

2015

Metacommunity Dynamics and the Biogeography of Central Louisiana Crayfishes

William Robert Budnick

Louisiana State University and Agricultural and Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_theses



Part of the [Environmental Sciences Commons](#)

Recommended Citation

Budnick, William Robert, "Metacommunity Dynamics and the Biogeography of Central Louisiana Crayfishes" (2015). *LSU Master's Theses*. 1706.

https://digitalcommons.lsu.edu/gradschool_theses/1706

This Thesis is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Master's Theses by an authorized graduate school editor of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.

METACOMMUNITY DYNAMICS AND THE BIOGEOGRAPHY OF CENTRAL
LOUISIANA CRAYFISHES

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Renewable Natural Resources

by
William R. Budnick
B.S., Auburn University, 2012
May 2015

My devotion and love for science and research was encouraged and nurtured by several key individuals in my life that have not once discouraged me in my endeavors. I therefore dedicate this thesis to my parents, Robert and Sharon Budnick and my brothers, Joe, John, and C.J. Without their support (both emotionally and financially) I would never have grown into the capable man I know I will become. Additionally, I hope to make proud with this thesis my two former bosses, Drs. Jim Stoeckel and Brian Helms. Without their guidance, mentoring, and criticism during my undergraduate research experience, I would have been extremely unprepared for my graduate degree and scientific research in general. I am forever grateful for everything you all have contributed to my life.

ACKNOWLEDGEMENTS

Several people played a key role in the development and execution of this thesis project, without whom I would never have been able to accomplish in two field seasons. First, I'd like to thank my committee members, and especially Dr. Kaller, for the time, energy, and patience in my professional development. You have all been critical in helping me along to the next step in my scientific career and I am glad to have been under your mentorship.

But, perhaps also as important are the dedicated members of my stream team. Thus, I thank Bradley Hester, Michael Baker, and Kayla Smith for their endurance during the days-long marathons of sampling during the hot Louisiana summers. You guys made some really long field days also some of the best I ever had, stinky waders and all. I am proud to have been able to supervise you and I hope you learned a lot from our experiences in the crayfish world.

I also thank my fellow labmates and co-workers for their emotional, technical, and physical support. Raynie Harlan, Tiffany Pasco, Josh Herron, Kristy Durham, Sam Lott, Catherine Murphy, Catherine Reuter, Tyler Loeb, Jesse Sabo, Claire LaBarbera, Corrine Tanner, and Chris Bonvillain, you guys are an awesome bunch of people to work and I hope to work with you again down the road.

Finally, let me show my undying appreciation towards my study organisms that not only helped me earn my degree, but also sent me around the world: the crayfishes of Louisiana. I still find it unfortunate that their critical position in the ecosystem is wholly unappreciated in favor of their culinary status.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	viii
ABSTRACT	ix
CHAPTER	
1 INTRODUCTION	1
1.1 THE CENTRAL LOUISIANA CRAYFISH METACOMMUNITY AND BIOGEOGRAPHICAL EXTENT	1
1.2 REFERENCES	3
2 DRAINAGE SCALE SPECIES-HABITAT RELATIONSHIPS OF CRAYFISHES (DECAPODA: CAMBARIDAE) IN A MIXED LAND USE REGION OF THE SOUTHEASTERN UNITED STATES	6
2.1 INTRODUCTION	6
2.2 METHODS	10
2.3 RESULTS	16
2.4 DISCUSSION	20
2.5 REFERENCES	25
3 SAMPLING CRAYFISH ASSEMBLAGES IN LOUISIANA COASTAL PLAIN STREAMS: GEAR, PROTOCOL, AND TIMING	30
3.1 INTRODUCTION	30
3.2 METHODS	33
3.3 RESULTS	36
3.4 DISCUSSION	41
3.5 REFERENCES	49
4 CHAPTER 4: THE INFLUENCE OF BIOLOGICAL FACTORS IN THE FORMATION OF INTRASPECIFIC DOMINANCE HIERARCHIES IN THE CRAYFISHES, <i>ORCONECTES HATHAWAYI BLACKI</i> AND <i>PROCAMBARUS PENTASTYLUS</i> (ASTACIDEA; CAMBARIDAE)	54
4.1 INTRODUCTION	54
4.2 METHODS	55
4.3 RESULTS	62
4.4 DISCUSSION	67
4.5 REFERENCES	73
5 CONCLUSION	76
5.1 DISCUSSION	76
5.2 REFERENCES	78

APPENDIX

A	CRAYFISH CAPTURE DATA AND ENVIRONMENTAL DATA	80
B	EXTENDED COMPETITION TRIAL DATA	94
VITA	97

LIST OF TABLES

2.1 MEANS AND STANDARD DEVIATIONS OF ENVIRONMENTAL VARIABLES FOR DRAINAGES SAMPLED (N=45, 9 SITES PER DRAINAGE) IN SUMMER 2013.....	19
2.2 TESTS OF NULL-HYPOTHESIS THAT THE CANONICAL CORRELATIONS IN THE CURRENT ROW AND ALL THAT FOLLOW ARE ZERO.....	19
2.3 ESTIMATES OF REGRESSION COEFFICIENTS AND FIT STATISTICS FROM GENERALIZED LINEAR MIXED MODELS	20
3.1 CRAYFISH SPECIES COMPOSITION FROM THE SUMMER 2014 STREAM SAMPLES.....	36
3.2 RESULTS OF ANALYSIS OF COVARIANCE ANALYZED WITH A NEGATIVE BINOMIAL GENERALIZED LINEAR MODEL TESTING FOR EFFECTS OF GEAR TYPE (BACKPACK ELECTROFISHER, DIPNET, AND BOTH) AND TIME OF DAY (DAY AND NIGHT) ON CRAYFISH CPUE.....	37
3.3 SUMMARY STATISTICS OF A TWO-WAY MIXED ANOVA TO TEST DIFFERENCES IN MEAN TOTAL LENGTH OF CRAYFISH BETWEEN TIMES OF DAY (DAY VERSUS NIGHT TRANSECTS), AND GEAR TYPES (BACKPACK ELECTROFISHERS VERSUS DIPNETS).....	39
3.4 LOGISTIC REGRESSION PERFORMED BY A GENERALIZED LINEAR MIXED MODEL TO DETERMINE THE ODDS OF SAMPLING AN INDIVIDUAL SEX WITH A GIVEN GEAR TYPE (BACKPACK SHOCKER OR DIPNET) AND TIME OF DAY (DAY OR NIGHT).....	40
4.1 ETHOGRAM FOR USE IN SCORING DOMINANCE INTERACTIONS BETWEEN INDIVIDUAL CRAYFISHES	59
4.2 MORPHOLOGICAL AND BIOLOGICAL SUMMARY DATA OF <i>ORCONECTES BLACKI</i> AND <i>PROCAMBARUS PENTASTYLUS</i> USED FOR INTRASPECIFIC DOMINANCE TRIALS.....	63
4.3 SUMMARY STATISTICS AND INCIDENT RATE RATIOS (IRR) OBTAINED THROUGH NEGATIVE BINOMIAL REGRESSION MODELLING THE COUNTS OF INTRASPECIFIC DOMINANCE BOUTS BETWEEN CONSPECIFIC CRAYFISH.....	65

