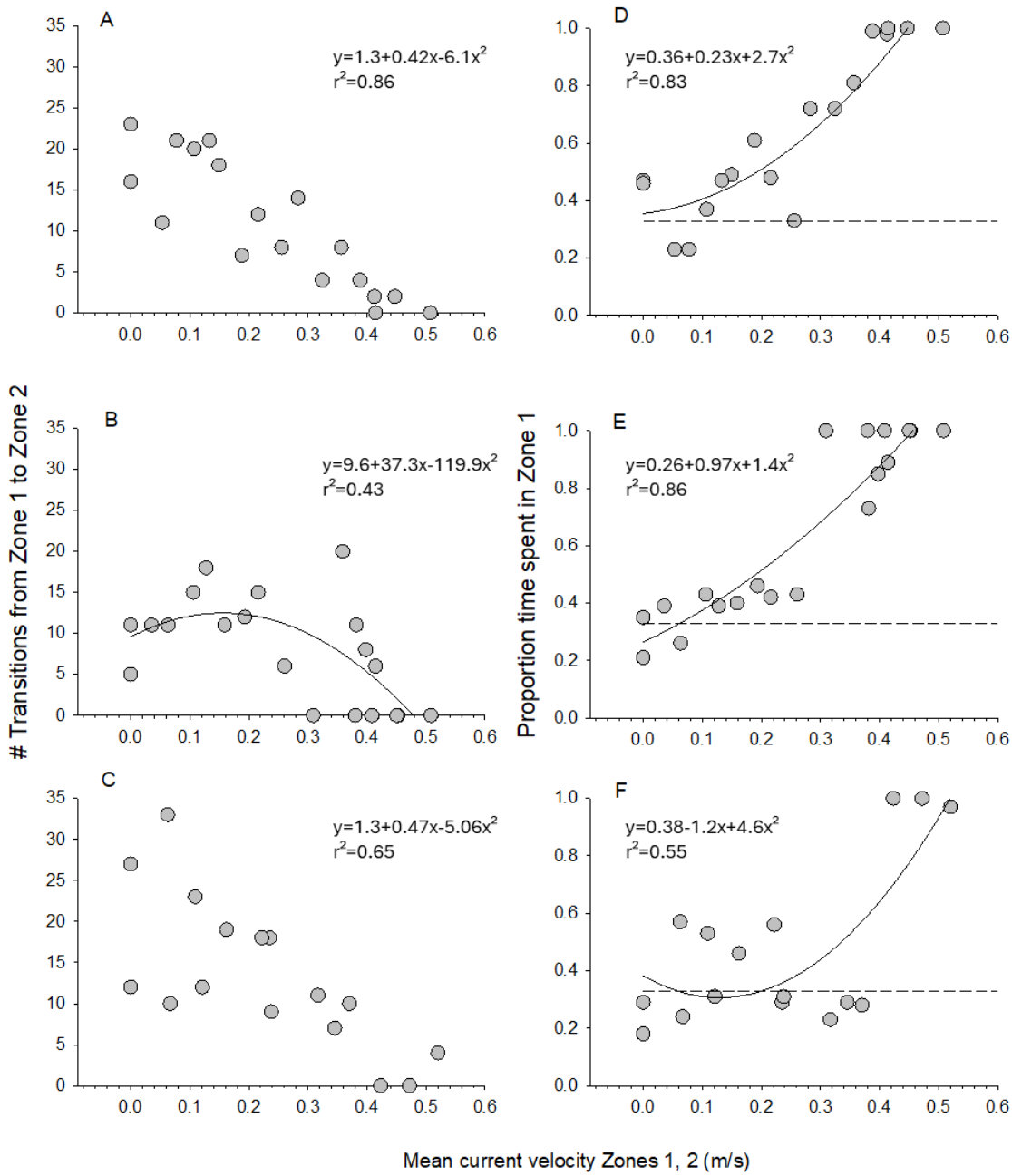

Figure 2.4 Relationships between mean current velocity and (A, B, C) number of transitions from zone 1 to zone 2 and (D, E, F) the proportion of time spent in the downstream zone when current velocity did not differ between upstream and downstream zones. Small crayfish are represented in the top panels, medium crayfish in the middle panels, and large crayfish in the bottom panels. Solid lines represent a quadratic regression through the data when assumptions of normality and constant variance were met by the raw data. Dotted lines indicate 33% threshold for proportion time spent downstream (zone 1).

