INVESTIGATING SALINITY TOLERANCE AND PARASITE DIVERSITY IN NATIVE NORTH CAROLINA ESTUARY PANOPEIDS (MUD CRABS)

By

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July, 2023

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Abstract

Investigating organismal response to invasions is increasingly important given human-mediated global change. Estuarine organisms face numerous biotic and abiotic factors that influence their ability to respond to invaders like parasites. When confronted with parasitic invaders, hosts can either develop resistance or expand distributions beyond the limits of the invader's tolerance. One such invader of US Atlantic estuaries is the Rhizocephalan (parasitic barnacle) *Loxothylacus panopaei*, which infects native panopeid crabs including the flatback mud crab *Eurypanopeus depressus*. Given that successful development of *L. panopaei* larvae is dependent on salinities >10 PSU, *E. depressus* may have a parasite refuge in lower salinity waters. However, in past field studies in North Carolina estuaries, the crab has not been detected at sites <10 PSU, and so it is unclear whether *E. depressus* would be able to tolerate salinities that would allow it to exploit a parasite refugia in low salinity waters. In Chapter 1, I aimed to answer this question by testing low salinity tolerance in adult *E. depressus* over a three-week exposure period. I examined mortality and righting response after exposure to salinity treatments (n=7) from fresh to moderate salinities (0–10 PSU) in *E. depressus* sourced from two Mid-Atlantic estuarine sites. I found higher

mortality and slower righting response in 0, 0.2 and 0.5 PSU, but high survival in salinities between 3-10 PSU, where past field surveys have not detected the crab. This investigation helps us determine the low salinity tolerance range of *E. depressus* in a laboratory setting, allowing us to resolve whether salinity is a key barrier to the species' ability to exploit parasite refugia in Atlantic estuaries. Though survival was relatively high except in near fresh salinities, trends for slower righting response times compared to the control salinity suggests some level of sub-lethal stress that may impact the crab's survival and competitive abilities. Future research should examine multiple stressors (e.g., salinity, competition, predation) on crab survival.

In addition, parasite infection prevalence and diversity can provide insight into the population dynamics of species inhabiting estuaries, as parasites may affect a species' ability to reproduce, survive, and even compete with other species. Chapter 2 of my thesis focused on identifying parasite infection prevalence and diversity in *E. depressus* compared to a co-occurring crab, *Rhithropanopeus harrisii*. I examined 2,022 crabs from 2016-2022 from 5 sites in which the crabs co-occur in the Pamlico and Neuse Estuaries, North Carolina for parasite composition, prevalence, and diversity. During my parasite surveys, I found two parasitic castrators, *L. panopaei* and Entoniscid isopods (possibly *Cryptocancrion brevibrachium* or *Cancrion carolinus*), as the major determinants of parasite prevalence in the two crab species. Total infection prevalence was found to be significantly higher in *R. harrisii* than *E. depressus,* as was Entoniscid prevalence, but *L. panopaei* prevalence did not significantly differ between the species. To our knowledge, our study is the first comprehensive investigation comparing parasite diversity in *R. harrisii* and *E. depressus* in North Carolina Estuaries.

Investigating Salinity Tolerance and Parasite Diversity in Native North Carolina Estuary Panopeids (Mud Crabs)

A Thesis

Presented to the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biology

By

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July, 2023

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LIST OF ABBREVIATIONS

1. SQ: This refers to the Swan Quarter Bell Island Pier in Swan Quarter, NC.
2. MD: This refers to Saint John's Pond in Saint Mary's City, MD.
3. CW: This refers to Carapace Width.
4. RR: This refers to Righting Response Time.
5. PSU: This refers to Practical Salinity Units.
6. ANOVA: This stands for Analysis of Variance.
7. CoxPH: This stands for Cox Proportional Hazards.
8. GLM: This stands for Generalized Linear Model.
9. LS_{50}: This stands for Lethal Salinity 50% Mortality.

CHAPTER 1: INVESTIGATING SALINITY TOLERANCE IN A NATIVE NORTH CAROLINA PANOPAEID, *E. depressus*

Introduction

There are many factors that can influence the distribution of species in an ecosystem. For organisms that live in estuaries, they must be able to tolerate a multitude of abiotic variables that fluctuate hourly, daily, monthly, and seasonally such as salinity, temperature, and oxygen (Hulathduwa *et al.,* 2007). Additionally, organisms face biotic pressures (e.g., predation, refuge, resource availability, etc.) that are often interpreted as the strongest driving forces of ecosystem structure (Armstrong & McGehee, 1976). However, abiotic and biotic factors likely interact in complex systems, influencing community structure and species distributions (Dunson & Travis, 1991).

From the biotic perspective, a major driver of species evolution and distribution is parasitism (Hatcher *et al.,* 2006; Tepolt *et al*., 2020). Parasites can greatly impact host fitness and behavior, and as such, are a major selective force on their hosts (Møller *et al.,* 1993; Tepolt *et al*., 2020). Hosts may respond to a parasite by engaging in an evolutionary arms race (e.g., The Red Queen Hypothesis), where the host must evolve resistance to infection by the parasite to stay extant (Hart, 1990; Morran *et al.,* 2011; Blakeslee *et al.,* 2021). On the other hand, some hosts may be able to expand their distribution into environment refuges (i.e., habitats in which the host can survive but that parasite cannot), thereby creating a type of 'parasite refuge' for the affected host (Tolley *et al.,* 2006; Blakeslee *et al.,* 2021). In estuarine bivalves, disease induced mortality can lead to the populations being restricted along spatial gradients in order to escape disease (Hofmann *et al.,* 2009). These parasite refugia can also be seen in native Hawaiian birds, where high-altitude

populations are able to escape malaria infection for most of the year (Samuel *et al.,* 2011). However, understanding the host's tolerance to certain abiotic factors is critical knowledge to begin to hypothesize whether an environmental refuge might exist under parasite pressure. The presence or absence of a refuge may also be characteristic of the ability of a host to cope with changing environmental conditions linked to invasive species and anthropogenic change (Little *et al.*, 2017).

In this study, we focused on a model system to examine how the introduction of a parasitic castrator would influence the salinity tolerance of an estuarine crab host. The Rhizocephalan, *Loxothylacus panopaei*, is native to the Gulf of Mexico, USA and was introduced to the Chesapeake Bay in the 1960s via the oyster aquaculture trade and has since expanded its invasive range southward to Florida (Van Engel *et al.,* 1966; Hines *et al.,* 1997; Carlton *et al.,* 2011; Kruse *et al.,* 2012) and northward with isolated populations in Long Island Sound (Freeman *et al*. 2013). *L. panopaei* has a direct life cycle, where the mud crab serves as the final and only host. *L. panopaei* can infect up to nine different mud crab species, including the white-fingered mud crab *Rhithropanopeus harrisii* and the flat-backed mud crab *Eurypanopeus depressus* (Hines *et al.,* 1997). Free-living female cypris larvae settle on an uninfected host and develop a kentrogon that proceeds to deposit the genetic material of the parasite into the host, which ultimately results in the formation of root-like structures (Høeg, 1992). After several molts, the internal parasite produces an external larval brood chamber where fertilized parasite larvae will be released (Reisser & Forward, 1991; Walker *et al.,* 1992). Once inside the crab, *L. panopaei* utilizes the host's osmoregulatory system to maintain its own osmolality (Reisser & Forward, 1991), but environmental stages of the parasitic larvae must use their own physiology to tolerate various abiotic factors like temperature and salinity that may impact survival.

A salinity refuge may exist for some mud crab host species faced with *L. panopaei* infection because successful development of *L. panopaei* larvae can only occur in salinities from 10 to 30 PSU (Walker & Clare, 1994), and *L. panopaei* adults are not found in waters below 10 PSU (Blakeslee *et al.* 2021). For example, *R. harrisii* appears to have a refuge from *L. panopaei* because the crab can survive in salinities ranging from approximately 1 to 40 PSU (Bousfield, 1955; Wurtz & Roback, 1955; Ryan, 1956; Rodriguez, 1963; Costlow *et al.,* 1966; Turoboyski, 1973; Boyle *et al.,* 2009). This refuge has been observed in Florida and North Carolina estuaries, where increased rainfall and reduced salinity during the wet season leads to significantly lower *L. panopaei* infection prevalence (Reisser & Forward, 1991; Tolley *et al*., 2006). However, not all mud crabs may have the same salinity tolerances and may not have the ability to use low salinity as a refuge from parasitism. For example, Blakeslee *et al.* (2021) did not find *E. depressus* in waters <10 PSU, paralleling the salinity tolerance for *L. panopaei*. However, the infection rate of *L. panopaei* in *E. depressus* is 22 times higher in the invasive range compared to the native range (Tepolt *et al.* 2020).

To date, there is limited literature surrounding *E. depressus'* distribution, salinity tolerance, and interactions with parasites. *E. depressus* individuals can often be found in euryhaline waters near oyster reefs from Massachusetts Bay to the east and west coasts of Florida, to Texas, the Indies and Bermuda (Rathbun, 1930; McDonald, 1982; Williams, 1984). Adult mud crabs of *E. depressus* have been found extensively around oyster bars in the Chesapeake Bay that were dominated by the oyster *Crassostrea virginica* (Gmelin, 1791) and this was likely due to the structure and refuge provided by the oyster reefs from predators and desiccation (Ryan, 1956; Grant & McDonald, 1979). *E. depressus* has recently been found to have invaded the Bulgarian coast of the Black Sea (Mitov, 2019), likely through ballast water exchange and hull fouling

actions. Established populations of *E. depressus* have also been discovered in the intertidal zone of Montevideo and Balnerio Costa Azul of Uruguay (Juanicó, 1978) as well as in Mar Chiquita Lagoon of Argentina (Spivak & Luppi, 2005). The occurrence of these populations in the southwestern Atlantic are believed to be anthropogenic due to the lack of natural connectivity with the northwestern Atlantic native populations (Tavares, 2011). An additional range extension for *E. depressus* has also been recorded on the southern coast of Brazil and was likely caused by ballast water exchange in Brazilian waters (Rodrigues *et al.,* 2014). The crab has been observed in the Caloosahatchee, Faka Union and Estero estuaries in southwest Florida, where salinities averaged from 37 to 33 PSU in the dry season and from 9.5 to 24.1 PSU during the wet season (Van Horn & Tolley, 2008). In prior lab studies using crabs from the Gulf of Mexico, *E. depressus* survived in salinities from 5 to 45 PSU (Garcés, 1987), with a lethal salinity of 0.2 PSU for 50% of crabs and showed high energy expenditures at low salinities (Hulathduwa *et al.* 2007). Further, *E. depressus* can hyper-osmoregulate when confronted with extreme decreases in salinity over short periods of time and is less resilient to desiccation stress beyond their favored habitat (oyster beds), which are subtidal in brackish waters and intertidal in higher salinities (Roegner & Mann, 1995; Van Horn & Tolley 2009; Johnson & Smee, 2014); this may represent a possible range constraining factor since *E. depressus* is much more common in habitats with available shelter (Meyer, 1994; Brown *et al.,* 2005).

As a result of the limited understanding of *E. depressus'* salinity tolerance and the role of salinity as a driver of the crab's distribution in estuaries (particularly in invaded regions where *L. panopaei* is now prevalent), we aimed to determine the lethal and sub-lethal effects of salinity on *E. depressus* from two Atlantic estuaries. Righting response (RR) is a common method used to assess sublethal stress in crabs (e.g., Rebach, 1974; Wilson, 1989; Blakeslee *et al.*, 2015; Blakeslee

et al., 2021; Lagos *et al.,* 2021; Ro *et al.*, 2022). Some examples of how RR has been used as a measure of various stressors include Rebach (1974) who found that hermit crabs lost mobility and RR at 1.6º ± 0.5º C and then regained RR when returned to the ambient (warmer) temperature. Further, Blakeslee *et al.* (2015) found a significant increase in RR time in trematode infected invasive green crabs (*C. maenas*) compared to the uninfected controls. RR has also been used in experiments involving the six-rayed starfish, *Leptasterias hexactis* to evaluate the effect of hyposmotic conditions (Shirley & Stickle, 1982). Alternative measures of non-lethal stress include feeding behavior, growth, and quantification of oxidative stress, which is capable of damaging various cellular components or cellular death (Shirley & Stickle, 1982; Elsayed & Gorbunov, 2003; Halliwell, 2007; Sies, 2015; Snitman *et al.,* 2022). To examine the potential influence of seasonality on crab response, we performed survival analyses in response to salinity stress for one population multiple times over the summer. Ultimately, we aimed to answer whether *E. depressus* would have the capacity to tolerate salinities <10 ppt that would allow it to exploit low salinity refugia to escape a parasitic castrator. Our results provide a baseline for understanding the role that salinity plays in the distribution of *E. depressus* and predict how *E. depressus* may be able to respond to the presence of the invasive Rhizocephalan, *L. panopaei*.

Methods

Field Sampling

To examine low salinity tolerance of *E. depressus* within the invasive range of *L. panopaei*, crabs were collected from the Bell Island Pier in Swan Quarter, NC, USA and Saint John's Pond in Saint Mary's City, MD, USA (Table S1, Appendix A). The Swan Quarter Bell Island Pier (herein referred to as SQ) is located in the Pamlico River which serves as a connection between the Tar River, a freshwater system, and the Pamlico Sound, an estuary,

resulting in a steep salinity gradient along the river from oligohaline to mesohaline (Copeland *et al*., 1984). To provide replication through time and determine whether there was a seasonal effect of sampling, SQ was sampled three times in 2022 (May, June, and July). Ten passive samplers (small plastic crates filled with oyster shells (Blakeslee *et al.* 2021)) were deployed for a month and redeployed after each sampling event. Salinity, water temperature and dissolved oxygen level were measured with a YSI Pro30 Conductivity Meter and a YSI Pro20i Dissolved Oxygen Meter (Xylem Inc.).

Saint John's Pond (MD) is along the eastern shore of the St. Mary's River which empties into the Potomac River. The pond is connected to the river by a small inlet that passes under Point Lookout Road which allows the pond to experience changes in tides daily. MD was sampled once in August to determine whether geographic differences in source location may affect salinity tolerance.

All *E. depressus* collected were transported to the laboratory at East Carolina University in plastic containers with damp towels and immediately processed. Specimens were measured (carapace width-CW), sexed and checked for *L. panopaei* infection prior to being randomly assigned a treatment group for the experiment. Any *E. depressus* with a visual infection by *L. panopaei* were excluded from this study because we were only interested in the effect that salinity has on survival and not parasitism itself. *E. depressus* females that were gravid at the beginning of the study were marked as gravid, but by the end of the exposure no females were gravid.

Laboratory Experimental Design

In late spring and summer 2022, we performed two survival experiments to explore *E. depressus* salinity tolerance: (1) a multi-site comparison (SQ and MD) at seven salinities, and (2)

a within-site comparison across months (May, June, July) for SQ only at four salinities. The multi-site analysis included seven salinities: 10 PSU (control), 5 PSU, 3 PSU, 1 PSU, 0.5 PSU, 0.2 PSU and 0 PSU, and were based on previous work (Pochtar personal communication) and a pilot trial we performed in March 2022. The within-site study included four salinities: 10 PSU (control), 3 PSU, 1 PSU and 0.2 PSU. Fixed-grid jewelry boxes were used to separate organisms, and each mud crab was given an individual ID to track survival. Organisms were maintained at 24°C on a 12-hour light cycle during each salinity trial within a self-contained Vivarium room at East Carolina University. Treatment salinities were prepared using Instant Ocean Sea Salt (Instant Ocean) and deionized water and checked with a YSI Pro30 Conductivity Meter. Wells in the jewelry boxes were filled with 60 mL of water at the respective salinity treatment. All individuals began the trials in 10 PSU salinity to reduce low-salinity shock; for specimens assigned to lower salinities, the salinity was decreased by 2.5 PSU every 48 hours until the treatment salinity was reached (Day 0 of the exposure period). Crabs were fed one Aqueon Cichlid Pellet (Aqueon) at the 36 hour-marker of the two-day cycle and allowed to feed for 12 hours. The exposure period lasted for 21 days for each treatment (Table S2 in Appendix A).

Righting Response

As a measure of sub-lethal stress, a "Righting Response" (RR) behavioral test was performed on each crab at three time points during the experimental period and measured the time required for a crab to right itself after being placed on the dorsal side of its carapace. The three separate RR tests performed included: Initial (the day immediately following processing), Exposure (after spending 24 hours in the assigned treatment) and Exit (Day 21 after the survival check). Organisms that expired prior to reaching the assigned salinity or the end of the salinity exposure trial were excluded from analyses. For each RR test, the individuals were observed for

a maximum of 120 seconds; the time at which the individual righted itself was recorded, and the trial was ended. Any individuals that did not flip at all during the trial were recorded at the maximum time (120 seconds).