4.4 SUMMARY STATISTICS AND REGRESSION COEFFICIENTS (CHANGE IN LOG ODDS) OBTAINED THROUGH LOGISTIC REGRESSION MODELLING FACTORS AFFECTING THE ODDS OF BEING DOMINANT IN A DOMINANCE BOUT BETWEEN CONSPECIFIC CRAYFISH.....	65
4.5 SUMMARY STATISTICS AND ODDS RATIOS OBTAINED THROUGH LOGISTIC REGRESSION MODELLING FACTORS AFFECTING THE ODDS OF BEING DOMINANT IN A DOMINANCE BOUT BETWEEN MATCH-SIZED (WITHIN 2MM) CONSPECIFIC CRAYFISH.....	66
4.6 SHELTER OCCUPANCY TIME QUANTILES, IN SECONDS, FOR CRAYFISHES OF <i>ORCONECTES BLACKI</i> AND <i>PROCAMBARUS PENTASTYLUS</i> DURING DOMINANCE TRIALS UNDER THE PRESENCE OR ABSENCE OF A PREDATOR CUE.....	68
A.1 LIST OF SPECIES EXPECTED TO RESIDE WITHIN THE DRAINAGES SAMPLED AND CAPTURE NOTES BASED ON COLLECTION EXPERIENCE AND THE LITERATURE.....	80
A.2 CRAYFISH COLLECTION DATA FOR THE 2013 AND 2014 COLLECTION YEARS IN STREAMS OF CENTRAL LOUISIANA.....	82
A.3 STREAM WATER QUALITY DATA FOR SITES SAMPLED FOR CRAYFISH IN SUMMER 2013.....	88
A.4 STREAM PHYSICAL HABITAT PROFILE DATA FOR SITES SAMPLED FOR CRAYFISH IN SUMMER 2013. DATA SHOWN ARE MEANS AND STANDARD DEVIATIONS OF THE HABITAT PARAMETERS.....	91
B.1 CONTINGENCY TABLE TABULATING THE FREQUENCIES OF INTRASPECIFIC SHELTER INTERACTIONS IN TWO SPECIES OF CRAYFISH WITHIN THE BIOLOGICAL VARIABLES: SIZE CLASS (SMALL, 32-45MM; MEDIUM, 46-59MM; AND LARGE, >60M), PRESENCE OF PHYSICAL ABNORMALITY, AND SEX.....	94
B.2 QUALITATIVE EXAMINATION BETWEEN TWO SPECIES OF CRAYFISH OF THE PROPORTION OF INTRASPECIFIC DOMINANCE INTERACTIONS THAT WERE SHELTER FOCUSED (I.E., CRAYFISH ATTEMPTED TO EVICT A CONSPECIFIC OR DEFENDED AGAINST AN EVICTION) UNDER PRESENCE OF PREDATOR CUE.....	96

LIST OF FIGURES

2.1 MAP SHOWING SITES SAMPLED AND DRAINAGE BOUNDARIES FOR THE SUMMER 2013 CRAYFISH COLLECTION EFFORT	9
2.2 TOTAL CATCH DATA BY DRAINAGE FOR CRAYFISH COLLECTED DURING THE SUMMER 2013 SAMPLING EFFORT DOCUMENTING SPECIES DIVERSITY IN THE CENTRAL LOUISIANA WATERSHEDS.....	15
2.3 PLOT OF SITE SCORES FROM A PRINCIPAL COMPONENTS ANALYSIS QUANTIFYING SIMILARITIES AMONG SAMPLE SITES IN CENTRAL LOUISIANA FROM THE 2013 CRAYFISH SAMPLE EFFORT (N=45).....	17
3.1 BARPLOTS OF MEAN CRAYFISH CATCH PER UNIT EFFORT (CPUE) AND STANDARD DEVIATION FOR DIFFERENT LEVELS OF TIME OF DAY (A) AND SAMPLING METHOD (B).....	38
3.2 BARPLOTS OF MEAN TOTAL LENGTH (MM) AND STANDARD DEVIATIONS (ERROR BARS) OF CRAYFISHES SAMPLED AT DIFFERENT TIMES OF DAY (A) AND WITH DIFFERENT SAMPLING METHODS (B)...	39
3.3 SPECIES ACCUMULATION CURVES SHOWING EXPECTED CRAYFISH RICHNESS AS A FUNCTION OF SITES RANDOMLY SAMPLED (FOR 100 PERMUTATIONS) WITH DIFFERENT COMBINATIONS OF GEAR TYPES AND TIMES OF DAY.....	42
3.4 SPECIES ACCUMULATION CURVES SHOWING EXPECTED CRAYFISH RICHNESS AS A FUNCTION OF INDIVIDUALS RANDOMLY SAMPLED (WITHOUT REPLACEMENT; RAREFACTION) WITH DIFFERENT COMBINATIONS OF GEAR TYPES AND TIMES OF DAY	43
4.1 SCHEMATIC OF THE CRAYFISH COMPETITION ARENAS.....	57

