

**EFFECTS OF LOCAL ADAPTATION ON INVASION SUCCESS: A CASE  
STUDY OF *Rhithropanopeus harrisii* (GOULD)**

A Dissertation

by

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## ABSTRACT

A major trend in invasion biology is the development of models to accurately predict and define invasive species and the stages of their invasions. These models focus on a given species with an assumed set of traits. By doing so, they fail to consider the potential for differential success among different source populations. This study looked at the inland invasion of *Rhithropanopeus harrisii* in the context of a current invasion model. This species has been introduced worldwide, but has only invaded freshwater reservoirs within the state of Texas (United States) indicating a potential difference amongst source populations. Previous studies indicate that this species should not be capable of invading inland reservoirs due to physiological constraints in the larvae. A more recent study gives evidence to the contrary. To investigate whether the inland populations are in fact successfully established, I attempted to answer the following questions: Can inland populations successfully reproduce in the inland reservoirs and rivers? If so, what factors in the native environment could have led to the evolution of this ability? What are the impacts of this species in the inland reservoirs and what is its potential spread? I combined a larval developmental study, conspecific and heterospecific crab competition trials, field collections, gut content analysis, shelter competition trials with crayfish, and larval and adult dispersal study to answer these questions.

I showed that *Rhithropanopeus harrisii* is established in the inland reservoirs and capable of spreading. I demonstrated that in the native populations along the Gulf coast

of the United States, this species is the least aggressive and is therefore likely excluded into lower salinity waters during reproductive periods. This likely led to a lowered salinity tolerance in the larval stages, which predispose these populations to successful introductions in inland freshwater bodies. I showed that the crabs are capable of outcompeting juvenile crayfish for shelter at high densities and therefore warrant management in order to reduce their effect. This study indicates a need for the invasion models to take the source population into account in order to ensure effective and prudent management strategies.

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## 1. INTRODUCTION

An introduced species is considered invasive when it spreads beyond the original site of introduction. Invasive species can have detrimental effects on an ecosystem. A recent survey of prominent invasion biologists found that invasive species are one of the top five threats to biodiversity (Young and Larson 2011). According to the Nature Conservancy, invasive species cost an estimated 1.4 trillion U.S. dollars to the world's economy due to crop destruction and elaborate control mechanisms such as import/export monitoring, manual eradication and pesticide application. Understanding the biology of invasive species is critical to thwarting the biological and economic damages done by these organisms.

There has been an emphasis in recent invasion biology literature in developing models to explain the invasion process (Blackburn *et al.*, 2011; Foxcroft, Pickett and Cadenasso, 2011; Gurevitch *et al.*, 2011), the impact of invasive organisms (Allstadt *et al.*, 2012; Thomsen *et al.*, 2011), and the potential spread of an invasive species (Dullinger *et al.*, 2009; Fitzpatrick *et al.*, 2012; Rodder and Lotters, 2010; Vaclavik and Meentemeyer, 2012). Models pertaining to the invasion process have aimed to generalize the vast number of studies across a multitude of plant and animal species into a generalized framework applicable to any organism. The simplest model, proposed by Foxcroft *et al.* (2011), stipulates that an invasion depends upon “(1) the characteristics of the introduced species, (2) system context, within which the invasion takes place, and (3) features of the receiving habitat.” This framework is useful across the breadth of

invasive species. However, it fails to include the impact of the invasive species upon a novel habitat.

A model described by Blackburn *et al.* (2011) identifies four stages of an invasion: Transport, Introduction, Establishment and Spread. The Transport and Introduction stages refer to the movement of a species out of its home range. Introduction is a separate stage in order to delineate those species that are directly introduced by human means (such as ornamental plants) versus those that invade by accidental means (such as a stowaway on the roots of an ornamental plant). Establishment refers to the initial growth of the introduced population until it reaches the equilibrium phase (defined as the point where the number of births and deaths are equal resulting in a relatively constant population). Finally, the Spread stage refers to the further invasion into new habitats from the original site of introduction. The stages are influenced by six filters: Geography, Captivity or Cultivation, Survival, Reproduction, Dispersal, and Environmental. Geography serves as a natural invasion barrier by keeping species within a native home range. Geographic isolation can be overcome by human transport of species, such as the unintentional transport of marine larvae in the ballast water of ships. Captivity or Cultivation acts as a filter for species that are directly introduced by man and that require human aid in order to survive and reproduce within their new environment. Prolonged duration in the novel environment may lead to local adaptations that allow the organisms to escape the Captivity/Cultivation filter. The Survival and Reproduction filters are linked. Species may be capable of surviving for extended periods of time in a new habitat but fail to reproduce and therefore never reach

the invasive status. Those that are capable of reproducing are much more likely to spread and invade new areas. Dispersal refers to an organism's ability to move away from its initial site of introduction. Species incapable of dispersing may stay viable in their introduced range but will fail to become invasive. The Environmental filter refers to all of the aforementioned filters occurring across multiple habitats as each is successively invaded. Even though the Blackburn *et al.* model does not explicitly model the impact of the organism, it provides a better framework than the Foxcroft *et al.* model for determining the success of an introduced species. Gurevitch *et al.* (2011) developed a framework similar to the Blackburn *et al.* model but utilized a web-like system to indicate the connectivity of the model components.

Allstadt *et al.* (2012) looked at interference competition, the inhibition of access to a critical resource by one species, and its role in invasion success. Their model indicated that interference is dependent on the spatial density of both the invader and the resident and is advantageous for the invader when it is rare in the novel environment. Thomsen *et al.* (2011) developed a framework meant to separate universal and unique characteristics, which directly influence the impact of invasive species, in order to better generalize invasions. The characteristics of the invading species, the native biota and the habitat are all important to the success of the invader. Thomsen *et al.* (2011) defined "unique" as those characters pertaining specifically to the species being investigated (e.g., a specific toxin produced by an invasive plant), whereas "universal" characters can be extrapolated to all invasions (e.g., abundance or fecundity).

Several authors have recently attempted to model the expansion of an invasive species into novel environments, utilizing type locality data from the native range and, in some cases, species' characteristics stemming from the species' realized niche. Dullinger *et al.* (2009) compared two species distribution models to determine which better predicted the factors allowing for the spread of an invasive species. The first used the native distribution only and the second incorporated the current invasive distribution as well. They showed that the second model was more informative. Rodder and Lotters (2010) argued that distribution alone was not enough to predict the spread of a species. They suggested that a thorough investigation of a species' ecology was a more informative method to identify meaningful predictors of a species potential to invade. Fitzpatrick *et al.* (2012) found similar results in their model. They used dynamic dispersal and population processes over fine-scale Global Information System (GIS) maps and were still not able to successfully predict the spread of hemlock woolly adelgid, an aphid like pest. They lacked specific details on timing and direction of long distance dispersal events, which could be ascertained with a better understanding of the local ecology. Vaclavik and Meentemeyer (2012) argued that if species distribution models are to be used, the stage of invasion must be considered to avoid underestimation of habitats at risk. They demonstrated that models built from realized niches were incapable of predicting invasion potential of *Phytophthora ramorum*, which causes Sudden Oak Death.

All of the aforementioned models are dependent upon the characteristics of the introduced organism. Even though these characteristics have been extensively

investigated, the construction of a generalized model predicting the success of invasive species has proven all but unobtainable. Successful vertebrate invasive species have been shown to possess the following characteristics: large native range with high abundance, vagility, broad diet, short generation times, ability to shift between r and K strategies, high genetic variation, gregariousness, ability of females to colonize alone, greater in size compared to relatives, an association with humans and broad physiological tolerances (Ehrlich, 1989). Resistant dormant eggs (in invertebrates) for dispersal and exploratory behavior (in skinks) are characteristics that also can explain the success of some invaders (Andonian and Hierro 2011; Briski *et al.*, 2011; Chapple, Simmonds, and Wong, 2011; Williamson and Fitter, 1996). Some studies have compared invasive species to congeners in their native habitat to determine why one species is invasive while another is not (Briski *et al.*, 2011; Burns and Winn, 2006; Deng *et al.*, 2004; Mihulka *et al.*, 2006). None have looked at differences that allow one population to be invasive while another population of the same species is incapable of invading similar habitats. It should be noted that Williamson and Fitter (1996) and Lee (2002) both mentioned the potential for individual populations of a species to be invasive while other populations are not but neither gave explicit examples and stated a lack of studies in this area.

The white-fingered mud crab, *Rhithropanopeus harrisi* (Gould, 1841), is a small xanthoid crab common throughout estuaries extending from the Miramichi estuary, Canada along the east coast of the United States to Veracruz, Mexico. According to the Global Invasive Species database, it has successfully invaded the west coast of North

America, Panama, Europe (including the Adriatic Sea, Baltic Sea, Belgium, Britain, Bulgaria, Denmark, Finland, France, Germany, Italy, Lithuania, Mediterranean Sea, Netherlands, North Sea, Poland, Portugal, Romania, Spain, and the Ukraine), Africa (Tunisia) and Asia (the Aral Sea, Azerbaijan, Azov Sea, Black Sea, Caspian Sea, Iran, Japan, Kazakhstan, Russia, Turkmenistan, and Uzbekistan). The crab has also invaded 10 freshwater reservoirs in Texas, the only known inland invasions. Most of the introductions have been attributed to ballast water transports with the exception of the west coast of North America and the lakes in Texas. Populations of *R. harrisi* on the west coast of North America are thought to have been introduced through oyster mariculture. Previous work attributed the inland introductions to fish stocking procedures. Fish stocking is common in the eastern United States, so why has this crab not successfully invaded any other freshwater lakes?

This study will address a lack in invasion literature: differential success within a species invading different habitats. This study will attempt to answer the following questions:

1. What unique traits exist in the likely source population of the invasive reservoir populations that does not occur in other native populations of *R. harrisi*?
2. What factors in the crabs' native environment may have led to this potential difference?
3. What impacts does this species have in its invaded habitat?

4. What is the potential for continued spread of this organism?

Addressing these questions with *R. harrisii* may provide a test of the Blackburn *et al.* model and will be the first study to address population differences allowing for differential invasion success in an individual species.



## **2. *Rhithropanopeus harrisi* (BRACHYURA: PANOPEIDAE), A MODEL ORGANISM**

### 2.1 Introduction

*Rhithropanopeus harrisi* is native to the Atlantic and Gulf coasts of North America. It occurs primarily in estuarine waters from intertidal to a maximum depth of 27 m (personal observation). It typically inhabits oyster reefs but will take refuge in and under any submerged structure. Due to its small size and hardiness, this species has been extensively studied since its description as *Pilumnus harrisi* by Gould in 1841. The following literature review is intended to demonstrate the usefulness of this species to biological studies and the current extent of our knowledge on the species.

### 2.2 General Biology

Individuals generally reach maturity within the first two years of life and are believed to live up to five years in the wild (based on personal observation of captive individuals). Mature males range from 4.4 to 26.1 mm in carapace width (around the 9<sup>th</sup> moult) and females are slightly smaller ranging from 4.4 to 19.0 mm in width (around the 10<sup>th</sup> moult) (Ryan, 1956; Turoboyski, 1973). The reproductive period of this species ranges from March to October depending on water temperature. Goy *et al.* (1985) were able to induce spawning year round in the lab by increasing water temperatures. Reproductive behavior in the wild is poorly studied due to poor visibility in their cryptic habitat. In the lab, individuals do not display any pre-mating behaviors to attract mates, suggesting the likelihood of chemical cues (Turoboyski, 1973). Females do not pass

through a pre-copulatory molt. The absence of pre-copulatory behavior is expected given the low visibility in the native habitats. A comparison of five brachyuran species by Rodgers *et al.* (2011) showed that *R. harrisii* averaged 79 minutes per copulation event compared to less than two minutes for two shore crabs which live in the upper intertidal zone and are more prone to predation by visual predators. *Rhithropanopeus harrisii* was also shown to have the highest sperm to egg ratio per copulation event. Females have previously been shown to store sperm and to be able to produce up to four subsequent broods of between 1000-4000 eggs each from a single mating event (Morgan *et al.*, 1983). The increased sperm and the sperm retention allow for the possibility of sperm competition in this species, however, to date this has not been investigated.

Adult *R. harrisii* are euryhaline, having been found in salinities from 0 to 40 practical salinity units (PSU) (Maes *et al.*, 1998; Jazdzewski and Konopacka, 1993; Cuesta *et al.*, 1991; Roche *et al.*, 2009; Turoboyski, 1973; Williams and Aladin, 1991; Wurtz and Roback, 1955). These crabs undergo four zoeal stages and one megalopal stage during development. Costlow, Bookhout, and Monroe (1966) investigated the salinity range for larval development of this crab. They were unable to find an optimal salinity but did show that the greatest survival to the first crab stage is between 15 and 25 PSU, with a minimum salinity of 2.5 PSU needed for development to this stage. They noted that at lower salinities, higher temperatures resulted in better survival.

Laughlin and French (1989) replicated the Costlow *et al.* study using populations from the east coast of Florida and an invasive population, which is thought to have

originated in the Chesapeake Bay area, from San Francisco Bay. These authors found similar results to the Costlow *et al.* study except the Florida population had significantly higher survival at 2 PSU and 30C with almost 80% survival. The Florida population was shown to have higher survival rates than the California population at all temperature salinity combinations except for the lowest combinations. The authors attributed this difference to local environmental adaptations. Goncalves *et al.* (1995a) conducted a similar study on an invasive population of *R. harrisii* from the Mondego River estuary in Portugal. Their results were similar to the Chesapeake Bay and Beaufort, North Carolina populations with optimal survival found to be at 25C and 15 PSU; however, they did not test salinities lower than 5 PSU. Schneider (1967) tested the temperature adaptations of three populations of *R. harrisii* (from Maine, North Carolina and Florida). He found no difference between the populations of Maine and North Carolina but showed that the Florida crabs were more tolerant to higher temperatures. This again suggests local adaptations to climate.

Many studies have focused on the larval behavior of *R. harrisii* (see review in Forward 2009) because *R. harrisii* larval stages can all be reared at the same salinity. This is due to strong larval retention behavior within estuaries, keeping *R. harrisii* larval stages near their hatching sites and in a relatively constant salinity (Cronin, 1982; Cronin and Forward, 1986). This requires osmoregulatory capabilities in all larval stages. In other crab species, like *Eurypanopeus depressus*, the zoeae experience larval transport out of the estuary (Christy and Stancyk, 1982) and may vary their osmoregulatory capabilities across larval stages.

## 2.3 Invasion History

*Rhithropanopeus harrisii* has a relatively long invasion history. This species was first documented in European waters (See Table 2.1 and Figure 2.1) in the late 1800s on the coast of the Netherlands (Maitland, 1874). It was recorded in Germany (Baltic Sea) and in the Ukraine (Black Sea) as early as 1936 (Schubert, 1936; Makarov, 1939). By 1948 it had expanded into the Azov Sea along the coasts of Russia and the Ukraine (Zaitsev and Ozturk, 2001) and was reported for the first time along the coast of Bulgaria in the Black Sea as well (Marchand and Saudray, 1971). It was first reported on the coast of Poland (Baltic Sea) and Romania (Black Sea) in 1951 (Nikolaev, 1951; Bacescu, 1967). Two years later it was found on the coast of Denmark (Baltic Sea) (Wolff, 1954). Saudray (1956) first reported the crab from the Atlantic coast of France in 1955. By 1990 the crab had been introduced to the Iberian Peninsula, specifically on the coast of Spain (Mariscal *et al.*, 1991). The following year, Goncalves *et al.* (1995b) reported it for the first time in Portugal. By 1994 the species had made it into the Mediterranean, where it was first reported from the Adriatic Sea on the coast of Italy in 1994 (Mizzan and Zanella, 1996). The same year it was also found on the North Sea coast of Belgium.

Continent	Country	Body of Water	First Reported
<b>Africa</b>	Tunisia	Mediterranean	<b>2003</b>
<b>Asia</b>	Russia	<u>Caspian Sea</u>	<b>1959</b>
Asia	Azerbaijan	Caspian Sea	1961
Asia	Turkmenistan	Caspian Sea	1961
Asia	Kazakhstan	<u>Aral Sea</u>	<u>1971</u>
Asia	Uzbekistan	Aral Sea	1971
Asia	Iran	Caspian Sea	2001
Asia	Kazakhstan	Caspian Sea	2001
Asia	Japan	W. Pacific	2000
<b>Central America</b>	Panama	Panama Canal	<b>1969</b>
<b>Europe</b>	Netherlands	<u>North Sea</u>	<b>1874</b>
Europe	Germany	<u>Baltic Sea</u>	<u>1936</u>
Europe	Ukraine	<u>Black Sea</u>	<u>1936</u>
Europe	Ukraine	<u>Azov Sea</u>	<u>1948</u>
Europe	Russia	Azov Sea	1948
Europe	Bulgaria	Black Sea	1948
Europe	Poland	Baltic Sea	1951
Europe	Romania	Black Sea	1951
Europe	Denmark	Baltic Sea	1953
Europe	France	N.E. Atlantic	1955
Europe	Spain	N.E. Atlantic	1990
Europe	Portugal	N.E. Atlantic	1991
Europe	Italy	<u>Adriatic Sea</u>	<u>1994</u>
Europe	Belgium	North Sea	1994
Europe	England	N.E. Atlantic	1996
Europe	Lithuania	Baltic Sea	2000
Europe	France	<u>Mediterranean</u>	<u>2000</u>
Europe	Finland	Baltic Sea	2008
North America	United States	<u>San Francisco Bay, California</u>	<u>1937</u>
North America	United States	Coos Bay, Oregon	1968
North America	United States	<u>Texas Reservoirs</u>	<u>1998</u>

Table 2.1. List of *Rhithropanopeus harrisi* invasions. Bold indicates the first introduction to the continent. Underlining indicates the first introduction to the body of water.

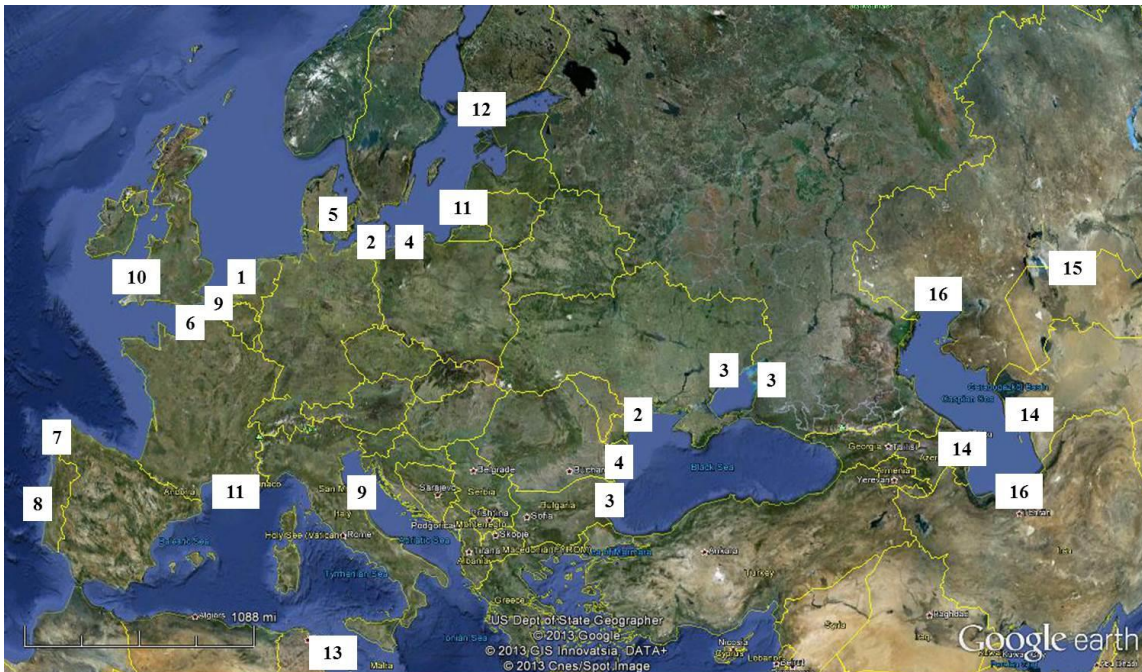


Figure 2.1. Map of European, African and Asian invasions. Numbers indicate the order of identified invasions. Map produced using Google Earth (6.1.0.5001).

In 1996, it was reported for the first time from the coast of England (Eno *et al.*, 1997). In 2000, the crab was reported for the first time from the Lithuanian coasts (Baltic Sea) and from the Mediterranean coasts of France (Bacevieius and Gasiunaite, 2008; Noel, 2001). The most recent European invasion was reported from the coast of Finland (Baltic Sea) in 2008 (Berezina and Petryashev, 2012).

The crab has only one report from the African continent. Ben Souissi *et al.* (2004) reported it from a lagoon south of Tunis, Tunisia in 2003. The first report of this crab from Asia occurred in 1959 in the Caspian Sea on the coast of Russia (Gadzhiev, 1963). It was reported from the coast of Azerbaijan and Turkmenistan in the Caspian Sea as early as 1961 (Gadzhiev, 1963; Zaitzev and Ozturk, 2001). By 1971, the crab had

invaded the Aral Sea on the coast of Kazakhstan and Uzbekistan (Andreyev and Andreyeva, 1988). It continued to spread in the Caspian Sea and by 2001 had been found on the coast of Iran and Kazakhstan (Zaitsev and Ozturk, 2001). The most recent introduction in Asiatic waters was in 2000 from the Nakagawa Canal on the western coast of Japan (Iseda *et al.*, 2007).

*Rhithropanopeus harrisi* has been introduced outside of its native range in North America as well (Figure 2.2). In 1937 it was first reported by Jones (1940) in San Francisco Bay, California and later in Coos Bay, Oregon in 1950 (Brockerhoff and McLay, 2011). The crabs were first reported from an inland reservoir (Possum Kingdom Reservoir, Texas) in 1998. The crabs have since spread in Texas to Lake Granbury, Lake Whitney, Tradinghouse Creek Reservoir, Lake Colorado City, E.V. Spence Reservoir, Lake Balmorhea, Squaw Creek Reservoir and Lake Texoma (Boyle *et al.*, 2010).

In Central America, the crab was first reported from the Panama Canal in 1969 but seems to have been absent for 38 years. It was re-identified by Roche and Torchin in 2007, after they found an established reproducing population in the Miraflores Third Lock Lake in western Panama near the Pacific coast.



Figure 2.2. Map of North and Central America. White line indicates the native range. Numbers indicate the sequential order of invasion. Map produced using Google Earth (6.1.0.5001).

Invasions of this species have primarily been attributed to shipping and ballast water. Introductions into San Francisco Bay and inland lakes of Texas seem to be exceptions. In the Bay, introductions have been attributed to oyster mariculture transfers from North Carolina in the 1920s and 1930s. In the lakes, introductions have been attributed to the stocking of saltwater fish species in inland lakes. Much work has been done to determine methods of clearing ballast tanks in order to minimize transfer of estuarine species. However, due to this species' broad salinity tolerance, adults that



attach to hulls or late larval stages taken up in the ballast waters are not likely to die during the ballast water exchanges (Briski *et al.*, 2012). The authors investigated ballast tanks of ships coming into U.S. ports to compare the number of species in those that did open ocean ballast water exchanges with coastal ships that do not undergo the exchange. They concluded that this method did not remove macro-invertebrate invaders.

## 2.4 Population Genetics

Recent studies have looked at the population genetics of invasive populations of *R. harrisii* to determine the number of introductions and identify a likely source population (Boyle *et al.*, 2010; Peterson 2006; Projecto-Garcia, Cabral and Schubart, 2010). Peterson compared three populations from California and Oregon (San Francisco Bay, California; Coos Bay and the Yaquina River, Oregon) with a population from the Neuse River Estuary, North Carolina. The author found five haplotypes amongst the invasive populations, all of which were nested with the haplotypes found in the Neuse River populations. Projecto-Garcia *et al.* (2010) compared populations from Poland, The Netherlands, Portugal, Spain, Bulgaria, New Jersey, Neuse River North Carolina and Louisiana. The authors found that the European populations shared haplotypes exclusively with the population from New Jersey. They also showed a strong differentiation of the Louisiana population from both the New Jersey and the Neuse River populations with four fixed differences. Boyle *et al.* (2010) compared four inland Texas lake populations (Possum Kingdom Reservoir, Lake Granbury, Lake Colorado City and Tradinghouse Creek Reservoir) with two populations from the Texas coast

(Garcitas Creek and Nueces River) to determine the number of invasions and possible movement between lakes. The authors identified 10 haplotypes from the reservoirs and three from the coastal sites. One haplotype was shared between the Nueces River population and all reservoirs. Results suggested that at least two different introductions had occurred and that some connections existed between populations in Possum Kingdom and Lake Granbury. Comparisons with data from the Projecto-Garcia *et al.* study indicated that the Texas haplotypes were nested within the Louisiana clade with shallow variation of typically one to two substitutions.

### 2.5 Impact of *Rhithropanopeus harrisi* in Invaded Regions

Little has been done to identify the biological impacts of this species in its introduced range. Turoboyski (1973) indicated that the crab was known to feed on “*Nereis diversicolor*, *Mytilus edulis*, *Dreissena polymorpha*, *Cordylophora caspia*, dead organic matter of animal origin, and the plants *Cladophora* sp. and *Enteromorpha* sp.” This study did not indicate a significant detrimental effect of *Rhithropanopeus harrisi* on any of these species. Howells (1998) indicated that introduced populations of *R. harrisi* in Possum Kingdom Reservoir, Texas had the potential to adversely affect the endemic Texas fawnsfoot bivalve (*Truncilla macrodon*) if it were to spread into the rivers below the reservoir. Boyle *et al.* (2010) reported that the crab clogged water intake pipes for lake homes, had been found in intake pipes of the Comanche Peak Nuclear Power plant on Squaw Creek Reservoir, and suggested that the crabs may be outcompeting native crayfish species in the inland Texas reservoirs. No further studies

have been done to assess the potential impact of this species on freshwater fishes or other endemic species.