Data Analysis

We used the Cox Proportional Hazards (CoxPH; survival) statistical model to evaluate survival as the response variable, with a continuous fixed variable of CW and categorial fixed variables of sex (male, female, gravid female), site (SQ, MD), month (only for within SQ: May, June, August) and salinity. Neither the multi-site nor within-site data ($p > 0.05$) violated the assumptions posed by the Cox Proportional Hazards assessment. We used Kaplan-Meier Survival Curves to visually demonstrate the probability that a group in a given treatment would survive over time (Kaplan & Meier, 1958). Lethal Salinity 50% (LS_{50}) Mortality (i.e., the salinity at which half of the tested population died) curves were also established for the multi-site (SQ and MD) trials and within site (SQ) trials.

A Generalized Linear Model (GLM) with a gamma distribution was used to evaluate non-lethal stress (Righting Response - RR in seconds) as the response variable. Separate models were created for only the "Exposure" and "Exit" RR because the "Initial" RR did not show any differential response across salinity treatments. For the Exit RR, data were included if there were more than five crabs surviving within a treatment. Predictor variables included the continuous fixed variable of CW and categorial variables of sex, site, month, and salinity as stated above. Bonferroni's pairwise comparisons evaluated significance for the RR results.

Data were analyzed using R Studio (Version 1.41717). Akaike Information Criterion (AICc) was used to compare CoxPH (Survival) models and GLM (RR) models for the multi-site and within-site data. R Studio packages used in these analyses were: eha, data.table, ggplot2,

survival, survminer, lubridate, ggsurvfit, performance, tidyverse, finalfit, dplyr, forcats, MASS, patchwork, and AICcmodavg. An ΔAICc of 2.0 was used as a cutoff value to determine the top models. Additive and interactive effects were considered when designing the models to evaluate survival and RR. Model formulas were determined based on predictors that could be considered ecologically significant.

Results

Multi-site Analysis (NC and MD sites)

Survival

Based on the CoxPH AICc assessment, there was no difference in survival based on site alone; therefore, data from each site were combined for the following analyses and summaries of results and discussion (Figure S1 in Appendix A shows the overall proportion of surviving crabs for each treatment). Adult *E. depressus* survived best at salinities at and above 3 PSU. After 21 days in the assigned treatment, survival probabilities ranged from 0 to 87.2% (Fig. 1.A). Salinity was a significant factor in determining the survival of uninfected adult *E. depressus* (Kaplan-Meier $p < 0.0001$). All of the *E. depressus* assigned to 0 PSU were dead within 24 hours of exposure and all but two crabs assigned to 0.2 PSU were dead within 10 days. The Kaplan-Meier curves (Fig 1.B) also graphically showed an increase in mortality risk as salinity was reduced to 0 PSU. The top performing model for predicting survival was an interaction between salinity and CW (AICc Wt. = 0.64, Table S3, S4, Appendix A). In CoxPH assessments, a crab's risk of death, when compared to the control treatment (10 PSU), increased as salinity declined. Individuals showed no significant difference in survival down to 3 PSU, but once salinity was reduced below 3 PSU, survival was significantly affected (Figure 2A). The LS_{50} plot showed 50% mortality at 2.32 PSU (Fig 3.A).

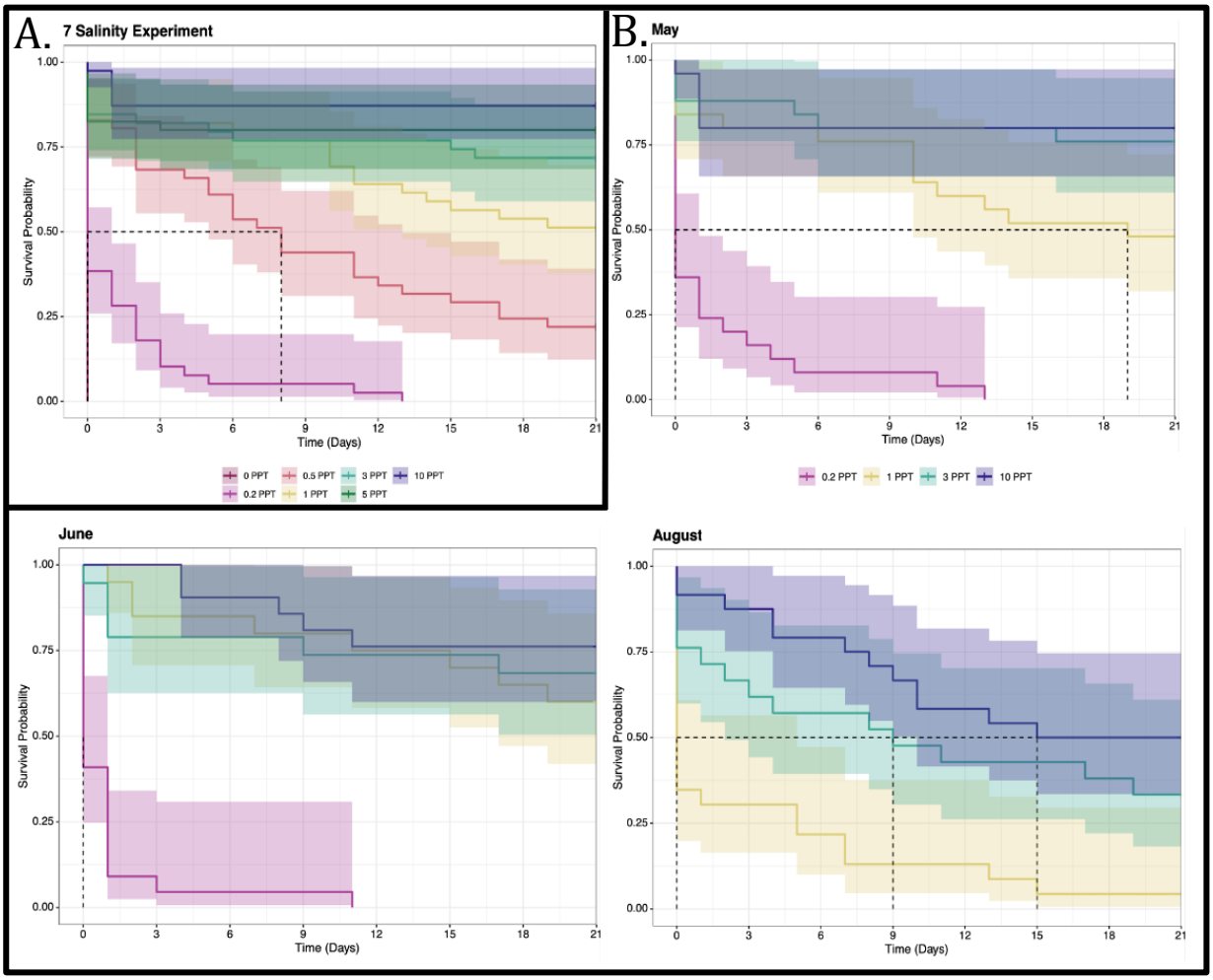

Fig. 1 Kaplan-Meier Survival Curves for Eurypanopeus depressus across salinity treatments; dotted lines show time at 0.50 Survival Probability for respective salinities. A) 7 salinity experiment showing combined SQ and MD (Saint John's Pond) survival across seven salinities B) Trials examining survival across season for SQ: May, June, August looking at survival probability for four different salinities.

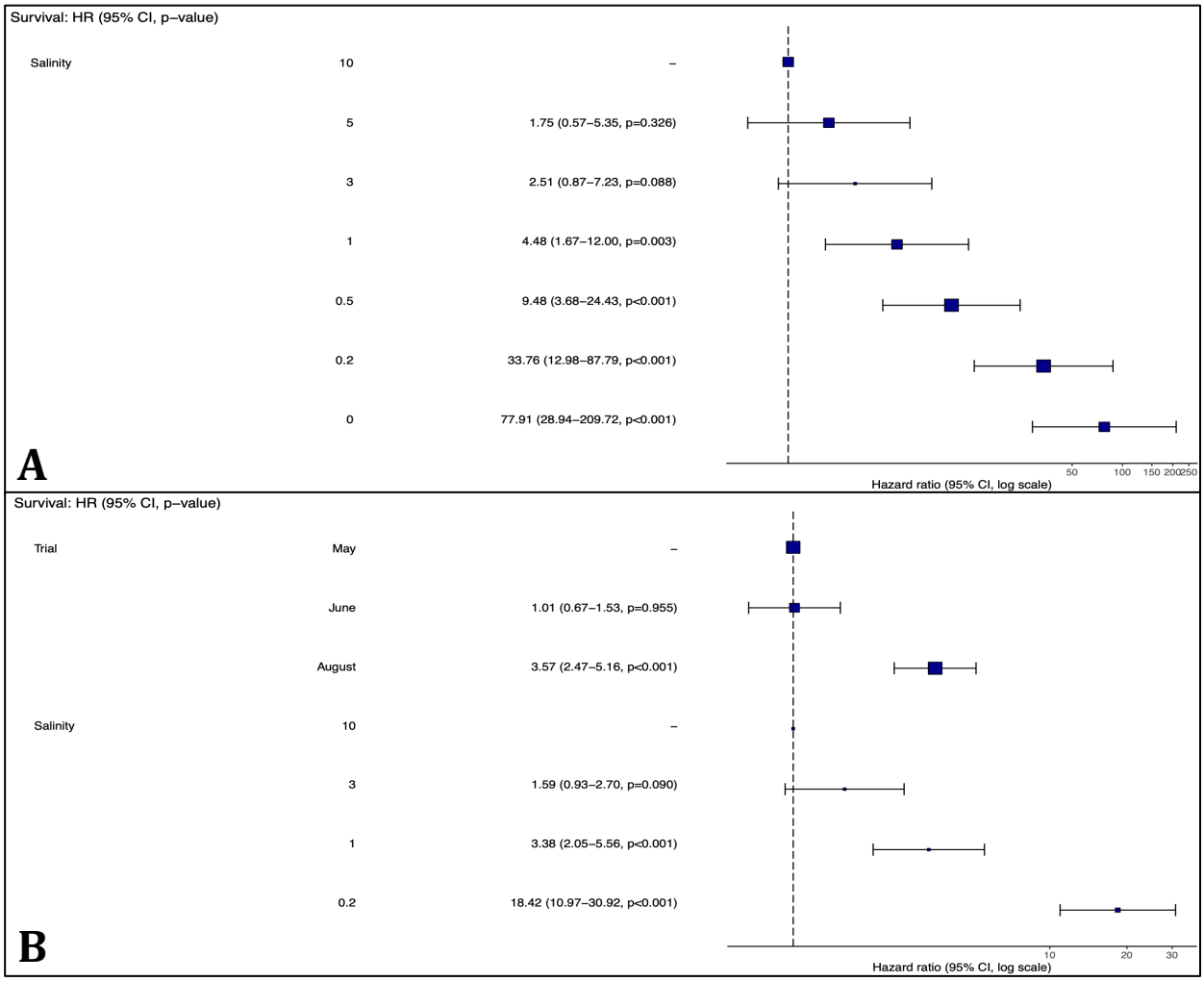

Fig. 2 Hazard Ratio Plots A) Hazard Ratio plot for SQ and MD B)Hazard ratio plot for Within SQ.

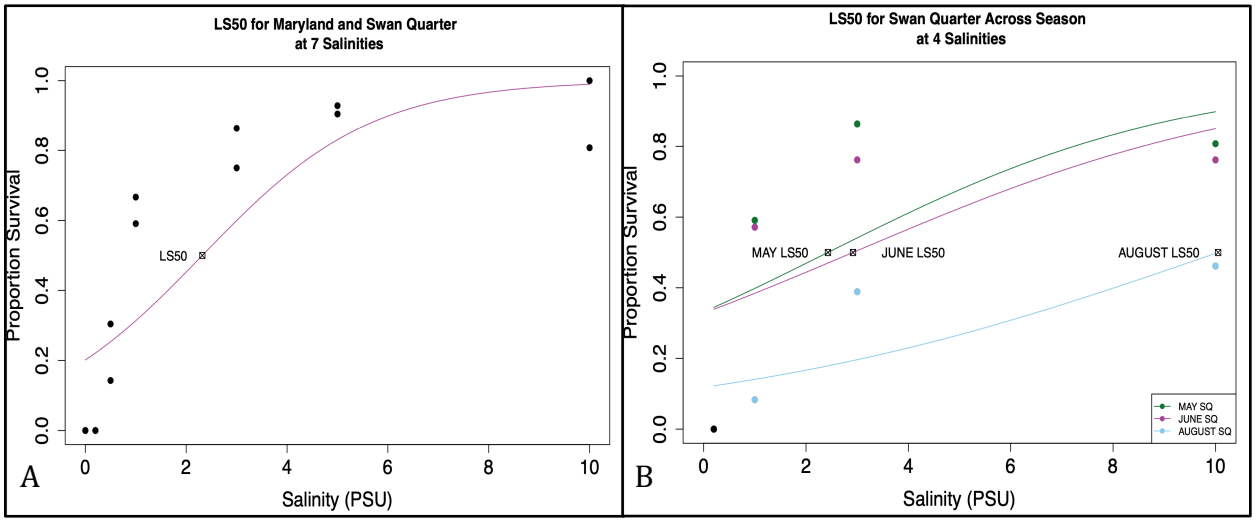

Fig. 3 Lethal Salinity 50% Mortality Plots. A) LS50 including the combined data from Saint John's Pond, MD and Swan Quarter, NC across seven salinities: 10 PSU, 5 PSU, 3 PSU, 1 PSU, 0.5 PSU, 0.2 PSU, 0

PSU, B) LS50 comparing SQ across season at four salinities: 10 PSU, 3 PSU, 1 PSU, 0.2 PSU, black points show where proportion survived was equivalent for LS50 curves.

Righting Response

The top performing model for predicting Exposure RR time included only salinity as a predictor (Table S9, S10, Appendix A). All salinities were significantly different ($p<0.001$) from the control (10 PSU) group (Table S11, Appendix A) (Fig. 4A), indicating that crabs took longer to right themselves at treatment salinities under 10 PSU. All individuals in the 0 PSU and 0.2 PSU for the Exit RR were dead by the end of the treatment and, as a result, were excluded from the analysis. The top performing model for predicting Exit RR time included only CW as a predictor, but the model results indicated that other variables were driving the significance (Table S12, S13, Appendix A). A linear regression indicated that no significant relationship was present between CW and Exit RR time (Figure S.6). The second-best model included only salinity, but only 3 PSU ($p = 0.00302$) and 1 PSU ($p = 0.00338$) were significantly different from 10 PSU (Table S14, Appendix A). Prolonged exposure to reduced salinity increases RR time at salinities below 5 PSU and above 0.5 PSU which is close to the LS_{50} value for *E. depressus* obtained from this study (2.32 PSU).