ABSTRACT

The greatest diversity of crayfishes, especially of rare *Orconectes* species, is found in the central Louisiana watersheds (Red River, Mermentau, Calcasieu, and Vermillion-Teche), and most species are widely distributed among the drainages. The purpose of this research was to lay groundwork for species distribution and metacommunity modelling for crayfishes in this region. To address this goal, two field studies were performed in the summers of 2013 and 2014, as well as an indoor laboratory study. Analyses of broadly distributed species indicated no significant correlations between species abundances and habitat variables nor any significant environmental gradients for those species. Within-drainage variability of habitat was substantial in this study and a large range of site conditions were sampled within any particular drainage, despite significant drainage differences. Subsequent analysis of drainage-restricted species indicated that drainage-scale modelling is suitable for endemic species such as *Procambarus pentastylus* and *P. natchitochae*. However, more restricted *Orconectes* *maletae*, *O. blacki*, and *O. hathawayi* could not be effectively modelled at the drainage scale, in part because they were not detected in the majority of samples. Examination of gear types and times of day indicated that catch per unit effort (CPUE) and average total length of crayfish was greater with electrofishers than with dipnets; however, no differences between time of day were detected for either CPUE or crayfish total length. Differences in estimates of sample diversity using different combinations of gears and times of day were detected when calculated on a per individual basis, but not on a per site basis. In intraspecific competition trials, body size and chelae width were found to be significant biological factors in determining the odds of dominance in *O. blacki* and *P.*

pentastylus. Presence of predator cue (water conditioned by *Micropterus salmoides*) generally reduced frequencies of dominance interactions in both species and generally increased median shelter occupancy times for both species. This research provides much needed information on the distributions and ecology of central Louisiana crayfishes, and future studies will be needed to quantify genetic units, dispersal corridors, and interspecific interactions between other co-occurring species and to characterize the central Louisiana crayfish metacommunity.

CHAPTER 1: INTRODUCTION

1.1 THE CENTRAL LOUISIANA CRAYFISH METACOMMUNITY AND BIOGEOGRAPHICAL EXTENT

Crayfish are unique in status in Louisiana because in addition to being a critical component of aquatic ecosystems (e.g. Womot 1995, Dorn and Wojdak 2004, Usio and Townsend 2004), two species are important cultural icons. Commercial production of crayfish is the dominant aquaculture enterprise in the state and focuses on two species, *Procambarus clarkii* and *Procambarus zonangulus*, with more emphasis on extensive production of the former (Huner and Romaine 1990). However, research on the ecology and biology of other crayfish species throughout the state is limited and antiquated with much work sourcing from the 1940s through the 60s. Although works by Walls, (2009, 1972, 1968), Black (1963, 1966, 1967, 1972), Fitzpatrick (1963, 1983, 1990, but see also Fitzpatrick and Sutkus 1992), and Penn (1942, 1950a, 1950b, 1956) represent some of the most in-depth, systematic studies of Louisiana crayfishes, they mainly focus on the taxonomic descriptions and distributions of myriad species. This represents a significant problem from both the basic science and management perspective, because almost no quantitative ecological information exists for any species within the state outside of *P. clarkii* and *P. zonangulus*.

Central Louisiana crayfish assemblages are defined herein as those occupying the Red, Mermentau, Calcasieu, Houston River branch of the Calcasieu River, and Vermillion-Teche river drainages. This region of Louisiana encompasses primarily streams of the Western Gulf Coastal Plain, and most streams are characterized as low-gradient and groundwater driven with diverse land-uses within each drainage (Hupp 2000, but see also Kaller et al. 2013). The majority of Louisiana's crayfish species are

widely distributed among the study region's drainages, with at least 20 of the 39 documented species occurring south of the Red River and west of the Mississippi River (Table A.1, see also Walls 2009). Within the region, 4 stream-dwelling species are considered endemic to Louisiana and are restricted to a single drainage.

The broad distributions of many crayfish species suggest metacommunity organization within drainages and across nearby drainages that should be further investigated (see Leibold et al. 2004). However, because research on Louisiana crayfish biogeography and dispersal is lacking, the metacommunities, or even the extent of local constituent communities, for this region cannot be reasonably determined among stream networks, drainages, or throughout the region. This is because documentation of species distributions and diversity, physical and chemical habitat assessments, and genetic analyses are required to characterize a metacommunity and its component local community patches with the appropriate scale (e.g., Chase and Ryberg 2004, but see also Leibold et al. 2010, Logue et al. 2011). Additionally, biotic interactions must also be taken into account for metacommunity characterization because local community dynamics and subsequent dispersal of crayfish species are affected by intraspecific and interspecific competition and predation, particularly by invertivorous fishes (e.g., Garvey et al. 1994).

Recent interest in the conservation of *Orconectes* crayfishes in this region has brought about questions concerning actual species distributions, distribution boundaries, and how species may disperse, all of which may actually help characterize the metacommunities. For example, Walls (1972) noted a zone of intergradation between *Orconectes hathawayi hathawayi* and *Orconectes hathawayi blacki* within the Calcasieu

River. Both species are rare in the state and of interest for possible listing for federal protection. However, the exact spatial extent, habitats used, and relationships with other co-occurring species are not up-to-date outside of their original species descriptions. Thus, opportunity exists to answer these questions and provide foundational frameworks for distribution models, diversity assessments, and ultimately a profile of the metacommunity dynamics within the study region.

This research focuses on quantifying species habitat-relationships among the Central Louisiana drainages that contain *Orconectes* species of interest, with the intention to build and calibrate species distributional models. In Chapter 2, I discuss a multivariate analysis of species-habitat relationships and note important gradients for species abundances in this region. Additionally, because of the scale at which I sampled and the methods I employed, Chapter 3 examines the efficiency of the sampling methods I employed in terms of number of crayfish and diversity of species captured by each of the gears. Finally, in Chapter 4, I discuss the results of a behavioral study that compared contest competition (i.e., agonism) within two species of crayfish (*O. h. blacki* and *P. pentastylus*).