## 2.6 Conclusion

Studies on *Rhithropanopeus harrisi* have ranged from occurrence records to the effects of potential pesticides in population control. Of particular interest to the development of invasion models are two sets of studies. The first studies consider the larval physiology of the species. Costlow *et al.* (1966) demonstrated that populations from North Carolina were unable to develop successfully below 2 PSU. This was reconfirmed by both Laughlin and French (1985a) and Goncalves *et al.* (1995a). The high levels of larval survival at 2 PSU in the Florida populations of the Laughlin and French study indicate a potential population difference.

The second set of key studies focused on the population genetics of invasive species. If introductions of *R. harrisi* into distant or inland habitats were occurring via ballast water transplants, then one might expect to see more shared haplotypes between the Gulf of Mexico and those on the east coast of the U.S.A and elsewhere. According to the American Association of Port Authorities, in 2011 six of the top 35 exporting ports were found in the Gulf of Mexico compared to 12 along the Atlantic coast of the U.S. Yet, Projecto-Garcia *et al.* (2010) found that European populations were more closely related to native populations from New Jersey. In contrast, Boyle *et al.* (2010) showed that the inland Texas populations sampled were more closely related to the Louisiana populations sequenced by Projecto-Garcia *et al.* (2010). The genetic studies

suggest that source populations differ for introduced populations and influence their survival in new environments.

*Rhithropanopeus harrisi* has the potential to spread to suitable lakes and estuaries across the entire planet. To model its invasive potential, further information is needed: (1) What unique traits exist in the likely source population of the invasive reservoir populations that does not occur in other native populations? and (2) What impacts does this species have in its invaded habitat?

### 3. LARVAL DEVELOPMENT OF INVASIVE POPULATIONS

#### 3.1 Introduction

An understanding of the characteristics that allow species to successfully invade is necessary for the management of invasive species. Ehrlich (1989) noted that invasive species have been shown to possess the following characteristics: large native range with high abundance, vagility, broad diet, short generation times, ability to shift between r and K strategies, high genetic variation, gregariousness, ability of females to colonize alone, greater size compared to relatives, an association with humans and broad physiological tolerances. Several studies have compared invasive species with congeners in their native habitat to determine why one is invasive while the other is not (Briski *et al.*, 2011; Burns and Winn, 2006; Deng *et al.*, 2004; Mihulka *et al.*, 2006), but none has looked at differences that allow one population to be invasive while another population of the same species is incapable of invading similar habitats. Such an analysis would be an innovative test of current models to explain known invasions.

In the Blackburn *et al.* (2011) model of invasive species establishment, each stage (Transport, Introduction, Establishment and Spread) of the invasion has several filters where a species may become excluded from the invaded habitat. Unique traits may allow one population of a species to bypass a filter when other populations of that species would be filtered out. As an example, *Rhithropanopeus harrisii* has become invasive along the Atlantic, Mediterranean and inland sea coasts of Europe and western Asia (See Section 2 for a more expansive discussion of known invasions). It has also invaded the west coast of the United States and Central America, yet it has only invaded

freshwater lakes in the state of Texas in the United States. Known larval physiological tolerances of this species suggest that it should only be capable of invading coastal estuaries, which indicates that the Blackburn *et al.* (2011) model would predict a failure for the invasive Texas populations to establish in the inland freshwater lakes. However, Boyle *et al.* (2010) found both zoeae and adult *R. harrisii* in Possum Kingdom Reservoir, Lake Colorado City, Tradinghouse Creek Reservoir, and Lake Granbury, Texas. They confirmed the presence of gravid females in all of the lakes with known inland populations at the time of the study indicating reproducing populations in each of these reservoirs. This suggests some difference exists between the inland source population and the populations in which the larval tolerances have been studied. In this species, the traits most frequently studied that may be relevant to the question of invasive capacity are salinity and temperature tolerance.

Adults of *R. harrisii* are capable of tolerating salinities from 0 to 44 PSU (Maes *et al.*, 1998; Jazdzewski and Konopacka, 1993; Cuesta *et al.*, 1991; Roche *et al.*, 2009; Turoboyski, 1973; Williams and Aladin, 1991; Wurtz and Roback, 1955) indicating that adult crabs can bypass the Establishment stage survival filter. Likewise larvae of *R. harrisii* have been used in numerous osmoregulatory studies over the last half-century due to their relatively large size and relative ease of rearing. Connoly (1957) was the first to describe the larval stages of *R. harrisii*, which consists of four zoeal stages followed by one megalops, from a population in Canada. Chamberlain (1962) was the first to successfully rear larvae from North Carolina populations at 6 and 10 practical salinity units (PSU) at 15°, 24°, and 30°C. He noted that there were four zoeal stages

followed by one megalopal stage, indicating no differences in the development between the Canadian and the North Carolina population. He also found that larvae were incapable of surviving at 1 PSU and 15 PSU at any temperature combination he tested. Hood (1962) mirrored Chamberlain's study using a population from Biloxi Bay, Mississippi. The author reared his larvae at  $10.83 \pm 0.2$  PSU and  $27^{\circ}\text{C} \pm 2^{\circ}$ . This study also confirmed the larval stages described by Connoly indicating that the larval development is the same across all the populations tested.

The Costlow *et al.* (1966) study is the most frequently cited paper on the effects of temperature and salinity on the developmental rate of this species. The larval stages were reared over 24 temperature ( $20^{\circ}$ ,  $25^{\circ}$  and  $30^{\circ}\text{C}$ ) and salinity (1.0, 2.5, 5.0, 15.0, 25.0, 33.0, 35.0 and 40.0 PSU) combinations. They successfully reared zoeae to the megalops stage at all combinations except for those at 1.0 PSU. Complete development to the first crab stage only occurred at salinities above 2.0 PSU. There did not appear to be an optimum salinity/temperature combination. However, at salinities less than 15 PSU, higher temperatures resulted in higher survival rates indicating greater salinity tolerance at higher temperatures. There were no changes in the developmental pathway across the temperature/salinity combinations however at  $30^{\circ}\text{C}$ , time to the first crab stage was about half that taken at  $20^{\circ}\text{C}$ . This suggests that populations at higher temperatures would have a more rapid development and be capable of surviving at lower salinities.

Subsequent studies have repeated the Costlow *et al.* study utilizing different populations of crabs to determine if physiological differences exist between populations

(Laughlin and French, 1989a; Goncalves *et al.*, 1995) and found similar results (Table 3.1). Interestingly Laughlin and French successfully reared zoeae from both the Florida and California populations to megalops at 2 PSU at all three temperatures except for the Florida population at 20°C. Both populations were capable of surviving to megalops, yet the Florida population had a much higher success rate at the higher temperatures. At 30°C, the Florida population was significantly more successful at 2 PSU than the California population indicating a difference between the populations. Laughlin and French (1989a) failed to attribute any significance to the higher success at those conditions because they had not been observed in the field during the breeding season of the Florida population. This suggests population differentiation at the physiological level which could allow for differential invasion success.

Previous genetic studies indicate that all of the coastal invasions of *R. harrisii* are likely from an east coast source population (Peterson, 2006; Projecto-Garcia, Cabral and Schubart, 2010). Boyle *et al.* (2010) indicated that the inland Texas populations were more closely related to individuals from the Gulf of Mexico. It is possible that the Florida population represents a Gulf of Mexico phenotype. Although the conditions may not have been present for the particular area Laughlin and French studied, they may be present in other areas of the Gulf. Salinities in the Texas reservoirs have the potential of reaching and exceeding 2.0 PSU during summer months and during droughts, typical salinities range from 0.5 to 2.0 PSU. Temperatures in the lakes range from 20° – 31°C during the summer months. If the populations are indeed reproductively active, then the physiological range of temperature and salinity tolerance for the Texas population differs



substantially from those previously tested. This study, set out to replicate the studies of Costlow *et al.* and others using inland populations of *R. harrisi* in order to determine if the larvae of the inland Texas populations differ from those from North Carolina and elsewhere in their ability to survive to the megalops and first crab stage at salinities lower than 2 PSU. This test will determine whether this species may possess the ability to bypass the reproductive filter of the Blackburn *et al.* (2011) model.

### 3.2 Methods

Twenty-five gravid female crabs were obtained from Lake Whitney, Texas between March and August of 2012. Gravid females were transported back to the lab in buckets of lake water maintaining the eggs at the salinity from which they were originally collected. Upon return to the lab, females were separated into individual 14 oz. plastic containers in approximately 350 ml of water (made from deionized water and Instant Ocean™ sea salts) corresponding to the salinity of the lake at the time of collection (0.8 to 1.5 PSU). A piece of potshard or small PVC pipe (cut in half) was placed inside the container with each gravid female. This served as a shelter and ensured that the female retained the eggs until hatching. Water was changed daily and the individuals were fed commercial food (crab and lobster bites) biweekly until the hatching of the larvae. Eight gravid females were also collected from the San Bernard River at a boat ramp just off of Texas Highway Farm-to-Market 521 approximately 25 river miles inland from the Gulf of Mexico, from a salinity of 20.3 PSU but efforts to rear the larvae of these females in the laboratory was unsuccessful.

Based upon the methods of Costlow *et al.* (1966) a total of 600 zoeae were used from each female's hatch. Upon hatching, 50 zoeae were transferred to pint glass jars with 300 ml water at one of 12 temperature/salinity combinations. Some trials had varying numbers at the start of the trial due to low hatching numbers. The following salinities were tested at both 20° and 30°C: 1.0, 2.0, 5.0, 15.0, 25.0 and 33.0. Trial water was made using deionized water and Instant Ocean™ salts. Water was premixed and stored in plastic containers in water baths. All trial waters stored at 30°C were aerated during storage. Jars of zoeae were kept in water baths at their respective temperatures. Water was changed daily and larvae were fed *Artemia* nauplii at a 3:1 density.

Upon molting to megalops, individual larvae were placed in small 120 ml plastic specimen cups with 90 ml of the corresponding test water and placed in water baths at the appropriate temperature. Water was changed daily and individuals were fed *Artemia* at a density of 10:1. Upon molting to first crab stage, individuals were placed in holding aquaria until the end of the experiment.

Statistical analysis was performed to determine if significant differences existed in the number of individuals that survived to megalops, the number of individuals that survived to the first crab stage, the first day to megalops, average number of days to megalops, the first day to first crab stage and the average number of days to first crab stage. All statistical analyses were performed in SPSS.

Average Duration of Zoael Development in Days												
Salinity in PSU	20				25				30			
	C	L		G	C	L		G	C	L		G
		FL	CA			FL	CA			FL	CA	
1.0	0	-	-	-	0	-	-	-	0	-	-	-
2.0	-	0	23	-	-	11.4	13.1	-	-	8.2	9	-
2.5	0	-	-	-	0	-	-	-	9.7	-	-	-
<b>5.0</b>	<b>0</b>	<b>19.7</b>	<b>19</b>	<b>0</b>	<b>13.5</b>	<b>10.6</b>	<b>11.7</b>	<b>12</b>	<b>8.6</b>	<b>7.4</b>	<b>9.3</b>	<b>10</b>
10.0	-	19.3	19.1	24	-	9.8	11.3	12	-	7.2	8.2	9
<b>15.0</b>	<b>21.6</b>	<b>19.5</b>	<b>20.3</b>	<b>24</b>	<b>11.4</b>	<b>9.8</b>	<b>11.4</b>	<b>12</b>	<b>8.1</b>	<b>7.1</b>	<b>7.9</b>	<b>9</b>
20.0	-	19.3	19	24	-	9.8	11.2	12	-	7.2	7.8	9
<b>25.0</b>	<b>20.9</b>	<b>18.8</b>	<b>19.7</b>	<b>24</b>	<b>11.4</b>	<b>10</b>	<b>10.2</b>	<b>12</b>	<b>8.3</b>	<b>7.3</b>	<b>7.5</b>	<b>9</b>
30.0	-	18.9	20.4	0	-	11.5	12.4	13	-	8	8.6	10
33.0	23.4	-	-	-	12.5	-	-	-	8.3	-	-	-
35.0	20.3	-	-	-	12.2	-	-	-	8.8	-	-	-
40.0	19.7	-	-	-	13.3	-	-	-	9.3	-	-	-

Average Duration of Megalopal Development						
Salinity in PSU	20		25		30	
	C	G	C	G	C	G
1.0	0	-	0	-	0	-
2.0	-	-	-	-	-	-
2.5	0	-	0	-	14.3	-
<b>5.0</b>	<b>0</b>	<b>0</b>	<b>21.6</b>	<b>18</b>	<b>12.1</b>	<b>14</b>
10.0	-	32	-	16	-	12
<b>15.0</b>	<b>36.3</b>	<b>32</b>	<b>18.5</b>	<b>17</b>	<b>11.8</b>	<b>13</b>
20.0	-	30	-	18	-	12
<b>25.0</b>	<b>30.3</b>	<b>24</b>	<b>19.5</b>	<b>17</b>	<b>11.8</b>	<b>13</b>
30.0	-	0	-	19	-	15
33.0	33.7	-	18.8	-	12.5	-
35.0	28.7	-	18.5	-	13.2	-
40.0	30.1	-	23.7	-	15	-

Table 3.1. Summary of prior larval studies. Salinities and durations in bold indicate those temperature combinations shared by the studies. The column designations are as follows: C = Costlow *et al.* (1966); L = Laughlin and French (1989a), FL = Florida population and CA = California population; G = Goncalves *et al.* (1995).

### 3.3 Results

A total of eight trials across the 12 temperature salinity combinations were completed using a total of 4737 zoeae (Table 3.2). At 20°C, larvae were reared from zoeae to megalops at all salinities except 1.0 PSU. Megalops were reared to first crab stage at this temperature at all salinities except 1.0 and 2.0 PSU. At 30°C, larvae were reared from zoeae to first crab stage at all salinities, marking the first time this species has ever been reared at a salinity below 2.0 PSU. Overall survival was much lower than that recorded by Costlow *et al.* (1966) across all experimental treatments. The lowest survival percentage occurred at 20°C and 1.0 PSU. The highest survival occurred at 30°C and 15.0 PSU (Table 3.3). The highest survival was seen at 15.0 PSU regardless of temperature, which agrees with previous studies. There were no significant differences between the survival numbers for any salinity across the two temperatures. Figure 3.1 shows the number of total number of individuals that survived to megalops and the first crab stage for each salinity tested. Mortality was higher for larvae from hatching to megalops, than from megalops to first crab across all salinity combinations that had full development. Other studies indicated higher incidences of malformed megalops as salinity increased which was also seen in this study. However, over the salinities tested here only two megalops were malformed (Figure 3.2).

A significant difference was observed in the developmental rate between the two temperatures. The first day to molt to a megalops and the average number of days to molt to megalops occurred significantly earlier at 30°C than at 20°C ( $p = 0.005$  and  $p =$

Salinity (PSU)	Total # of Trials	20 C			30 C		
		Original # of Zoeae	# of Megalops	# of Crabs	Original # of Zoeae	# of Megalops	# of Crabs
1	8	500	0	0	475	18	10
2	8	400	11	0	362	64	39
5	8	400	79	55	350	105	98
15	8	400	166	139	350	144	136
25	8	400	149	99	350	74	48
33	8	400	20	13	350	8	0
Totals		2500	425	306	2237	413	331

Table 3.2. Summary of larval trials.

### Comparison of Survival Between Salinities

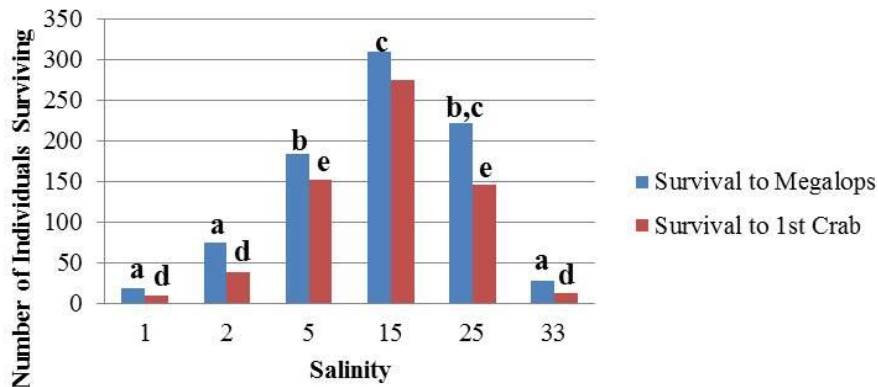


Figure 3.1. Comparison of survival between salinities. Letters indicate non-significant differences with the exception that Survival to Megalops and Survival to 1<sup>st</sup> Crab were not compared.

0.001 respectively). The first day to molt to the 1<sup>st</sup> crab stage and the average number of days to 1<sup>st</sup> crab stage also occurred significantly earlier at 30°C than at 20°C ( $p = 0.018$  and  $p = 0.012$  respectively). Results (Table 3.4 and Figures 3.3 – 3.6) were similar to

that seen in all previous studies with typical zoeal development lasting between 19 and 24 days at 20°C and 9 and 15 days at 30°C. Development to first crab stage had similar differences between the temperatures with development taking 26 to 32 days at 20°C and 12 to 24 days at 30°C.

Overall development is more prolonged at 20°C than at 30°C, which can be seen in Figures 3.3-3.7. The maximum variation was seen at 15 PSU and 20°C, where it took from 23 to 47 days to develop to the first crab stage, whereas at 30°C and the same salinity it only varied by four days. It took a significantly longer time for larvae to molt to megalops and to the 1<sup>st</sup> crab stage at 20°C ( $p = 0.005$  and  $p = 0.018$  respectively). The average number of days to molt to both megalops and the 1<sup>st</sup> crab stage was also significantly longer at 20°C ( $p = 0.001$  and  $p = 0.012$  respectively). For each of the comparisons just listed, there was a significant difference between the 20°C and 30°C at both 5 ( $p = 0.007$ ,  $p = 0.007$ ,  $p = 0.007$  and  $p = 0.007$ ) and 25 PSU ( $p < 0.001$ ,  $p = 0.007$ ,  $p < 0.001$  and  $p = 0.007$ ).

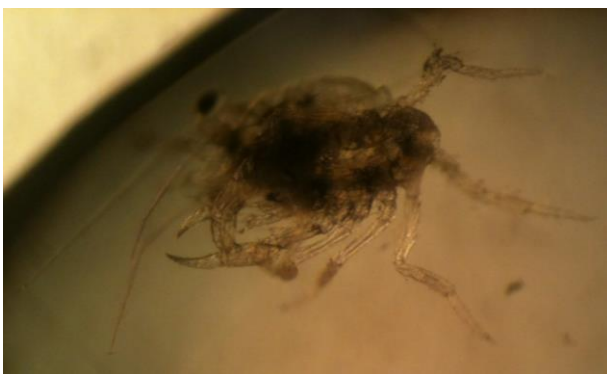


Figure 3.2. Malformed megalops. Notice the presence of the spines on the anterior portion of the megalops. These spines are typical of zoeae but are not present in normal megalops upon molting from zoeae stage 4.

	20						30					
	1	2	5	15	25	30	1	2	5	15	25	30
Survival to Megalops	0	11	79	166	149	20	18	64	105	144	74	8
Survival to 1st Crab	0	0	55	139	99	13	10	39	98	136	48	0
% Survival to Megalops	0%	3%	20%	42%	37%	5%	4%	18%	30%	41%	21%	2%
% Survival to 1st Crab	0%	0%	15%	35%	25%	3%	2%	11%	28%	39%	14%	0%
% of Megalops to 1st Crab	0%	0%	76%	84%	68%	65%	56%	61%	93%	94%	65%	0%

Table 3.3. Summary of mortality rates. Rate given for each temperature/salinity combination.

	20						30					
	1	2	5	15	25	30	1	2	5	15	25	30
Days to 1st Megalops	0	22	<b>20</b>	19	<b>18.8</b>	22	10.3	9.8	<b>10.3</b>	9.3	<b>9.5</b>	11
Average Days to Megalops	0	24	<b>23</b>	21	<b>21.1</b>	25	10.8	10	<b>9.5</b>	10.3	<b>10</b>	15
Days to 1st Crab	0.0	0.0	<b>28.4</b>	26.0	<b>26.6</b>	28.0	12	13	<b>13.1</b>	12.4	<b>13.8</b>	24
Average Days to 1st Crab	0	0	<b>31.3</b>	29.0	<b>29.4</b>	30.2	15	13.8	<b>14.0</b>	13.8	<b>13.8</b>	24

Table 3.4. Summary of developmental rates. Rates given for each temperature/salinity combination. Trials at both 5 and 25 PSU were significantly different between 20°C and 30°C.

### 1st Day to Molt to Megalops

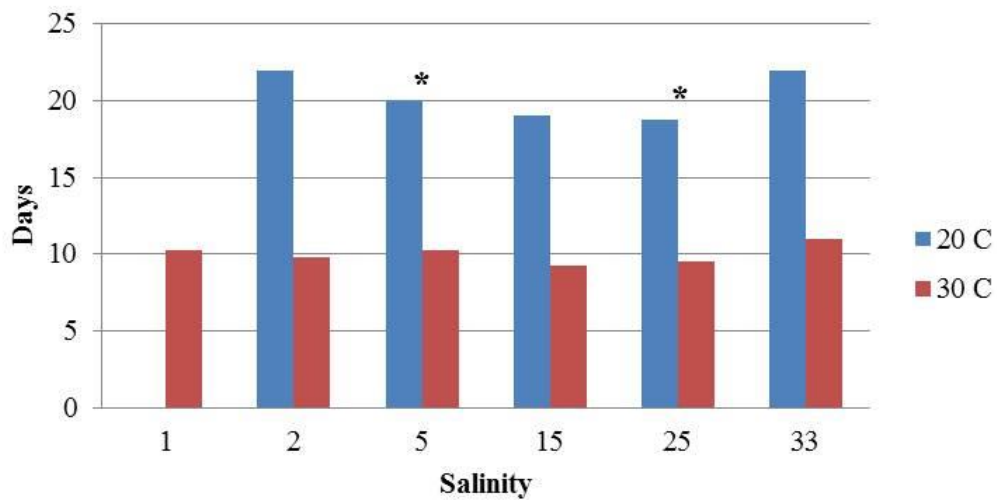


Figure 3.3. Number of days to molt to megalops at 20° and 30°C. Comparison of the 1<sup>st</sup> day to molt to megalops for each salinity across the two temperatures. \* indicate significant differences between the two temperatures for a given salinity.

### 1st Day to Molt to 1st Crab Stage

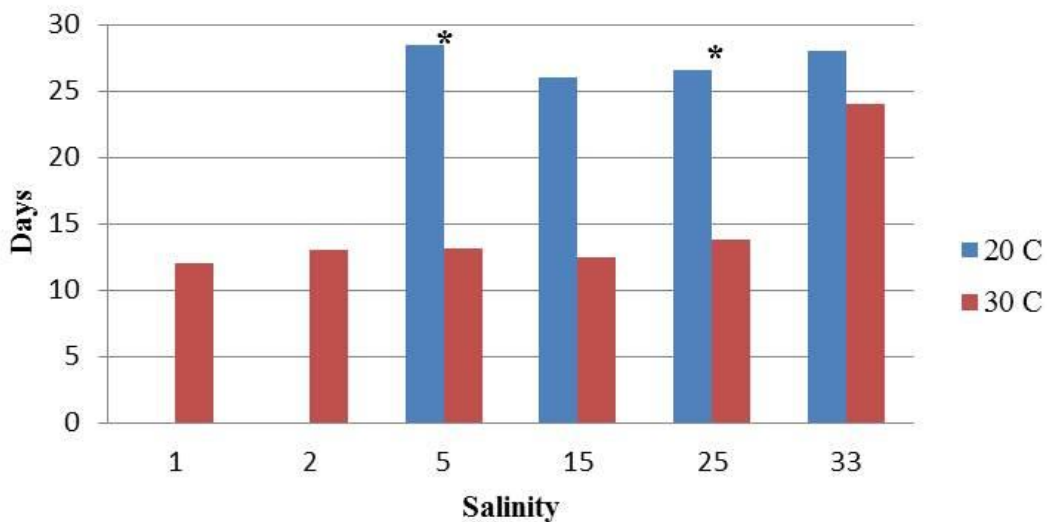


Figure 3.4. Number of days to molt to 1<sup>st</sup> crab stage at 20° and 30°C. Comparison of the 1<sup>st</sup> day to molt to 1<sup>st</sup> crab stage for each salinity across the two temperatures. \* indicate significant differences between the two temperatures for a given salinity.



## Average Number of Days to Megalops

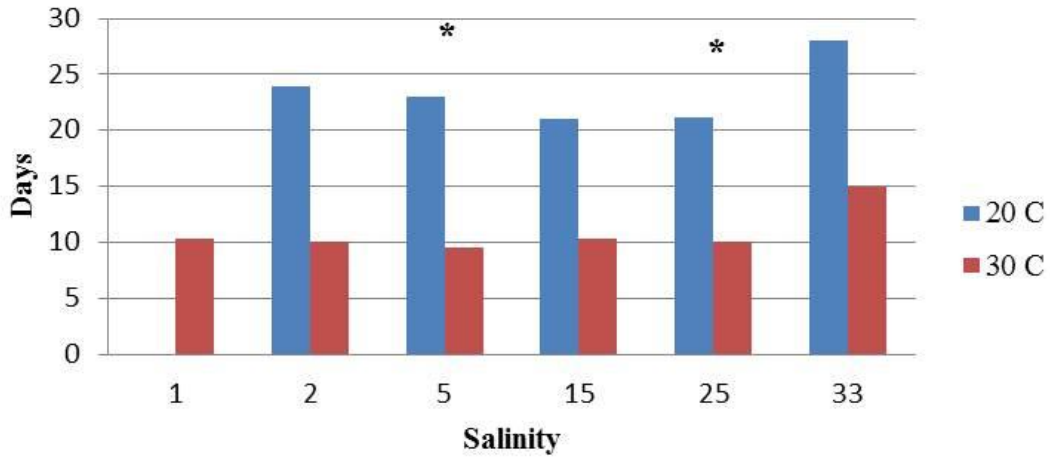


Figure 3.5. Comparison of the average number of days to molt to megalops. \* indicate significant differences between the two temperatures for a given salinity.