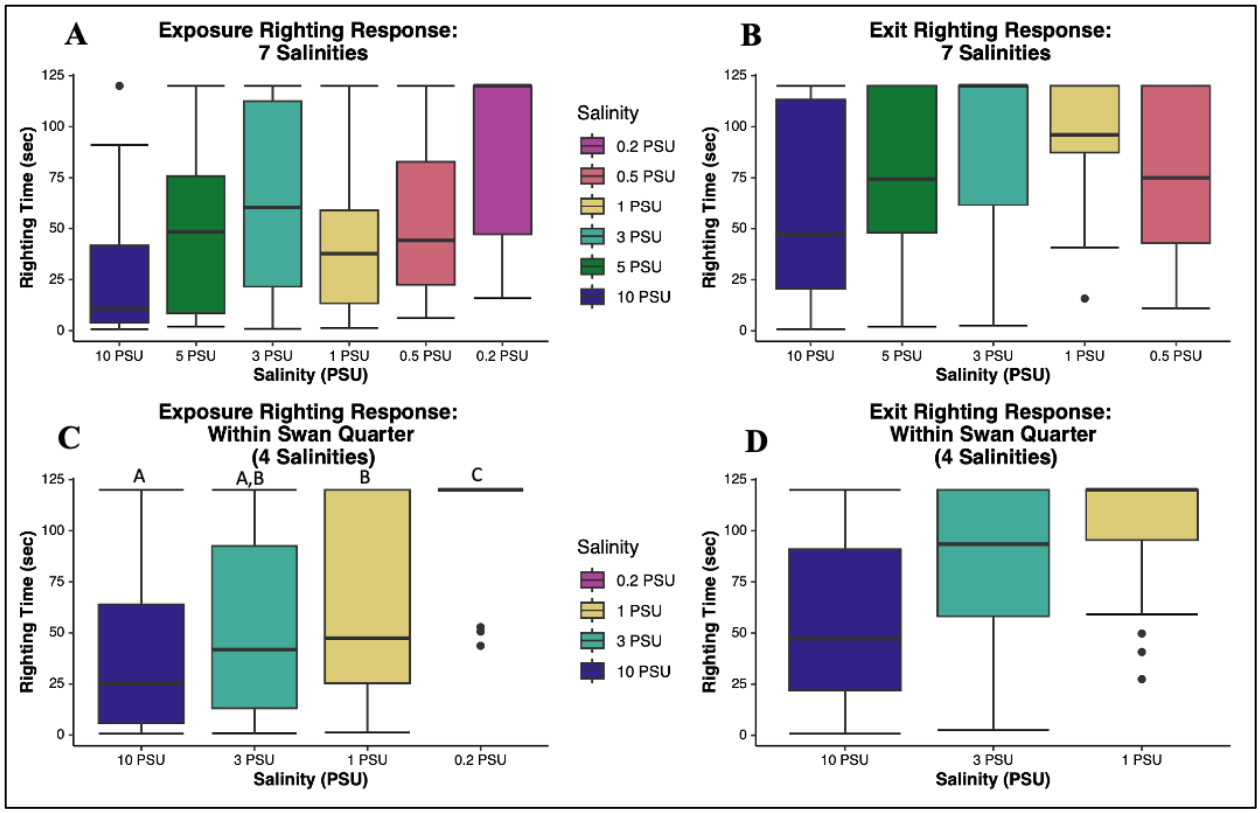

Fig. 4 A) SQ & MD Exposure Righting Response across seven salinities: 0 PSU (all dead), 0.2 PSU (n=15), 0.5 PSU (n=34), 1 PSU (n=34), 3 PSU (n=33), 5 PSU (n=33), 10 PSU (n=39). B) SQ & MD Exit Righting Response across seven salinities: 0 PSU (all dead), 0.2 PSU (all dead), 0.5 PSU (n=9), 1 PSU (n=21), 3 PSU (n=28), 5 PSU (n=32), 10 PSU (n=35).C) SQ Exposure Righting Response (completed after 24 hours) at four salinities: 0.2 PSU (n=18), 1 PSU (n=52), 3 PSU (n=59), 10 PSU (n=70). D) SQ Exit Righting Response (completed on Day 21) at four salinities: 0.2 PSU (all dead), 1 PSU (n=26), 3 PSU (n=41), 10 PSU (n=49). Bonferroni's pairwise comparison was used to distinguish groups, only Exposure Within SQ showed significant differences using Bonferroni's.

Seasonal Differences at Swan Quarter

Survival

Adult *E. depressus* survived best at experimental salinities at and above 3 PSU. The survival probability across treatments after 21 days (irrespective of month) varied from 0 to 76.2% (Fig 1. B-D). For the control salinity (10 PSU), survival probability after 21 days ranged from 50 to 76.2%, with August having the lowest survival probability. For each month, salinity was a significant factor in determining the survival of adult *E. depressus* (Kaplan-Meier $p < 0.0001$). For May, only two *E. depressus* in the 0.2 PSU treatment survived beyond 10 days and

only one crab survived past 10 days in June for the same treatment. All of the 0.2 PSU crabs died within 24 hours of exposure for August. Based on the CoxPH assessment, the additive model of individual factors salinity, month, CW and sex were the best predictors of *E. depressus* survival (AICc Wt. = 0.52; Table S6, Appendix A). Gravid females had significantly higher survival compared to males and females (Figure S5, Table S8, Appendix A). The Hazard Ratios for May and June were not significantly different, but crabs in the August trial were 3.57 times more at risk of mortality compared to that of those from May (control) (Fig 2.B). In general, August had the lowest number of *E. depressus* alive at the end of the experiment compared to May and June, impacting the LS_{50}s for each month (Figure S2, Appendix A). August had the highest LS_{50} of 10.05 PSU, compared to May (2.43 PSU) and June (2.92 PSU) (Fig 3B), indicating that crabs collected in August died at higher salinities. Irrespective of month, the lowest salinities - 1 PSU and 0.2 PSU - had significantly higher mortality than the control (Figure 2B).

Righting Response

The top performing model for predicting Exposure and Exit RR time included only salinity as a predictor (Tables S15, S16, S17, S18 Appendix A). For the Exposure RR, all crabs in the 0 PSU treatment died within 24 hours and were excluded from this analysis. Irrespective, crabs in lower salinities (0.2 PSU, 1 PSU, 3 PSU) took a significantly ($p<0.01$) longer time to right as compared to the control (10 PSU) (Table S15, Figure 4.C) For the Exit RR, all crabs in the 0 PSU and 0.2 PSU treatments died by the end of the experiments and were excluded from this analysis. Irrespective, crabs in lower salinities (1 PSU, 3 PSU) took a significantly ($p<0.01$) longer time to right as compared to the control (10 PSU) (Table S16, Figure 4.D).

Discussion

As anthropogenic change continues to impact terrestrial and marine systems alike, it is increasingly important to understand biotic and abiotic drivers of species distributions, in order to predict how community composition may shift with time. In estuaries, salinity is considered a driving force behind observed species distributions in estuaries alongside other key abiotic factors and the optimum range for survival can be different depending on the species (Anger, 2003; Hulathduwa *et al.,* 2007). Biotic factors like species interactions or parasitism can also drive the distribution of organisms (Armstrong & McGehee, 1976). In this system, an invasive Rhizocephalan, *L. panopaei,* poses a threat to native mud crabs, like *E. depressus*, because it is a parasitic castrator, resulting in the host effectively becoming biologically dead since it can no longer contribute to the gene pool (O'Brien & Van Wyk, 1985). Prior lab and field work have shown that *L. panopaei* is not capable of tolerating salinities below ~10 PSU (Walker & Clare, 1994; Tepolt *et al.*, 2020; Blakeslee *et al*., 2021). In addition, recent work in North Carolina estuaries has shown that *E. depressus* is also not observed at sites <10 PSU (Blakeslee *et al*., 2021), prompting our question of whether salinity is a primary driver of this observation. Such an understanding of the salinity tolerance range of *E. depressus* could provide an indication of whether this crab species can exploit low salinity refugia to escape its invasive parasitic castrator (Blakeslee *et al.,* 2021). We examined this question by investigating both lethal (mortality experiment) and sublethal effects (RR) of salinity using salinity treatments at and below 10 PSU. Below we discuss our main findings.

Lethal Effects

Previous studies have examined the osmoregulatory ability of *E. depressus* and found it to be hyperosmotic at 15 PSU and 5 PSU; *E. depressus* is also capable of stabilizing its osmolality within 4 hours to respond to acute salinity shock, which is important due to the rapid

changes in salinity that can occur in estuaries (Walls 2006; Van Horn & Tolley, 2009). Our results examining mortality using important demographic predictors (size, sex) and salinity determined that *E. depressus* is capable of tolerating salinities well below 10 PSU in the laboratory. LS_{50} results demonstrated 50% survival at 2.43 PSU and 1.87 for individuals from two different locations. Populations from southwest Florida were observed in salinities down to 9.5 PSU in the wet season by Van Horn & Tolley (2008) and down to 5 PSU near Boca Raton, FL, USA (Garcés, 1987). Although Hulathduwa *et al.* (2007) found that Gulf of Mexico *E. depressus* had an LS_{50} of 0.2 PSU, those populations have a different coevolutionary history with *L. panopaei* (host and parasite have coevolved together) than the *E. depressus* used in this study, where the parasite is invasive (Hines *et al.,* 1997; Kruse *et al.,* 2011).

Even though we found *E. depressus* was capable of high survival at salinities below 10 PSU (particularly 5 and 3 PSU) over three weeks in the lab, our experiment only involved changes in salinity and did not include other ecologically important factors that may be limiting the distribution of *E. depressus* in low salinity waters. Notably, the preferred habitat of *E. depressus* is oyster reefs, and crabs are impacted more strongly by desiccation stress outside of this habitat (McDonald, 1982; Van Horn & Tolley, 2009). Oysters have been found to have a wide salinity tolerance, but field and laboratory studies have indicated differences in survival and condition depending on the source population and local conditions (Breuer, 1962; Lowe *et al.,* 2017; Marshall *et al.,* 2021). Moreover, *E. depressus*, along with other mud crabs, are important predators of oyster reefs and compete with one-another for resources in this preferred habitat (Brown *et al.,* 2005; Grabrowski *et al.,* 2008). Other studies on blue crabs, hermit crabs and stone crabs have shown that survival is associated with the ability to exploit available shelter refuge to escape predation. This evidence may suggest that a lack of shelter availability,

particularly preferred oyster reef habitats, in low salinity (< 10 PSU) sites of Atlantic estuaries could be influencing the distribution of *E. depressus* (Bertness, 1981; Beck, 1995; Heck & Coen, 1995; Shervette *et al.,* 2004). *E. depressus* along with at least two other species of mud crabs have been found to be directly reliant on shell-based habitats for various life history traits and *E. depressus* abundance is significantly related to oyster reef health and biomass (Meyer, 1994; McDonald, 1982; Menendez, 1987; Weber & Epifanio, 1996; Searles *et al.,* 2022). Furthermore, Hulathduwa *et al.* (2011) showed that as salinity increased, *E. depressus* showed increased dominance for shelter refuges compared to other mud crabs, notably *R. harrisii*, which is typically found in areas that are near fresh to moderate in salinity, and coincidentally, where there are less predators.

There were differences in the LS_{50} for populations collected from NC across different months; in particular, individuals collected in August had higher mortality rates at higher salinities than previous months. We believe this higher mortality rate may be due to temperature stress naturally experienced at the field site. The sea temperature at collection in August was 7 ºC higher than May and 3 ºC higher than June. Like other aquatic invertebrates, the oxygen consumption rate in *E. depressus* increases as temperature increases, and oxygen consumption can be significantly lower at 24ºC compared to 33ºC (Neurohr, 2013). Further, Garcés (1987) found an upper thermal limit of 30ºC for *E. depressus*, but field observations from Florida document the species in water temperatures up to 31ºC (Grizzle, 1974). Because crabs sourced during August were at temperatures approaching the thermal limit of the species (29.4 ºC), we suspect that these crabs likely had an underlying level of stress that was higher than the earlier trials and resulted in a greater risk of mortality. Even our control treatment (10 PSU), which demonstrated high survival in all other month, experienced relatively high levels of mortality.

However, as we did not directly measure oxygen consumption or other methods of assessing thermal stress, we can only speculate that the high field water temperatures the crabs were experiencing affected laboratory survival rates. Future studies should evaluate the interactive effects of low salinity and high temperature on survival to establish the limitations of *E. depressus* survival in these conditions.

Sub-Lethal Effects

In low salinity water, the concentration of salts in the medium is reduced and as a result, estuarine organisms may show various adaptive responses to cope with osmotic stress (Mantel & Farmer, 1983; Rainbow & Black, 2001). Osmotic stress can affect the acid-base status of the haemolymph in osmoregulating crabs through physiological modifications in the cell (Truchot, 1981; Whiteley *et al.,* 2001). However, these physiological modifications can require substantially more energy, particularly in hypo-osmotic conditions (Setiarto *et al.,* 2004; Silvia *et al.,* 2004, Tseng & Hwang, 2008). The increase in required energy can lead to lower energy availability for development and depletes lipids found in the hepatopancreas of mud crabs (Li *et al.,* 2017; Luo *et al.,* 2023). This reduction in energy availability could lead to longer RR times, since the crabs are likely attempting to maintain homeostasis. Therefore, RR time provides a qualitative measure of non-lethal stress that *E. depressus* may be experiencing in hypo-osmotic environments due to greater energy expenditure. Righting response has been used in several studies to evaluate the level of non-lethal stress experienced by crabs (e.g., Rebach, 1974; Wilson, 1989; Blakeslee *et al.*, 2015; Blakeslee *et al*., 2021; Ro *et al.*, 2022) because longer righting response times renders the individual more susceptible to predation (Wilson, 1989).

Despite being able to tolerate salinities well below what is observed in the field, there does appear to be a trend for low-salinity sub-lethal stress on *E. depressus* (Figure 4). Inevitably,

there may be a trade-off associated with exploiting low salinity refugia to escape *L. panopaei* through increased osmotic stress (Blakeslee *et al.,* 2021). Parasite avoidance can take on many forms (e.g. infection source avoidance, infected mate & conspecific avoidance), and is generally less energetically expensive than immune responses to infection (Hamilton & Zuk, 1982; Dugatkin *et al.,* 1994; Karvonen *et al.,* 2004; Curtis, 2014). Exploitation of salinity refugia to escape parasitism has been seen in desert stream pupfish to avoid trematode infection despite costs associated with hyper-osmotic conditions (Kinne, 1960; Haney *et al.,* 1999). However, parasitism may be more costly than salinity as shown by Rogowski & Stockwell (2005) by comparing pupfish condition at high, low and intermediate salinity sites. Due to *L. panopaei* castrating its hosts (rendering them biologically dead post-infection) it is possible that parasitism may become more costly than osmotic stress (Alvarez *et al.,* 1995).

Conclusion

In our study, we determined that *E. depressus* is capable of tolerating and surviving for extended periods of time (21 days) at salinities well under the observed field distribution of the organism in North Carolina Estuaries. Our within-site study at Swan Quarter represents the first, to our knowledge, seasonal examination evaluating consistency in response over time in this system. This new data describing populations of *E. depressus* in North Carolina and Maryland and is different from the low-salinity tolerance previously reported for populations of *E. depressus* from the Gulf of Mexico (Hulathduwa *et al.,* 2007). Since the seven-salinity study provided a higher resolution investigation into low salinity tolerance for *E. depressus*, future studies should aim to establish why it is not found in the lower salinity sites in North Carolina estuaries via *in situ* and *transplant* field experiments of *E. depressus* survival across salinities. Past work has found that the favored habitat for *E. depressus* is oyster reefs (McDonald, 1982;

Van Horn & Tolley, 2009) and that it competes for resources with other mud crabs and likely also for favored habitat to escape predation (Brown *et al.,* 2005; Grabrowski *et al.,* 2008). A competition experiment should be established in the future to evaluate the dominance of *E. depressus* against competitors (i.e. *R. harrisii*) in salinities under 10 PSU since the righting response trials showed increase stress at lower salinities. The increased stress at salinities < 10 PSU will likely impact the ability of *E. depressus* to outcompete co-occurring mud crabs in favored habitat (Rebach, 1974; Wilson, 1989; Blakeslee *et al.*, 2015; Blakeslee *et al*., 2021; Ro *et al.*, 2022). Future studies should also aim to determine the differences in salinity tolerances between adult and juvenile *E.* depressus because although adults may be capable of tolerating a broad range of salinities, several studies investigating crustacean reproductive success and larval/juvenile survival and development in low salinities have observed reduced success or different optimum ranges than the adult counterparts (Steele & Steele, 1991; Brown & Bert, 1993; Charmantier *et al.,* 2002; Anger, 2003).

Our study shows that *E. depressus* in Mid-Atlantic estuaries may be capable of exploiting low-salinity refugia to escape its parasitic castrator, *L. panopaei.* However, any available refugia may have an associated trade-off of increased non-lethal stress and rates of oxygen consumption (Walls 2006; Van Horn & Tolley, 2009; Neurohr, 2013; Blakeslee *et al.,* 2021). Further investigations into *E. depressus* will be necessary to fully tease-apart the question of whether *E. depressus* truly has a refugia in low-salinity sites since there is a small amount of literature directly relating to the ecology of this mud crab species.

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CHAPTER 2: PARASITE DIVERSITY AND PREVALENCE IN NATIVE PANOPAEIDS OF TWO NORTH CAROLINA ESTUARIES

Introduction

Despite many parasites being invisible to the naked eye, they are still important components of all types of communities, both terrestrial-based and marine-based. There is research that suggests that parasites can impact the structure of communities by impacting species abundance and these parasites act as strong biotic factors in helping to create biodiversity (Minchella & Scott, 1991; Price *et al.,* 1986; Freeland, 1983). Parasites are able to impact these communities in different ways: they may decrease the abundance of some species more than others based on species-specific susceptibility, they may decrease functional importance of the host species through lowering host health, or they may increase functional importance of their host (Poulin, 1999). Furthermore, the successful establishment of a parasite within different hosts may be impacted by how taxonomically related the different species are to one another (Cameron, 1964; Jenkin, 1963). In addition, since many parasites have complex life cycles (Auld & Tinsley, 2015; Parker *et al.,* 2003; Orlofske *et al.,* 2012), understanding parasite composition can further expand the knowledge of food web interactions within communities (Parker *et al.,* 2015; Lafferty *et al.,* 2008).