1.2 REFERENCES:

- Black, J. B. 1972. Life history notes on the crayfish *Orconectes lancifer*. *Proceedings of the Louisiana Academy of Science*. 35: 7-9.
- Black, J. B. 1967. A new crayfish of the genus *Cambarus* from southwestern Louisiana (Decapoda: Astacidae). *Proceedings of the Biological Society of Washington*. 80: 173-178.
- Black, J. B. 1966. Cyclic male reproductive activities in the dwarf crayfishes *Cambarellus shufeldtii* (Faxon) and *Cambarellus puer* (Hobbs). *Transactions of the American Microscopical Society*. 85(2): 214-232.
- Black, J. B. 1963. Observations on the home range of stream-dwelling crawfishes. *Ecology*. 44(3): 592-595.

- Chase, J. M. and W. A. Ryberg. 2004. Connectivity, scale-dependence, and the productivity-diversity relationship. *Ecology Letters*. 7(8): 676-683.
- Dorn, N. J. and J. M. Wojdak. 2004. The role of omnivorous crayfish in littoral communities. *Oecologia*. 140:150-159.
- Fitzpatrick, JR. J. F. 1963. Geographic variation in the crayfish *Faxonella clypeata* (Hay) with the definition and defense of the genus *Faxonella* Creaser (Decapoda, Astacidae). *Tulane Studies of Zoology*. 10(1): 57-79.
- Fitzpatrick, JR. J. F. 1983. A revision of the dwarf crayfishes (Cambaridae, Cambarellinae). *Journal of Crustacean Biology*. 3(2): 266-277.
- Fitzpatrick, JR, J. F. 1990. *Procambarus (Pennides) vioscai paynei*, a new subspecies from east of the Mississippi River (Decapoda, Cambaridae). *American Midland Naturalist*. 123(2): 259-267.
- Fitzpatrick JR, J. F. and R. D. Sutkus. 1992. Environmental notes on the recently described crayfish, *Procambarus (Girardiella) kensleyi* Hobbs. *Southwestern Naturalist*. 37(3): 328-330
- Huner, J. V. and R. P. Romaine. 1990. Crawfish culture in the southeastern USA. *World Aquaculture*. 21(4): 58-65.
- Hupp, C. R. 2000. Hydrology, geomorphology, and vegetation of Coastal Plain rivers in the south-eastern USA. *Hydrological Processes*. 14: 2991-3010.
- Kaller, M. D., C. E. Murphy, W. E. Kelso, and M. R. Stead. 2013. Basins for fish and ecoregions for macroinvertebrates: Different spatial scales are needed to assess Louisiana wadeable streams. *Transactions of the American Fisheries Society*. 142(3): 767-782.
- Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loureau, and A. Gonzales. 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters*. 7(7): 601-613.
- Leibold, M. A., E. P. Economo, and P. Peres-Neto. 2010. Metacommunity phylogenetics: separating the roles of environmental filters and historical biogeography. *Ecology Letters*. 13: 1290-1299.
- Logue, J. B., N. Mouquet, H. Peter, and H. Hillebrand. 2011. Empirical approaches to metacommunities: A review and comparison with theory. *Trends in Ecology and Evolution*. 26(9): 482-491.

- Penn, G. H. 1942. Observations on the biology of the Dwarf Crayfish, *Cambarellus shufeldtii* (Faxon). *American Midland Naturalist*. 28(3): 644-647.
- Penn, G. H. 1950a. A new crayfish of the genus *Orconectes* from Louisiana (Decapoda: Astacidae). *Journal of the Washington Academy of Science*. 40(5): 166-169.
- Penn, G. H. 1950b. Utilization of crayfishes by cold blooded vertebrates in the eastern United States. *American Midland Naturalist*. 44(2): 421-426.
- Penn, G. H. 1956. The genus *Procambarus* in Louisiana (Decapoda, Astacidae). *American Midland Naturalist*. 56(2): 406-422.
- Usio, N. and C. R. Townsend. 2004. Roles of crayfish: Consequences of predation and bioturbation for stream invertebrates. *Ecology*. 85(3): 807-822
- Walls, J. G. 1972. Three new crayfishes related to *Orconectes difficilis* (Faxon) (Decapoda: Astacidae). *Proceedings of the Biological Society Washington*. 84(53): 449-458
- Walls, J. G. 1968. A new *Faxonella* species from northeast Louisiana (Decapoda: Astacidae). *Proceedings of the Biological Society Washington*. 81: 413-418.
- Walls, J. G. 2009. The crawfishes of Louisiana. Louisiana State University Press.
- Womot, W. T. 1995. Redefining the role of crayfish in aquatic ecosystems. *Reviews in Fisheries Science*. 3(1): 33-63.

CHAPTER 2: DRAINAGE-SCALE SPECIES-HABITAT RELATIONSHIPS OF CRAYFISHES (DECAPODA: CAMBARIDAE) IN A MIXED LAND USE REGION OF THE SOUTHEASTERN UNITED STATES

2.1 INTRODUCTION:

Characterizing species distributions is integral to the development and improvement of conservation actions for imperiled populations in areas with localized hotspots of species richness and abundance (Loiselle et al. 2003, Cayuela et al. 2009). However, of equal importance is understanding the factors that ultimately determine the local and landscape distributions of species of conservation concern. Species distributions manifest in multiple arrangements such that they may be contiguous about the landscape, patchy with limited corridors for dispersal between suitable habitats, or constrained and disjunct because of the inability of species to emigrate from their current territory (MacArthur 1972, Picket and Thompson 1978). Variations in distributions often stem from synergistic global and local effects, such as climate change, interspecific competition for resources, and anthropogenic alterations to habitat, all of which may affect dispersal to, colonization of, and extinction rates in suitable habitat patches (Arujo and Luoto 2007, Frey et al. 2011, Calabrese et al. 2013).