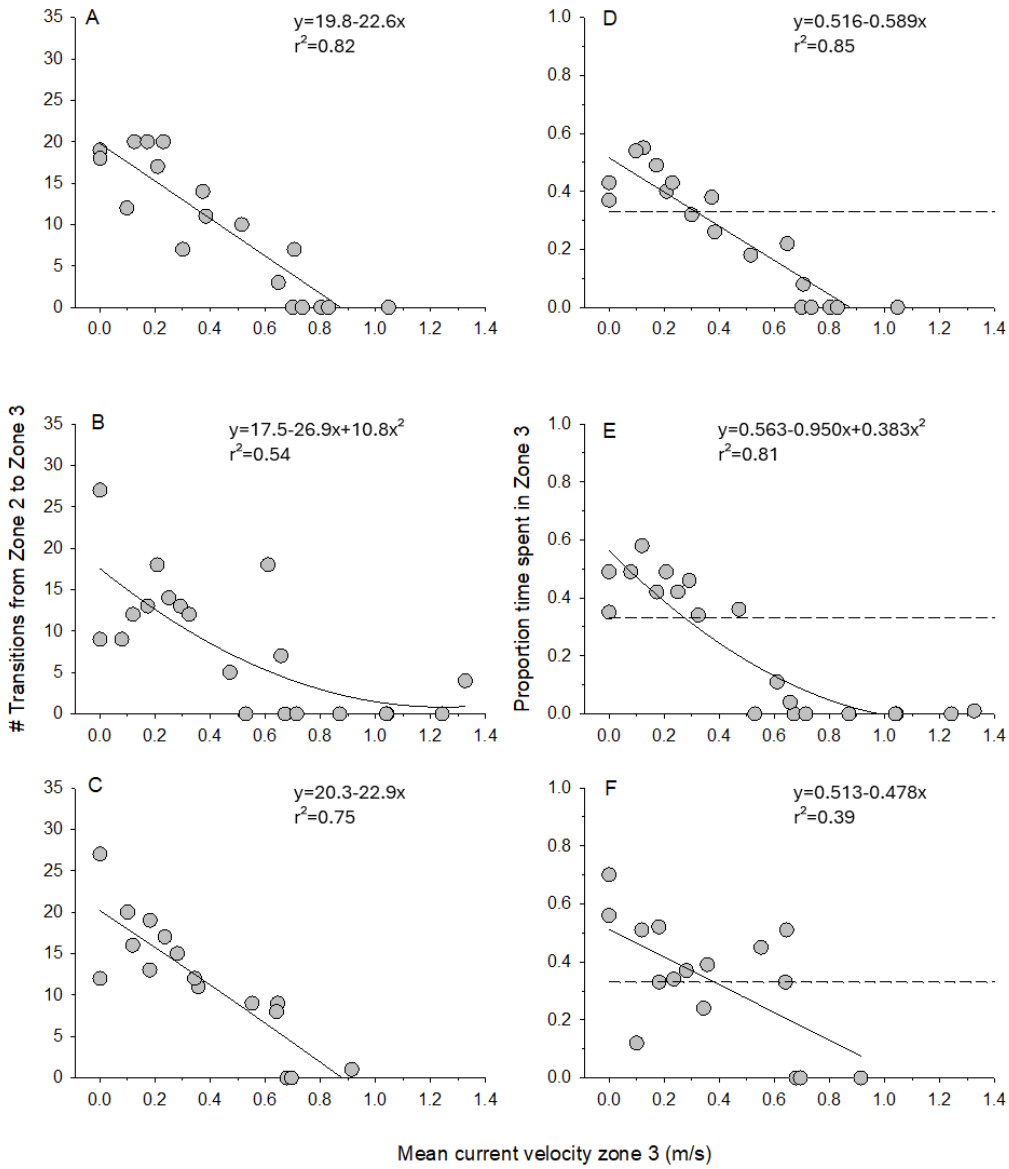

Figure 2.5 Relationships between mean current velocity and (A, B, C) number of transitions from zone 2 to zone 3 and (D, E, F) the proportion of time spent in the downstream zone when current velocity was higher in the upstream zone. Small crayfish are represented in the top panels, medium crayfish in the middle panels, and large crayfish in the bottom panels. Solid lines represent a linear regression through the data when assumptions of normality and constant variance were met by the raw data. Dotted lines indicate 33% threshold for proportion time spent upstream (zone 3).