This chapter is focused on identifying the various parasites that infect *Rhithropanopeus harrisii* and *Eurypanopeus depressus* at sites where the two species co-occur in two North Carolina estuaries: the Pamlico and Neuse Rivers. These specimens were collected during prior investigations in the Blakeslee Lab (e.g., Blakeslee et al. 2021) from 2016 – 2022. This research works to leverage the past data (2016-2021) with newly collected data (2022) to gain a broader

understanding of the influence of host-preference and seasonality in host-parasite composition. In addition, while I was examining *E. depressus* in Chapter 1, I did not detect any macro-parasites , which was unexpected especially as I did find parasitic infections in *R. harrisii* in my pilot investigations for Chapter 1. During these analyses, any individuals that were found to be infected with *Loxothylacus panopaei* during processing were excluded from the study since my study was focused on uninfected crabs. However, all *E. depressus* were dissected and evaluated for metazoan endoparasites after exiting the assigned treatment or after death. Moreover, as above, I analyzed 6 years' worth of field collected crabs from sites where the two species co-occur. Aside from a direct comparison of the two crab species in terms of prevalence of *L. panopaei* specifically (Tepolt et al. 2020), to our knowledge, no studies have yet performed a comprehensive comparative study of the metazoan macroparasite diversity in these two crab species, which act as important members of these estuarine communities by serving as predators and prey (Kulp *et al.*, 2016; Puntila-Dodd *et al.,* 2019).

There were several parasites that we expected to observe infecting *R. harrisii* and *E. depressus* in these estuaries. The first of which was *L. panopaei*, which is invasive along the East Coast of the United States (Hines *et al*., 1997). *L. panopaei* can infect a variety of Xanthid mud crabs (Hines *et al.,* 1997; Kruse *et al.,* 2011), including *Panopeus lacustris, Panopeus simpsoni, Panopeus obesus, Eurypanopeus depressus, Dispanopeus sayi,* and *Rhithropanopeus harrisii*. However, in the parasite's invasive Atlantic populations, only *E. depressus* and *R. harrisii* have been identified with infections of *L. panopaei* (Tepolt et al., 2020). Infection by *L. panopaei* has been shown to have a negative effect on survival of the host (Alvarez *et al.,* 1995) and it eliminates reproductive capabilities of the host by castration (O'Brien & Van Wyk, 1985). Another anticipated parasite was the entoniscid isopod, which are endoparasites of anomuran and

brachyuran crabs (Shields & Earley, 1993). Entoniscid isopods in our region belong to the genera *Cancrion* and *Cryptocancrion,* and have also been found to infect Xanthid crabs in North and South America (Shields & Earley, 1993; Moore et al. 2020; Williams et al., 2023; Greenberg et al., in prep). Like the rhizocephalan *L. panopaei*, entoniscids can castrate their hosts (Giard, 1887; Blakeslee, unpublished; Greenberg et al., in prep) and can feminize male crabs (Reinhard, 1956).

In addition, a prior survey by Kroft & Blakeslee (2016) showed the infection of three other parasites (aside from *L. panopaei*) in native panopeid mud crabs, which were trematodes, nematodes, and acanthocephalans. Trematodes are a type of parasite with a complex life cycle, in which various stages of the parasites occur in different hosts (Poulin & Cribb, 2002). Crustaceans can act as the second intermediate hosts for trematode infections, during which time cercaria develop into a metacercaria which is typically within a cyst inside the host (Galaktionov *et al.,* 1996). A review done by Thieltges *et al.* (2009) found that crustacean hosts have a limited richness of trematode species, where many crab hosts only show infection by at most 2 trematode species, likely due to a limited number of trematode species that utilize crabs as intermediate hosts.

Crabs have also shown infection by acanthocephalans and nematodes as well, both of which have complex life cycles (Poulin *et al.,* 2003). In addition, parasites belonging to Digenea, Cestoda, Acanthocephala and Nematoda are highly likely to utilize crustaceans as intermediate hosts in their life cycles (Marcogliese, 1995). Acanthocephalans occur within the body cavity of the crabs (Brockerhoff & Smales, 2002) and have been shown to affect activity levels of infected crustaceans (Haye & Ojeda, 1998). This alteration of behavior may be related to the fact that the transmission of acanthocephalan from crustacean to the final host is dependent on predation, so

the parasite alters the host behavior to increase vulnerability (Holmes & Bethel, 1972). Many nematode species require the use of an invertebrate intermediate host for the completion of their life cycles (Marcogliese, 1996). Juvenile acuariid nematodes can also be found in the body cavity of crustacean hosts and the haemocoel (Klimpel & Palm, 2011), which then mature in birds, fish or mammals following predation of crabs (Poulin *et al.,* 2003; Anderson, 1996). Cestodes also have a complex life cycle and are endoparasites within the intermediate and final hosts (Caira & Reyda, 2005). The presence of cestodes within crustaceans is not well documented (Gurney *et al.,* 2006), but infection by some cestode species may lead to a decrease in the digestive enzymes produced in digestive glands of the crab host (Gurney *et al.,* 2006).

In this study, we evaluated *E. depressus* and *R. harrisii* from the Pamlico and Neuse Rivers (2016-2022 collections) for metazoan macroparasites. The goals of this study were to establish the parasite prevalence and diversity between these two species at sites where they co-occur along these estuary systems. To our knowledge, this is the first long-term study evaluating parasites between these two species and provides an insight into the parasites that may impact native North Carolina mud crabs.

Methods

Study Sites

All organisms were collected from sites located along the Pamlico River and Neuse River. *R. harrisii* and *E. depressus* have been found to co-occur at these locations in previous field efforts by the Blakeslee Lab at East Carolina University (Blakeslee et al. 2021). Figure 5 shows a map of the sites used in this study. Wright's Creek and Swan Quarter Bell Island Pier (Swan Quarter) are both located on the Pamlico River. Matthews Point (now called Bishop's

Marina), Pin Oak Court and Cedar Island are all located on the Neuse River. The salinities at each of these sites range from approximately 10-15 PSU on average (Blakeslee *et al.,* 2021).

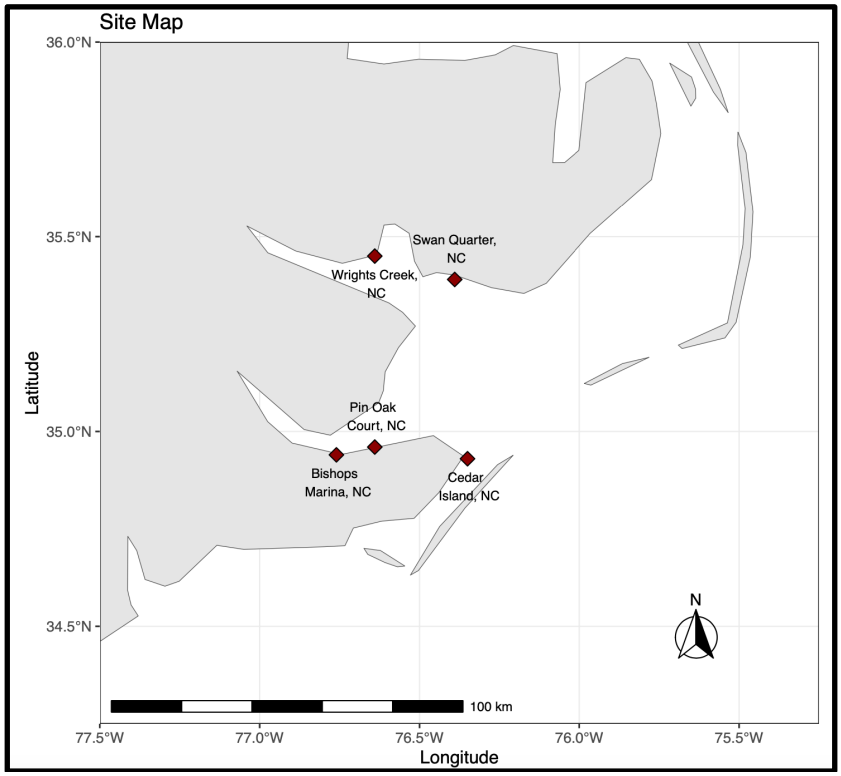


Fig. 5 Location of field sites used for collection of R. harrisii and E. depressus.

Field Sampling

Organisms were collected using standard sampling procedures by utilizing small plastic crates (19 x 22 x 16 cm) filled with oyster shells and covered with a mesh netting on the top opening (Fig. 6A), as in Chapter 1. Collectors were re-deployed after sampling to recruit more individuals for the study. Any organisms besides those utilized in the study were identified and counted prior to being released back into the estuary. The panopeids collected from each site were transported in small plastic containers (one container per site, Fig. 6B) back to the lab to record population demographics and establish parasite prevalence and diversity. In addition, prior collected specimens retained by the Blakeslee Lab from collections during 2016-2022 on

the Pamlico River and Neuse River were used to compare seasonality in the parasite composition as well.

Fig. 6 ***A)*** *Passive Sampler filled with autoclaved oyster shell (Photo credit: Dr. April Blakeslee)* ***B)*** *Transport container with paper towel dampened with water from the site and filled with R. harrisii and E. depressus individuals.*

Abiotic data recorded from sampling events consisted of: time of day, water temperature, air temperature and weather conditions at the time of collection. Other abiotic data consisted of the salinity and temperature of the water using a YSI Pro30 Conductivity Meter and dissolved oxygen will be measured with a YSI Pro20i Dissolved Oxygen Meter.

Population Demographics and Dissections

Carapace width (CW) was measured using calipers by measuring the farthest points across the carapace of each individual (Fig. 7). Each panopeid was sexed (Fig. 8) and checked for infection by *Loxothylacus panopaei. R. harrisii* (Fig. 9) can be identified by white tips on the claws and characteristic markings on the dorsal side of the carapace and lacks any red coloring inside the mandibles (Williams 1984). Conversely, *E. depressus* (Fig. 10) has small red rectangular markings on the inside of the mandibles and has darker claws than *R. harrisii*. The

red markings in *E. depressus* are present on both sexes and at all sizes. Some *Panopeus herbstii* were included in samples from previous years and those panopeids were distinguished by a small red marking on the mandibles of males and a larger knob-shaped tooth on the dominant claw of the crab (Williams, 1984); however, this species was not used in this study.

Fig. 7 Calipers are used to measure the carapace width of each panopeid.

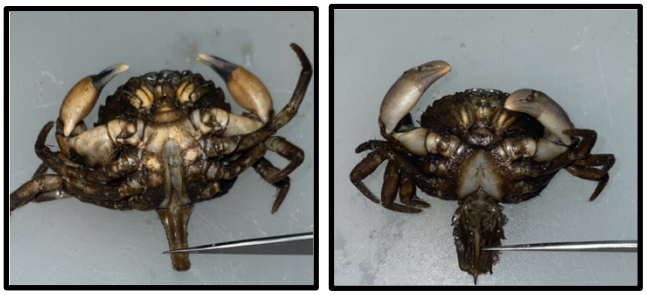


Fig. 8 Male (left) and female (right) E. depressus.

Fig. 9 Male R. harrisii with characteristic white claws (left) and indentation pattern on carapace (right).

Fig. 10 Typical E. depressus individual with black-tipped claws.

Live panopeids were euthanized prior to dissection. This was accomplished by organizing specimens into small plastic bags and placing them in a -80C freezer. This allowed the panopeids to gradually slow down metabolic processes until they were ready to be dissected. This is a more humane process than euthanizing the crabs via 100% Ethanol or by live dissections. Each dissection began by inserting a scalpel into the posterior end of the carapace and separating the dorsal and ventral pieces of the carapace. The hepatopancreas, gonads and ganglia (if able to be obtained) were removed from the interior cavity and placed on a slide for viewing under a microscope (Figure 11).

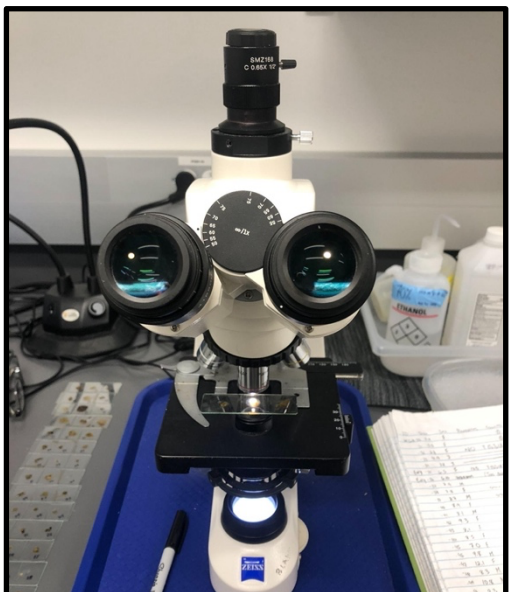


Fig. 11 Typical microscopy set up with slide containing hepatopancreas and gonads from a mud crab.

Parasite Identification

The parasites surveyed for were metazoan macroparasites. Figure 12 shows a *R. harrisii* individual with a mature larval sac protruding from its abdomen. Figure 13A shows what the internal organ of a crab would look like if they are infected with *L. panopaei* as well, and Fig. 13B shows what healthy hepatopancreas tissue looks like in comparison to the internal root structures.

Fig. 12 R. harrisii infected with L. panopaei. Two mature larval sacs are visible near the anterior portion of the abdominal flap.

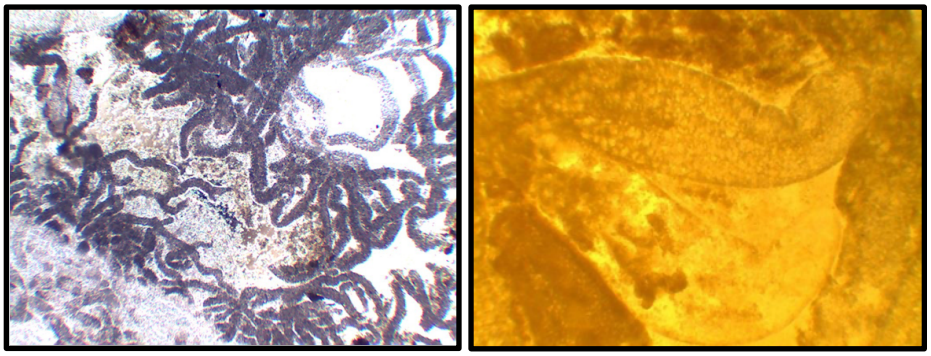


Fig. 13 A) Left, Internal root structures produced by the Rhizocephalan parasite, L. panopaei B) Right, Healthy hepatopancreas tissue from a crab that is not infected with L. panopaei. (Photo Credit: Dr. A.M.H. Blakeslee)

The entoniscid isopod encountered during dissection was likely *Cryptocancrion brevibrachium* (Williams *et al.,* 2023) found commonly in NC estuaries. The female entoniscid

(Figure 14A) is visible to the naked eye, but the male entoniscid (Figure 14B) looks more characteristic of a typical isopod.

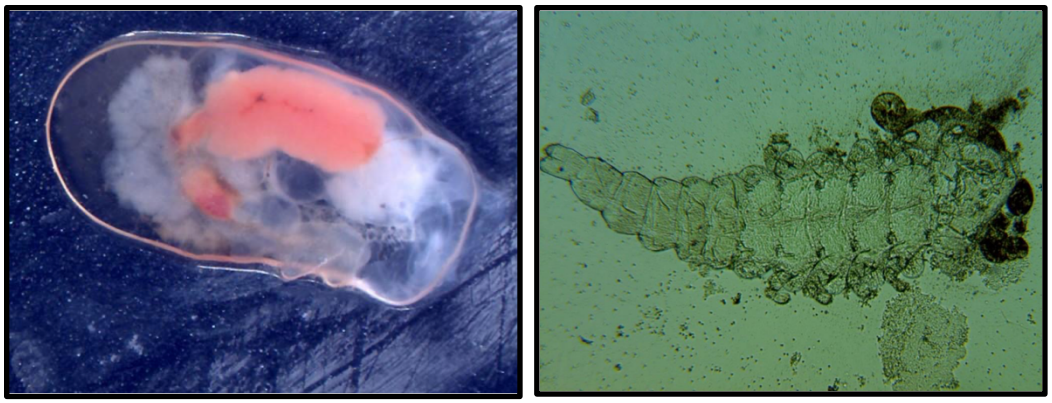


Fig. 14 A) Left, female entoniscid isopod Cancrion spp. This is visible to the naked eye. B) Right, male entoniscid isopod Cancrion spp. (Photo Credits: Dr. A.M.H. Blakeslee and Jason Williams)

Aside from entoniscids and *L. panopaei,* other anticipated metazoan macroparasites were: Nematoda or parasitic roundworms (Figure 15), metacercarial cysts of Trematoda (Figure 16), Cestoda or tapeworms (Figure 17) and Acanthocephalans in the acanthella stage of infection (Figure 18). Any parasites found outside of these groups were recorded and saved for genetic sequencing later to confirm species identity down to the lowest taxonomic level possible.