Modelling distributions of imperiled species can both characterize and predict distributions of species through incorporation of relevant biological and environmental variables (Guisan and Thuiller 2005, Elith and Leathwick 2009). Such models allow for effective range delineations, calculations of occurrence probabilities based on site-specific habitat characteristics, identification of geographically isolated populations and subpopulations, and quantification of environmental-species relationships (Kearney and Porter 2009, Godsoe and Harmon 2012). However, selecting suitable predictor variables for incorporation into analyses without introducing analytical noise or clouding

interpretation of results is often problematic (Austin and van Niel 2010). Furthermore, choice of an appropriate scale is important because one must decide whether to model distributions at small scales, such as within streams in a single drainage, or at larger, cross-drainage scales; this decision is important because not accounting for spatial scale may introduce geographical bias in the overall implementation of conservation plans (see Guisan and Thuiller 2005, Cayuela et al. 2009). Questions of community composition, habitat relationships, scale, and species distributions have been investigated with several groups of model organisms such as fish (Rahel and Hubert 1991), mosquitos (Juliano 2010), and birds (Chapman and Reich 2007). Importantly, all of these studies found scale-dependent patterns of species richness, relative abundances, and habitat correlations.

Crayfish diversity in the southeastern United States is the highest in the world (Hobbs 1974). High beta diversity among congeners at drainage and stream reach scales is prevalent in all southeastern states, especially within Appalachian Mountain stream drainages (Crandall and Buhay 2008). However, important biological and ecological data on these species are often limited; 60% of all crayfishes in North America are characterized mostly by species descriptions and characterizations of habitats and species-associates that are often collected as part of other, non-crayfish research efforts (Taylor 2007, Kaller et al. 2013). Louisiana crayfishes are culturally and economically important, with many seasonal festivals celebrating wild and aquacultural production of *Procambarus clarkii* and *P. zonangulus* that exceeded 46,000 metric tons in 2013 (Louisiana Summary Agricultural and Natural Resources, 2013). Unfortunately, the cultural and economic focus on these species has not resulted in a similar research

emphasis on the ecology and distribution of other Louisiana crayfishes. State and federal imperiled species programs have highlighted the need to document Louisiana crayfish ranges and distribution patterns, especially in mixed land-use drainages that include municipalities, national forests, managed pine plantations, farmland, and wetlands.

The purpose of my study was to define appropriate spatial scales, habitat characteristics, and relevant biological variables for construction of crayfish species distribution models in central Louisiana Coastal Plain streams. We focused our sampling within the Mermentau, Red, Calcasieu, Houston (a subdrainage of the Calcasieu River), and Vermillion-Teche river drainages, which were selected because of recent state interest in documenting rare *Orconectes* species (*Oroconectes hathawayi blacki*, *Orconectes h. hathawayi*, *Orconectes maletae*). These species are endemic to streams in the study drainages, and have poorly documented, apparently non-overlapping ranges. Here, we quantify relationships among crayfishes along species and habitat gradients to identify the effects of biotic and abiotic factors on distributions of Louisiana crayfish species of concern. In addition, we also address the effects of spatial scale on assessments of population and species status within these Coastal Plain drainages.

2.1.1 STUDY AREA:

The streams of the central Louisiana drainages are encompassed by three ecoregions based on similarity of ecosystems within the boundaries (see Daigle et al. 2006). Stream sites in the Red River and Calcaiseu drainages and Houston system are in the West Gulf Coastal Plain ecoregion and are best characterized as sandy-bottom streams having low specific conductance, low agricultural influence, and variable levels of forest cover. In sharp contrast, tributaries of the Mermentau River are within the