## Average Number of Days to 1<sup>st</sup> Crab Stage

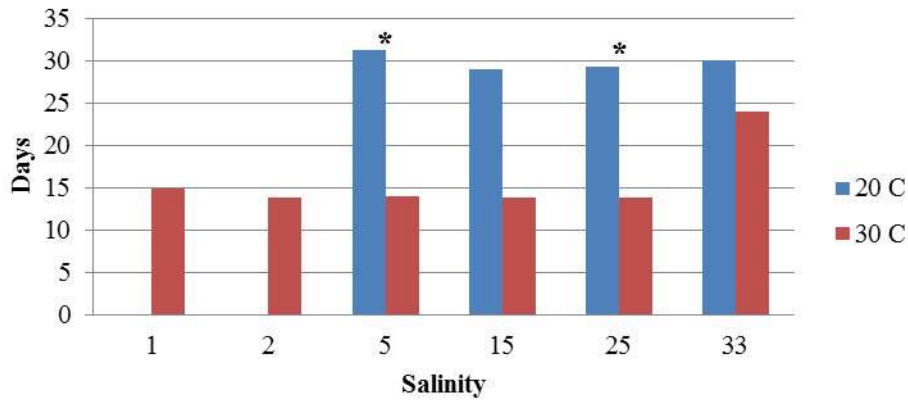


Figure 3.6. Comparison of average number of days to molt to 1<sup>st</sup> crab stage. \* indicate significant differences between the two temperatures at a given salinity.

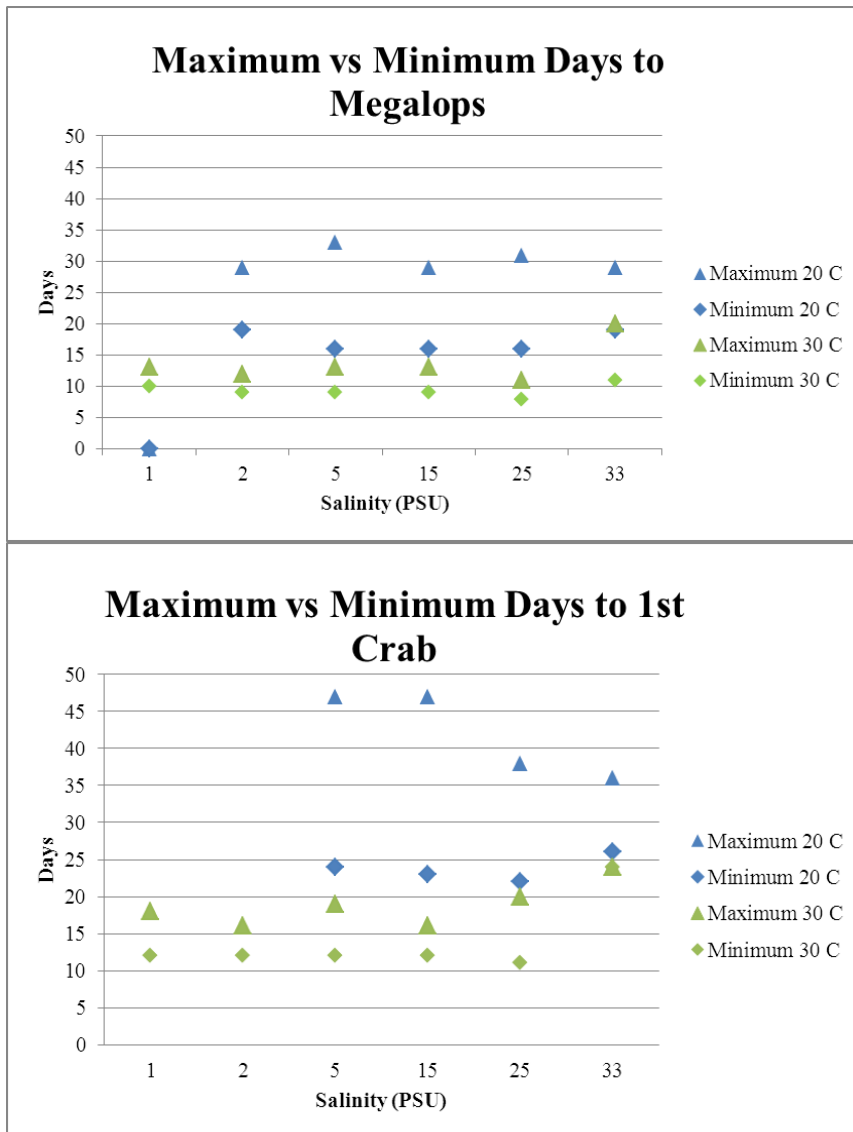


Figure 3.7. Maximum vs. minimum number of days of development. a. Development to Megalops at all temperature and salinity combinations. b. Development to the first crab stage at all temperature and salinity combinations.

### 3.4 Discussion

The objective of this study was to determine if larvae of *Rhithropanopeus harrisi* had the ability to reproduce in salinities below 2PSU, which would enable it to overcome the Reproduction filter, explaining the differential success in invading low

salinity habitats between the introduced versus the source population. This study demonstrates the successful rearing of larvae to the first crab stage at 1.0 PSU, below the previous reported limit of 2.0 PSU (Costlow *et al.* 1966; Laughlin and French, 1989a; Goncalves *et al.*, 1995).

The results demonstrate a physiological difference between *R. harrisii* in inland Texas populations and estuarine populations from Florida, California, North Carolina and Spain. Laughlin and French (1989a) observed a much higher survival of larvae of *R. harrisii* at 2.0 PSU and 30°C in a Florida population than in a California population. These authors, however, noted that the wild crabs typically do not reproduce during times when they experience these conditions. The California population, located near San Francisco Bay, rarely would encounter water temperatures > 20 °C. This, however, is not the case for either inland or coastal Texas populations.

Several authors have reviewed the ontogeny of osmoregulation in decapod larvae over the last twenty years (Pequeux, 1995; Charmantier, 1998; Charmantier and Charmantier-Daures, 2001; Anger, 2003; Anger *et al.*, 2008 Charmantier *et al.*, 2009; Torres *et al.*, 2011, Romano and Zeng, 2012). Charmantier (1998) was unable to classify *R. harrisii* into one of the three osmoregulatory pathways he proposed. Based on my results, however, it appears that this species appears to fit his type 2 pattern. In this pattern, larval stages are capable of hyper-iso- or hyper-hypo-osmoregulating from hatching throughout development, which is a pattern typical of euryhaline species with high levels of larval retention. Osmoregulation appears to be controlled by ionocytes

that are found on the branchiostegites of zoeae, and the posterior gills of megalops and juvenile crabs (Charmantier *et al.*, 2009).

Although I was able to collect ovigerous female crabs from the inland lakes, I found only eight females in coastal estuaries, which had small brood sizes of ~ 300-600 eggs. These coastal females, however, failed to hatch any larvae at all or only hatched a few individuals that failed to survive longer than two days at any salinity. With the reduced number of gravid females collected and low number of larvae that hatched in the laboratory, it is difficult to determine whether the failure to raise larvae was due to pre-existing conditions within the wild population, effects of collecting and transportation, or rearing techniques in the laboratory. The eight gravid females collected from the coast were found under some rocks along the shoreline. Most of the coastal sites I visited have soft mud substrates with minimum cover and murky water, which makes collection by hand very difficult. Individual crabs could be caught in traps with oyster shell (see Section 4), but these traps apparently did not attract gravid females.

*Rhithropanopeus harrisi* populations along the Texas coast extend further inland than most other native populations of this species in other locations across its range. Individuals of this species do not make migrations upriver, as has been observed in the Chinese mitten crab (*Eriocheir sinensis*), which is known to migrate hundreds of kilometers upstream (Panning, 1939; Sui *et al.*, 2009; Veldhuizen and Stansih, 1999). The lack of this migration pattern in *R. harrisi*, suggests that if populations along the

Texas coast have the ability to develop at the lower salinities, then some other mechanism is preventing their movement further upstream. Compared to *E. sinensis*, which has a typical carapace width of 25 – 40mm (Veldhuizen and Stansih, 1999), *R. harrisii* is much smaller, thus, size may be a limiting factor that prevents free movement of crabs upstream.

It is possible that this low salinity tolerance phenotype may be in extremely low numbers in the natural populations of *R. harrisii*. Dr. Don Keith, professor emeritus Tarleton State (personal communication) hypothesized that *R. harrisii* had been introduced to Texas lakes through stocking procedures of saltwater fish (i.e. Red Drum, *Sciaenops ocellatus*). These stocking procedures require hatching of *S. ocellatus* larvae at 15-25 PSU. Therefore, to stock fish in an inland freshwater reservoir, they must be acclimated first to the lower salinity (Wilson, 1990). If crab larvae were introduced into the stocking tank along with the fish, and underwent the same acclimatization process, it could result in the selection of larvae capable of developing at lower salinities.

The introductions of *R. harrisii* into inland lakes occurred < 20 years ago. Boyle *et al.* (2010) suggests that at least two different introductory events had occurred based on mtDNA haplotype frequencies. These authors found relatively high diversity in the inland reservoirs compared to the coastal sites they examined. It should be noted, however, that they had limited samples from the coastal sites, which potentially biased their results. High levels of genetic diversity in some invasive populations suggest they

did not experience strong bottlenecks during invasion (Rytönen *et al.*, 2011; Kirk *et al.*, 2011; Boubou *et al.*, 2009).

*De novo* mutations do not seem an explanation for the increase in tolerance to freshwater salinity conditions in *R. harrisii*, given the short lapse since they invaded Texas lakes (i.e., < 20 years). Strong directional selection also does not appear to be occurring. In the marine copepod *Eurytemora affinis*, a physiological transition from saline to freshwater conditions occurred within few generations in invasive populations (i.e., in the Great Lakes) and in the lab, which was attributed to selection of pre-existing variation (Lee *et al.* 2011). Furthermore, this copepod shifted its optimal development to freshwater conditions, as expected under strong directional selection. In the case of *R. harrisii*, however, I observed that although larvae are capable of surviving to the first crab stage at 1 PSU (i.e. freshwater), the optimal salinity was still more similar to the one experienced by the coastal populations. Therefore, this suggests that strong directional selection has not shifted the salinity optimal in *R. harrisii* inland populations.

Other mechanisms may explain the absence of a shift to optimal development in freshwater conditions in *R. harrisii* inland populations. Phenotypic plasticity may be one possibility. For example, salinity tolerance has been observed in the crab *Callinectes sapidus*, which has been shown to be regulated by an increase or decrease in the number of ion transporters in posterior gill epithelia (Towle *et al.* 2001). Phenotypic plasticity may also explain the maintenance of the optimal salinity around 15 PSU in *R.*

*harrisii*. Natural estuarine populations may be exposed to wide fluctuations in salinity and larvae having a broad physiological response be selected.

Balancing selection of a pleiotropic gene(s) could limit the positive selective pressure on larval salinity tolerance if the gene(s) involved affect other traits that are negatively selected for (Levin, 2003; Sih, Bell and Johnson, 2004; Stearns, 2010; Karl and Avise, 1992; Lafuma and Maurice, 2007; Frankham, 2008). Thus any positive gain in the direction of lowered salinity tolerance would result in a negative gain in the corresponding trait. Lawson *et al.* (2011) illustrated the maintenance of genetic variation by antagonistic pleiotropy in a MetS quantitative trait loci study in mice due to context dependent interactions. Balancing selection could also be at work in a non-pleiotropic gene. For instance, if the upregulation of ion transporters is energetically costly, then balancing selection would select for a suboptimal phenotype to minimize energy costs. Continued investigation into the mechanism of low salinity tolerance in *R. harrisii* could determine whether balancing selection was acting or not.

Finally, additive genetic variation/hybrid vigor could explain the low levels of this adaptive trait. Considering that Boyle *et al.* (2010) found evidence of multiple introductions and females of this species can store sperm (Morgan *et al.* 1983), it is possible that the variation seen here is the result of mixed parentage within the individual broods. This implies that the trait is not necessarily present in the native environments but instead is the result of the admixture of multiple populations within the inland lakes.

Interestingly, the optimal salinity of 15 PSU seen in this study appears to be the optimal salinity across all populations regardless of temperature. This reflects the natural habitat of this species as an estuarine species. The difference in the lower salinity range of this species does not reflect differences in habitats. Although it is true that waters off the east coast of North America are cooler than those throughout the Gulf of Mexico, the salinity regimes in estuaries of both areas are similar due to freshwater inputs through rivers and streams. There are several possible explanations (competitive exclusion, predator/parasite avoidance, prior distribution during glacial refugia, historic river outflow to the Gulf region) for why this adaptation may have developed in the Gulf of Mexico and not on the east coast.

One possibility for the evolution of low salinity tolerance in the larvae of *R. harrisii* in the Gulf of Mexico estuaries may be competitive exclusion from higher salinity waters. A literature search of crab species native to the Gulf of Mexico and the east coast of the United States indicates that the estuaries of the Gulf of Mexico are home, on the average, to twice as many species of xanthoid crabs that overlap in habitat with *R. harrisii*. *Rhithropanopeus harrisii* may largely be confined to the upper reaches and consistently lower salinities in order to escape competition for shelter, food, etc.

A second possibility is that movement of the crabs into low salinity waters reduces or avoids parasitism by the rhizocephalan barnacle, *Loxothylacus panopaei*. Kruse *et al.* (2012) looked at the distribution of this parasitic species (which is native to the Gulf of Mexico) along the southeast Atlantic coast of the United States and the Gulf



of Mexico. The barnacle was shown to infect *Eurypanopeus depressus*, *Panopeus spp.* and *R. harrisi*. Of the 20 sites investigated by these authors, only two had populations of *R. harrisi* with infections. Grosholz and Ruiz (1995) reported infections in 80% *R. harrisi* collected in the lower Chesapeake Bay. Their study also noted the absence of infected *R. harrisi* in the northern Chesapeake Bay, where salinities rarely top 5.0 PSU. Tolley *et al.* (2006) showed that freshwater inflow created potential refuges for the crab *Panopeus obesus* from the barnacle parasite. The prevalence of the parasite fell below 10% once observed salinities were below 15 PSU. Considering that the parasite is native to the Gulf of Mexico, it can be assumed that populations of *R. harrisi* in the Gulf of Mexico have been under pressure from this parasite and may have adapted to the lower salinities.

Regardless of the potential causes for this physiological difference, the lower salinity tolerance of the invasive populations is of major concern. It highlights the potential of this species to spread to even more freshwater habitats. I conducted a brief search of the United States Geological Society's National Water Information System (National Water Information System Web), and surveyed 44 lakes from Texas. Of those 44, thirteen had maximum salinities greater than 0.7 PSU and seven had average salinities greater than 0.7 PSU. It should be noted that the availability of data was unequal for each lake due to unequal sampling by the reporting agencies. Some of these lakes lacked data during years of drought, which would potentially increase the number of lakes with salinities greater than 0.7 PSU. Currently, breeding populations of *R. harrisi* are only known from nine lakes, seven of which were sampled. Therefore, there

are at least six more lakes that may be capable of supporting a reproducing population of *R. harrisii*. This does not take into account the miles of river connecting the different lakes or the potential to spread into other states. The small size (0.5 – 1.0mm) of these larval stages increases the likelihood of their going undetected in live bait wells and ballast water, thus increasing the potential of this crab to spread throughout the state of Texas and potentially into other states as well (Connolly, 1925).

## **4. POTENTIAL FACTORS INFLUENCING THE RANGE OF *Rhithropanopeus harrisii* IN ESTUARIES OF THE GULF OF MEXICO**

### 4.1 Introduction

An emphasis on species characteristics in invasion models is critically important in the management of invasive species. Models currently assume that species traits are homogenous throughout the range of the species. This assumption ignores local adaptation, which could potentially “pre-adapt” a species to successful invasion. Species descriptions, including their type locality and habitat, initially are based on a holotype specimen from a single population. Over time, information from individuals of different populations usually is incorporated into descriptions and studies of their life history and habitat. However, the individuals or populations that have been studied previously may not necessarily be the source population. Therefore, it becomes imperative that the models incorporate a new variable, the source population.

Phenotypic differences in source populations are likely to due to local ecological and evolutionary factors. These differences can cause differential invasion success. If the differences are unknown, false assumptions can be made. Invasive species have the potential to undergo selection during the invasion process through stochastic events such as founder effects and bottlenecks. The assumption of a bottleneck has been shown to be violated by introductions from multiple sources (Boyle *et al.*, 2010). There also is the potential for natural selection to act on existing and novel traits within the new environment. A trait may appear novel in an invaded habitat because it has not been previously described in the literature. Finding such a trait would then immediately lead

the researcher to assume natural selection was acting on a novel mutation or new combination of genes due to multiple introduction sources. However, if the trait does exist in the source population(s), then a stochastic event is the more likely selective agent. In either case, an understanding of the factors that lead to this innovation are warranted in order to understand why one population/species may develop the traits necessary for invasion.

Most papers on *Rhithropanopeus harrisii* have focused on populations exclusively from the east coast of the United States (Bentley and Sulkin, 1977; Costlow *et al.*, 1966; Fitzgerald *et al.*, 1998; Forward *et al.*, 1982; Forward and Wellins, 1989; Frank *et al.*, 1975; Kalber and Costlow, 1966; Reisser and Forward, 1991; Ryan, 1956). Although the studies are statistically rigorous, they are focused on only a subset of the populations of the entire range because of ease of collection as most of the authors were located in the area.

*Rhithropanopeus harrisii* has now become invasive across the globe, most recently in several lakes in Texas. Boyle *et al.* (2010) found evidence of reproducing populations in all of the inland populations they examined. Boyle *et al.* (2010) also found that inland lake populations shared more cytochrome oxidase subunit 1 haplotypes with Gulf coast individuals than they did with Atlantic coast populations. The Gulf of Mexico coast, and not the east coast of the U.S.A., is thus the likely home to the source population(s) for this invasion.

In section 3, I demonstrated the ability of the inland populations to survive and reproduce at salinities lower than those reported for east coast populations. Whether the ability of larvae to develop at lower salinities is a novel phenotype that evolved within the lakes or a phenotype that occurs in the source coastal populations remains uncertain. Because optimum survival was found at 15 PSU, I assume that the phenotype is more likely to already exist in the source population(s). If the phenotype does exist in the source coastal populations, what potential factors are acting on those populations that do not exist elsewhere? Several potential possibilities come to mind: competitive exclusion, lower average salinity, and past range reductions/expansions during times of glaciation and melting. Of these, the first two possibilities are explored herein. No fossil or “relic” populations of *R. harrisii* are known. Inland populations are associated with reservoirs of recent human construction.

Competitive exclusion in its simplest form means that two sympatric species with completely overlapping niches cannot exist together (Hardin, 1960). Where there is not a complete overlap of niches, then the species have been shown to shift their realized niche away from that of the competition (Hutchinson, 1957). Increasing numbers of sympatric species with overlapping niches would cause a greater reduction in the realized niche away from the fundamental niche (Morse, 1974; Branch, 1976; Ricklefs, 2010). Many studies have looked at competition and personal space in crustaceans (Alonso and Martinez, 2006; Aspey, 1978; Barbaresi and Gherardi, 1997; Blank and Figler, 1996; Breen and Metaxas, 2012; Bubb *et al.*, 2009, Emperanza, 2007; Figler, Cheveton and Blank, 1999; Garvey, Stein and Thomas, 1994; Gherardi and Cione, 2004;

Heck Jr. and Hambrook, 1991; Huber, 1987). Crustacean antagonism is highly ritualized and personal territory is often highly defended. The meral spread is the most common display, typically the first action done to illustrate hostile intentions (Dingle and Caldwell; 1969, Caldwell and Dingle, 1975; Gherardi and Cione, 2004). Many species such as crayfish, lobsters and porcelain crabs also will use antennae whipping as a means of threat display (Solon and Cobb, 1980), but the antennae of *R. harrisii* are too short to be useful for such a display.

In this study, I looked at the potential for competitive exclusion of *Rhithropanopeus harrisii* from preferred niche space within the primary estuary into more freshwater upriver locations. Exclusion of *R. harrisii* could explain the lowered salinity tolerance of the larval stages of this species from Gulf coast populations. I first gathered data on the number of sympatric species with similar niches to *R. harrisii* within the Gulf of Mexico region and the Atlantic coast of the United States (Rathbun, 1930; Felder, 1973; Powers, 1977). The following nine estuarine crab species have ranges and occupy similar niches to *R. harrisii* (Table 4.1): *Eriphia gonagra*, *Menippe adina*, *Menippe mercenaria*, *Eurytium limosum*, *Eurypanopeus depressus*, *Eurypanopeus abbreviatus*, *Dyspanopeus texanus*, *Eurypanopeus turgidus* and *Panopeus* spp. (Rathbun, 1930; Felder, 1973; Powers, 1977). The *Panopeus* species group is comprised of two species *Panopeus simpsoni* and *Panopeus obesus*, which were formally united as *Panopeus herbstii* (Williams, 1983). In older literature, it may be impossible to determine which of the two species was studied. All nine species are found in Florida. All but *Menippe mercenaria* are common throughout the northern

Gulf of Mexico. Of the nine, only five occur alongside *R. harrisii* up the east coast to North Carolina and only two or three of those, *E. depressus* and *Panopeus* spp., are found as far north as Massachusetts. The species that occur throughout the range of *R. harrisii* are *Eurypanopeus depressus* and *Panopeus* spp.

The most well studied population of *R. harrisii* on the east coast of the United States is in Chesapeake Bay (Ryan 1956). Ryan collected approximately 1000 individuals from 113 stations. Salinities at these stations ranged from 2.8 to 18.6 PSU. The most inland of his collecting sites was approximately 30 miles (48 km) inland. The GBIF website identified 94 collecting sites from the Atlantic coast and 74 from the Gulf of Mexico. The most inland of these collecting sites was approximately 40 miles inland on the Hudson River in New York. Wurtz and Roback (1956) found *R. harrisii* in the Neches River near Beaumont, Texas (approximately 60 miles) at a salinity of 0.34 PSU. My Colliers Ferry Park is near this site. Wurtz and Roback (1956) also collected near my Orange City collection site (approximately 40 miles). Other collections of *R. harrisii* on the Texas coast have historically come from the bays. Collection sites indicate a potential exclusion of *R. harrisii* into more fresh water as this is the maximum distance they have been found upriver in natural habitats.

The first possible factor influencing the local distribution and larval physiology tolerances of *R. harrisii* is the direct competition between this species and similar xanthoid species that occupy similar niches. I identified eight species that occupy similar niches within Gulf of Mexico estuaries. *Rhithropanopeus harrisii* is the only one

of these species that is capable of surviving for extended periods of time at salinities below 2 PSU. It is also the only species to show the ability to hatch its eggs at a salinity as low as 2 PSU. This opens the possibility that *R. harrisii* may inhabit the upper reaches of the estuaries and further upriver here in Texas due to interspecific competition. Hulathduwa *et al.* (2011) looked at shelter competition between *R. harrisii*, *E. depressus*, and *P. simpsoni* in the presence of a blue crab, *Callinectes sapidus*. They found that *R. harrisii* was outcompeted by both of the other species and was therefore more likely to be eaten by the blue crab. They speculated that this species may be moving into lower salinity waters to avoid predation.

Species	States within Range															
	Gulf of Mexico					Atlantic Coast										
	TX	LA	MS	AL	FL	GA	SC	NC	VA	MD	DE	NJ	NY	CT	RI	MA
<i>Rhithropanopeus harrisii</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Eriphia gonagra</i>	X				X			X								
<i>Menippe adina</i>	X	X	X	X	X											
<i>Menippe mercenaria</i>					X	X	X	X								
<i>Eurytium limosum</i>		X			X	X	X	X	X	X	X	X	X			
<i>Eurypanopeus depressus</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Eurypanopeus abbreviatus</i>	X	X					X									
<i>Dyspanopeus texana</i>	X	X	X	X	X											
<i>Eurypanopeus turgidus</i>	X	X	X	X	X											
<i>Panopeus spp</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<b>TOTAL</b>	<b>7</b>	<b>7</b>	<b>5</b>	<b>5</b>	<b>8</b>	<b>4</b>	<b>5</b>	<b>5</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>2</b>	<b>2</b>	<b>2</b>

Table 4.1. Sympatric crab species. A list of xanthoid crab species that occur within estuaries of the contiguous United States and overlap with *Rhithropanopeus harrisii* ecologically.

In order to determine if *R. harrisii* is less aggressive and therefore more likely to be excluded, I performed personal space trials to determine how close individuals of four xanthoid species would permit other individuals to approach to them. Previous studies on agonism in crustaceans have focused on interactions solely in the context of a



resource. I chose to not use a resource in the first set of competition trials in order to determine if an agonistic interaction would still occur, thus indicating the baseline of aggression for each of the four species.

Shelter is a limiting resource for the crab species, which have sandy or muddy substrates. An exposed crab in the bays is in danger of predation by bony fishes and larger crustaceans. If a small crab such as *R. harrisii* were less agonistic towards others, it could be more likely to share a shelter and have a better chance of avoiding predation or desiccation. I then performed shelter competitions to determine if competitive exclusion was taking place between the separate species. I hypothesized based on Hulathduwa *et al.* (2011) that *R. harrisii* is being excluded from shelter by sympatric species.