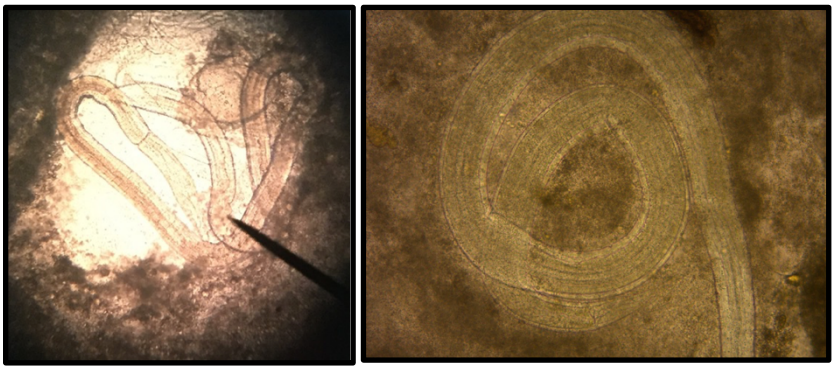


Fig. 15 Images of Nematoda (Parasitic roundworms) found in the hepatopancreas of a dissected crab. (Photo Credit: Dr. A.M.H. Blakeslee)

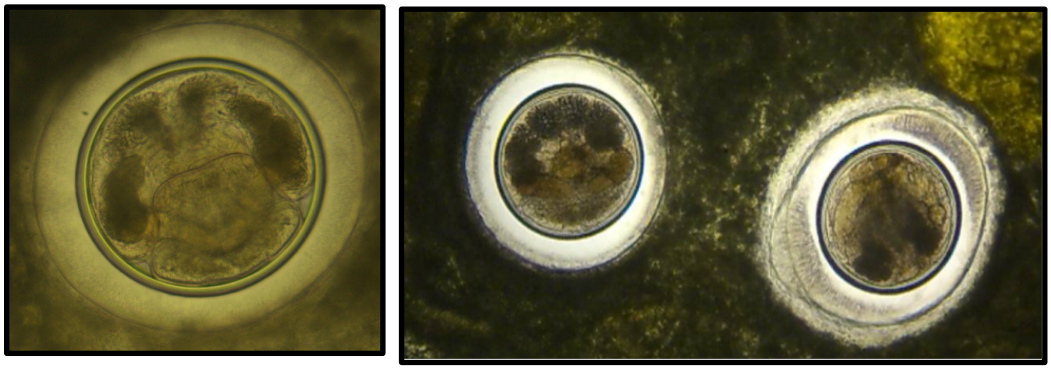


Fig. 16 Images of the metacercarial cyst stage of Trematoda (fluke) Microphallidae. (Photo Credit: Dr. A.M.H. Blakeslee)

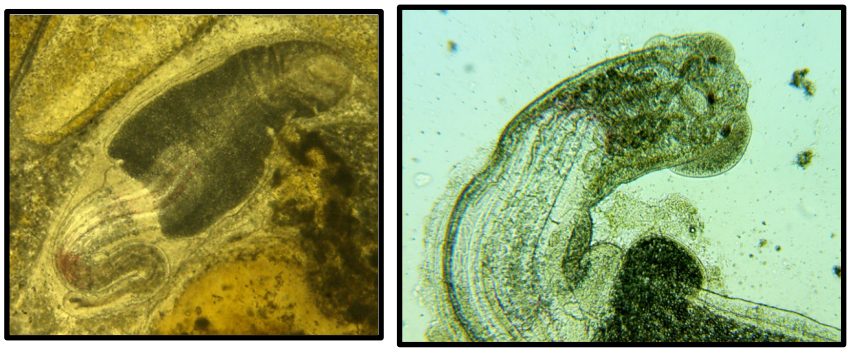


Fig. 17 Images of Cestoda (Tapeworms) found in a dissected crab. (Photo Credit: Dr. A.M.H. Blakeslee and Connor Hinton)

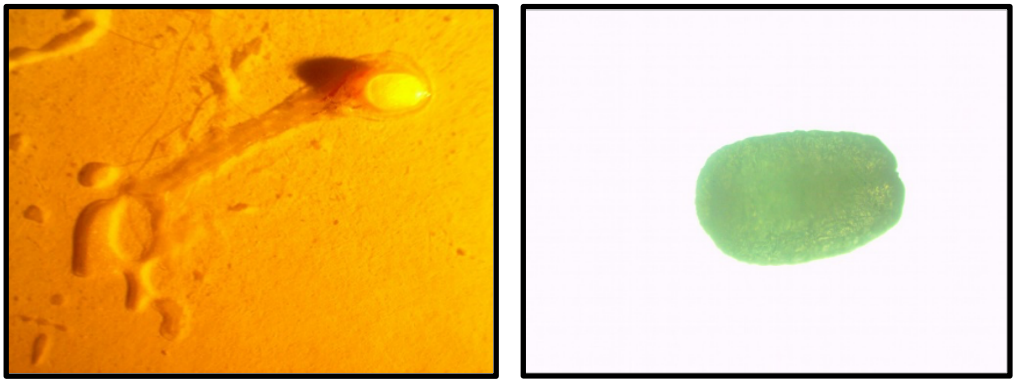


Fig. 18 Photos of the Acanthella stage of the spiny-headed worm parasite, Acanthocephala. (Photo Credit: Dr. Carolyn Tepolt)

Data Analysis

The data were analyzed using RStudio (Version 1.41717). The Kruskal-Wallis rank sum test was performed on data comparing total infection prevalence, entoniscid infection prevalence and *L. panopaei* infection prevalence between *R. harrisii* and *E. depressus*. Comparisons between the two species were also run using One-Way ANOVA for each specific site, by month and across years. Significant ANOVA model results were then evaluated using Tukey HSD post-hoc test to check for differences between pairs as a preliminary step in analysis. Parasite richness, diversity (down to the lowest taxonomic level possible) and prevalence were recorded during this data analysis.

Results and Discussion

Prevalence

Overall infection prevalence (Fig. 19A) for *R. harrisii* (mean prevalence = 0.207, n = 1615 crabs) and *E. depressus* (mean prevalence = 0.049, n = 407 crabs) was found to be significantly different (p = 0.0002051, Table S19 Appendix A). *L. panopaei* prevalence (Fig. 19B) was not significantly different between the two species; however, the prevalence of the Entoniscid (Fig. 19C) endoparasite was found to be significantly different (p = 0.004134, Table S19 Appendix A) which is likely what is driving the overall difference in prevalence between the two species. An important note is that we found 4 times as many R. harrisii at our site than E. depressus ((*E. depressus* n=407, *R. harrisii* n=1615); however, the strong differences in prevalence between the two species and the overall large sample size in our study suggest this difference in infection prevalence is not biased by the difference between the two species sample sizes.

A study by Eash-Loucks *et al.* (2014) examined long-term changes in a northeast Florida mixed oyster habitat due to *L. panopaei* and noticed a reduction in the *E. depressus* population after the first incidence of *L. panopaei* infection. They reported that the population of *E. depressus* was unable to recover over the course of the study and that *L. panopaei* appears to prefer infecting *E. depressus* to other similar mud crabs (*R. harrisii* was not found during their study period). It is possible that the comparatively low *E. depressus* populations to *R. harrisii* populations in the Pamlico and Neuse Rivers is due to this seemingly increased susceptibility to infection. A susceptibility experiment evaluating host preference for *L. panopaei* cyprids could provide insight to this phenomena. It is also possible that infection by *L. panopaei* is influencing predator-prey dynamics in these sites. *Callinectes sapidus* has been found to preferentially feed on *E. depressus* infected by *L. panopaei*; other predators were also more likely to feed on infected *E. depressus* compared to conspecifics, this could mean that predators may be impacting the abundance of *E. depressus* (Gehman & Byers, 2017). The lower parasitism prevalence in *E. depressus* compared to *R. harrisii* observed here may be an example of the 'healthy-herd' hypothesis; this suggests that predators can reduce the transmission of disease in prey when infected prey are preferentially consumed to uninfected prey although the opposite situation can occur (Packer *et al.,* 2003; Cáceres *et al.,* 2009; Duffy *et al.,* 2011).

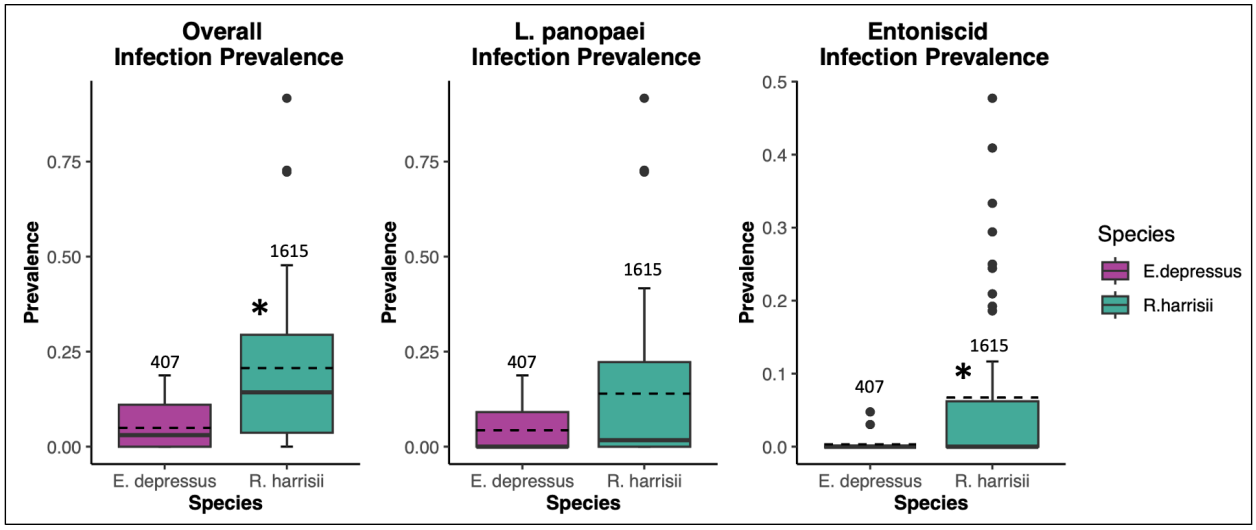


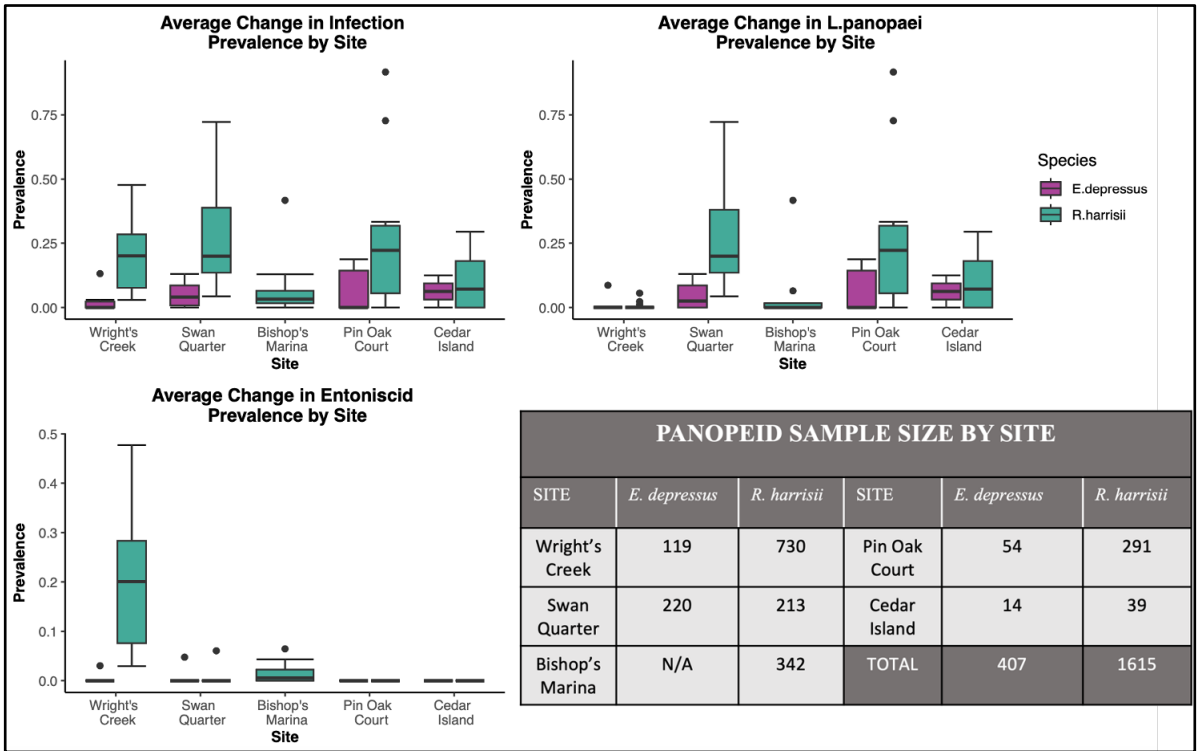
*Fig. 19 Comparison of infection prevalence for E. depressus and R. harrisii for **A)** Overall infection prevalence **B)** The invasive Rhizocephalan, L. panopaei infection prevalence and **C)** Entoniscid infection prevalence.*

Although we have yet to confirm the exact species of entoniscid observed in this study Williams *et al.* (2023) confirmed the presence of a new species of entoniscid, *Cryptocancrion brevibrachium* which is capable of infecting both *E. depressus* and *R. harrisii*. The other possible species would be *Cancrion carolinus* which has been shown infecting *R. harrisii*; both species of entoniscid have been found in North Carolina; therefore the parasite tissue will need to be evaluated for spp. confirmation (William *et al.,* 2023). While not much is currently known about the new species of entoniscid (*C. brevibrachium*), the internal parasite *Portunion sp*. (Isopoda: Entoniscidae) shows differential castration in intertidal crabs from New Zealand by fully castrating female hosts but not male hosts (Brockerhoff, 2004). Two of the four species of intertidal crabs from the same site also showed different rates of parasitism by the entoniscid, the authors suggested 1) inhabiting high-shore versus mid- to low- shore 2) length of time exposed to infective stages or 3) host cleaning as possible mechanisms for differential infection prevalence (Brockerhoff, 2004).

As outlined in the methods, there are five sites at which *R. harrisii* and *E. depressus* co-occur (Wright's Creek and Swan Quarter on the Pamlico; Bishop's Marina, Pin Oak Court and Cedar Island on the Neuse). The One-Way ANOVA (Table S20, Appendix A) was significant for each of the prevalence comparisons by site in Fig. 20. When evaluating the Tukey HSD output for *L. panopaei* prevalence by site, *R. harrisii* from Swan Quarter and from Wright's Creek were found to be significantly different ($p = 0.0106163$). Wright's Creek is typically at a lower salinity than Swan Quarter (Blakeslee *et al.,* 2021) and may experience salinities below 10 PSU (the low-salinity threshold for *L. panopaei*) due to freshwater input during the wet season which could result in lower *L. panopaei* infection prevalence. *R. harrisii* from Wright's Creek and Pin Oak Court were also found to be significantly different ($p = 0.0029399$), although because these two sites are on two different estuaries and are likely unconnected it is likely not an ecologically important comparison.

The ecologically important Tukey HSD results for Entoniscid prevalence were for *R. harrisii* from Wright's Creek compared to those from Swan Quarter ($p = 0.0000002$) and *E. depressus* from Wright's Creek versus *R. harrisii* from Wright's Creek ($p = 0.0000018$). However, because Wright's Creek *R. harrisii* have a high prevalence rate compared to the other sites (zero to low infection prevalence, Fig. 20C), the comparisons involving *R. harrisii* Wright's Creek were all significant ($p << 0.05$). Other studies have found that sheltered sites have a reduced prevalence for a rhizocephalan than exposed sites due to differences in current pattern which may affect exposure to infective stages (Alosairi & Pokavanich, 2017; Al-Wazzan *et al.,* 2021). Sheltered versus exposed sites may also allow for increased self-cleaning and different environmental conditions which could affect infection prevalence as well (Al-Yamani *et al.,* 2004; Høeg *et al.,* 2005). These factors could also impact the infection prevalence by site in this

study, but analysis of the average temperatures and salinities over the course of 2016-2022 for each site would be necessary.