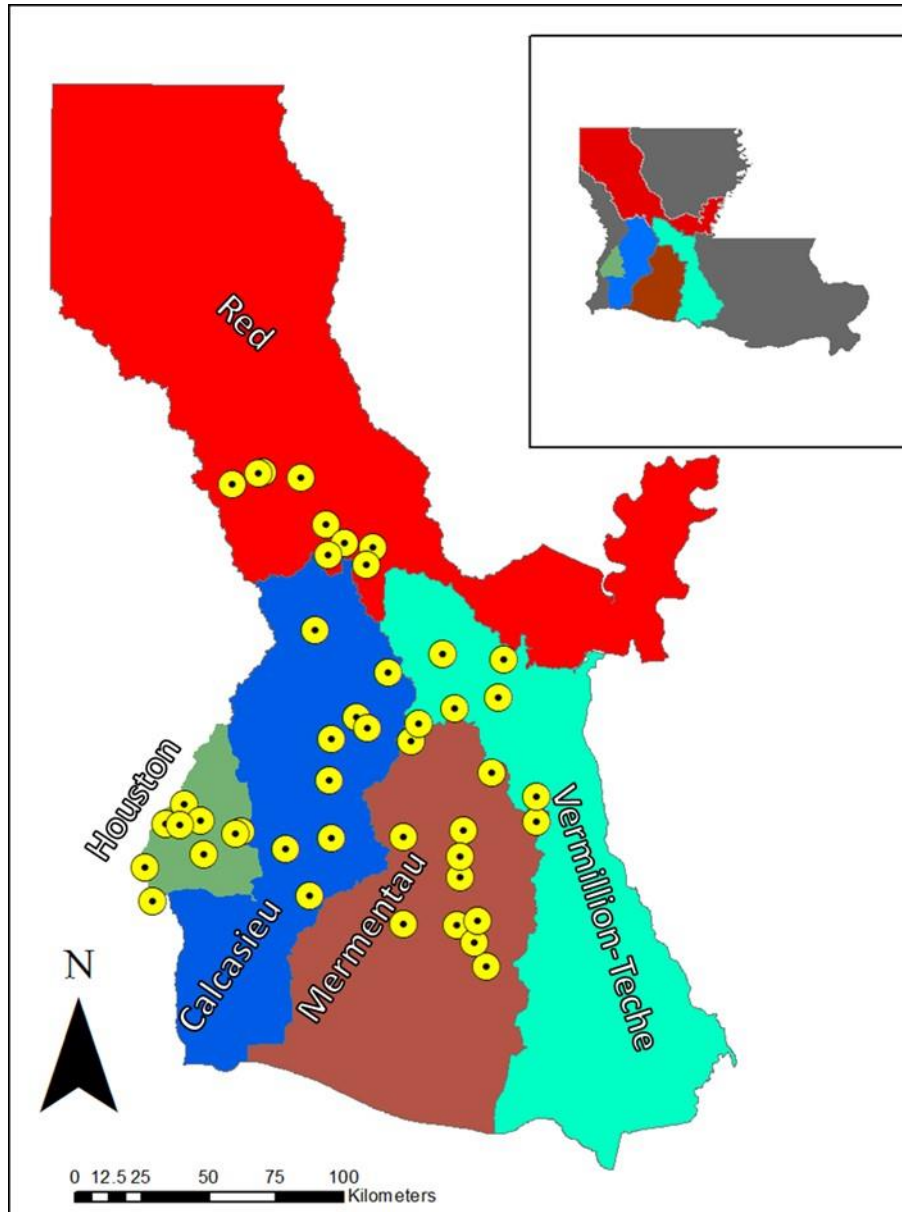


Figure 2.1: Map showing sites sampled and drainage boundaries for the summer 2013 crayfish collection effort. Each site is represented by a yellow centroid (n=45). The Houston system is actually a tributary to the Calcasieu River and is not considered to be a full drainage. However, it was sampled as a drainage because of species that are restricted to this system (*Orconectes blacki*).

South Coastal Plain ecoregion and are characterized by clay-bottom streams characteriuzed by high specific conductance and high agricultural impacts. Sites within the Red River and Vermillion-Teche drainages are also within the West Gulf Coastal Plain ecoregion; however Vermillion-Teche sites tend to be in more agricultural regions and included characteristics very similar to those of the Mermentau River basin. Characteristics of the streams of the Red River drainage tend to vary geographically based on proximity to the Vermillion-Teche and Calcasieu Drainages (i.e., streams closer to Vermillion-Teche drainage boundary were more agriculturally-impacted, whereas streams closer to the Calcasieu boundary tended to be less-agriculturally impacted). All sampled sites were in 1st, 2nd, or 3rd order streams with permanent standing water (Strahler 1957).

2.2 METHODS:

Within each of the 5 sample drainages, we chose 9 permanent streams that had average wetted widths > 3 m, depths of no more than 1 m, and were accessible from public roads (45 sites total, Figure 2.1). Within each stream, length of the sample reach was standardized as 30 times the average stream width (5 measurements at 10-m intervals) to account for possible stream size effects on crayfish density. Sampling reaches were divided into 6 equal length sub-reaches for ease of sampling.

Collection of physicochemical and crayfish data took place over two consecutive days at each stream. On the first day, sub-reaches were marked and single mid-channel measurements of pH, water temperature (°C), dissolved oxygen concentration (mg/L), oxygen percent saturation, turbidity (NTU), and specific conductance (µS/cm) were

recorded with a YSI® Sonde 9130. A 1-L water sample was taken at each site and returned to the laboratory for determination of calcium, total hardness, and alkalinity (mg/L) with wet chemical titration methods (HACCH Total and Calcium Hardness, Cat. No.: 1456-00; HACCH Alkalinity, Cat. No.: 20637-00).

Wetted channel width for each subreach was measured, and stream velocity was recorded with a Sontek Flowtracker® Doppler flow meter at 3 points (25%, 50%, 75% wet channel-width) across the stream at approximately 75% depth. Three wood counts (0.5m–diameter circle) were completed at each flow point in the subreach, and percent canopy coverage was measured in the middle of the channel with a concave spherical densiometer. Finally, visual estimates of stream bank height, dominant-vegetation type, and stream substrate were recorded as physical profile characteristics. After completing measurements in each subreach, one minnow trap per subreach (Frabill® Deluxe Minnow Trap, 6.35mm steel mesh) baited with approximately 100g of fish (*Oreochromis* species) was anchored and allowed to soak overnight.

Crayfishes were actively collected on day two by three people, one carrying a backpack DC electrofisher (either a Smithroot® LR-24 or a Halltech® HT200B), and two people carrying dipnets. Voltage on the electrofishing units was regulated to maintain a consistent output amperage between 1.2-1.5 amps to reduce crayfish limb-loss associated with higher amperages (see Rabeni et al. 1997, Price and Welch 2009). One person with a dipnet worked with the electrofisher to help scoop individuals stunned with the electrofisher while the other person with the dipnet collected crayfish away from the electrofishing unit (usually opposite side of the channel), and also attempted to collect individuals by scooping through deeper pools and undercut banks that could not be

effectively electrofished. Two-pass removals in each subreach were conducted for 10 minutes total (5 minutes per pass, 20 person-minutes total effort) with both the electrofisher and the dipnet, switching sides after each pass. Traps were collected after each sampling effort. Vouchered specimens were stored at the LSU School of Renewable Natural Resources.

2.2.1 STATISTICAL ANALYSIS:

Total numbers of individuals of each species were summed across subreaches, resulting in grand total for each reach. Because many traps yielded few to no specimens, individuals captured in traps were excluded from analysis, and catch per unit effort (CPUE) was calculated as the number of individuals captured per person-hour (total abundance / 2 person-hours). All statistical analyses were performed with SAS 9.3.

We used principal components analysis (PCA) and broken stick regression analysis to characterize habitat variation among sample sites. Other ordinations were evaluated (e.g., detrended correspondence analyses and non-metric multidimensional scaling), however, PCA best fit the criteria for selecting an ordination for these data (ter Braak 1995, Lepš and Smilauer 2003, Hirst and Jackson 2007). Prior to conducting ordinations, variables were evaluated with pair-wise correlations, and one variable was removed from pairs exhibiting correlations $>|0.70|$ to reduce multicollinearity. Significant principal components were identified with broken stick regression (Jackson 1993), and variables exhibiting twice the minimum correlation required for statistical significance (Stevens 2002) were used to interpret each component.