## 4.2 Methods

### 4.2.1 Field Collections and Site Comparisons

I first compared localized distributions from the literature to determine *Rhithropanopeus harrisii* occupied different upriver localized distributions within Atlantic coast estuaries from those on the Texas coast. Collection data from the Global Biodiversity Information Facility (Atlantic Reference Centre (OBIS Canada), 2013; EPA's EMAP Database, 2013; National Benthic Inventory, 2013; Conkright *et al.*, 2002; The SERTC Invertebrate Database: Invertebrates of the southeastern United States, 2013; USGS Nonindigenous Aquatic Species Database, 2013) was used to populate a

Google Earth (6.1.0.5001) map showing known collecting sites for each state with collections (see Appendix 2).

I personally collected from 28 sites (Table 4.2) on the Texas coast to determine how far up river this species extends from the coastal estuaries. Sites were limited to streams and tributaries that could be accessed from a public roadway without trespassing onto private property. Salinity and temperature were measured at each site using a YSI 30 meter (model 30-10FT) during trap deployment and collection. GPS for each site was found using a hand held Garmin (model gps72) global positioning unit. I deployed traps consisting of a black sterilite mini crate, 22.9 cm x 20 cm x 15.6 cm (Model number 16959012), filled with oyster shells and covered by black mesh with 2.5 cm openings. The traps were soaked for approximately two months. It should be noted that the traps did not prevent individuals from entering and leaving the traps at any time, but instead acted more like a temporary shelter from which I could remove and collect crabs.

I also looked at published salinity data for North Carolina and Virginia compared to that for Texas coastal sites (Deaton *et al.*, 2010; Kuhn and Chen, 2005; Schoenbaechler and Guthrie, 2011a; Schoenbaechler and Guthrie, 2011b; Guthrie, Matsumoto and Solis, 2012; Johnson *et al.*, 1993; Guthrie, 2010a; Guthrie 2010b). North Carolina and Virginia data were chosen because most of the papers on east coast populations are from either Chesapeake Bay or from the Beaufort area of North Carolina.

#### 4.2.2 Interpersonal Space and Shelter Competition

Studies of interpersonal space are critical in aggressive species, such as river shrimps (*Macrobrachium* spp.) or crayfish (*Procambarus* spp.). I followed protocols suggested by such studies (Mariappan and Balasundaram, 2003; Figler *et al.*, 1999; Blank and Figler, 1996; Larson and Magoulick; 2009). I performed shelter competition and agonistic trials to determine what effect, if any, the three most common sympatric species had on *R. harrisii*. I collected *Panopeus simpsoni*, *Eurypanopeus depressus* and *Dyspanopeus texanus* from Galveston Bay, Texas (29° 15' 20.46" N, 94° 55' 06.71" W) at a salinity of 26 PSU. *Rhithropanopeus harrisii* was collected from Lake Whitney, TX (31° 51' 47.01" N, 97° 23' 12.29" W) at 2 PSU. All species were maintained in separate aquaria. Individuals of *R. harrisii* were adjusted over a one-week period from 2 PSU to 26 PSU for subsequent trials. Prior to the start of trials, all crabs were placed in individual plastic containers with 300 ml of 26 PSU water and a piece of PVC pipe for shelter. Two sets of trials were conducted. For all trials, individuals were size matched except for individuals of *P. simpsoni*, which are larger on average than all other species. The first trial (interpersonal space) consisted of two crabs placed at opposite ends of a rectangular arena (Figure 4.1) that was submerged in a larger tank. The arena restricted lateral movement of the crabs. The test crab was placed at one end of the arena and the "opponent" was placed at the opposite end. Trials lasted until the crabs came into contact or passed each other. When necessary the opponent was gently pushed towards the test crab using a lever within the arena. All trials were filmed and the test crab responses were recorded as aggressive (raised chelae) or non-aggressive (no raised

chela). All species combinations were tested. Individual crabs were only used in one trial per day but due to collecting difficulties were used in multiple trials. No trial lasted longer than one minute to insure that no crabs would kill each other. A Chi-square analysis was performed to determine whether a species was significantly aggressive towards conspecifics or heterospecifics.

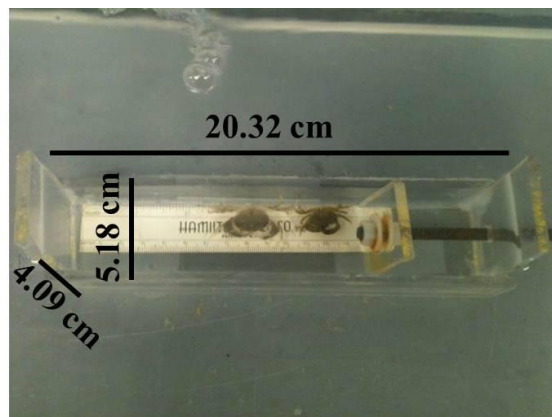


Figure 4.1. The test arena used for the interpersonal space trials.



Figure 4.2. The test arena for the shelter competition trials.

Sites	GPS	
	North	West
Low water Dam on Nueces	27°53.065'	97°37.559'
Aransas River off HWY 77	28°07.575'	97°25.664'
Mission River at Refugio Park	28°17.489'	97°16.680'
Mission River off of Rd 2678	28°11.038'	97°12.845'
West Copano Bay Mouth of Aransas River	28°04.648'	97°13.279'
Salt Creek off Hwy 35	28°16.341'	96°57.660'
Guadalupe River off HWY 35	28°28.681'	96°51.780'
Hog Bayou off HWY 35	28°29.814'	96°50.624'
West Caranchau Creek off FM 616	28°50.755'	96°24.727'
Tres Palacios River off Hwy 35	28°53.163'	96°10.940'
Colorado River East of Power Plant on HWY 521	28°47.252'	95°59.748'
Colorado River West of Bay City off HWY 35	28°59.055'	96°00.220'
Colorado River at Wharton off HWY 59	29°18.511'	96°06.029'
Brazos River off of HWY 521	29°03.301'	95°33.411'
San Bernard River off 521	29°00.668'	95°35.265'
San Bernard River off FM 2611	28°57.047'	95°33.323'
Brazos River off of HWY 36	28°56.824'	95°22.846'
Oyster Creek off Hwy 523	29°00.823'	95°19.771'
Austin Bayou off CR 227	29°05.665'	95°17.032'
CR 227 at left turn sign	29°07.700'	95°14.556'
North Side of Chocolate Bayou off FM 2004	29°12.702'	95°12.466'
Chocolate Bayou off 171 near Liverpool	29°18.160'	95°16.035'
Halls Bayou off FM 2004	29°17.202'	95°07.879'
Jack Brooks Park	29°22.034'	95°02.954'
Trinity River, Wallisville Park	29°48.548'	94°43.828'
Trinity River of HWY 90 in Liberty	30°03.491'	94°49.130'
City of Orange Boat Ramp on Sabine River	30°05.782'	93°43.486'
Colliers Ferry Park in Beaumont, Neches River	30°07.835'	94°05.641'

Table 4.2. A list of Texas coastal trapping sites.

The second set of trials (shelter competition) was conducted in a small aquarium (14.5 cm x 22 cm x 9cm) (Figure 4.2). A PVC shelter was placed against one wall of the arena and the tank was filled to a height of 3cm with 26 PSU water from a tank

containing a stone crab, *Menippe adina*, which induced a shelter seeking response in all test species. The arena was wrapped with brown paper to minimize the effect of external movement on the behavior of the crabs. A *R. harrisii* individual and a second crab were placed inside the arena and filmed for 30 minutes. All trials were analyzed for the amount of time each individual spent under the shelter alone or together. Each crab was used in multiple trials. No crab was used in more than one trial per day. A Wilcoxon Signed Rank test performed using SPSS 20 to determine if the proportion of time *R. harrisii* spent under the shelter was significantly different from its heterospecific competitors.

## 4.3 Results

### 4.3.1 Results of Field Collections

Of the 28 traps I deployed, I was able to retrieve 14 (Table 4.3). I found *R. harrisii* in seven of the 14 retrieved traps and along the shoreline at a site where I was unable to retrieve the trap. The minimum salinity for a site with *R. harrisii* was 0 PSU and the maximum was 34 PSU. The San Bernard River site is the most inland coastal site examined in this study.

Site	Miles From Bay	Salinity PSU		Temperature °C	
Aransas River off HWY 77	26.26	1.1	1	17.5	26.8
Salt Creek off Hwy 35	0.79	20.2	31.3	21.6	25.7
Guadalupe River off HWY 35	7.43	0.3	0.3	16.7	26
Tres Palacios River off Hwy 35	18.11	0.1	0.5	17.3	25.6
Brazos River off of HWY 521	24.24	0.1	0.4	15.7	23.1
CR 227 at left turn sign	4.92	1.4	0.4	14.9	19.5
Jack Brooks Park	8.73	1.3	0.2	15.2	18.2
Low water Dam on Nueces	12.22	1.3	7.6	17.8	26
Mission River off of Rd 2678	3.76	27.9	<b>33.9</b>	18.8	26.5
San Bernard River off 521	18.23	0.1	0.3	17	21.3
Brazos River off of HWY 36	4.91	1.8	2.5	15	23.5
Oyster Creek off Hwy 523	4.5	4	0.1	15.4	21
Halls Bayou off FM 2004	11.27	0.3	0.2	17.3	23.1
Colliers Ferry Park in Beaumont, Neches River	44.57	<b>0</b>	0.1	15.9	20
City of Orange Boat Ramp on Sabine River	33.51	<b>0</b>	0.1	16.3	21

Table 4.3. List of collection sites from the Texas coast. Collections occurred from 2/18/12 through 4/21/12. *Rhithropanopeus harrisii* was collected from all of the unshaded sites. Salinities in bold represent the minimum and maximum.

#### 4.3.2 Results of Interpersonal Space and Shelter Competition Trials

32 conspecific interpersonal space trials were conducted (Table 4.4). There were only 7 aggressive (raised chelae) displays between conspecific individuals.

*Eurypanopeus depressus* and *E. turgidus* were both shown to be significantly non-aggressive (no raised chelae) ( $p = 0.05$  and  $0.002$  respectively). *Panopeus simpsoni* had the greatest variation with a 3:2 ratio of aggressive to non-aggressive acts.

*Rhithropanopeus harrisii* had 1 aggressive and 6 non-aggressive interactions but was not found to be significantly non-aggressive.

Conspecific Interpersonal Space Trials					
Species	Aggressive	Non-Aggressive	Total Trials	Chi-Square	p-value
<i>R. harrisii</i>	1	6	7	4.17	0.06
<i>E. depressus</i>	0	4	4	4	<b>0.05</b>
<i>E. turgidus</i>	0	10	10	10	<b>0.002</b>
<i>P. simpsoni</i>	6	4	10	0.4	0.53
<i>D. texanus</i>	0	1	1	1	0.32

Table 4.4. Conspecific interpersonal space trials. Bold indicates significant p-values.

1086 heterospecific interpersonal space trials were conducted (Table 4.5). 346 aggressive displays and 740 non-aggressive displays were observed. No species was found to be significantly aggressive. *R. harrisii* was found to be significantly non-aggressive when interacting with all four of the other species. *Eurypanopeus depressus* was significantly non-aggressive when interacting with *P. simpsoni*, *E. turgidus* and *R. harrisii*. *Eurypanopeus turgidus* was significantly non-aggressive when interacting with *P. simpsoni* and *E. depressus*. *Panopeus simpsoni* was found to be significantly non-aggressive when interacting with *E. depressus*. Lastly, *D. texanus* was found to be significantly non-aggressive when interacting with *E. depressus*. No trials were run between *D. texanus* and *E. turgidus* because the *D. texanus* died before the trial began.



Trial	Heterospecific Interpersonal Space Trials				
	Aggressive	Non-Aggressive	Total Trials	Chi-Square	p-value
<i>R. harrisii</i> vs. <i>E. depressus</i>	2	53	55	40.36	< <b>0.001</b>
<i>R. harrisii</i> vs. <i>E. turgidus</i>	2	68	70	62.23	< <b>0.001</b>
<i>R. harrisii</i> vs. <i>P. simpsoni</i>	0	70	70	70	< <b>0.001</b>
<i>R. harrisii</i> vs. <i>D. texanus</i>	0	10	10	10	<b>0.002</b>
<i>R. harrisii</i> vs All	4	203	207	191.3	< <b>0.001</b>
<i>E. depressus</i> vs. <i>R. harrisii</i>	19	50	69	13.928	< <b>0.001</b>
<i>E. depressus</i> vs. <i>E. turgidus</i>	29	71	100	17.64	< <b>0.001</b>
<i>E. depressus</i> vs. <i>P. simpsoni</i>	37	61	98	5.88	<b>0.015</b>
<i>E. depressus</i> vs. <i>D. texanus</i>	2	6	8	2	0.16
<i>E. depressus</i> vs All	87	188	275	37.09	< <b>0.001</b>
<i>E. turgidus</i> vs. <i>R. harrisii</i>	37	33	70	0.229	0.63
<i>E. turgidus</i> vs. <i>E. depressus</i>	39	61	100	4.84	<b>0.028</b>
<i>E. turgidus</i> vs. <i>P. simpsoni</i>	21	78	99	32.82	< <b>0.001</b>
<i>E. turgidus</i> vs. <i>D. texanus</i>	9	11	20	0.2	0.65
<i>E. turgidus</i> vs. All	106	183	289	20.52	< <b>0.001</b>
<i>P. simpsoni</i> vs. <i>R. harrisii</i>	42	28	70	2.8	0.09
<i>P. simpsoni</i> vs. <i>E. depressus</i>	29	71	100	17.64	< <b>0.001</b>
<i>P. simpsoni</i> vs. <i>E. turgidus</i>	44	56	100	1.44	0.23
<i>P. simpsoni</i> vs. <i>D. texanus</i>	9	11	20	0.2	0.65
<i>P. simpsoni</i> vs. All	124	166	290	6.08	<b>0.014</b>
<i>D. texanus</i> vs. <i>R. harrisii</i>	1	6	7	3.56	0.06
<i>D. texanus</i> vs. <i>E. depressus</i>	3	5	8	0.5	<b>0.048</b>
<i>D. texanus</i> vs. <i>E. turgidus</i>	NA	NA	NA	NA	NA
<i>D. texanus</i> vs. <i>P. simpsoni</i>	1	6	7	3.56	0.06
<i>D. texanus</i> vs. All	6	19	25	6.76	<b>0.009</b>

Table 4.5. Heterospecific interpersonal space trials. Values in the cells refer to the actions of the first species in the species pairs. Bold indicates significant p-values.

64 shelter competition trials were conducted (Figure 4.3 and Table 4.6).

*Rhithropanopeus harrisii* occupied the shelter in each species interaction for about 10

percent of the time. It was typically the loser as the competitor usually occupied the shelter for longer periods of time. *E. depressus* was the only species to spend a significantly more amount of time under shelter than *R. harrisii*.

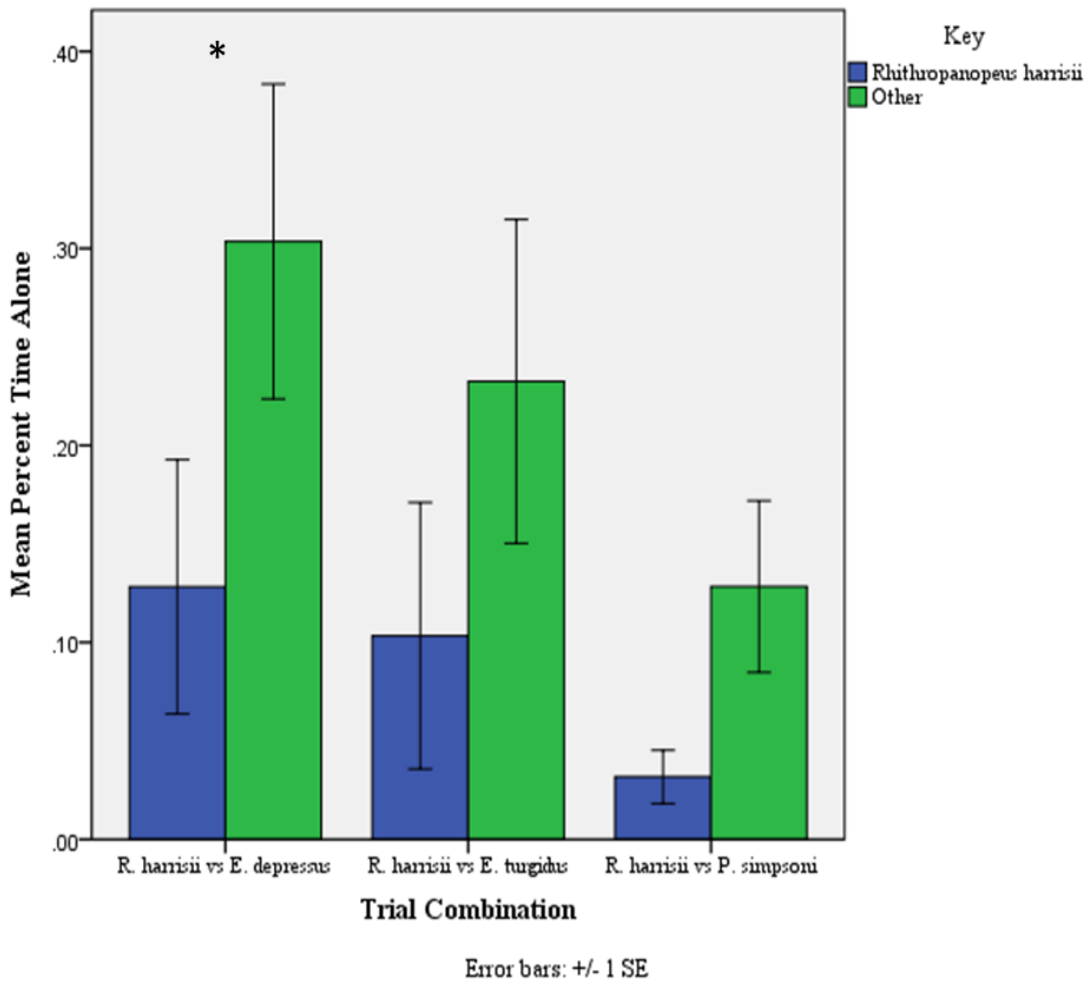


Figure 4.3. Heterospecific shelter competition. Comparison of the proportion of time spent alone under shelter during competition. ‘\*’ – indicates  $p < 0.05$ .

	p-value
<i>R. harrisii</i> vs. <i>E. depressus</i>	.023
<i>R. harrisii</i> vs. <i>E. turgidus</i>	.314
<i>R. harrisii</i> vs. <i>P. simpsoni</i>	.151

Table 4.6. P-values for the Wilcoxon Signed Rank Test.

#### 4.4 Discussion

According to the reports in the Global Biodiversity Information Facility, many collections of *Rhithropanopeus harrisii* are shown to be in the main bodies of bays or in the Gulf of Mexico. These collecting sites may be in error due to mistakes in the reporting of GPS coordinates, differences in the map coordinate systems used, or assumptions based on old records. Many older records from the 1800's have scant data, providing as little as the name of a bay and a state. When better data are not provided, it is common for the GPS coordinates to be given as a more or less central point within a bay (M. Wicksten, personal communication). Published collections indicate that *R. harrisii* is typically found in the upper reaches of estuaries.

Personal collections from the lower reaches of bays of Texas have never produced an *R. harrisii* but I have collected individuals from six of the other eight xanthoid species occupying similar niches. I have never collected *Eurytium limosum* or *Eriphia gonagra*, which are typically found in deeper waters than I was able to sample.

Another explanation for the reported collections from the main bodies of bays may be misidentification. The xanthoid crabs are notoriously difficult to distinguish between species especially for biologists that do not continuously work with the group. *R. harrisii* and *E. depressus*, on cursory examination, are very similar to the naked eye. The primary distinguishing characteristic is a raised orange/red spot on the inside of the ischium of the third maxilliped, a trait that is only visible in mature adults.

Two gravid females were collected from traps from two different sites (Oyster Creek and Orange Boat Ramp). Both sites had a salinity of 0.1 PSU. This is well below the published range of reproduction for this species and is less than I have observed with the lake populations. Neither of the females kept the egg mass in the transport back to the lab so I was unable to attempt to rear the larvae. It is possible that the salinity measured at the time was below average. The Oyster Creek site had a salinity of 4 PSU at the time of deployment but had dropped to 0.1 PSU at the time of collection. The Orange site had actually risen to 0.1 PSU from 0 PSU at the time of deployment. This marks the lowest recorded salinity for a gravid female throughout the range of this species.

Comparison of salinity data proved difficult because of unequal sampling across the regions as well as over time. Table 4.7 provides the minimum and maximum salinities for Chesapeake Bay, the bays of North Carolina and six of the major Texas bays. The bays from the Atlantic coast averaged 14 PSU whereas those from Texas averaged 19 PSU. There were several bays in both Texas and North Carolina that were

either below or above this average, however, in general the bays in Texas were found to be more saline and to have higher maximums. My personal observations are within the known ranges for each of the bays. All of the new collections sites are around the minimum salinities with the exception of the Mission River site.

The salinity regime is very similar among all of the estuaries examined here with the Atlantic coast estuaries having a lower average salinity. This is likely a result of localized droughts in Texas raising the maximum salinities and thus skewing the averages towards the maximum. The Texas estuary with the lowest salinity is the Sabine Lake estuary, which averages around 9 PSU throughout the length of the lake. Two of my personal collecting sites where *R. harrisii* was found are up river of this estuary and have consistently lower salinities than the average of 9 PSU. Another site, the Nueces River site, was also considerably lower than the average for the Nueces Bay estuary. Even though the Texas coast had similar salinity ranges to the Atlantic coast, it also had higher maximums during *R. harrisii*'s peak breeding season. Higher salinities would allow sympatric species to move into preferred regions and thereby displace *R. harrisii* further upriver into a more freshwater environment.

The competition trials were the most illuminating. *Rhithropanopeus harrisii* was shown to be the least aggressive in each of the interactions that were tested. This agrees with a study by Hulathduwa *et al.* (2011) that found that *R. harrisii* was subordinate to *E. depressus* and *P. simpsoni*. However, they found that *E. depressus* was dominant over both *P. simpsoni* and *R. harrisii* in competition for shelter. I did not test shelter

competition between *E. depressus* and *P. simpsoni*, however, I did test direct interactions between the two species and neither showed significant aggression towards the other.

Bay	State	Salinities (PSU)		
		Minimum	Maximum	Average
Chesapeake Bay	VA,MD	0.5	30	13-17
Chowan River Estuary	NC	0.5	5	3
Roanoke River Estuary	NC	0.5	5	3
Albemarle Sound	NC	0.5	25	5
Pamlico River Estuary	NC	0.5	25	11
Neuse River Estuary	NC	0.5	25	12
Pamlico Sound	NC	0.5	30	19
Core Sound & Bogue Sound	NC	5	30	24
New River & White Oak River Estuaries	NC	0.5	30	20
Cape Fear	NC	0.5	30	16
Southern Estuaries	NC	15	30	27
Sabine Lake	TX	0	20	9
Galveston Bay	TX	0	30	17
Guadalupe Bay	TX	0	37	9
Colorado-Lavaca Estuary	TX	0	37	21
Aransas Bay	TX	1	40	21
Nueces Bay	TX	0	43	25
Corpus Christi Bay	TX	0	41	30

Table 4.7. Salinity comparison of several bays. A list of all bays with known populations of *Rhithropanopeus harrisii*.

The reduction in the number of xanthoid species occupying similar niches with *R. harrisii* north of Florida would result in a reduction in interspecific competition. Even though there are other families of crabs that may be replacing the missing xanthoid species, niche overlaps between different families of crabs are greatly reduced. In the Chesapeake, Ryan (1956) reported *R. harrisii* and *E. depressus* from several of the same sites. Hulathduwa *et al.* (2011) noted that in field sampling in Barataria Bay, Louisiana,

*R. harrisii* was more common in low salinity waters with fewer predators. My collections agree with the increased numbers of *R. harrisii* as one moves into less saline waters. It should also be noted that *E. depressus* predominantly utilizes oyster reefs as cover whereas *R. harrisii* has been shown to utilize almost anything. I have personally collected *R. harrisii* out of beer cans, beer bottles, submerged tires, bricks, plastic wrap, and vegetation of all kinds. Ryan (1956) found a similar trend in Chesapeake Bay. It would thus seem likely that *R. harrisii* is avoiding competition with sympatric xanthoid species.

Hulathduwa *et al.* (2011) showed *E. depressus* to be the more dominant species, yet my results did not detect a significant difference in aggression between that species and *R. harrisii*. It could be possible that like *R. harrisii*, *E. depressus* is utilizing the lower end of its salinity tolerance in order to avoid other xanthoid species. Both *P. simpsoni* and *E. turgidus* showed potentially higher levels of aggression towards *E. depressus*. If this is occurring then *R. harrisii* would have to be moving into waters with even lower salinities in order to avoid competing with *E. depressus*. If the adaptation to reproduce at lower salinities does occur in the coastal populations, it would increase the likelihood of successful invasion into freshwater reservoirs.

Hulathduwa *et al.* (2011) postulated that *R. harrisii*, due to their reduced ability to find shelter, was moving into the lower salinity waters in order to escape predation. At five of the seven locations where I collected *R. harrisii*, I also found evidence of *Callinectes sapidus*, a common predator of xanthoid crabs. The salinity of these five

sites ranged from 0.1 to 33.9 PSU and averaged 7.6 PSU. In aquaria, both *C. sapidus* and *Menippe adina* will kill and eat *R. harrisii*. Both *C. sapidus* and *Menippe adina* require waters greater than 7.6 PSU for larval development and thus cannot maintain populations in salinities that *R. harrisii* inhabits. The upriver portions of the estuary where I collected are also under a less developed tidal regime, which reduces the likelihood of *R. harrisii* facing desiccation and predation by birds (i.e. willets, night herons and rails). Upriver habitats could provide *R. harrisii* with freedom from some predation and competition with sympatric xanthoid species by moving upriver.