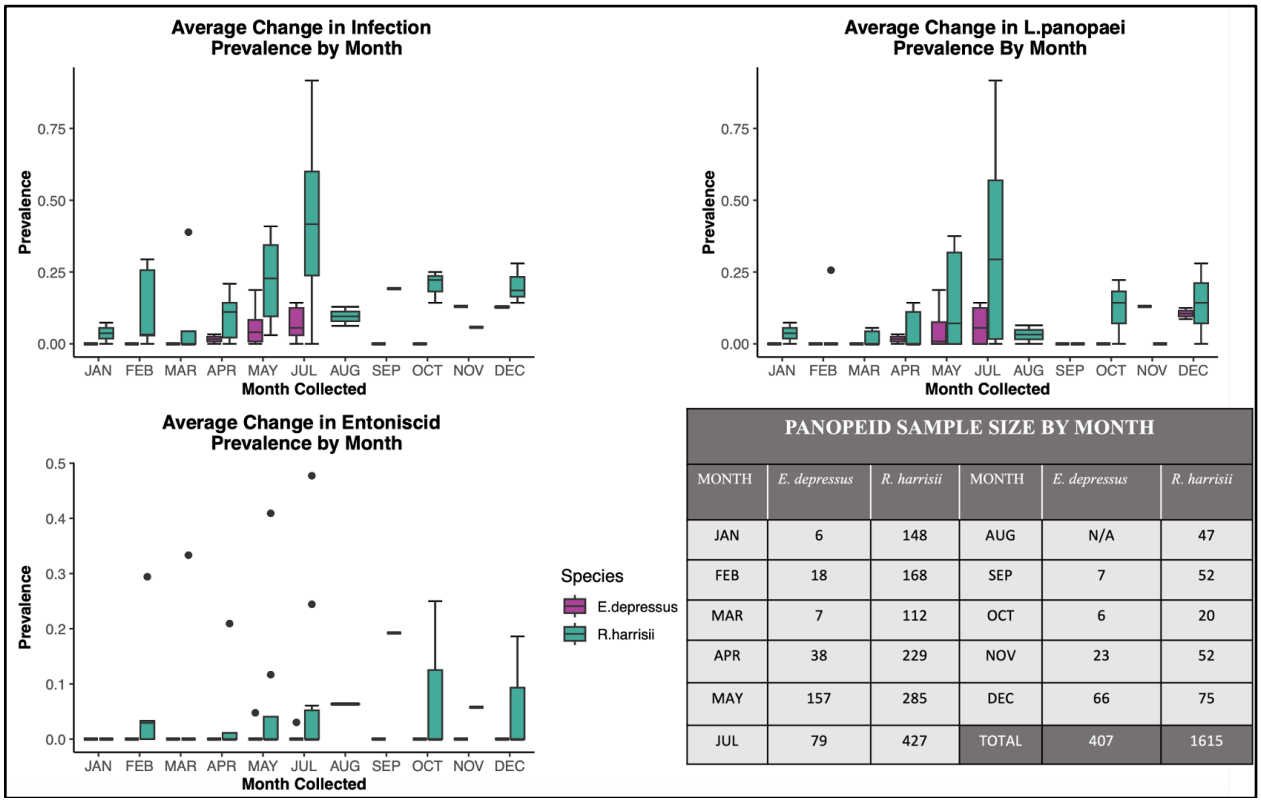
PANOPEID SAMPLE SIZE BY SITE

SITE	*E. depressus*	*R. harrisii*	SITE	*E. depressus*	*R. harrisii*
Wright's Creek	119	730	Pin Oak Court	54	291
Swan Quarter	220	213	Cedar Island	14	39
Bishop's Marina	N/A	342	TOTAL	407	1615

*Fig. 20 Change in Infection Prevalence by Site and grouped by Panopeid species **A)** Overall change in prevalence **B)** Change in L. panopaei by site **C)** Change in Entoniscid prevalence by site. Wright's Creek and Swan Quarter are located on the Pamlico Estuary. Bishop's Marina, Pin Oak Court and Cedar Island are on the Neuse Estuary. Table provides the number of replicates.*

Efforts by the Blakeslee Lab over the past several years (2016-2022) have consisted of sampling the established sites at least 3-4 times per year (approximately once per season). Infection status may change across the seasons in some species although host-parasite relations may not be cyclical and is likely taxon or habitat specific (Poulin, 2020), and as such we decided to evaluate the change in infection prevalence from month to month for both species. Figure 21 shows the infection prevalence distributed by month for both *R. harrisii* and *E. depressus*. Based on the general trend observed in Figure 21A, infection prevalence tends to peak in mid-summer (July) before tapering back down by winter when temperatures are much colder (One-Way ANOVA, $p = 0.0165$, Table S21 Appendix A). The same general trend can be observed for *L.*

panopaei in *R. harrisii* although the trend is less obvious for *E. depressus* (Fig. 21B). The One-Way ANOVA's were not found to be significant for *L. panopaei* infection prevalence by month and Entoniscid infection prevalence by month. Tukey's HSD did show a significant difference for *E. depressus* (July) compared to *R. harrisii* (July) ($p = 0.0439724$), but no other comparisons were found to be significant. It is worth noting that the large number of comparisons could be reducing the statistical power of the test, so it is likely necessary that a different post-hoc test will need to be completed for an accurate representation of the data.



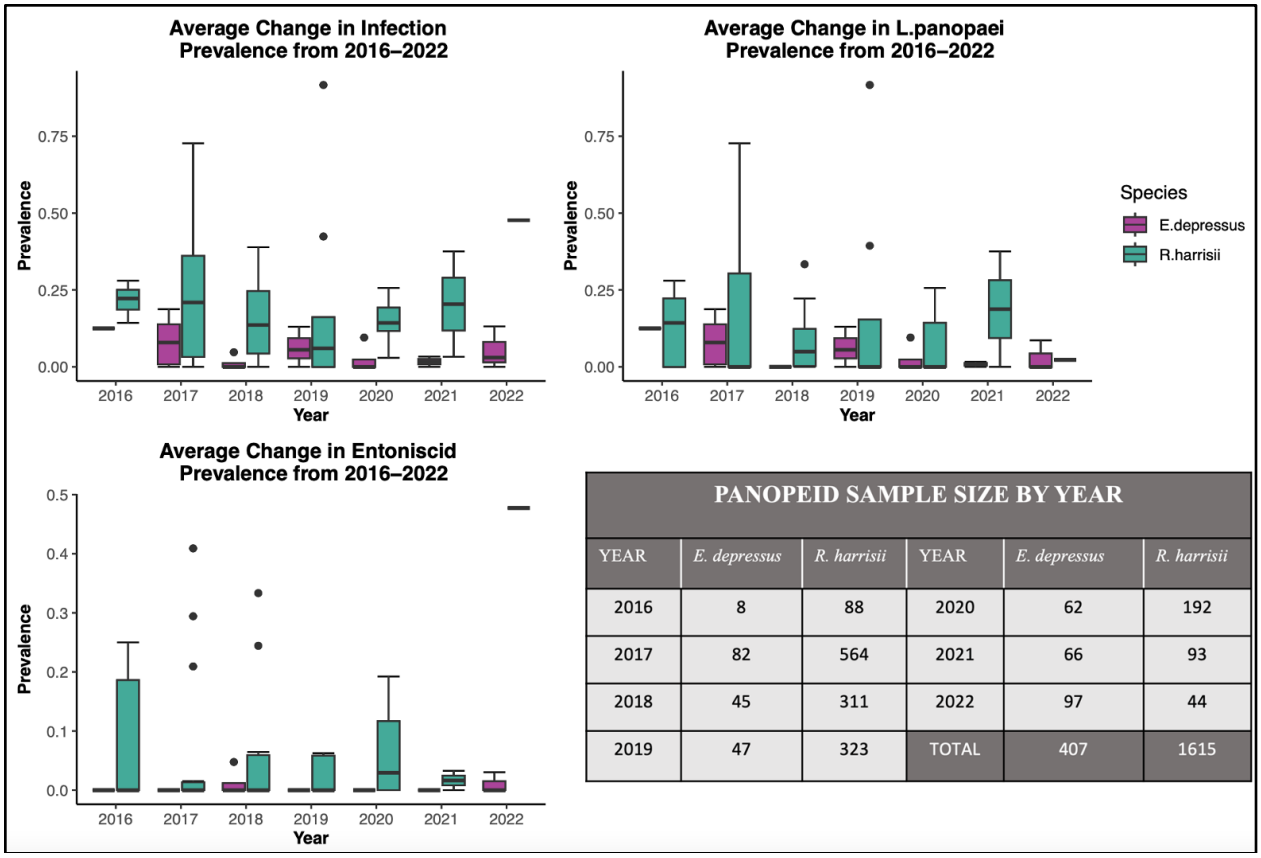
PANOPEID SAMPLE SIZE BY MONTH					
MONTH	*E. depressus*	*R. harrisii*	MONTH	*E. depressus*	*R. harrisii*
JAN	6	148	AUG	N/A	47
FEB	18	168	SEP	7	52
MAR	7	112	OCT	6	20
APR	38	229	NOV	23	52
MAY	157	285	DEC	66	75
JUL	79	427	TOTAL	407	1615

*Fig. 21 Change in Infection Prevalence by month and grouped by Panopeid species **A)** Overall change in prevalence **B)** Change in L. panopaei prevalence by month **C)** Change in Entoniscid prevalence by month. Table provides the number of replicates.*

The general trend observed for parasite prevalence throughout the year is an increase in prevalence from January through July, after which the prevalence drops again. This is likely linked closely with increasing water temperatures in the estuary which is complementary to a

study done in 2012 at Clambank Creek, North Inlet, South Carolina where prevalence of infection by *L. panopaei* peaked in June (O'Shaughnessy *et al.,* 2014). Another study (Davies *et al.,* 2019) found that *Hematodinium* sp. prevalence and intensity was greater in the spring but with lower intensity and infection prevalence was lower in autumn but had greater severity; they also noted that seasonal patterns seemed to be host-specific.

Figure 22 shows the overall change in infection prevalence for the three categories (total, *L. panopaei* and Entoniscid prevalence) from 2016-2022. Of which, only the One-Way ANOVA for Entoniscid prevalence was found to be significant ($p = 0.0159$, Table S22 Appendix A). *R. harrisii* from 2022 were significantly different from *E. depressus* from 2022 ($p = 0.0036650$, Tukey HSD) with *R. harrisii* having nearly a 50% infection rate (one site, Wright's Creek July 2022 n=44). Of the 97 *E. depressus* dissected from that year, 33 of the samples were also from Wright's Creek in July (prevalence = 0.03). The other 58 *E. depressus* were from Wright's Creek in December (prevalence = 0). Also, compared to 2016, *R. harrisii* in 2022 had significantly different Entoniscid infection prevalence ($p = 0.0184347$, Tukey HSD) although the samples from 2016 were from December and October of that year (Wright's Creek, Pin Oak Court, and Cedar Island).



YEAR	*E. depressus*	*R. harrisii*	YEAR	*E. depressus*	*R. harrisii*
2016	8	88	2020	62	192
2017	82	564	2021	66	93
2018	45	311	2022	97	44
2019	47	323	TOTAL	407	1615

*Fig. 22 Change in Infection Prevalence from 2016-2022 grouped by species **A)** Change in overall infection prevalence from 2016-2022 **B)** Change in L. panopaei infection prevalence from 2016-2022 **C)** Change in Entoniscid infection prevalence from 2016-2022. Table provides the number of replicates.*

R. harrisii (2022 versus 2017) were also significantly different in the Tukey HSD results ($p = 0.0040767$). 2017 was a particularly productive year for sampling with all five sites being represented at least twice over the course of the year and one-third of the total number of *R. harrisii* (2016-2022) are represented by 2017 (n=564, Fig. 22). By comparison there were only 44 *R. harrisii* collected in 2022 (Fig. 22). *R. harrisii* 2022 was also significantly different from each other year for *R. harrisii* (2018 $p = 0.0061184$, 2019 $p = 0.0015591$, 2020 $p = 0.0103710$, 2021 $p = 0.0095819$) although this once again may only be statistically relevant for years with similar sample sizes (Fig. 22). While there does not appear to be much overall change in prevalence from year to year for *R. harrisii* and *E, depressus* it is possible that a longer-term study may yield results. Quinn *et al.* (2021) compared historical (1969-1970) to contemporary

(2018-2020) parasite abundance and found that a parasitic isopod varied around a stable mean but trematodes increased in abundance over the 50 year span. They also did not observe a change in abundance for larval acanthocephalans. This provides evidence that temporal variation in abundance and prevalence may depend on the parasite and can make short term predictions difficult. For our study, entoniscid prevalence varied from year to year for *R. harrisii*, but it is possible that it may be moving about a stable mean that we are unable to observe due to the fairly short-term dataset. In this situation, continued observation would be necessary to draw any noteworthy conclusions about year to year prevalence in the two species.

Diversity

The two main contributors to overall infection prevalence for both *R. harrisii* and *E. depressus* were the invasive Rhizocephalan, *L. panopaei* and an Entoniscid. It is suspected that the species of Entoniscid observed is possibly *Cryptocancrion brevibrachium* which has been observed in North Carolina Estuaries and is a "short-armed" species capable of infecting *R. harrisii*; however it has not yet been confirmed if it is capable of infecting *E. depressus* (Williams *et al.,* 2023). Conversely, *E. depressus* and *R. harrisii* have both been found to be hosts for *Cancrion carolinus* which also occurs in NC Estuaries (Williams *et al*., 2023).

The only other metazoan macroparasite taxa observed at these five sites where the crabs co-occurred was Nematoda. However, unpublished data in the Blakeslee lab has found Digenean trematode cysts in *R. harrisii*, and Moore et al. (2020, 2021) also found trematode cysts in panopeid mud crabs at sites off the coast of Beaufort, NC.

There was one female *E. depressus* from Swan Quarter (May 2021) that was found to have one nematode present in the hepatopancreas. One *R. harrisii* that was already infected with *L. panopaei* (mature external sac) from Swan Quarter (July 2017) was also found to have a nematode present in what remained of the hepatopancreas. One possible explanation for the drastically low infection prevalence of nematodes in the panopeids could be the absence of one or more hosts that are required for the nematode's complex life cycle (Marcogliese, 1996). Although there were no other metazoan macroparasites observed for these two species at the study sites, there were two suspected cases of a microsporidian (a parasitic protist) in *R. harrisii* obtained from Bishop's Marina in April and May of 2017 (Hirt *et al.,* 1999). Spore morphology (Fig. 23) in the sample did appear to be similar to the description of microsporidian morphology provided by Refardt *et al*. 2008. Microsporidian infection has not yet been confirmed in either case but both samples were sent to an expert (J. Bojko) for analysis.

Fig. 23 Possible spores of a Microsporidian infection in R. harrisii from Bishop's Marina in April 2017. (10x Magnification, Sample ID: RHMPApr17-49)

Conclusion

This work represents the first, to our knowledge, comprehensive study of parasite diversity for *R. harrisii* and *E. depressus* from North Carolina Estuaries. Prevalence was primarily determined by *L. panopaei* and Entoniscid infection status for both species at sites where they co-occur and there appears to be a general trend for increased infection prevalence in the summer for both *R. harrisii* and *E. depressus*. Although the two species do show similar trends, it is important to note that *R. harrisii* tends to show higher infection rates for overall prevalence and for the Entoniscid. Thus, the absence of parasites observed in *E. depressus* from Chapter 1 of this thesis is consistent with the data presented herein and *E. depressus* is likely parasitized at lower rates than its panopeid compatriot.

There are multiple potential explanations for this observation of reduced parasitism rates in *E. depressus.* First, increased parasitism may have resulted in lower overall parasitism rates ('healthy herd' hypothesis) (Packer *et al.*, 2003). Parasitism could also be impacted by self-cleaning, habitat differences and exposure to infective stages of parasite larvae (Brockerhoff, 2004). Dittmer *et al.* (2011) found differences in parasite prevalence between two sympatric species of New Zealand shore crabs and observed differing immune responses between the two species; both species are of similar body size and co-occur at the sites in the study, but one showed higher rates of parasitism. Although the results did not explicitly show a correlation between immune response and infection prevalence, the two crabs do show different behaviors in the habitat. Differences in site prevalence could be driven by environmental factors or shelter from currents (Al-Yamani *et al.,* 2004; Høeg *et al.,* 2005; Alosairi & Pokavanich, 2017; Al-Wazzan *et al.,* 2021). In addition, variation in prevalence across seasons may be specific to the organism although rhizocephalans have been shown to have peak prevalence in the summer which is consistent with our data (O'Shaughnessy *et al.,* 2014; Davies *et al.,* 2019).

Future steps for this study include combining data sets from previous Blakeslee Lab investigators to obtain a more accurate representation of parasite prevalence for *R. harrisii* and *E. depressus* in the Pamlico and Neuse Estuaries. A comparison of parasite diversity and prevalence along a salinity gradient for *R. harrisii* would also be possible given that *R. harrisii* is abundant from near fresh to moderate salinities (Blakeslee *et al.,* 2021); although, *E. depressus* would need to be excluded in that analysis due to its absence from waters with <10 PSU salinity. Finally, a formal statistical analysis with more representative tests and comparisons would improve our understanding of the differences in parasite diversity and prevalence between the two mud crab species, and how this is influenced spatially and temporally.