Generalized linear mixed models were used to assess whether environmental factors retained from the PCA differed among drainages. Laplace approximation of

maximum likelihood estimates was used for all models, and random factors included parish (parish political boundaries generally follow watercourses and fit hydrologic units well) and the type of land use around the sampling site. AIC and X^2/df criteria were used to determine goodness of fit and least overdispersion in the case of fitting of an exponential family of models. Autoregressive versions of models also were evaluated to test for autocorrelation among sampling sites. Dissolved oxygen, pH, and temperature were best fit with an identity link function and normal distribution, whereas calcium hardness was best fit with a log link function and negative binomial distribution.

Canonical correlation analysis was performed with CPUE of the broadly distributed species (i.e., species that occurred in all drainages; *P. clarkii*, *P. acutus*, *C. puer*, and *O. lancifer*) and the environmental variables obtained from PCA. This analysis used data from all sites and therefore quantified these data at the study region scale. We removed species with a single representative among all of the sites, species that were restricted to a single drainage, and non-typical stream-dwellers (i.e., primary burrowing species such as *C. ludovicianus*) to reduce the effects of unequal weighting. This analysis produced linear combinations of variate pairs which consisted of a response matrix (the set of species variables) and a constraining matrix (the set of environmental predictors). The analysis differs from PCA in that the set of variables within each component have loadings calculated such that correlation between the variate pair is maximized. The canonical variate pairs are then interpreted through the variables with the largest correlations to their respective variate component. Afterwards, other variables in the variate component with strong correlations above 0.32 were examined and compared as one would in a PCA, with the added ability to make cross-variate comparisons of the

correlations (e.g., interpretations of the species-species abundance correlations along on an environmental gradient of interest). This analysis was chosen over other ordinations because: 1) inspection of the CPUE data indicated numerous zeros, precluding unimodal techniques that employ chi-square distance metrics that will fail to compare sites with 0 CPUE data (Leps and Smilauer 2003), and 2) exploratory analyses did not meet the criteria needed to use canonical correspondence analyses or non-metric multidimensional scaling (Jongman et al. 1995, ter Braak 1995, Hirst and Jackson 2007). Redundancy analysis was performed *post hoc* with the resulting canonical variates and variable loadings in order to estimate the proportion of variance explained within the set of variables of one variate component and the variance explained by the opposing set of variables. This subsequent analysis provided a measure of predictive power of the canonical variates to the individual environmental and crayfish assemblage components. Generalized linear mixed models, which analyzed the data at the drainage scale, were used to examine the relationship between CPUE of the drainage-restricted yet common species, *Procambarus pentastylus* and *P. natchitochae*, and the environmental variables obtained from the PCA. These models focused on determining important factors that predict CPUE specifically within a single drainage as opposed to the canonical correlation analysis that examined simultaneous relationships across all drainages. To include these species in the canonical correlation analysis would confound the analysis through improbable correlations (e.g., a significant correlation of *P. pentastylus* CPUE with *P. natchitochae* CPUE when both species' ranges do not cross drainage boundaries).

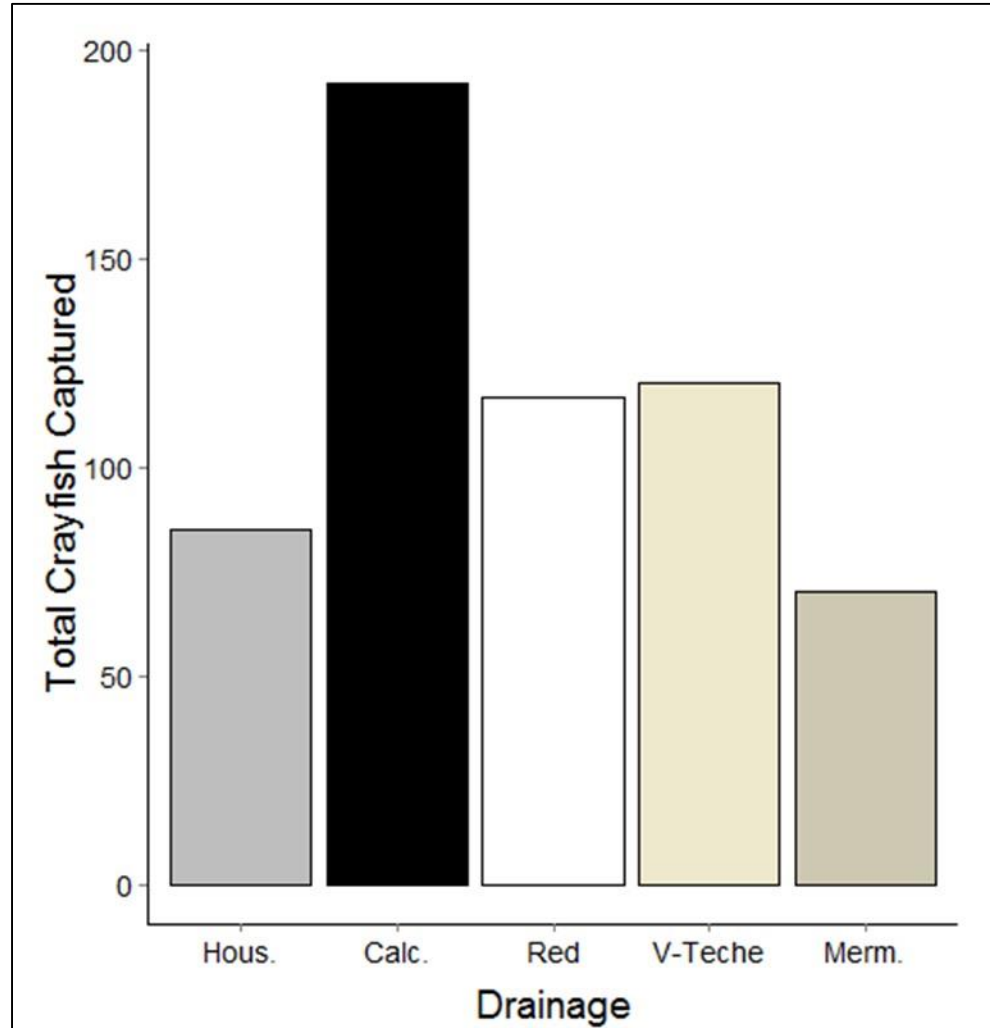


Figure 2.2: Total catch data by drainage for crayfish collected during the summer 2013 sampling effort documenting species diversity in the central Louisiana watersheds. Hous. = Houston river, Calc. = Calcasieu, Red = Red River, V-Teche = Vermillion-Teche, Merm. = Mermentau. The Houston drainage is actually the western subdrainage of the Calcasieu drainage, but was sampled as its own drainage for this study. Each drainage sample consists of 9 sites and the totals for each drainage represent the sum of the total number of crayfish captured among all 9 sites. Drainages are sorted from west to east.