The increased number of sympatric xanthoid species, the higher average salinities within the bays, the reduced aggression and exclusion from shelter in *R. harrisii* are all possible reasons for the increased numbers of this species that occur upriver of the bays and estuaries of Texas. The localized distribution of this species occurring upriver due to the above reasons could potentially lead to the ability to reproduce at lower salinities. The adaptation to reproduce in even lower salinity waters would allow the crabs to escape competition with other xanthoids. It would also “pre-adapt” these populations to being invasive in inland reservoirs, whereas those populations on the Atlantic coast are not.



## 5. IMPACT OF *Rhithropanopeus harrisii* IN INLAND RESERVOIRS AND POTENTIAL SPREAD

### 5.1 Introduction

Invasive species are known to have devastating ecological effects. The brown tree snake, *Boiga irregularis*, has been responsible for the decimation of 22 species of birds on the island of Guam (Wiles *et al.*, 2003). Cane toads, *Bufo marinus*, have affected large numbers of anuran predators in Australia (Shine, 2010). The zebra mussel, *Dreissena polymorpha*, has been shown to be responsible for the ecological collapse of many native bivalve species in its march through the Great Lakes (Sousa *et al.*, 2013). The fire ant, *Solenopsis invicta*, and the Argentine ant, *Linepithema humile*, have both been shown to devastate native arthropods and ground dwelling vertebrate species in their invasions worldwide (Porter and Savignano, 1990; Vogel *et al.*, 2010; Holway *et al.*, 1998; Suarez *et al.*, 1998). These examples represent some of the most devastating animal invasions of the last century, but they also represent devastation to easily visible victims. Many invasions may occur for multiple years prior to identification and even then the effects remain unknown, as in the case of red tomato spider mite, *Tetranychus evansi*, (Boubou *et al.*, 2011) and the freshwater amphipod, *Gammarus varsoviensis* (Grabowski *et al.*, 2012).

Invasions may be cryptic for several reasons. The first is that the species may be morphologically similar to a native species as in the case of *G. varsoviensis* (Grabowski *et al.*, 2012). This makes it difficult for the casual observer to differentiate between the

two thus causing an invasion to go unnoticed for many years. A second reason is the size of the actual invader. In the case of *T. evansi*, individuals of this species are typically less than 0.5mm in length making them extremely difficult to see with the naked eye (Gotoh *et al.*, 2009). A third reason is an invasion by a non-native genotype into a conspecific. For instance, populations of the common reed in North America, *Phragmites australis*, have been invaded by a more euryhaline genotype. The more euryhaline genotype has allowed expansion of the species into new habitats causing species displacement (Silliman and Bertness, 2004). A final potential reason for cryptic invasions is a seeming lack of impact by the invasive species. In the case of the brown tree snake, everyone living in the area will notice a loss of bird life. In the case of the amphipod, *G. varsoviensis*, it may only be replacing a morphologically similar native species. This would almost certainly have gone unnoticed without the genetic analysis performed by Grabowski *et al.* (2012).

The white fingered mud crab, *Rhithropanopeus harrisi*, was first identified in Possum Kingdom reservoir, Texas, during the summer of 1998. Since that time, it has spread to the following nine Texas reservoirs: Lake E.V. Spence, Lake Colorado City, Lake Balmorhea, Lake Granbury, Squaw Creek Reservoir, Lake Whitney, Lake Texoma, Tradinghouse Creek reservoir and most recently was confirmed by this author in Lake Hubbard Creek. Since that time only two published studies have discussed any aspects of the invasion, Howells (1998) and Boyle *et al.* (2010).

Howells (1998) noted the introduction and the possibility of *R. harrisii* to impact native unionid mussels if it were to spread into the Brazos River below Possum Kingdom reservoir. *Rhithropanopeus harrisii* has been shown to feed on the following mytiliform bivalves: *Mytilus edulis*, *Dreissena polymorpha*, *Ischadium recurvum*, *Mytilopsis leucophaeata*, and the spat of *Crassostrea virginica* (Turoboyski, 1973; Milke and Kennedy, 2001; Kulp *et al.*, 2011). Milke and Kennedy (2001) found that *R. harrisii* were successful only when preying on smaller individuals. Milke and Kennedy (2001) also postulated that larger bivalve species would have a size refuge from *R. harrisii* as predation decreased once the bivalves reached a certain size. Thus it is possible that *R. harrisii* could adversely affect bivalve species in the invaded areas.

Boyle *et al.* (2010) indicated that the crabs were indeed moving into the waters below Possum Kingdom reservoir but did not investigate any effects the crabs may be having on local organisms. They did note that the crabs were impacting water pumps and had been found in the intake grates of the nuclear power plant at Squaw Creek Reservoir. Boyle *et al.* (2010) also noted that they were first made aware of the crabs' invasion into Lake Whitney, Texas, by fishermen who had noted the presence of the crabs in the stomachs of catfish that they had landed. I have seen the crabs eaten by blue gill sunfish (*Lepomis macrochirus*), channel catfish (*Ictalurus punctatus*) and the yellow bullhead catfish (*Ameiurus natalis*). The first two I have seen while scuba diving in Possum Kingdom reservoir. The yellow bullhead and channel catfish were both observed to consume *R. harrisii* in aquaria (Boyle, personal observation). These observations suggest that they may be a substitute for normal prey.

*Rhithropanopeus harrisi* is a small crab with adults reaching a maximum size of 19 – 26.1 mm (Ryan, 1956; Turoboyski, 1973). It has cryptic coloration, which can vary with the substrate type. In my personal collections of this species from inland lakes I have noted that in Possum Kingdom Reservoir, Lake Whitney and Lake Granbury, all of which have a large amount of limestone rock substrate, the crabs tend to have a light gray and tan coloration pattern. In Lake Colorado City and Lake E.V. Spence, which have more sandstone substrate, the crabs tend to have a reddish coloration. Crabs found below any of the dams tend to be dark brown to black like the anoxic muds they are found in. All of these coloration patterns along with their small size make the crabs exceedingly difficult to spot in the invaded reservoirs. Thus the public is relatively unaware as to the impact of this species warranting a more direct study of its impact.

The purpose of this study was to elucidate what impacts the crab may be having in the reservoirs and to try and determine the potential spread of this species. First, I analyzed the stomach contents of crabs from several reservoirs to identify potential prey items. This species has previously been shown to be an omnivorous species eating primarily vegetative material and opportunistically eating animal matter (Turoboyski, 1973).

I next looked at shelter competition with a native crayfish (*Procambarus clarkii*). Boyle *et al.* (2010) commented on the paucity of crayfish and suggested that competition with the crayfish may be occurring. Shelter competition was analyzed since previous work has shown the importance of shelter to crayfish and other species (Alonso and

Martinez, 2006; Barbaresi and Gherardi, 1997; Blank and Figler, 1996; Bubb *et al.*, 2009; Figler, Cheveton and Blank, 1999; Gherardi and Cione, 2004; Hanshew and Garcia, 2012; Holbrook and Schmitt, 2002; Larson and Magoulick, 2009; McDonald, Jensen and Armstrong, 2001; Rosson *et al.*, 2011; Usio, Konishi and Nakano, 2001; Vorburger and Ribi, 1999). Larson and Magoulick (2009) specifically looked at the role shelter played in the survival of juvenile crayfish and showed that those individuals excluded were more susceptible to predators. Body size has been shown to be a primary factor in aggression in crustaceans (Pavey and Fielder, 1996). *Procambarus clarkii* is a much larger decapod than *R. harrisii*. There is an overlap in size between the juveniles of *P. clarkii* and the adults of *R. harrisii*. Therefore, shelter competition was investigated using only juvenile *P. clarkii* (less than 30mm) and adult *R. harrisii*.

Finally, to determine the dispersal ability of larval stages I looked at flow rates below the dams of Possum Kingdom reservoir, Lake Granbury, Lake Whitney, Lake Texoma, Lake Colorado City, and E.V. Spence Reservoir. In the Blackburn *et al.* (2011) model of invasions, if a species makes it through the first three stages of invasion, then the last stage of invasion is the spread of the species. *Rhithropanopeus harrisii* is thought to be dispersed primarily via ballast water (Turoboyski, 1973; Roche and Torchin, 2007). Because there is no large-scale transport by shipping into the inland lakes and reservoirs of Texas, introduction by ballast water is highly unlikely to occur there. In Section 3, I described the developmental duration of *R. harrisii* from inland reservoirs. These data were combined with flow rate data to give a maximum potential dispersal capability of this species. The larvae were treated as particles due to their

planktonic nature and susceptibility to any currents in the environments. This protocol has been used extensively in the literature (Aiken *et al.*, 2007; Cowen and Sponaugle, 2009; Hamer, McCollin and Lucas, 1998; Horvath and Lamberti, 1999; Johnson and Hester, 1989; Largier, 2003; Levin, 2006; Metaxas and Saunders, 2009; Schultz and Cowen, 1994; Siegel *et al.*, 2003). This protocol does discount the movement of the larvae up and down within the water column but the constant flow in one direction is going to cause a continuous downstream movement of this species. Hence these estimates will be considered the maxima of larval dispersal, as larval movements as well as uneven flow rates along the river will shorten this distance. This will allow for the development of management strategies to minimize movement of this species between reservoirs.

## 5.2 Methods

### 5.2.1 Stomach Contents

Crabs were collected by hand along the shore and by scuba diving from Lake Whitney, Possum Kingdom Reservoir, Lake Colorado City, and Lake E.V. Spence. Individuals were immediately preserved in a 70% ethanol solution. The dorsal carapace was removed and the digestive tract was extracted and placed on a slide. Each digestive tract was examined using a compound light microscope and contents were identified to the lowest possible taxonomic level.

### 5.2.2 Shelter Competition with *Procambarus clarkii*

Crabs were collected from Lake Whitney and returned to the lab in an aerated ice chest with water from the lake. The crabs were placed in 20-gallon aquaria up to a density of 15 crabs per aquarium for up to one month prior to being used in the experiments. Juvenile red swamp crayfish, *Procambarus clarkii*, were collected while scuba diving from the Comal River in Prince Solms Park, New Braunfels, Texas because of ease of collection. Individuals were measured with vernier calipers to the nearest mm. *Procambarus clarkii* individuals were returned to the lab in the same manner as the crabs and kept in 20-gallon aquaria up to a density of 10 crayfish for up to one month prior to being used in the experiments. A week prior to the beginning of the trials, individuals were isolated in plastic containers with 300 ml of 2 PSU water with a small piece of PVC pipe for shelter.

Methodology was adapted from Figler, Cheveton and Blank (1999). All trials were conducted in a small aquarium (14.5 cm x 22 cm x 9cm) (Figure 4.2). The aquarium was wrapped with brown paper to minimize the effect of external movement on the behavior of the test individuals. A PVC shelter was placed in the center of the aquarium and the tank was filled to a height of 3cm with clean 2 PSU water or 2 PSU water from a tank containing a yellow bullhead catfish, *Ameiurus natalis*. Preliminary investigations showed that both species spent more time under shelter in water with cues from the catfish, so water from the catfish aquarium was used to induce a shelter seeking response. The use of the water with cues instead of the presence of the actual catfish

was also used to minimize loss of test subjects. In the control treatment, the PVC shelter from the test individual was used. In the Crab versus Crab and the Crayfish versus Crayfish, the shelter was randomly chosen from one of the test individual's isolation container. In all other treatments, the shelter was from the test crayfish's isolation container or randomly chosen from the two crayfish being tested. Nine treatments (Table 5.1) were tested. All trials were analyzed for the amount of time each individual spent under the shelter alone or together. Individuals were used in multiple trials. No individual was used in more than one trial per day. Previous studies have focused on a 1:1 ratio of test subjects (Alonso and Martinez, 2006; Barabresi and Gherardi, 1997; Blank and Figler, 1996; Figler, Cheveton and Blank, 1999). Breen and Metaxas (2012) point out this reduces the potential for additive and substitutive or redundant effects between the two species. In Section 4, I showed that *R. harrisii* displays low levels of agonism with both conspecifics and heterospecifics. This reduces the likelihood of direct interference competition between the two and emphasizes the potential for additive effects with increasing density.

Shelter Competition Treatments		
Crab Control	Crayfish Control	Crab vs. Crab
Crayfish vs. Crayfish	Crab vs. Crayfish	2 Crabs vs. 1 Crayfish
Crab vs. 2 Crayfish	2 Crabs vs. 2 Crayfish	6 Crabs vs. 2 Crayfish

Table 5.1. A list of shelter competition treatments.



A t-test was performed to determine if there were any significant differences between the control water and predator water trials within each treatment. One-way ANOVAs were performed to determine if there were any significant differences between the amount of time each species spent alone, with conspecifics, and together across all treatments.

### 5.2.3 Larval Dispersal Ability

Larval dispersal ability was determined using the larval development rates as described in Section 3 and river flow rates obtained from the United States Geological Survey National Water Information system. River flow rates were obtained for the Red River, Brazos River and the Colorado River. The rates were given in  $\text{ft}^3/\text{s}$ . To convert to velocity, the area of the river at the site of the gage was determined from gage height and from measurements taken from Google Earth (6.1.0.5001). Rates were converted to  $\text{km}/\text{day}$ . Dispersal was determined by multiplying the minimum and maximum days (9 and 24 respectively) to develop to the megalops stage based on the results reported in Section 3.

### 5.2.4 Adult Dispersal Ability

Adult dispersal ability was determined using individuals collected from Lake Whitney. A tag and recapture study was not possible owing to the small size of this species. Preliminary efforts to mark the crabs with a waterproof paint in aquaria were ineffective due to the crabs scouring their carapace while moving in and under the gravel and large rocks. Four bottom substrates (glass, sand, mud and gravel) were tested.

Glass was used as a control and the other three substrates typify the substrates found in the various inland reservoirs. For each substrate, 10 trials were conducted using 6 crabs for each trial. Individuals were placed in a 50-gallon aquarium filled to a height of 5 cm with 2 PSU water. Five large flat rocks were placed around the interior perimeter of the tank and were placed in the same position during each treatment (Figure 5.1). The crabs were placed in the front left corner each time and were left for at least 11 consecutive hours of light. At the end of this time, the crabs were collected and their location was noted. They were then placed back into the left corner and were left for at least 11 consecutive hours of darkness. Light and dark trials were conducted as I have personally seen the crabs active during the day and at night while scuba diving. It should also be noted that in many of the inland lakes, appreciable levels of light are not found deeper than 3-5 m. At the end of the dark trial, the crabs were removed and a new set of six crabs were used for the next trial.

At the end of each trial the average distance moved by an individual was calculated by the total distance moved divided by the number of individuals in the trial. This is a conservative estimate of adult dispersal as it only takes into account the net movement of the individual. It is also more realistic as the lakes in which it has invaded are typified by gravel substrates with larger rocks dispersed throughout. In areas with muddy or sandy substrates, other cover types like tree stumps, plant roots or trash are found dispersed throughout.

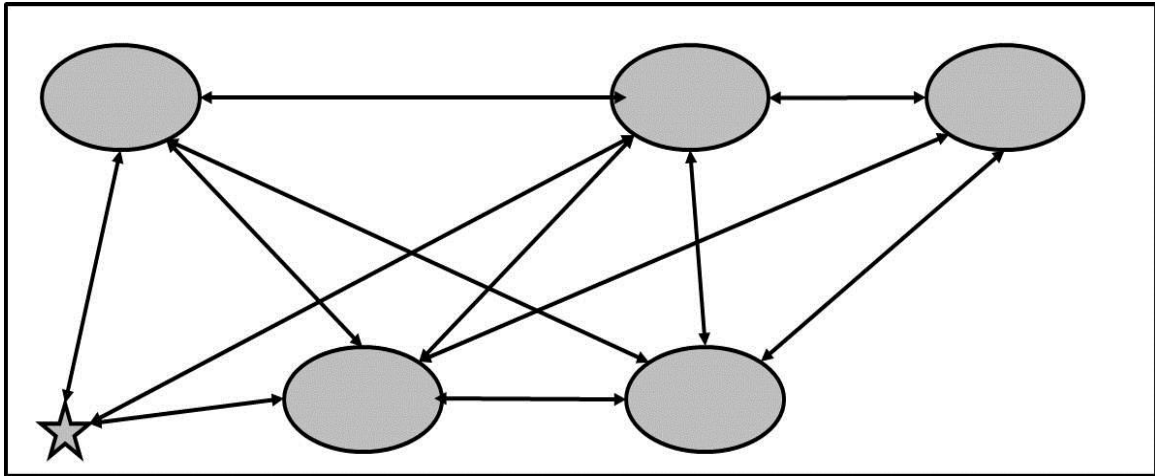


Figure 5.1. Adult dispersal map. Map of the aquarium indicating the location of the large flat rocks. The star indicates the front left corner.

<b>Stomach Contents</b>	<b>Number of Crabs with Specific Contents</b>	<b>Percent Total Crabs with Specific Contents</b>
Unidentifiable Animal Tissue	7	14%
Plant Tissue	50	98%
Green Algae	47	92%
Diatoms	32	63%
Bryozoan Statoblasts	9	18%
Stalked Ciliate	1	2%

Table 5.2. Proportion of stomach contents.

## 5.3 Results

### 5.3.1 Stomach Content Results

The stomach contents of 51 crabs were examined from Lake Whitney, Possum Kingdom Reservoir, Lake Colorado City, and E.V. Spence Reservoir. Stomach contents included unidentifiable animal tissue, plant tissue, green algae, diatoms, bryozoan statoblasts, and stalked ciliates (Table 5.2). The bryozoan statoblasts were identified to the genus *Pectinatella*, which is known from Texas freshwater bodies. The majority of the stomach contents were comprised of plant tissue (98%) and green algae (92%). Unidentifiable animal tissue, bryozoan statoblasts and stalked ciliates were found in less than 20% of the crabs examined.

### 5.3.2 Shelter Competition with Prior Residence

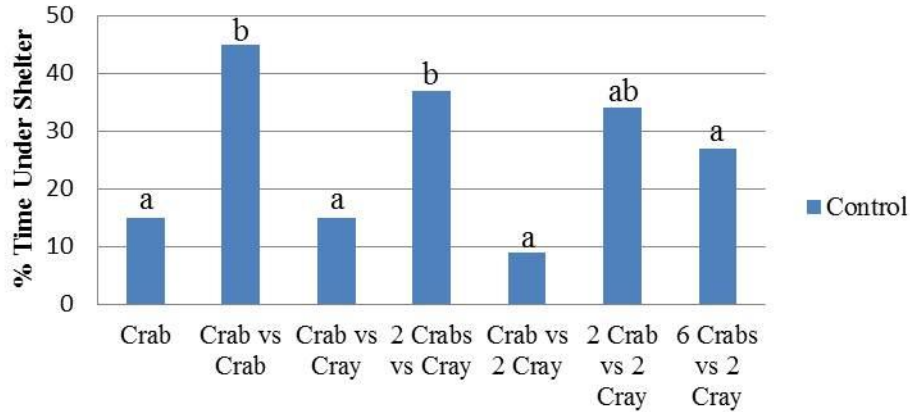
173 trials were videotaped and analyzed. There were no significant differences between the amounts of time either species spent under the shelter between control and predator waters when only one individual was in the test aquarium. In the Crab versus Crab treatment, individuals spent significantly more time under the shelter in the control water than in the predator water ( $p = 0.002$ ). There were no significant differences between any of the comparisons in the Crayfish versus Crayfish treatment. In the Crab versus Crayfish treatment, crabs spent significantly more time under the shelter in the control water than in the predator water ( $p = 0.001$ ). In the 2 Crabs versus Crayfish treatment, crabs spent significantly more time together under the shelter in the control water than in the predator water ( $p = 0.003$ ). In the Crab versus 2 Crayfish, there were

no significant differences between any of the comparisons. In the 2 Crab versus 2 Crayfish, crayfish spent significantly more time together in the predator water ( $p = 0.007$ ). In the same treatment, crabs and crayfish spent significantly more time together under the shelter in the control water ( $p = 0.006$ ).

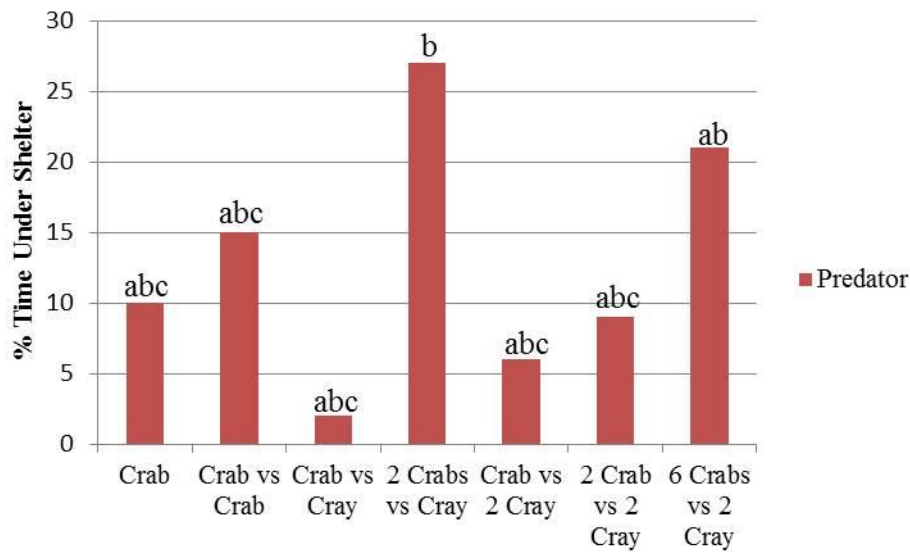
Crabs spent significantly more time under the shelter alone when there were two or more crabs in the treatment in control water (Figure 5.2a). A similar trend was seen in the predator water with the exception of the 2 Crab versus 2 Crayfish treatment which was not significantly higher than those treatments with only one crab (Figure 5.2b). Crayfish spent significantly less time alone under the shelter in all treatments with two or more crabs in control water (Figure 5.3a). Like the crabs, this trend was upheld in the predator water with the exception of the 2 Crab versus 2 Crayfish treatment (Figure 5.3b).

In the control water, the crabs shared the shelter with other crabs significantly more often when there were only two crabs in the arena (Figure 5.4a). Crabs shared the shelter with other crabs significantly more time when they outnumbered the crayfish by a factor of 3:1 in the predator water or were by themselves (Figure 5.4b). Across all treatments and water types, crayfish were significantly more likely to share a shelter in the absence of crabs than when in the presence of crabs (Figure 5.5a and 5.5b). In both control and predator water crabs and crayfish shared the shelter significantly more time in the 6 Crabs versus 2 Crayfish treatment (Figure 5.6a and 5.6b). There was no significant difference amongst all other comparisons.

## Percent Time Crab Spent Alone Under Shelter



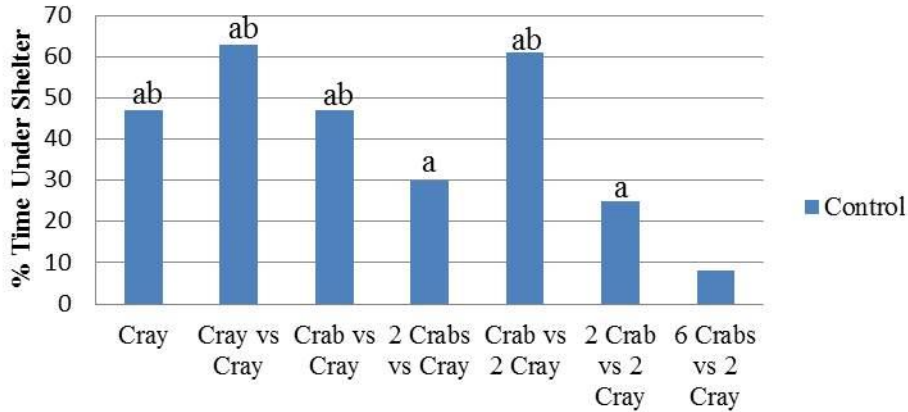
a.



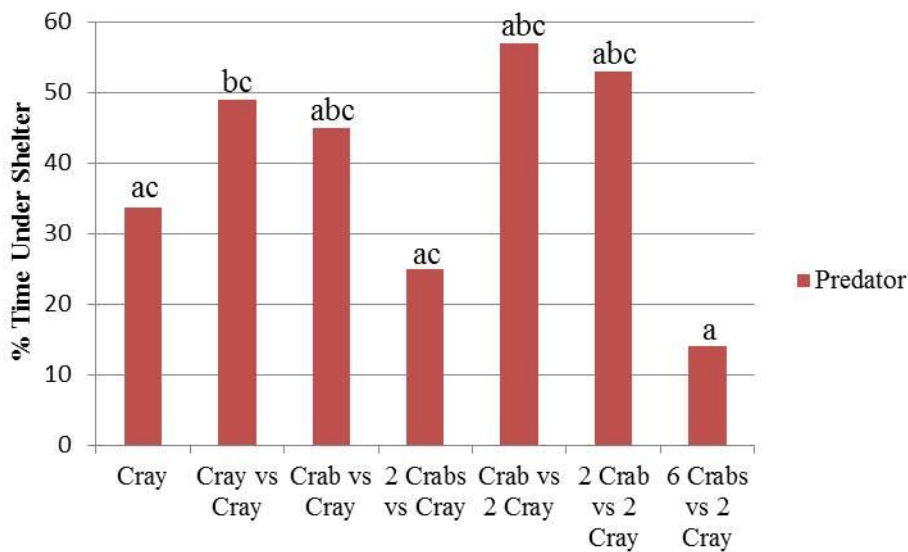
b.