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APPENDIX A: SUPPORTING INFORMATION

Table S 1: Sampling locations and their region, sample dates and coordinates.

Site	Region	Date	Latitude	Longitude
Swan Quarter Bell Island Pier	NC	05/11/2022	35.435715	-76.399092
Swan Quarter Bell Island Pier	NC	06/22/2022	35.435715	-76.399092
Swan Quarter Bell Island Pier	NC	07/29/2022	35.435715	-76.399092
Saint John's Pond	MD	08/01/2022	38.189361	-76.431676

Table S 2: Sample size for each treatment and trial used in the Low-Salinity Tolerance Experiment that were alive after the ramping period and were included in the analysis. SQ = Swan Quarter, NC, MD = Saint John's Pond, MD. M = Male, F = Female, G = Gravid Female.

Site	Trial	10 PSU	5 PSU	3 PSU	1 PSU	0.5 PSU	0.2 PSU	0 PSU
SQ	May	26 (M = 13, F = 7, G = 6)	N/A	22 (M = 12, F = 2, G = 8)	22 (M = 7, F = 9, G = 6)	N/A	26 (M = 11, F = 10, G = 5)	N/A
SQ	June	21 (M = 6, F = 8, G = 7)	N/A	21 (M = 3, F = 18, G = 0)	21 (M = 4, F = 14, G = 3)	N/A	22 (M = 6, F = 13, G = 3)	N/A
SQ	August	26 (M = 13, F = 11, G = 2)	N/A	18 (M = 4, F = 11, G = 3)	12 (M = 5, F = 6, G = 1)	N/A	15 (M = 6, F = 6, G = 3)	N/A
SQ/ MD	7 Salinities	40 (M = 22, F = 9, G = 9)	35 (M = 16, F = 12, G = 7)	33 (M = 18, F = 6, G = 9)	34 (M = 11, F = 15, G = 8)	37 (M = 14, F = 15, G = 8)	40 (M = 18, F = 13, G = 9)	35 (M = 12, F = 11, G = 12)

Table S 3: Survival AICc table for CoxPH testing response variable "survival" against various predictor variables for Swan Quarter versus Maryland.

Model	AICc	DAICc	AICc Wt.	Cumulative Wt.
Salinity*CW	1436.13	0.00	0.64	0.64
Salinity*CW + Site	1438.05	1.92	0.25	0.89
Salinity + Site + CW + Sex	1440.64	4.51	0.07	0.95
Salinity*Sex	1442.40	6.27	0.03	0.98
Salinity*Sex + Site	1444.12	7.99	0.01	0.99
Site*CW + Salinity	1445.45	9.32	0.01	1.00
Salinity	1449.15	13.02	0.00	1.00
Salinity + Site	1451.14	15.01	0.00	1.00
Salinity + CW	1451.25	15.12	0.00	1.00
Salinity*Site	1454.89	18.76	0.00	1.00
Salinity*Site + CW	1456.97	20.84	0.00	1.00
Site*CW	1641.69	205.56	0.00	1.00
Site	1652.20	216.07	0.00	1.00

*Table S 4: Model output for top performing model for survival in Table S3. Formula = Survival ~ Salinity*CW. (n = 281, number of events = 156). Base Salinity for the model was 10 PSU. Significance Codes = 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1*

	Hazard Ratio	**Standard Error**	**z-value**	**Pr(>|z|)**	**Lower 95% CI**	**Upper 95% CI**
Salinity 5 PSU	5.08797	2.36602	0.688	0.49170	4.927e-02	525.4165
Salinity 3 PSU	0.00418	2.11419	-2.591	0.00958 **	6.631e-05	0.2635
Salinity 1 PSU	0.07529	2.01950	-1.281	0.20029	1.438e-03	3.9420
Salinity 0.5 PSU	0.05395	1.98023	-1.474	0.14037	1.113e03	2.6156
Salinity 0.2 PSU	0.47298	1.96440	-0.381	0.70310	1.006e-02	22.2303
Salinity 0 PSU	1.09892	1.95408	0.048	0.96150	2.386e-02	50.6159
CW	0.66335	0.19951	-2.057	0.03965 *	4.487e-01	0.9808
Salinity 5 PSU : CW	0.85985	0.26480	-0.570	0.56851	5.117e-01	1.4448
Salinity 3 PSU : CW	1.76755	0.20893	2.726	0.00641 **	1.174e+00	2.6620
Salinity 1 PSU : CW	1.47857	0.20737	1.886	0.05931 .	9.848e-01	2.2200
Salinity 0.5 PSU : CW	1.61837	0.20462	2.353	0.01863 *	1.084e+00	2.4168

Salinity 0.2 PSU : CW	1.50609	0.20388	2.009	0.04458 *	1.010e+00	2.2459
Salinity 0 PSU : CW	1.50751	0.20291	2.023	0.04309 *	1.013e+00	2.2438

*Table S 5: Model output for second-best performing model for survival in Table S3. Formula = Survival ~ Salinity*CW + Site. (n = 281, number of events = 156).Site: SQ = Swan Quarter, NC, MD = Saint John's Pond, MD. Base Salinity for model was 10 PSU, base Site for model was SQ. Note that Site is not considered significant. Significance Codes = 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1*

	Hazard Ratio	**Standard Error**	**z-value**	**Pr(>|z|)**	**Lower 95% CI**	**Upper 95% CI**
Salinity 5 PSU	5.277094	2.380836	0.699	0.48477	4.964e-02	561.0043
Salinity 3 PSU	0.004298	2.123732	-2.566	0.01029 *	6.692e-05	0.2761
Salinity 1 PSU	0.074923	2.029612	-1.277	0.20169	1.403e-03	4.0015
Salinity 0.5 PSU	0.053242	1.990634	-1.473	0.14066	1.076e-03	2.6344
Salinity 0.2 PSU	0.458118	1.977162	-0.395	0.69297	9.506e-03	22.0770
Salinity 0 PSU	1.099011	1.965789	0.048	0.96169	2.332e-02	51.7946
CW	0.670384	0.201770	-1.982	0.04748 *	4.514e-01	0.9956
Site: MD	0.874067	0.247010	-0.545	0.58581	5.836e-01	1.4184

Salinity 5 PSU : CW	0.856752	0.266497	-0.580	0.56182	5.082e-01	1.4444
Salinity 3 PSU : CW	1.763727	0.210189	2.700	0.00694 **	1.168e+00	2.6628
Salinity 1 PSU : CW	1.479280	0.208607	1.877	0.06052 .	9.828e-01	2.2265
Salinity 0.5 PSU : CW	1.621066	0.205899	2.346	0.01897 *	1.083e+00	2.4270
Salinity 0.2 PSU : CW	1.510009	0.205296	2.007	0.04470 *	1.010e+00	2.2580
Salinity 0 PSU : CW	1.507469	0.204259	2.009	0.04450 *	1.010e+00	2.2496

*Table S 6: Model output for top performing model for survival in Table S8. Formula = Survival ~ Salinity + Trial + CW + Sex. (n = 293, number of events = 176). Trial = month trial was run (1 = May, 2 = June, 3 = August), CW = Carapace Width, FG = Female Gravid, M = Male, base Salinity for model was 10 PSU. Significance Codes = 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1*

	Hazard Ratio	**Standard Error**	**z-value**	**Pr(>|z|)**	**Lower 95% CI**	**Upper 95% CI**
Salinity 3 PSU	1.62023	0.27394	1.762	0.0781 .	0.9471	2.7718
Salinity 1 PSU	3.32939	0.25687	4.683	2.83e-06 ***	2.0124	5.5082
Salinity 0.2 PSU	18.94350	0.26645	11.040	< 2e-16 ***	11.2372	31.9346
Trial 2 (June)	0.92060	0.23109	-0.358	0.7203	0.58528	1.44802

Trial 3 (August)	3.25798	0.19276	6.127	8.93e-10 ***	2.23291	4.75364
Carapace Width	0.98670	0.02801	-0.478	0.6327	0.93398	1.04239
Sex FG	0.54966	0.25583	-2.339	0.0193 *	0.33291	0.90752
Sex M	0.98124	0.17690	-0.107	0.9147	0.69374	1.38788

*Table S 7: Model output for the second-best model for survival in Table S8. Formula = Survival ~ Salinity + Trial. (n = 293, number of events = 176). Trial = month trial was run (1 = May, 2 = June, 3 = August), base salinity for model was 10 PSU. Significance Codes = 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1*

	Hazard Ratio	**Standard Error**	**z-value**	**Pr(>|z|)**	**Lower 95% CI**	**Upper 95% CI**
Salinity 3 PSU	1.58643	0.27222	1.695	0.090 .	0.9305	2.705
Salinity 1 PSU	3.37526	0.25481	4.774	1.81e-06 ***	2.0484	5.562
Salinity 0.2 PSU	18.42152	0.26431	11.023	< 2e-16 ***	10.9735	30.925
Trial 2 (June)	1.01182	0.20972	0.056	0.955	0.67080	1.52620
Trial 3 (August)	3.57287	0.18732	6.798	1.06e-11 ***	2.47495	5.15785

Table S 8: Survival AICc table for CoxPH testing response variable "survival" against various predictor variables for Swan Quarter across season.

Model	**AICc**	**DAICc**	**AICc Wt.**	**Cumulative Wt.**

Salinity + Trial + CW + Sex	1672.46	0.00	0.52	0.52
Salinity + Trial	1673.68	1.22	0.28	0.80
Trial*CW + Salinity	1675.56	3.10	0.11	0.90
Salinity*CW + Trial	1677.11	4.65	0.05	0.96
Salinity*Trial	1678.63	6.17	0.02	0.98
Salinity*Trial + CW	1680.19	7.73	0.01	0.99
Salinity*Sex + Trial	1680.25	7.79	0.01	1.00
Salinity	1729.23	56.77	0.00	1.00
Salinity*Sex	1731.17	58.71	0.00	1.00
Salinity*CW	1732.17	59.71	0.00	1.00
Trial*Sex	1827.60	155.14	0.00	1.00
Trial	1832.42	159.97	0.00	1.00
Trial*CW	1832.97	160.51	0.00	1.00

*Table S 9: Model output for second-best model for Exposure Righting Response in Table S10. Formula = Exposure Righting Response ~ Salinity + Site. Base Salinity for model was 10 PSU, Base Site for model was Swan Quarter, NC. Null Deviance = 212.71 (187 degrees of freedom), Residual Deviance = 189.42 (181 degrees of freedom). Number of Fisher Scoring Iterations: 6. Significance Codes = 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1*

	Estimate	**Standard Error**	**t-value**	**Pr(>|t|)**

Intercept	0.037104	0.005177	7.167	1.88e-11 ***
Salinity 5 PSU	-0.018378	0.005880	-3.126	0.002067 **
Salinity 3 PSU	-0.022486	0.005593	-4.021	8.51e-05 ***
Salinity 1 PSU	-0.015888	0.006026	-2.637	0.009103 **
Salinity 0.5 PSU	-0.020175	0.005728	-3.522	0.000541 ***
Salinity 0.2 PSU	-0.026793	0.005669	-4.726	4.58e-06 ***
Site: MD	0.003298	0.002520	1.309	0.192197

Table S 10: AICc table for Gamma GLM models examining Exposure Righting Response for Swan Quarter versus Maryland.

Model	**AICc**	**DAICc**	**AICc Wt.**	**Cumulative Wt.**
Salinity	1844.38	0.00	0.35	0.35
Salinity + Site	1845.13	0.75	0.24	0.58
Salinity*Site + CW	1846.47	2.09	0.12	0.71
CW + Sex + Salinity + Site	1846.63	2.25	0.11	0.82
Salinity*Site	1846.73	2.35	0.11	0.92
CW*Salinity	1847.46	3.08	0.07	1.00
CW	1856.15	11.77	0.00	1.00
CW + Site	1858.22	13.84	0.00	1.00

Site	1858.50	14.12	0.00	1.00
CW*Site	1860.31	15.93	0.00	1.00
CW + Sex	1860.74	16.36	0.00	1.00

*Table S 11 Model output for top performing model for Exposure Righting Response in Table S10. Formula = Exposure Righting Response ~ Salinity. Base Salinity for the model was 10 PSU. Null Deviance = 212.71 (187 degrees of freedom), Residual Deviance = 190.67 (182 degrees of freedom). Number of Fisher Scoring Iterations: 6. Significance Codes = 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1*

	Estimate	**Standard Error**	**t-value**	**Pr(>|t|)**
Intercept	0.038224	0.005140	7.436	3.93e-12 ***
Salinity 5 PSU	-0.018228	0.005913	-3.082	0.002373 **
Salinity 3 PSU	-0.022650	0.005622	-4.029	8.22e-05 ***
Salinity 1 PSU	-0.015950	0.006059	-2.632	0.009209 **
Salinity 0.5 PSU	-0.020170	0.005761	-3.501	0.000582 ***
Salinity 0.2 PSU	-0.026806	0.005706	-4.698	5.16e-06 ***

Table S 12: AICc table for Gamma GLM models examining Exit Righting Response for Swan Quarter versus Maryland.

Model	**AICc**	**DAICc**	**AICc Wt.**	**Cumulative Wt.**
CW	1333.07	0.00	0.33	0.33
Salinity	1333.46	0.39	0.28	0.61
CW + Site	1335.12	2.04	0.12	0.73

Salinity + CW	1335.36	2.29	0.11	0.84
Salinity + Site	1335.71	2.63	0.09	0.93
CW*Site	1337.02	3.95	0.05	0.97
CW + Sex	1338.76	5.68	0.02	0.99
CW*Salinity	1342.88	9.81	0.00	0.99
Salinity*Site	1342.88	9.81	0.00	1.00
CW + Sex + Salinity + Site	1343.41	10.34	0.00	1.00
Salinity*Site + CW	1345.05	11.98	0.00	1.00

*Table S 13: Model output for top performing model for Exit Righting Response in Table S12. Formula = Exit Righting Response ~ CW. Null Deviance = 90.877 (124 degrees of freedom), Residual Deviance = 90.626 (123 degrees of freedom). Number of Fisher Scoring Iterations: 5. Significance Codes = 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1*

	Estimate	**Standard Error**	**t-value**	**Pr(>|t|)**
Intercept	0.0106825	0.0023948	4.461	1.82e-05 ***
CW	0.0001830	0.0001965	0.932	0.353

*Table S 14: Model output for second-best model for Exit Righting Response in Table S12. Formula = Exit Righting Response ~ Salinity. Base Salinity for the model was 10 PSU. Null Deviance = 90.887 (124 degrees of freedom), Residual Deviance = 86.724 (120 degrees of freedom). Number of Fisher Scoring Iterations: 6. Significance Codes = 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1*

	Estimate	**Standard Error**	**t-value**	**Pr(>|t|)**

Intercept	0.016741	0.001605	10.430	< 2e-16 ***
Salinity 5 PSU	-0.003157	0.002105	-1.500	0.13630
Salinity 3 PSU	-0.005983	0.001976	-3.028	0.00302 **
Salinity 1 PSU	-0.006188	0.002069	-2.991	0.00338 **
Salinity 0.5 PSU	-0.003474	0.002978	-1.167	0.24567

*Table S 15: Model output for top performing model for Exposure Righting Response in Table S17. Formula = Exposure Righting Response ~ Salinity. Base Salinity for the model was 10 PSU. Null Deviance = 255.03 (198 degrees of freedom), Residual Deviance = 233.88 (195 degrees of freedom). Number of Fisher Scoring Iterations: 6. Significance Codes = 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1*

	Estimate	**Standard Error**	**t-value**	**Pr(>|t|)**
Intercept	0.027153	0.002717	9.993	< 2e-16 ***
Salinity 3 PSU	-0.007516	0.003459	-2.173	0.03102 *
Salinity 1 PSU	-0.010744	0.003319	-3.237	0.00142 **
Salinity 0.2 PSU	-0.017909	0.003273	-5.472	1.36e-07 ***

*Table S 16: Model output for top performing model for Exit Righting Response in Table S18. Formula = Exit Righting Response ~ Salinity. Base Salinity for the model was 10 PSU. Null Deviance = 85.011 (115 degrees of freedom), Residual Deviance = 78.976 (113 degrees of freedom). Number of Fisher Scoring Iterations: 6. Significance Codes = 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1*

	Estimate	**Standard Error**	**t-value**	**Pr(>|t|)**
Intercept	0.017084	0.001375	12.429	< 2e-16 ***
Salinity 3 PSU	-0.005141	0.001730	-2.972	0.00362 **

Salinity 1 PSU	-0.007342	0.001746	-4.206	5.22e-05 ***

Table S 17: AICc table for Gamma GLM models examining Exposure Righting Response for Swan Quarter across season.