Figure 5.2 a&b. Comparison of time crabs spent alone under shelter. a) The percent time crabs spent alone under the shelter across all treatments in control water. b) The percent time crabs spent alone under the shelter across all treatments in predator water. Letters above bars indicate non-significant differences ( $p \leq 0.05$ ).

## Percent Time Crayfish Spent Alone Under Shelter



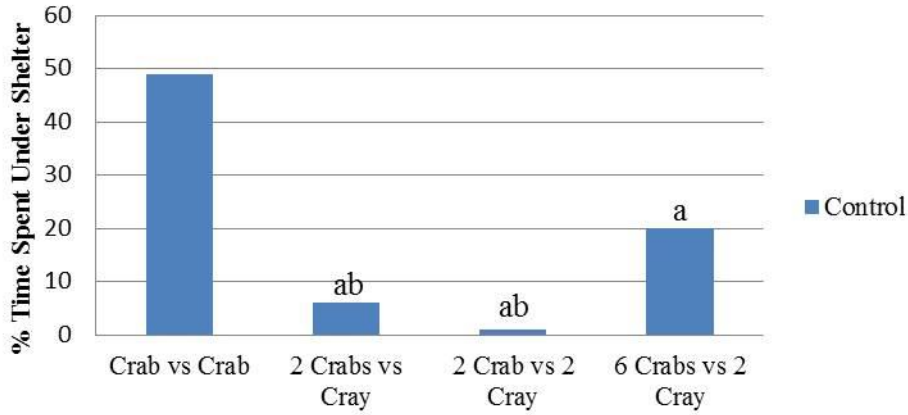
a.



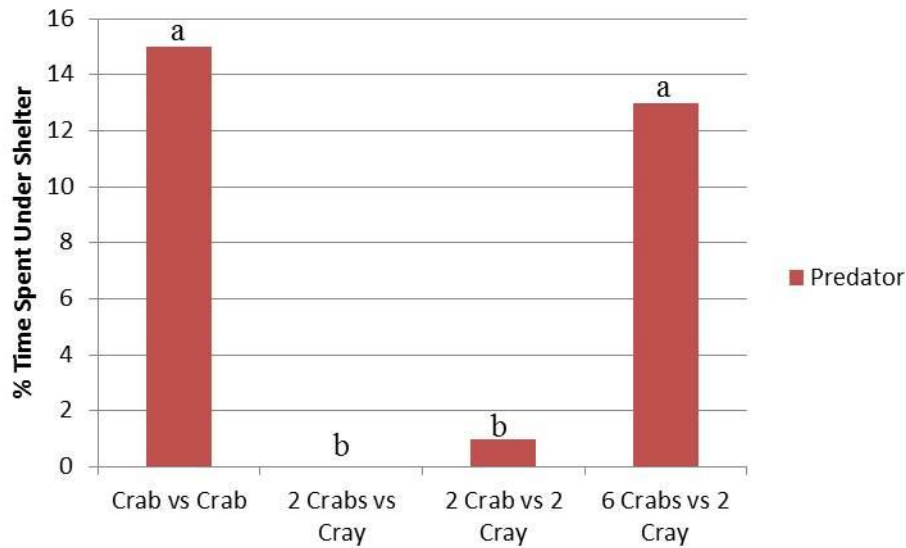
b.

Figure 5.3 a&b. Comparison of the time crayfish spent alone under the shelter. a) The percent time crayfish spent alone under the shelter across all treatments in control water. b) The percent time crayfish spent alone under the shelter across all treatments in predator water. Letters above bars indicate non-significant differences ( $p \leq 0.05$ ).

## Percent Time Crabs Shared the Shelter with other Crabs



a.

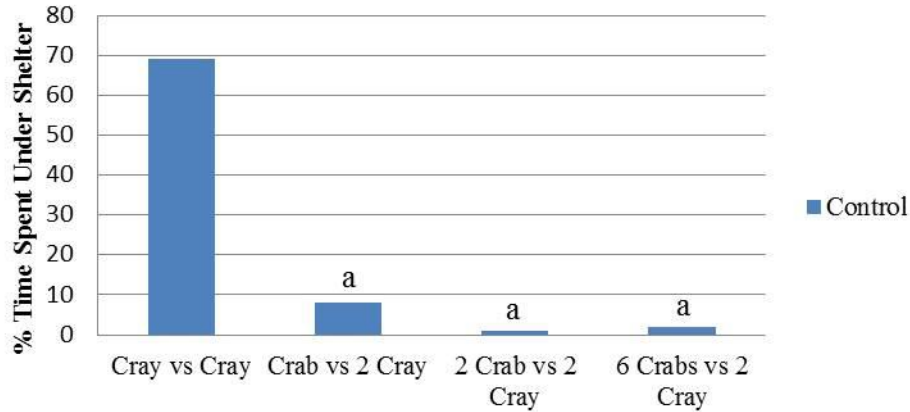


b.

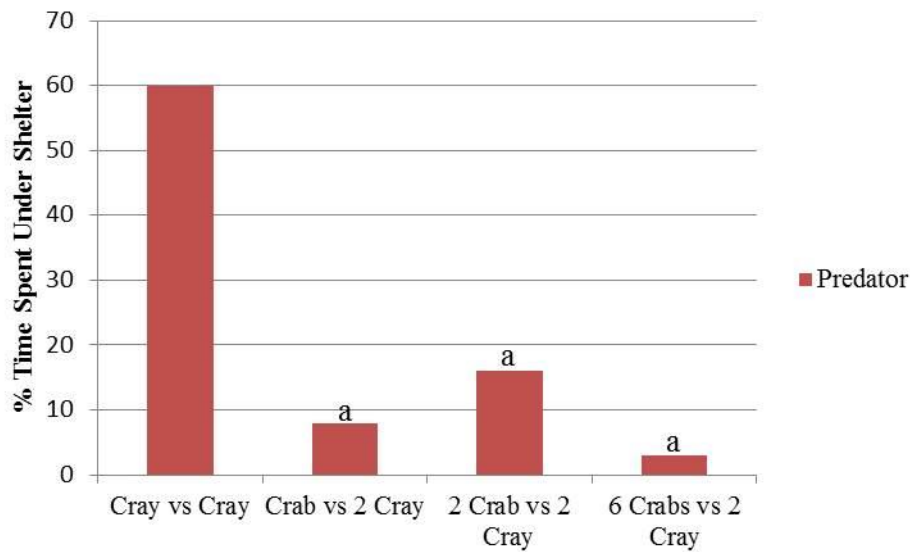
Figure 5.4 a&b. Comparison of the time crabs shared the shelter. a) The percent time crabs shared the shelter with at least 1 other crab across all treatments in control water. b) The Percent time crabs shared the shelter with at least 1 other crab across all treatments in predator water. Letters above bars indicate non-significant differences ( $p \leq 0.05$ ).



## Percent Time Crayfish Shared Shelter with other Crayfish



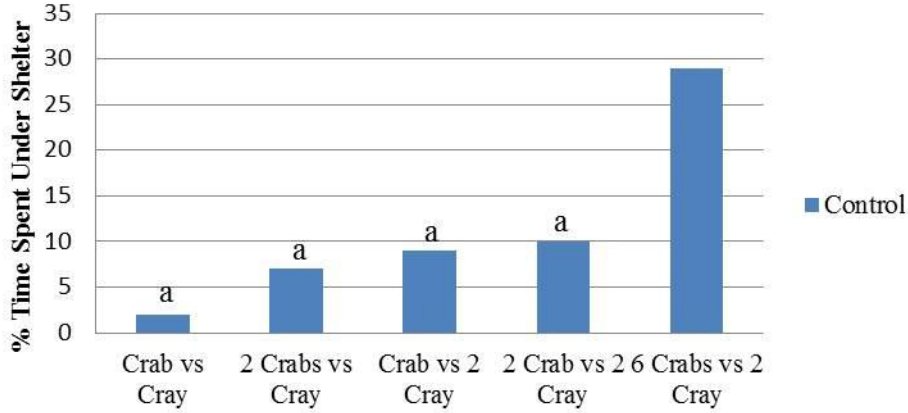
a.



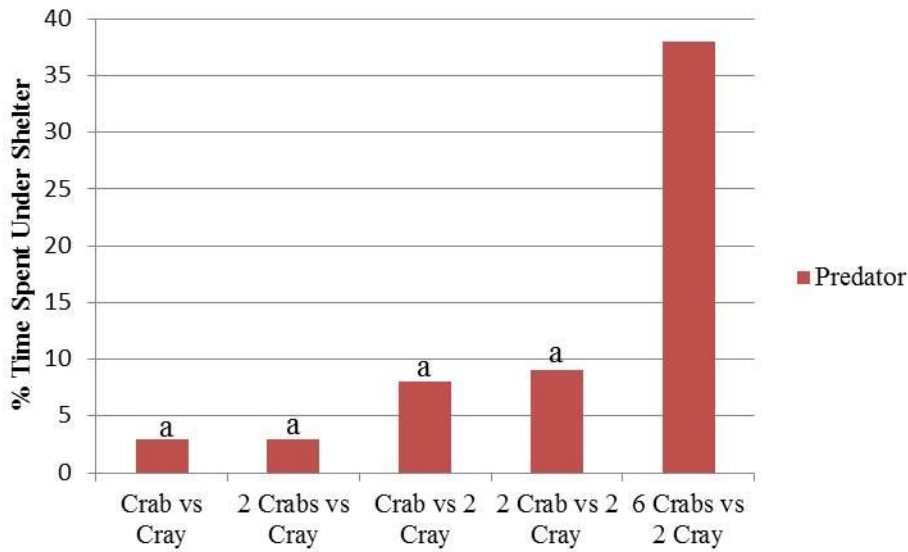
b.

Figure 5.5 a&b. Comparison of the time crayfish shared the shelter. a) The percent time crayfish shared the shelter with at least 1 other crayfish across all treatments in control water. b) The percent time crayfish shared the shelter with at least 1 other crayfish across all treatments in predator water. Letters above bars indicate non-significant differences ( $p \leq 0.05$ ).

## Percent Time Crabs Shared Shelter with Crayfish



a.



b.

Figure 5.6 a&b. Comparison of the time crabs and crayfish shared the shelter. a) The percent time crabs and crayfish shared the shelter across all treatments in control water. b) The percent time crabs and crayfish shared the shelter across all treatments in predator water. Letters above bars indicate non-significant differences ( $p \leq 0.05$ ).

### 5.3.3 Larval Dispersal Ability

For the Brazos River, three different velocities were found and the potential larval dispersal was determined for each. For each velocity a minimum of 9 days and a maximum of 24 days were used based on the time it took for development to the megalops stage. This stage represents the first benthic phase in this species. The Brazos River below Possum Kingdom Reservoir has an average velocity of 21.89 km/day. Larvae hatching at this point have the potential of dispersing between 197 and 525 km. The Brazos River below Lake Granbury has an average velocity of 21.25 km/day. Larvae hatching at this site have the potential of dispersing between 191 and 510 km. The Brazos River below Lake Whitney has an average velocity of 15.1 km/day. Larvae hatching at this site have the potential to spread between 135 and 362.4 km.

The velocity of the Red River below Lake Texoma was determined to be 25 km/day. Larvae hatching at this site have the potential to disperse between 225 and 600 km. Two sites on the Colorado River were used. The first site below Lake Colorado City has an average velocity of 9.06 km/day. The larvae have the potential to spread between 81.5 and 217 km. The site below Lake E.V. Spence had the slowest velocity of the six sites analyzed. The river here moved at 7.45 km/day and could result in movement of larvae from 67 and 179 km.

### 5.3.4 Adult Dispersal

40 trials were conducted across the four substrates during daylight and darkness. Individual crabs moved an average of 31.36cm during daylight and 33.96cm during the

darkness (Figures 5.7 & 5.8). They moved an average of 2.67cm/hr during the daylight and 2.82cm/hr during the darkness (Figures 5.9 & 5.10). No significant difference was found across substrates or time of day. Based on the results above adults have the potential to move a maximum of 67.68cm per day and up to 0.247km per year.

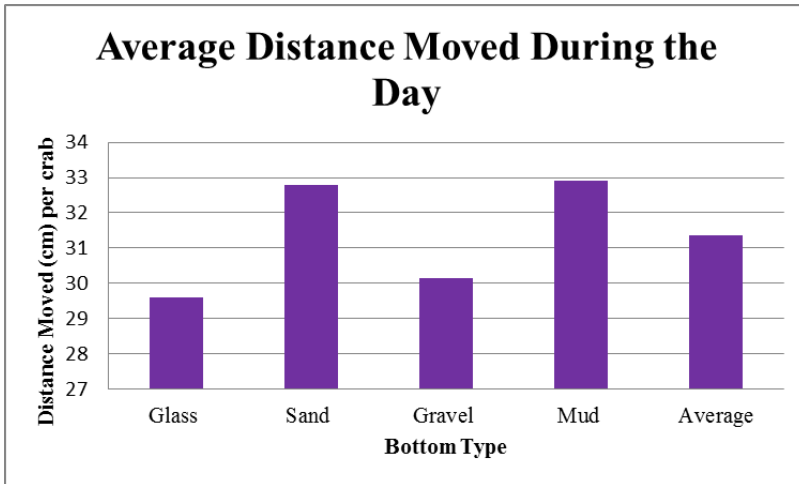


Figure 5.7. Average distance moved during the day. There were no significant differences across all treatments.

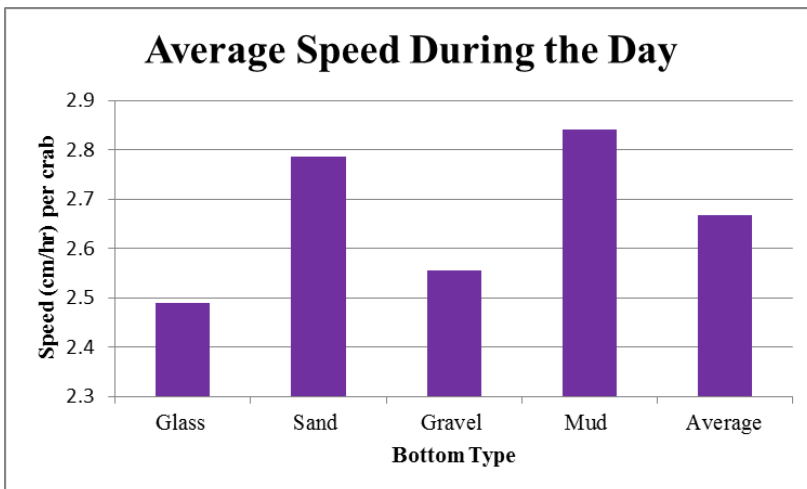


Figure 5.8. Average crab speed during the day. There were no significant differences across all treatments.

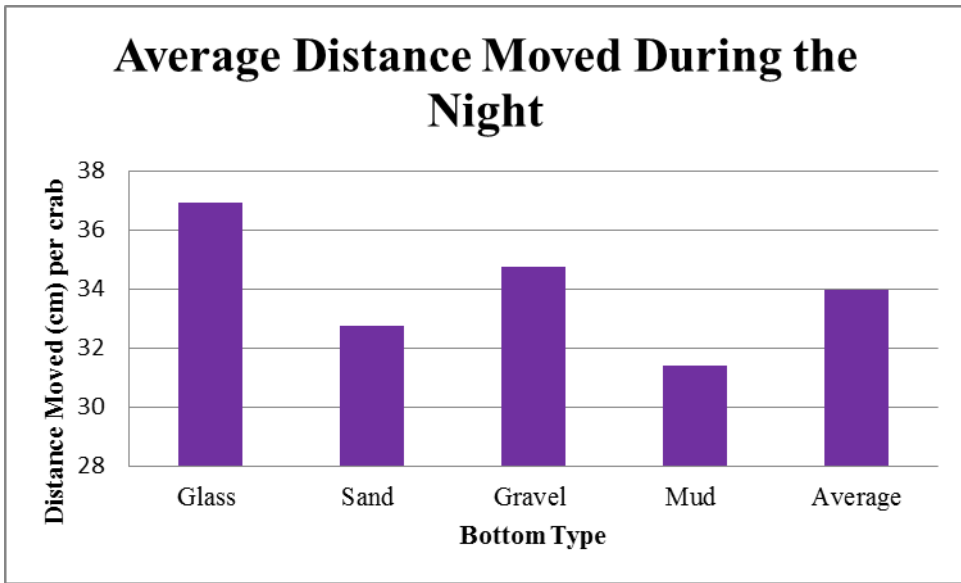


Figure 5.9. Average distance moved during the night. There were no significant differences across all treatments.

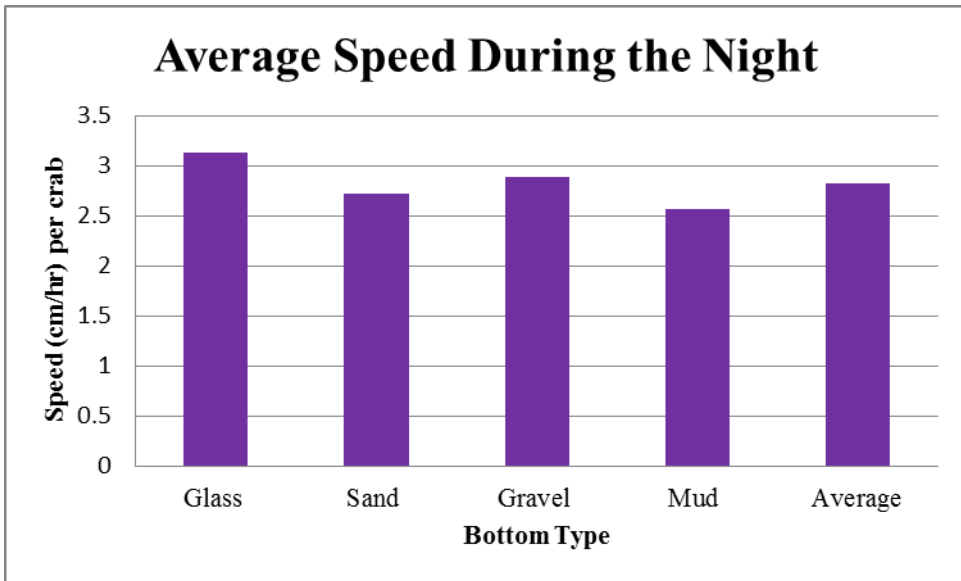


Figure 5.10. Average crab speed during the night. There were no significant differences across all treatments.

## 5.4 Discussion

The objective of this study was to identify some of the biological impacts of *Rhithropanopeus harrisii* as well as to determine its dispersal ability. The Blackburn *et al.* (2011) model does not take the impact (positive or negative) of an invasive species directly into account. However, the model's final stage of the invasion process directly determines the impact of an invasive species by looking at the potential spread. While an invader may be detrimental to a native species during its invasion, if its presence is short lived because of a failure to reproduce and spread then it may not warrant mediation. However, if an invader is capable of spreading and reproducing then it is likely to have a larger impact and therefore warrant management.

To date, there has been very little work on the impact of the invasive populations of *R. harrisii* in Texas reservoirs (Howells, 1998; Boyle *et al.*, 2010). Literature searches indicated that Howells's (1998) concern for the endemic unionids of the Brazos River have experimental validity (Turoboyski, 1973; Milke and Kennedy, 2001; Kulp *et al.*, 2011). The surrounding area was surveyed and the presence of annelid worms, bivalves and aquatic insect larvae was noted. However, the gut content analysis performed here found animal tissue to be relatively rare in *Rhithropanopeus harrisii*. The bulk of the content was plant or algal in nature. The mixture of gut contents confirms that *R. harrisii* is primarily an omnivorous scraper, feeding indiscriminately on whatever settles out on the substrate. This indicates that this species is not likely to cause the extirpation of any native flora/fauna through predation. It is possible that

when bivalve larvae become more prevalent in the crabs' surroundings they may become a viable food source and thus the crabs could potentially impact bivalve populations through consumption of larval stages.

Prior to the start of the shelter competition, several trials were conducted to determine if the crabs could in fact compete with the crayfish (data not shown). In those trials, crabs were placed in an aquarium with either adults or juveniles of *P. clarkii*. In all cases where adults were used, the larger crayfish killed the crabs. Only in competitions with juveniles were the crabs able to compete for shelter. However, the competition was rarely seen to be direct physical interactions except in one trial where a large crab ate a small crayfish (length = 15mm). Crabs were found to share the shelter with either conspecifics or heterospecifics significantly more often in the presence of predators.

Juvenile crayfish up to 20mm appear to respond to any interaction with a tail flip, the known escape response. Edwards *et al.* (1994) found that the lateral giant (LG) interneuron had a very low threshold and was the first interneuron stimulated up to the crayfish reaching 20mm. At this length, Edwards *et al.* (1994) demonstrated habituation in the LG interneuron, which allowed the crayfish to interact in other ways. Heberholz *et al.* (2004) looked at interactions between dragonfly nymphs and juvenile crayfish up to 26mm in length. Heberholz *et al.* (2004) found that the crayfish used tail flips to escape every interaction they faced with the nymphs. This behavior would likely prevent a crayfish from sharing a shelter with another individual. The results above

suggest this is the case. As the number of individuals were increased, crayfish became significantly less likely to share a shelter with either a crab or crayfish. The opposite trend was seen in the crabs, which, based on the conspecific interpersonal space trials (See Section 4), are significantly unaggressive towards each other. Boyle *et al.* (2010) noticed a paucity of crayfish in lakes where the crabs had been introduced. My results suggest that *R. harrisii* can successfully compete with *P. clarkii* for shelter in areas where they achieve densities of 3:1. Larson and Magoulick (2009) found that juvenile *P. clarkii* excluded from shelter suffered a higher risk of predation. In those areas where *R. harrisii* is capable of excluding the crayfish, higher predation would cause the apparent decline in crayfish.

The only direct study on the dispersal pathway of this crab was done by Briski *et al.* (2012). They found an adult gravid *R. harrisii* female in sediment of the ballast compartment of a large container ship off the Atlantic coast of the United States. No studies have analyzed the dispersal capabilities of this species in an invasive context. Cronin (1982) commented that *R. harrisii* was not likely to have high levels of larval connectivity between neighboring estuaries due to strong larval retention strategies. The inland lakes provide a new context within which to view the dispersal capabilities of the larvae of this species. Comparison of the larval dispersal to the adult dispersal indicates that larval dispersal is likely to be a more effective means of dispersal. Adult movement was relatively restricted and appeared to be influenced by the amount of cover available. Yet larval dispersal is restricted only by the amount of time spent in the water column. Based on the results of the larval development studies (Section 3), the larvae will spend



more time in the cooler waters of the spring during the earliest months of reproduction. This also coincides with a period of higher average rainfall for the state of Texas. This increased rainfall would increase the flow of the rivers and likely increase the dispersal of the larvae further downstream. On the Brazos River, it is approximately 180 km from Possum Kingdom Reservoir to Lake Granbury. Even at the fastest development rate, it is possible for larvae to disperse between the lakes based on the average flow rate. This allows for rapid dispersal from lake to lake similar to the dispersal seen in the larvae of the two fish species (*Coregonus albula* and *C. larvaretus*) (Naesje *et al.*, 1986).

Direct larval dispersal is restricted between reservoirs not directly connected by a water way and will always occur in a downriver fashion. Larvae are still capable of dispersing to other water bodies through transport in ballast waters of personal watercraft as well as through stocking procedures. Adult dispersal could potentially account for dispersal from one water body upriver to another reservoir. However, it would be a considerably longer dispersal time. For instance, based on the average rate it would take a crab 2,739 years to walk from Lake Granbury to Lake Whitney. These are the closest two reservoirs directly on the Brazos River. Adult dispersal cannot be ruled out completely as it is still possible that they could cling to an anchor line or underside of a boat and be transported between lakes.

In conclusion, the crabs have the potential to impact the natural biota of the invaded areas. They are eating whatever food particles are available and previous studies by other authors indicate the potential to adversely affect local bivalve

populations. The crabs are capable of outcompeting crayfish in laboratory studies due to the crabs increased sharing of shelter as the number of individuals increased in relation to the crayfish. This may explain the decreased numbers of crayfish observed by Boyle *et al.* (2010) in crab-infested lakes. The dispersal abilities of this species indicate that it is capable of expanding rapidly between reservoirs connected by water ways. This will certainly make control of this species difficult.

## 6. CONCLUSIONS

Invasive species are a unique and troubling problem for modern day ecologists. Much effort has been put forth to identify and control potential or current invasive species through modeling efforts. These models rely on the assumption that a species' traits are ubiquitous across the entire range of that species, ignoring the role local adaptation may play in invasion success. Several models have shown increased success in identifying source populations when working backwards from the invasive organism to the source population (Dullinger *et al.*, 2009; Rodder and Lotters, 2010; Fitzpatrick *et al.*, 2012; Vaclavik and Meentemeyer, 2012). The most comprehensive of the models to date is that of Blackburn *et al* (2011). This study focused on examining how that model fit the current invasions of *Rhithropanopeus harrisii*.

A major concern with the Blackburn *et al.* (2011) model is the assumption that species traits are the same across the entire range of a species. This removes the potential for local adaptation through changes to the realized niche. In his concluding remarks to the 1957 Cold Springs Harbor symposia on quantitative biology, G. Evelyn Hutchinson coined the terms fundamental niche and realized niche. These two terms have permeated ecological literature and guided the exploration of species interactions since their introduction. The fundamental niche, as defined by Hutchinson (1957), is the ecological space defined by the range of all variables affecting the species. The author defined the realized niche in terms of competition. If two species within the same habitat have overlapping niches and therefore compete, then the space within their

fundamental niche that does not overlap and thereby reduces competition is the realized niche. Thus the realized niche is a function of competition and thereby has the potential to be selectively changed over time. This has implications in all fields of biology.

Species characteristics, which are assumed to be ubiquitous across the entire range of the species, are potentially under selective forces through competition with overlapping species. This has large implications in the management of invasive species. In today's political climate, money is a very sensitive subject and increasingly so for the scientific community. In the case of invasive biology, much of the money is dedicated to eradication and prevention. The success of these programs is dependent upon how well the invasive organism's characteristics are understood. By including the realized niche of the invasive organism into the models, one could better predict the extent of future invasion. This would allow for the creation of management strategies for specific areas instead of across its entire range.

The invasion of inland reservoirs by *Rhithropanopeus harrisi* is a good example of this phenomenon. This crab has successfully invaded estuarine habitats throughout Europe, the Mediterranean and as far east as Japan. Yet the crabs have only invaded freshwater habitats in Texas. The limited genetic analyses have indicated that those crabs in European waters are more closely related to individuals from the east coast of the United States than to those of the Gulf Coast. Boyle *et al.* (2010) showed a closer genetic relationship between inland populations and those of the Texas coast.

This study was the first to show that the inland populations of *Rhithropanopeus harrisi* are capable of successful reproduction at a salinity of 1 PSU. Despite my inability to rear larvae from the limited number of females I obtained from the coast, it can be assumed that the adaptation to lower salinity waters is more likely to have occurred in the source population. The physiological change and genetic difference demonstrate differences between populations from the Gulf Coast and those from the East Coast of the United States. Future studies should focus on determining the extent of the low salinity tolerance amongst Gulf Coast populations. Another line of studies should focus on identifying the actual physiological change between the East Coast and inland populations as well as the genetic basis for the physiological change. One possibility is an upregulation of the Na<sup>+</sup>,K<sup>+</sup>-ATPase or Arginine Kinase both of which have previously been documented in the gills of blue crabs when moved to lower salinity waters (Towle *et al.*, 2001; Kotlyar *et al.*, 2000).

In Section 4, I suggested two factors that could potentially have led to the adaptation to lowered salinities during development. The first factor was increased competition with sympatric species. *Rhithropanopeus harrisi* lives sympatrically with eight other species of xanthoid crabs along the Gulf Coast of the United States compared to four species along the East Coast. Of the eight species, *R. harrisi* has the lowest salinity tolerance. Increased competition with the other species could potentially cause the exclusion of *R. harrisi* to lower salinity waters in the upper reaches of the estuaries. I looked at direct competition between *Rhithropanopeus harrisi* and four other sympatric species. Of the five, *R. harrisi* was the only crab to be significantly non-

aggressive in each of the treatments examined here. This non-aggression would make the exclusion to less saline waters more likely.

The second factor was differences in the salinity regimes of the native estuaries. There did not appear to be a significant difference in the average salinities for any of the estuaries examined, however, it was noted that the upper extremes were always seen in the Texas estuaries. These upper limits would allow the sympatric species that are more aggressive than *R. harrisi* to push further into the estuary driving *R. harrisi* even further up river from the estuary. *Rhithropanopeus harrisi* is also the only species of xanthoids along the Gulf Coast that has strong larval retention strategies. These strategies retain larvae in the areas where they hatched. This would increase the likelihood of this species becoming established in areas of the estuaries that have consistently lower salinities and are much more likely to see the extremely low salinities because of little tidal input.

All of these seem likely to have influenced the evolution of this species along the Gulf Coast and are plausible causes for an adaptation to lower salinities during larval development. Future studies that determine the source population of the inland crabs would allow the direct investigation of each of these factors on that source population compared to one on the East Coast. This would allow for the determination of which factor has had the greatest selective force in generating the lowered salinity tolerance.

Section 5 illustrated the impact and potential spread of this species in Texas reservoirs. The gut content analysis demonstrated that *R. harrisi* is an omnivorous

species that appears to feed primarily by the scraping of settled out particulates from nearby substratum. Howells's (1998) concerns over the impact of this species on endemic unionids are still untested. Literature suggests that the crabs could potentially eat bivalves but would be more likely to impact the successful recruitment of the bivalve larvae and juveniles. The competition studies of *R. harrisii* and *Procambarus clarkii* demonstrated the ability of the crab to outcompete the crayfish for shelter. The crabs were more likely to share the shelter especially as the number of individuals in the test arena increased, whereas the crayfish showed the exact opposite trend. This would preclude the crayfish in areas of high crab densities to be more susceptible to predation.

The dispersal of this species had previously been attributed to bait bucket and ballast water movement of adults. The successful rearing of larvae at 1 PSU demonstrated the ability of this species to spread by larval dispersion as well. Based on the flow rates and the length of development, I showed that the crabs were capable of dispersing between the two most disparate lakes within one developmental cycle. This species has the ability to spread to any reservoir connected via water way very rapidly and could explain the observations of Boyle *et al.* (2011) that documented the spread of *R. harrisii* to Lake Whitney sometime in 2005 where it had previously not been seen.

Using the Blackburn *et al.* (2011) model and current published larval physiological tolerances, the invasions of *R. harrisii* in Texas reservoirs would only be able to reach the survival filter in the third stage of the model. Based on my current study, I conclude that *R. harrisii* is in fact an established invasive species in the inland

reservoirs and has moved onto the fourth and final stage of dispersal. This species demonstrates that knowledge of the realized niche of the invasive species is essential to understanding the potential for a species to spread. This knowledge will assist in identifying source populations and thus better mediate future introductions. The increased knowledge could also allow the identification of more effective strategies of eradication or mitigation of current invasive populations. Current invasion models lack the inclusion of population specific data, which is a potential limitation in the effectiveness of each of these models.



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## **APPENDIX 1**

### **SUPPLEMENTAL DEVELOPMENTAL FIGURES**

The following are supplemental figures representing the days to development to both megalops and the first crab stage across all salinities and temperatures.

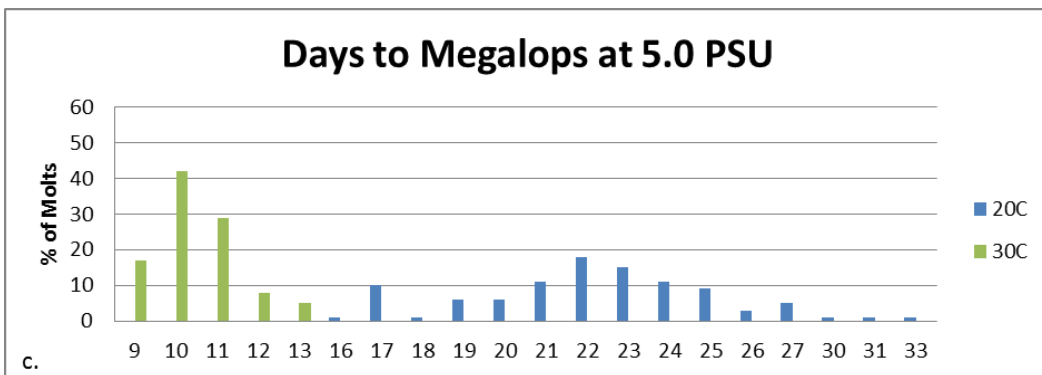
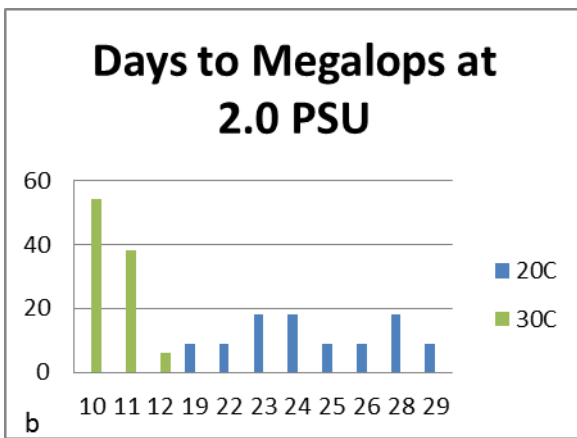
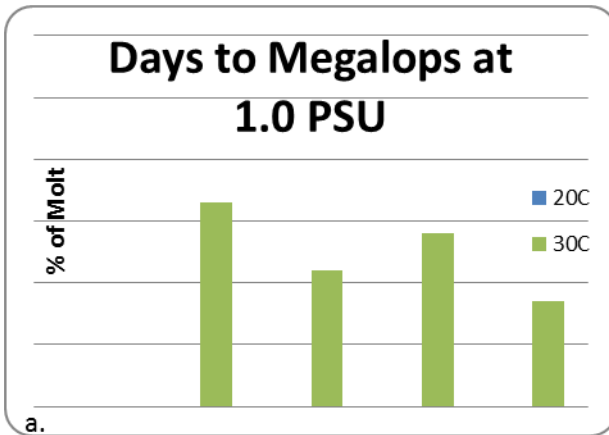


Figure A1-1. Days to Megalops at 20° and 30°C. Bars indicate the percentage of molts that occurred at each day.

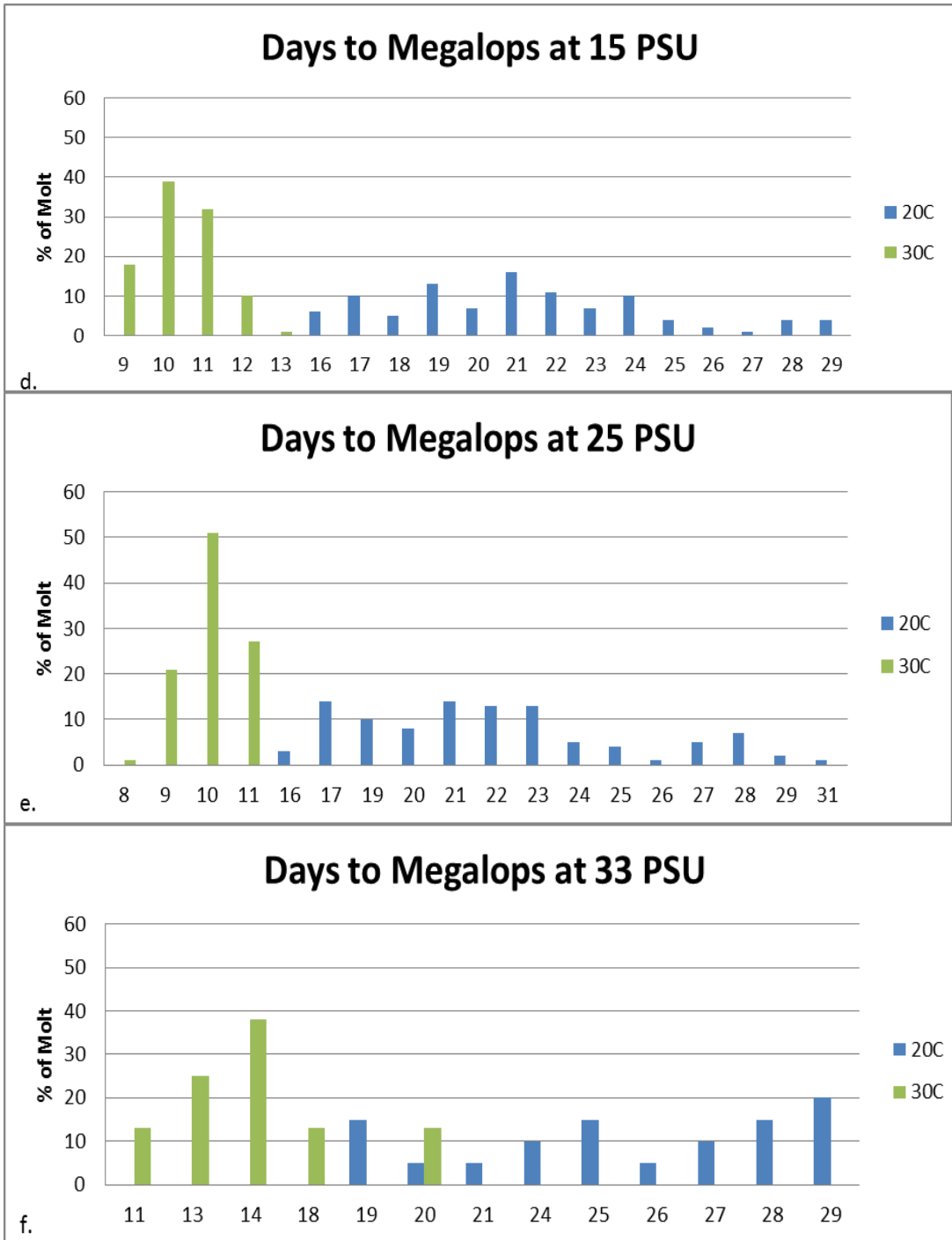


Figure A1-1 continued. Days to Megalops at 20° and 30°C. Bars indicate the percentage of molts that occurred at each day.



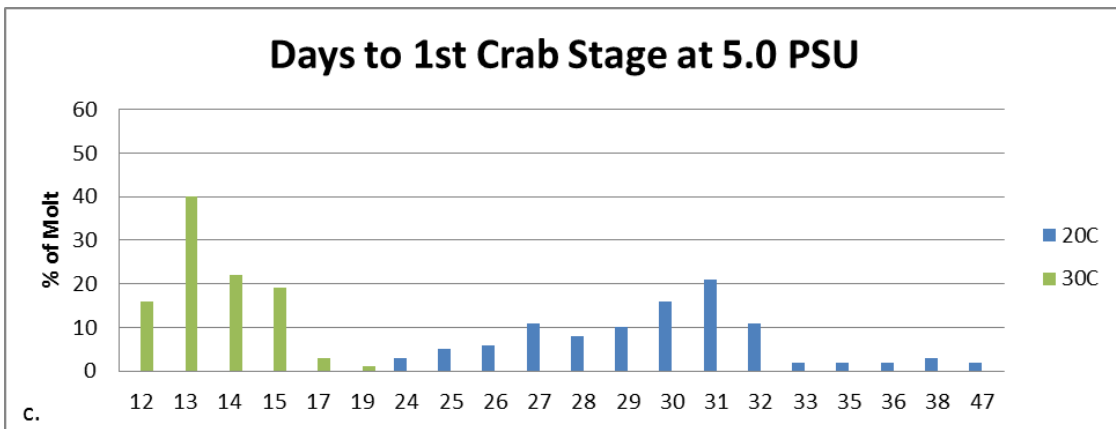
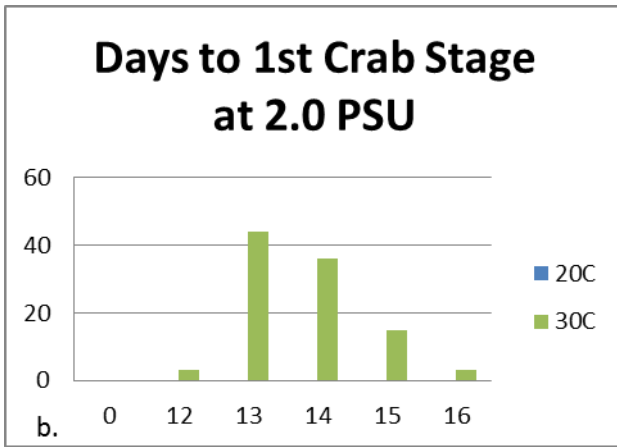
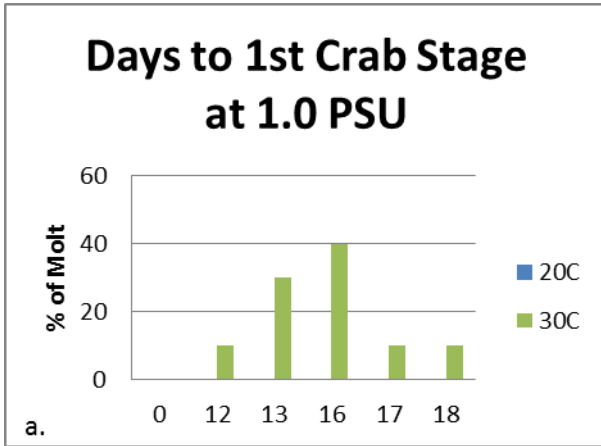


Figure A1-2. Days to 1<sup>st</sup> Crab Stage at each temperature salinity combination. Bars indicate the percentage of molts that occurred at each day.

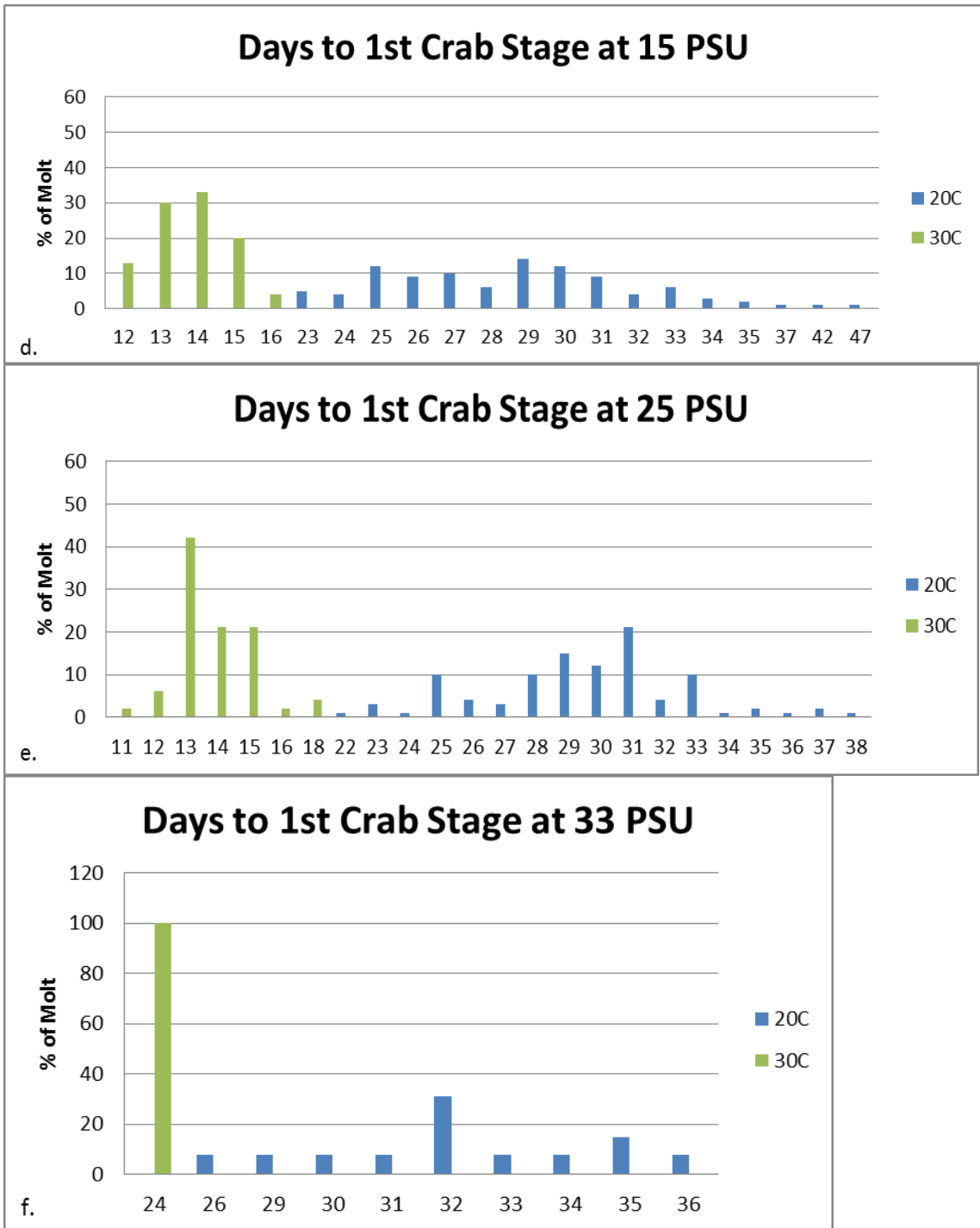


Figure A1-2 continued. Days to 1<sup>st</sup> Crab Stage at each temperature salinity combination. Bars indicate the percentage of molts that occurred at each day.

## APPENDIX 2

### NATIVE RANGE COLLECTION MAP STATE BY STATE

The following are maps created from Google Earth (6.1.0.5001) and the collection information I gathered from GBIF or through my own personal collections. The red pins represent sites less than 5 miles from the nearest bay. Blue pins represent sites between 5 and 10 miles from the nearest bay. Yellow pins represent sites between 10 and 15 miles from the nearest bay. The green pins represent sites greater than 15 miles from the nearest bay.

#### Gulf Coast Collecting Sites

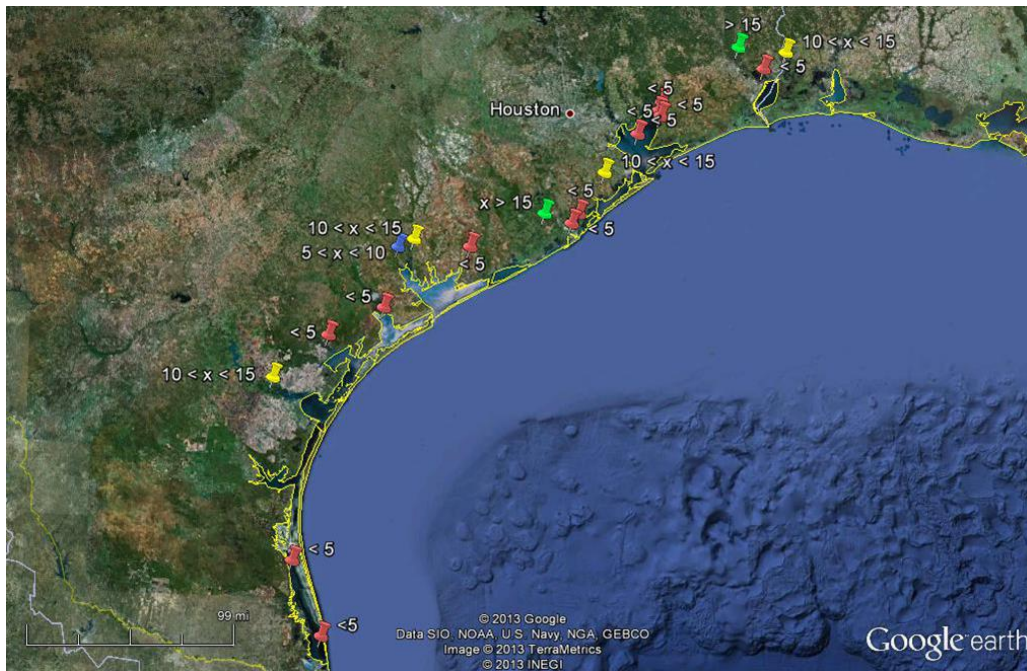


Figure A2-1 Texas collecting sites.

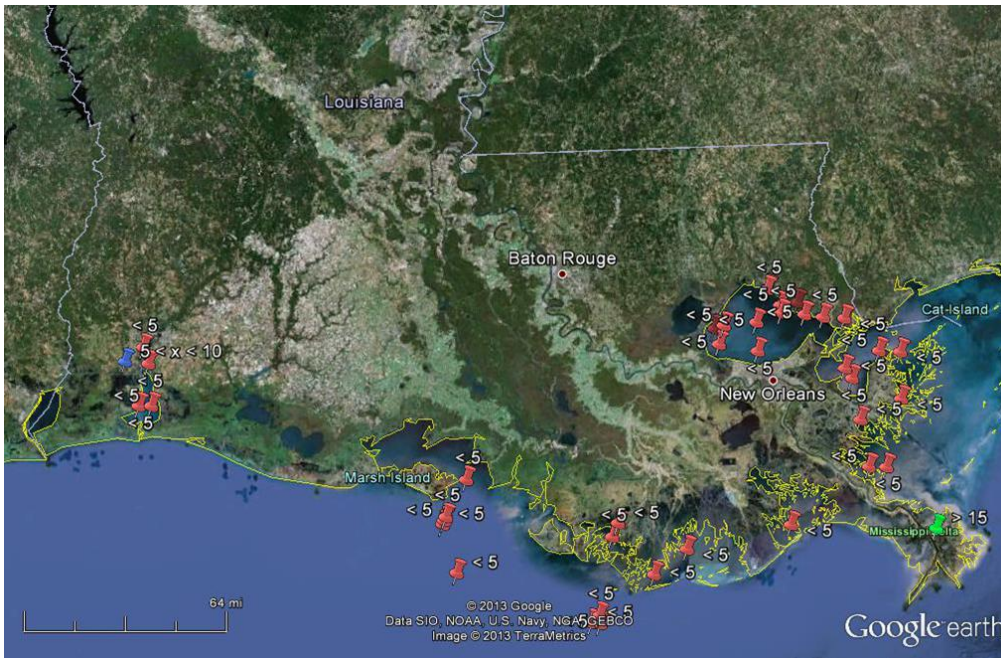


Figure A2-2. Louisiana collecting sites.