Model	AICc	DAICc	AICc Wt.	Cumulative Wt.
Salinity	1975.60	0.00	0.79	0.79
Salinity + Trial	1978.97	3.37	0.15	0.94
CW*Salinity	1981.50	5.90	0.04	0.98
Salinity*Trial	1984.32	8.72	0.01	0.99
Salinity*Trial + CW	1984.60	9.00	0.01	1.00
CW + Sex + Salinity + Trial	1989.00	13.40	0.00	1.00
CW	1990.31	14.71	0.00	1.00
Trial	1993.64	18.04	0.00	1.00
CW + Trial	1993.92	18.32	0.00	1.00
CW + Sex	1997.99	22.40	0.00	1.00
CW*Trial	1998.03	22.44	0.00	1.00

Table S 18: AICc table for Gamma GLM models examining Exit Righting Response for Swan Quarter across season.

Model	AICc	DAICc	AICc Wt.	Cumulative Wt.

Salinity	1229.67	0.00	0.78	0.78
Salinity + Trial	1233.99	4.32	0.09	0.87
CW*Salinity	1234.76	5.08	0.06	0.93
CW	1236.21	6.53	0.03	0.96
CW + Sex	1238.17	8.50	0.01	0.98
CW + Sex + Salinity + Trial	1238.22	8.55	0.01	0.99
Trial	1238.99	9.32	0.01	0.99
CW + Trial	1240.23	10.56	0.00	1.00
Salinity*Trial	1242.53	12.85	0.00	1.00
Salinity*Trial + CW	1244.33	14.66	0.00	1.00
CW*Trial	1244.51	14.83	0.00	1.00

Table S 19: Kruskal-Wallis Rank Sum Test Results for total prevalence, L. panopaei prevalence and Entoniscid prevalence comparing E. depressus to R. harrisii.

Prevalence Type	**χ^2**	**Degrees of Freedom**	**p-value**
Total	13.784	1	0.0002051
L. panopaei	2.1145	1	0.1459
Entoniscid	8.2239	1	0.004134

*Table S 20: One-Way ANOVA Test Results evaluating Change in Infection Prevalence for R. harrisii and E. depressus by Site for total prevalence, L. panopaei prevalence and Entoniscid prevalence. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 '', 1*

Prevalence Type	Df	Sum Sq	Mean Sq	F-value	Pr(>F)	Residuals Df	Residuals Sum Sq	Residuals Mean Sq
Total	8	0.6299	0.07874	2.616	0.0159 *	60	1.8057	0.03010
L. panopaei	8	0.7983	0.09979	3.9	0.000926 ***	60	1.5353	0.02559
Entoniscid	8	0.4563	0.05703	12.93	1.29e-10 ***	60	0.2647	0.00441

*Table S 21: One-Way ANOVA Test Results evaluating Change in Infection Prevalence for R. harrisii and E. depressus by Month for total prevalence, L. panopaei prevalence and Entoniscid prevalence. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 '', 1*

Prevalence Type	Df	Sum Sq	Mean Sq	F-value	Pr(>F)	Residuals Df	Residuals Sum Sq	Residuals Mean Sq
Total	20	1.145	0.05728	2.131	0.0165 *	48	1.290	0.02688
L. panopaei	20	0.8459	0.04229	1.365	0.187	48	1.4877	0.03099
Entoniscid	20	0.0918	0.004589	0.35	0.994	48	0.6292	0.013109

*Table S 22: One-Way ANOVA Test Results evaluating Change in Infection Prevalence for R. harrisii and E. depressus from 2016-2022 for total prevalence, L. panopaei prevalence and Entoniscid prevalence. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 '', 1*

Prevalence Type	Df	Sum Sq	Mean Sq	F-value	Pr(>F)	Residuals Df	Residuals Sum Sq	Residuals Mean Sq
Total	13	0.5484	0.04218	1.229	0.285	55	1.8872	0.03431
L. panopaei	13	0.2704	0.02080	0.555	0.879	55	2.0631	0.03751
Entoniscid	13	0.2546	0.019481	2.309	0.0159 *	55	0.4664	0.008481

Figure S 1: Proportion of Eurypanopeus depressus alive at the end of each experiment for respective treatments for comparing Swan Quarter and Maryland. Since site was not found to be a significant predictor of survival, proportions have been combined for this figure. Treatments were 10 PSU, 5 PSU, 3 PSU, 1 PSU, 0.5 PSU, 0.2 PSU and 0 PSU.

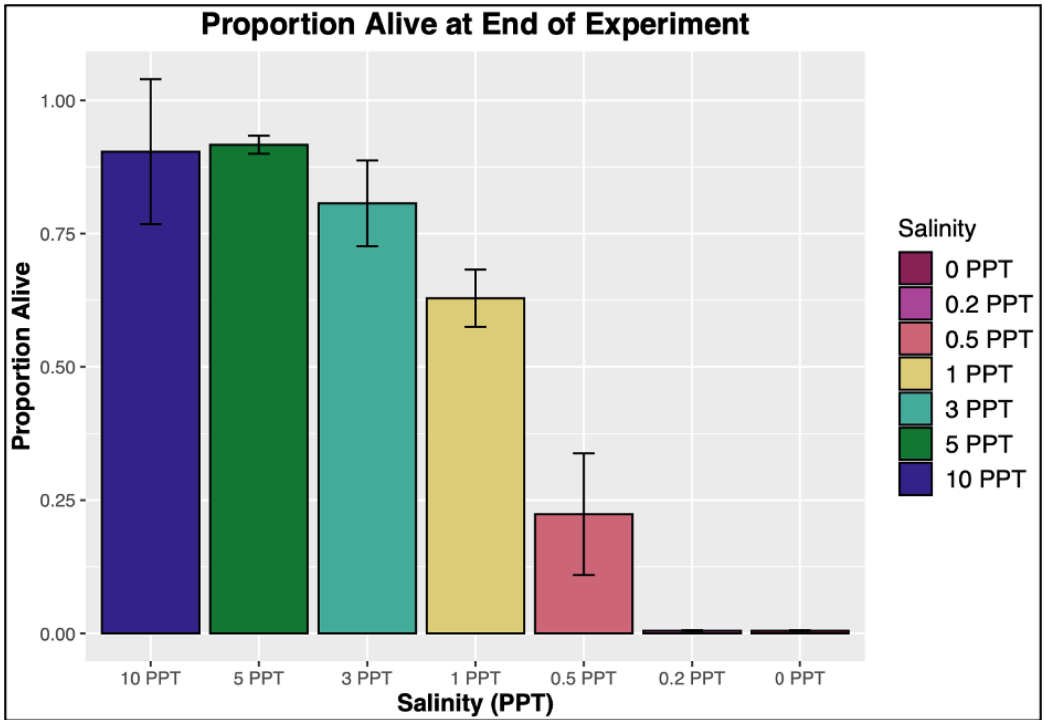

Figure S 2: Proportion of Eurypanopeus depressus alive at the end of each experiment for respective treatments for Swan Quarter across season. Treatments were 10 PSU, 3 PSU, 1 PSU and 0.2 PSU.

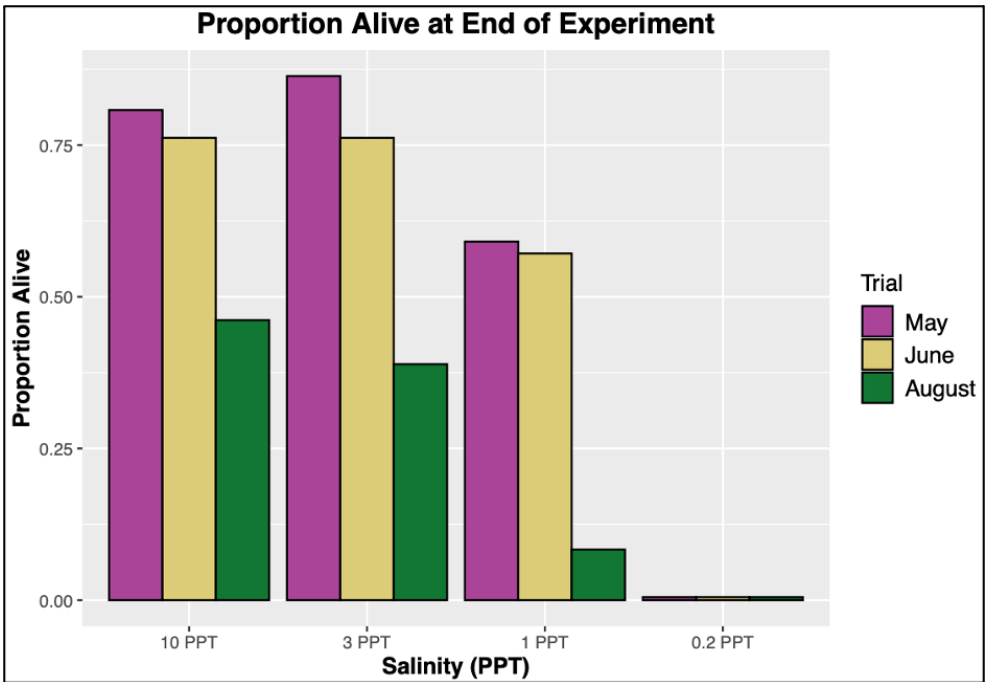

Figure S 3: Boxplot of Carapace Width (CW) by Salinity for SQ versus MD. An interaction between CW and Salinity was found to be the top model for Survival.

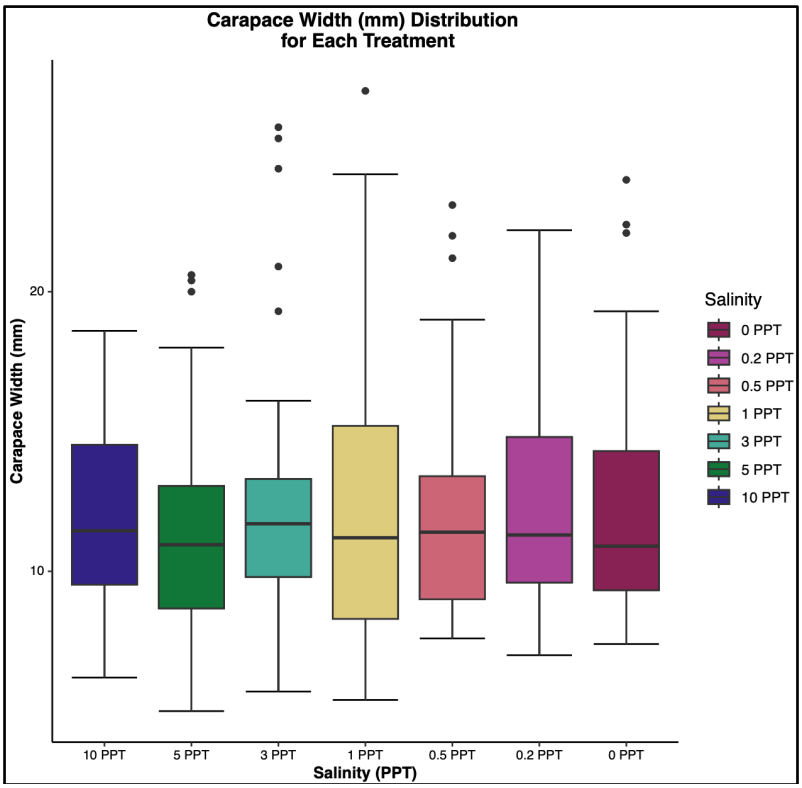

Figure S 4: Boxplot of Carapace Width (CW) by Salinity grouped by site for SQ versus MD. An interaction between CW and Salinity was found to be the top model for Survival.

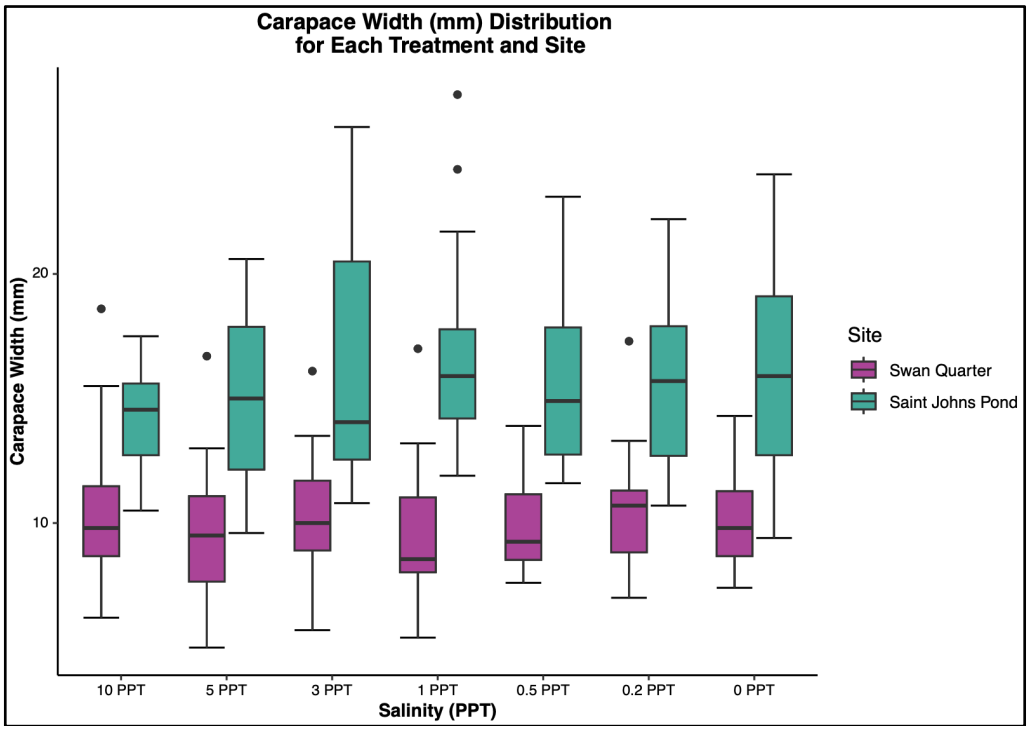

Figure S 5: Proportion of E. depressus alive at the end of the experiment for Swan Quarter across season sorted by sex. This figure is a reference for the significance code from ***TABLE S6****.*

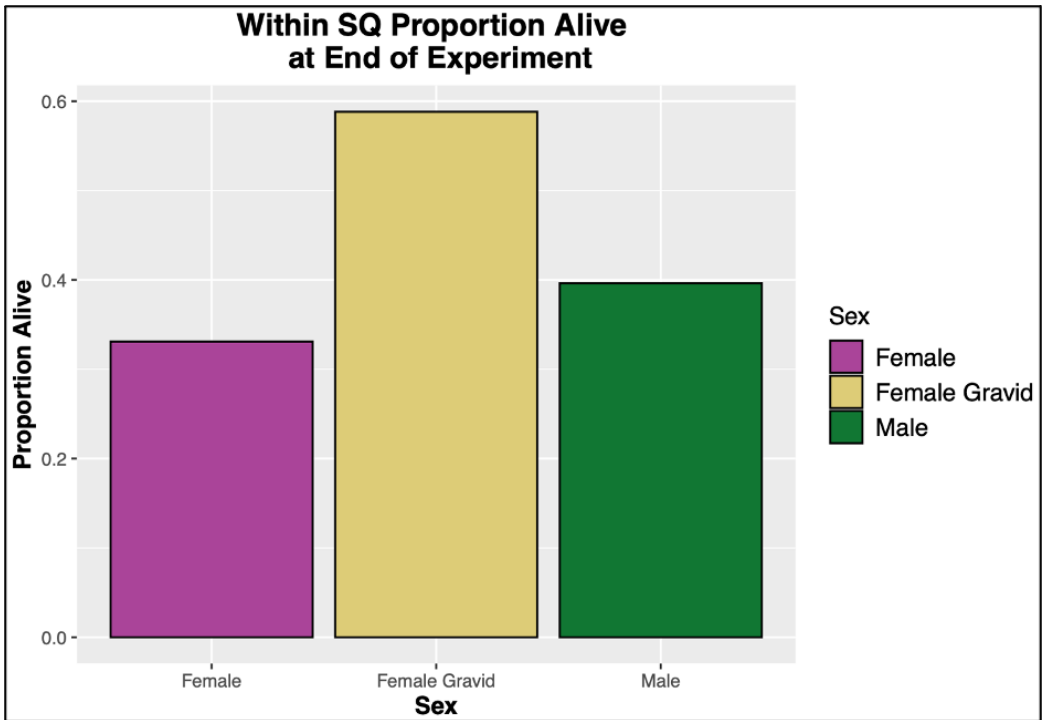

Figure S 6: Linear regression of Carapace Width (mm) against Exit Righting Response Time (sec) for all salinities and both sites from SQ and MD.