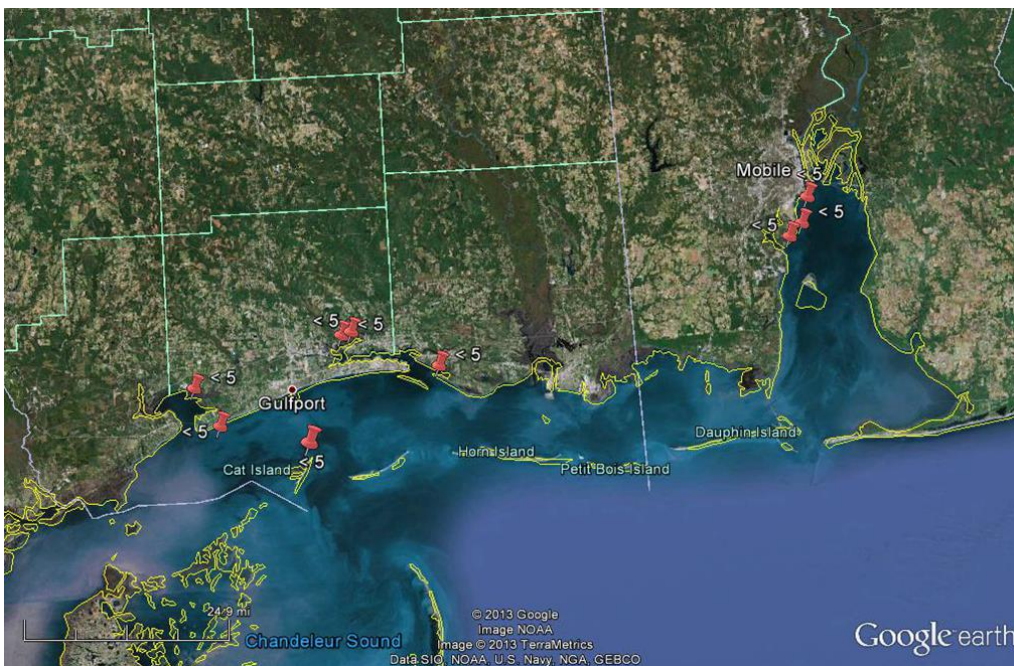


Figure A2-3. Mississippi and Alabama collecting sites.



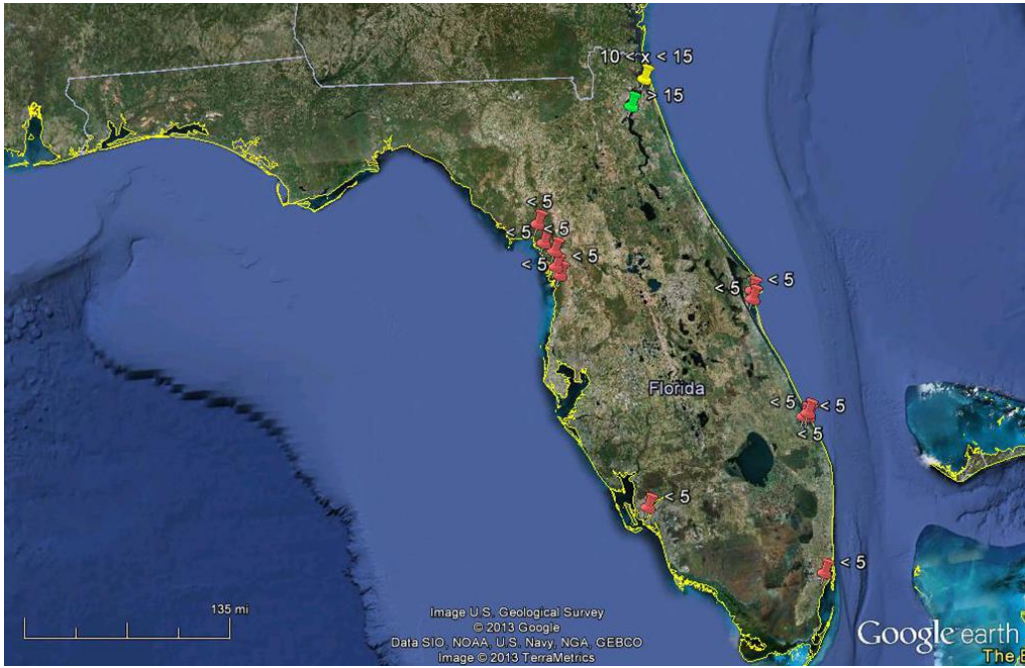


Figure A2-4. Florida collecting sites. Represents the split between the Gulf coast and the Atlantic coast.

Atlantic Coast Collecting Sites

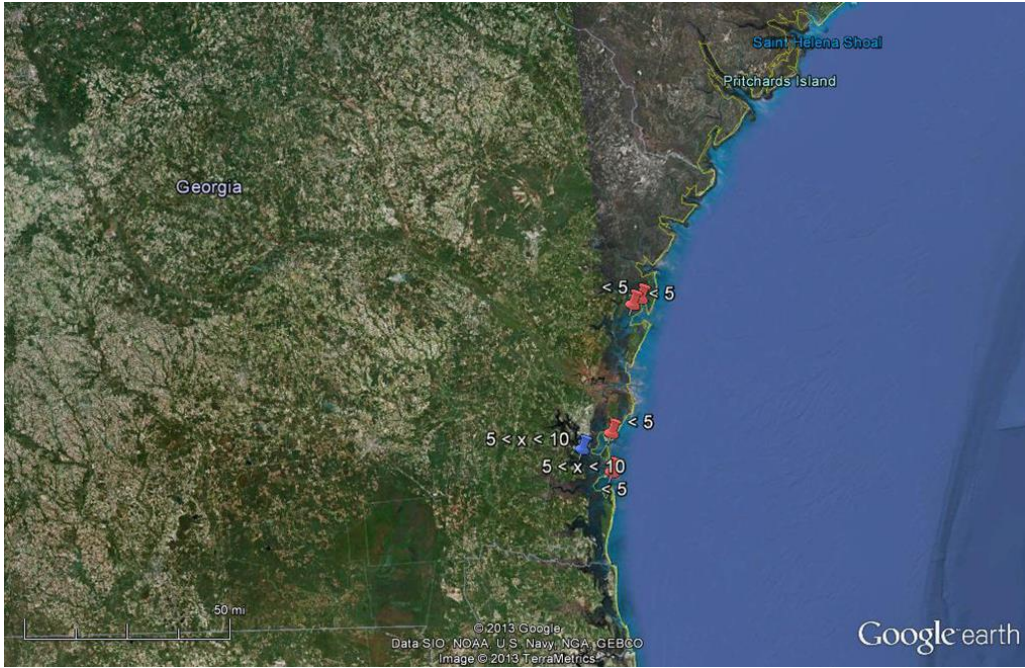


Figure A2-5. Georgia collecting sites.

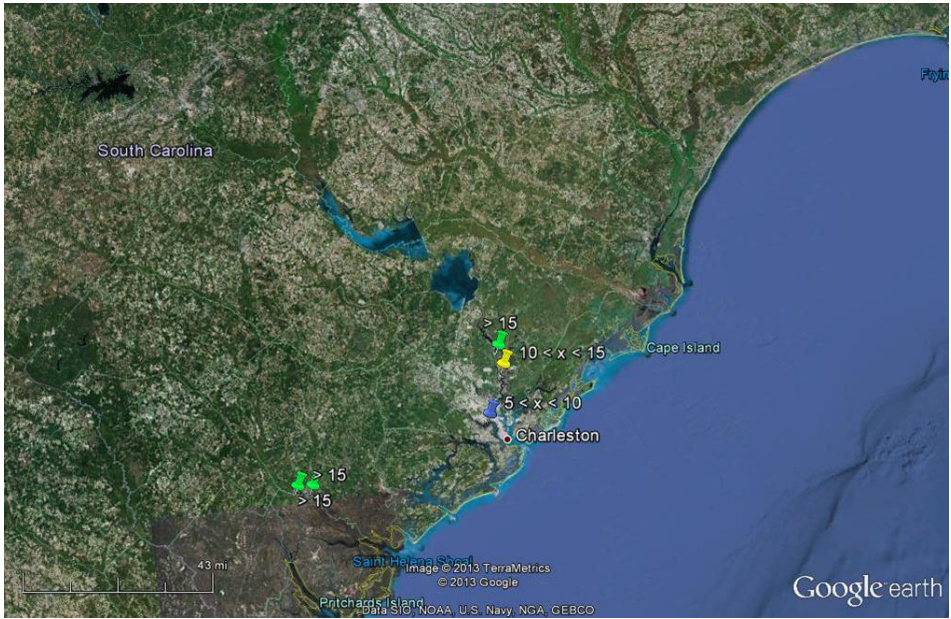


Figure A2-6. South Carolina collecting sites.

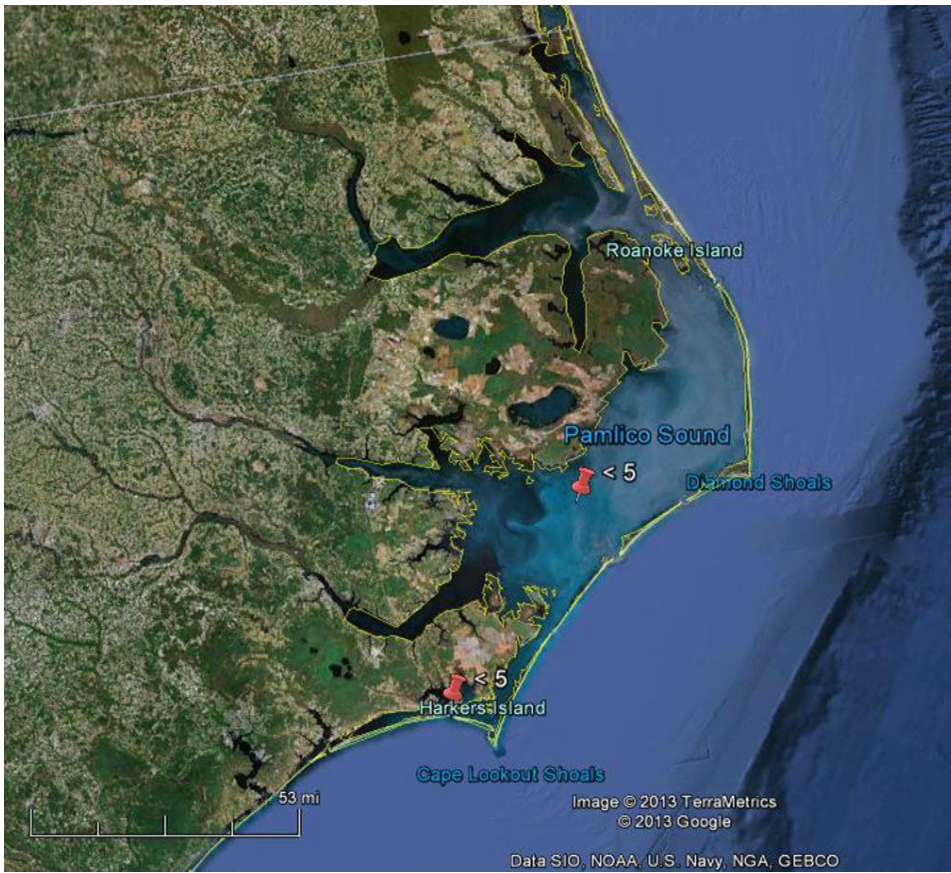


Figure A2-7. North Carolina collecting sites.



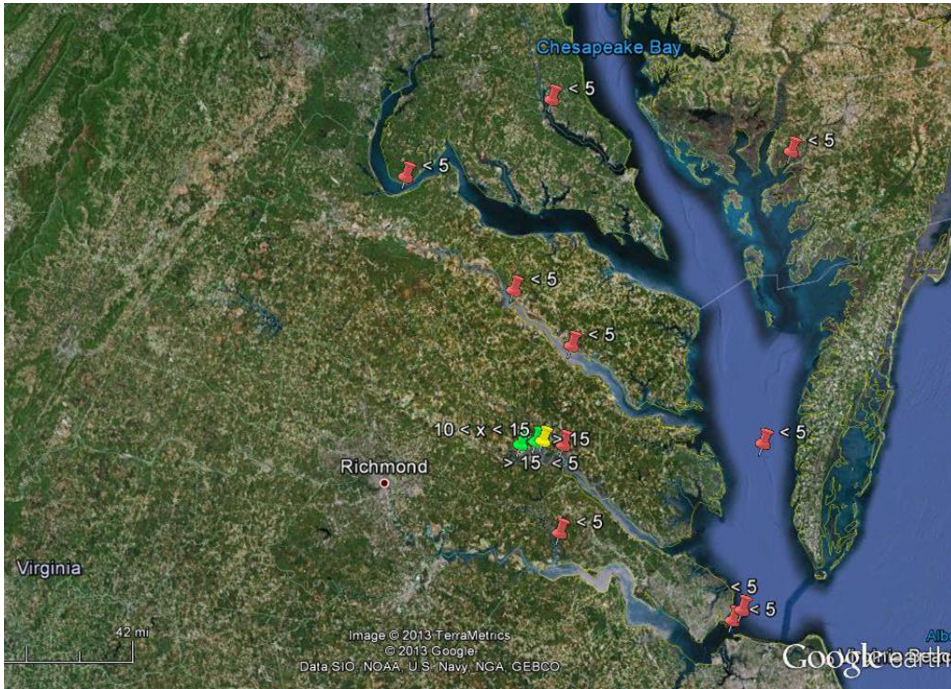


Figure A2-8. Virginia collecting sites.

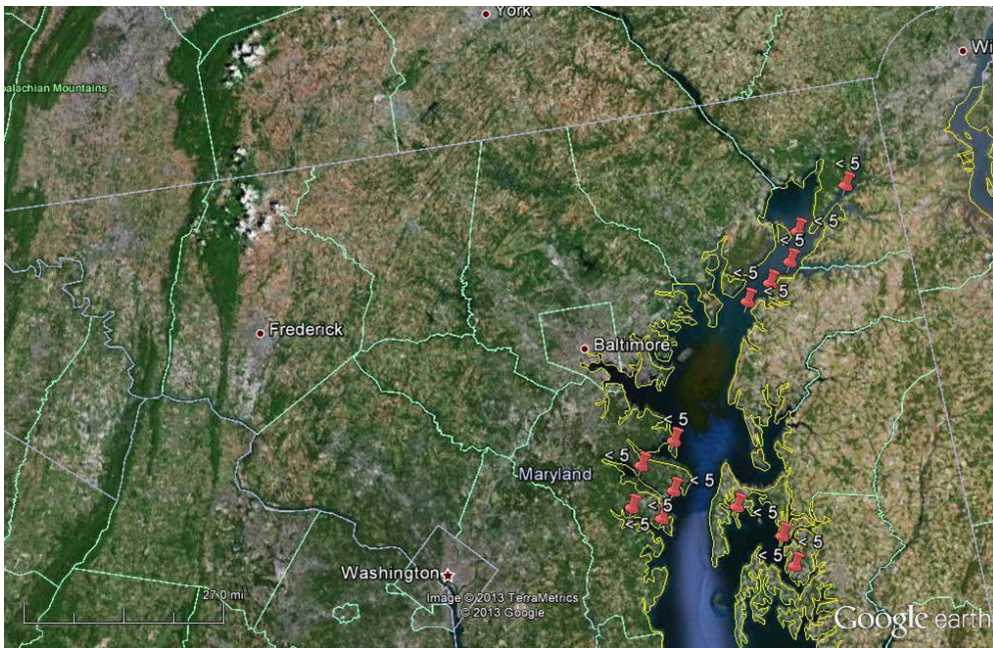


Figure A2-9. Maryland collecting sites.



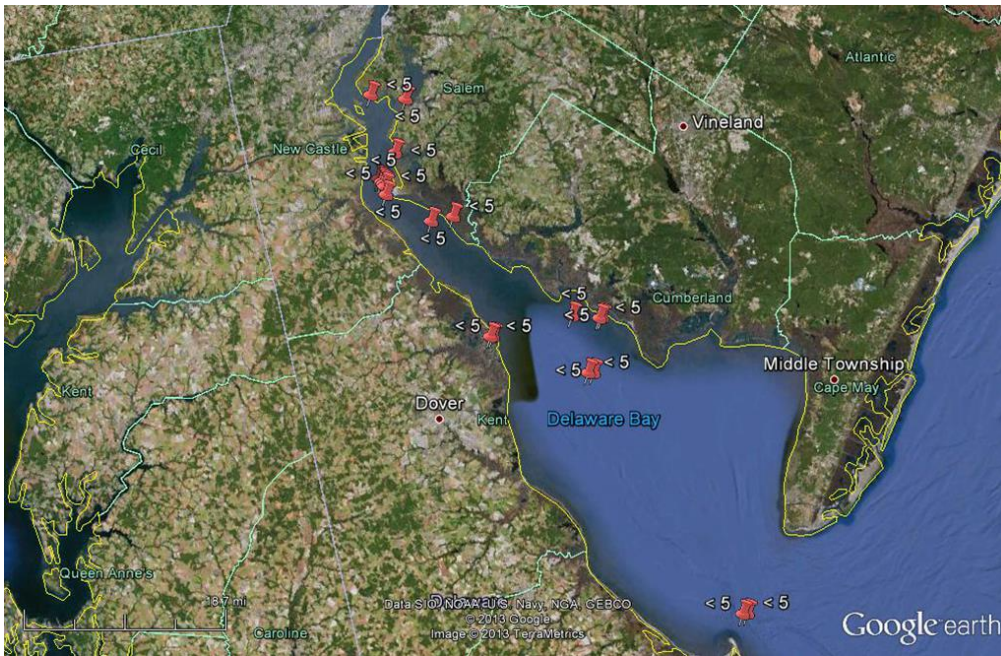


Figure A2-10. Delaware collecting sites.

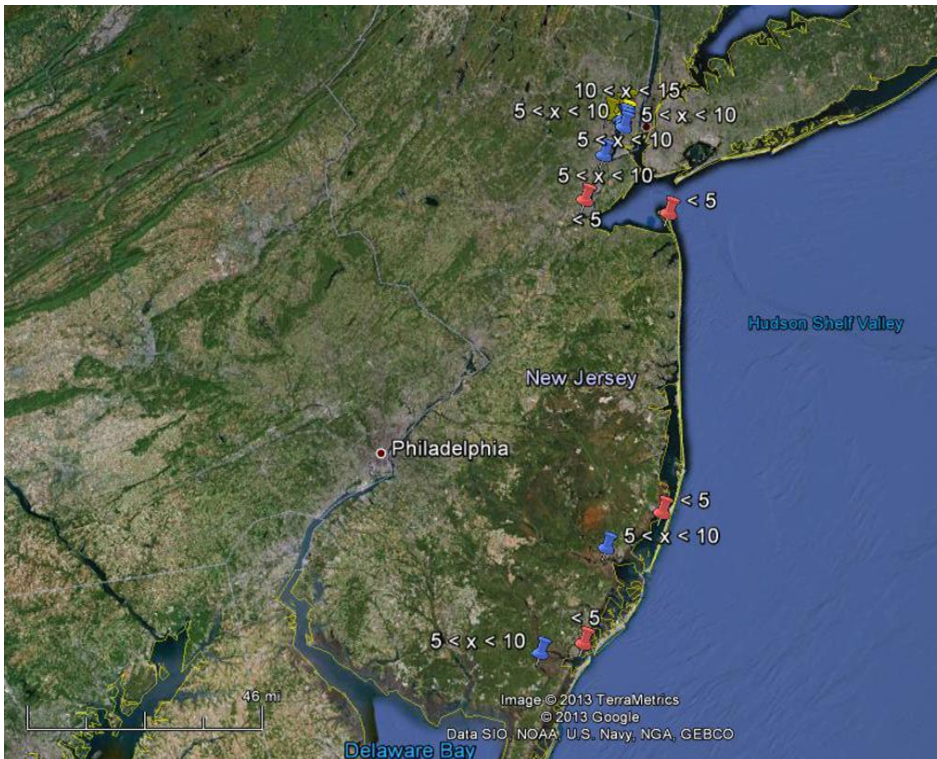


Figure A2-11. New Jersey collecting sites.



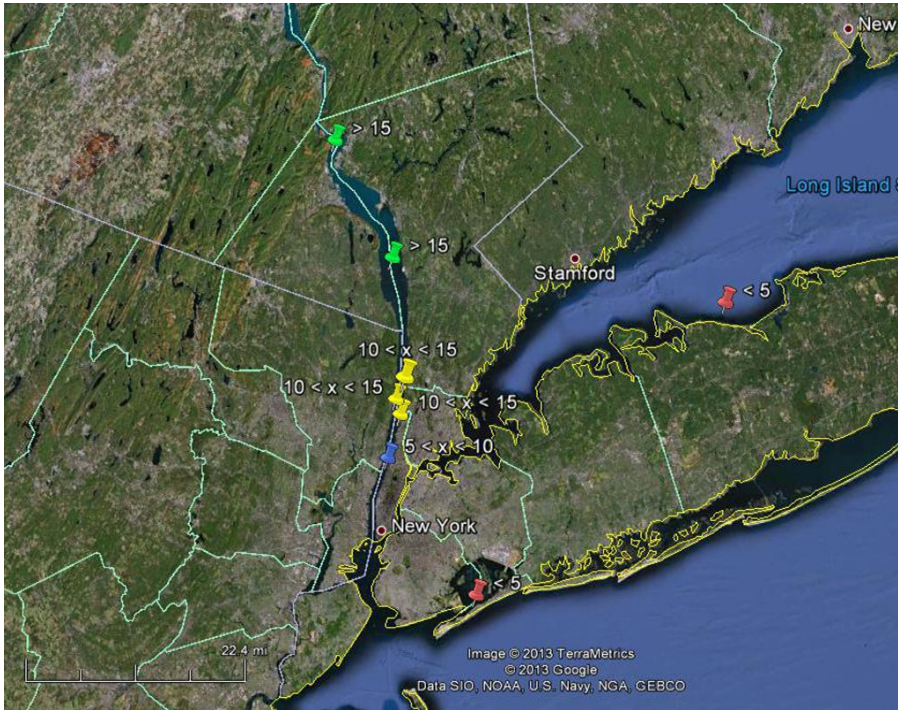


Figure A2-12. New York collecting sites.

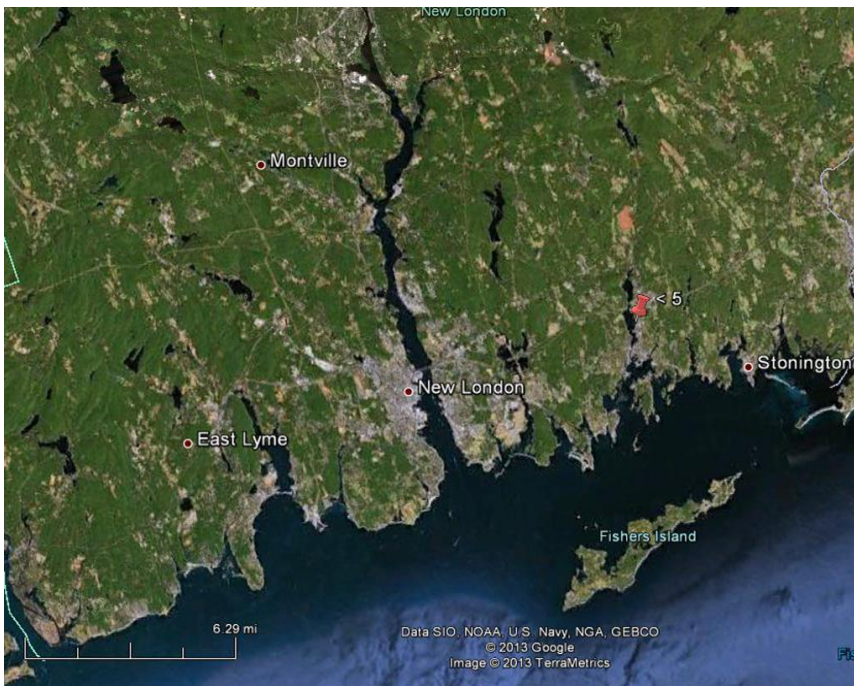


Figure A2-13. Connecticut collecting sites.

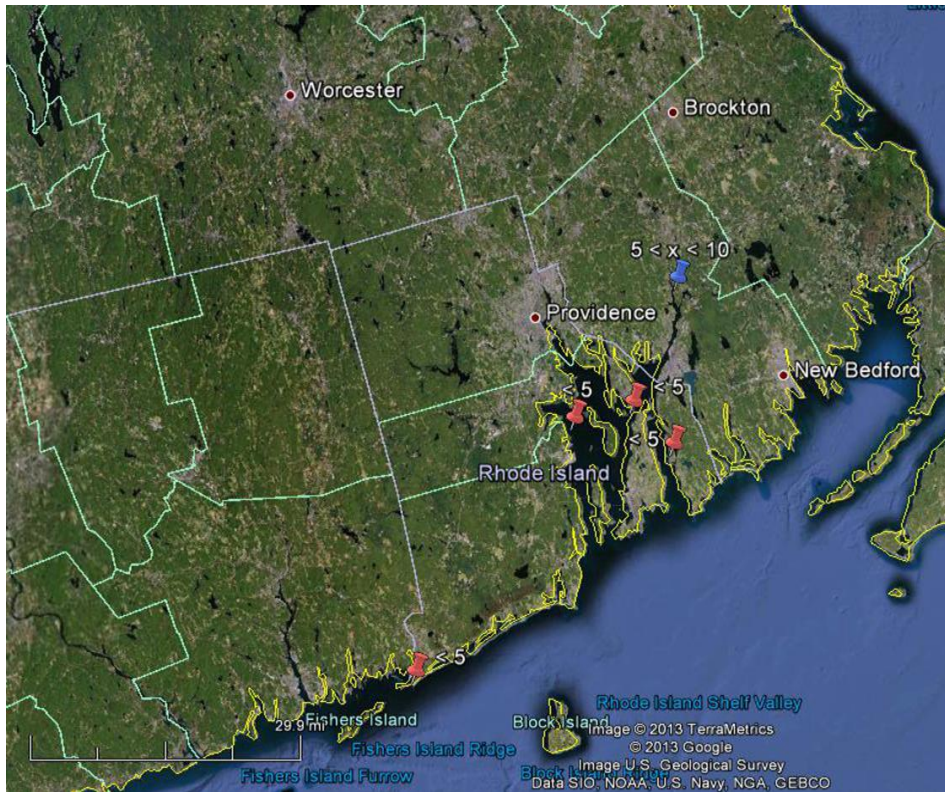


Figure A2-14. Rhode Island and Massachusetts collecting sites.