The same model framework was applied to *P. natchitochae*, a similarly common species but restricted to the Red River drainage, but we found that the model fit the CPUE data poorly. It should be noted that analysis of *P. natchitochae* consisted of data from 8 sites rather than 9 sites because one site yielded 81 individuals of *P. natchitochae* and substantially inflated the sample variance. Thus the site was considered an outlier relative to the rest of the sites in this drainage and was removed from the analysis. Drainage-scale generalized linear mixed modelling was also applied to the three rare *Orconectes* species that were also drainage-restricted. Analysis of candidate models, including zero-inflated models, with chi-square/degrees of freedom fit statistics indicated that the best model for *P. pentastylus* used a log link function and a Poisson error distribution. However, due to their rarity and low CPUE in the study, no models, including zero-inflated models, fit these data well.

2.3 RESULTS:

We collected 1040 crayfish from the 45 sites during 2013, with 390 of the individuals excluded from analyses because they were too small or immature for confident species identification. The majority of crayfishes were captured from the Calcasieu drainage, whereas the fewest were collected from the Mermentau drainage (Figure 2.2, but see also Table A.2). Rare *Orconectes* species were found in all drainages, with the exception of the Mermentau river drainage; however, no Form I males of *O. hathawayi* were found.

Variables removed due to high correlations with each other and other variables included percent dissolved oxygen saturation, total hardness and magnesium hardness. Inspection of PCA output and broken stick regression supported one significant principal

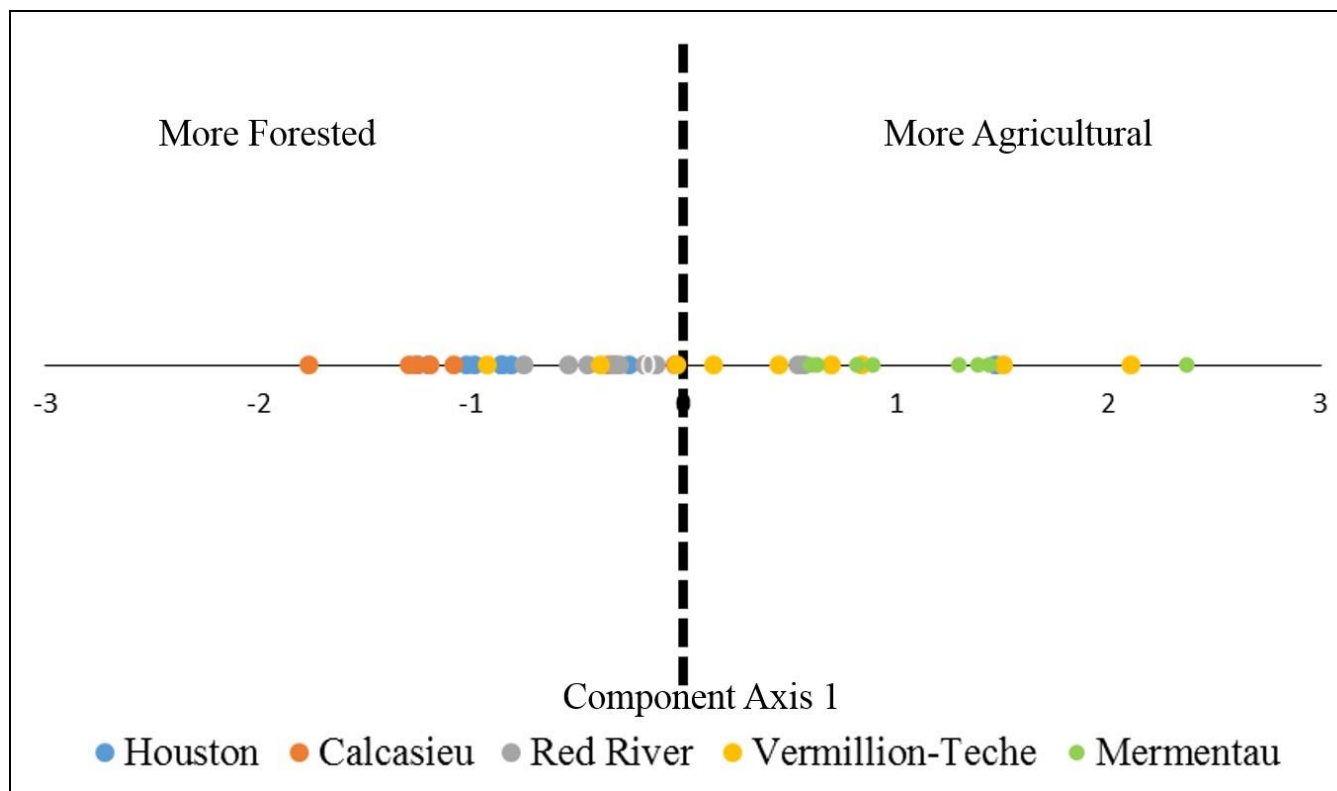


Figure 2.3: Plot of site scores from a principal components analysis quantifying similarities among sample sites in Central Louisiana from the 2013 crayfish sample effort (n=45). Each site is represented by a centroid and the color corresponds to the drainage identity. The vertical dashed line separates the sites into either forested sites (negative scores) or agricultural sites (positive scores). Only the first principal component was found to be interpretable is representative of a water quality gradient. Positive scores indicate sites with higher levels of dissolved oxygen, temperature, calcium hardness, and pH. Sites with similar scores are similar in water quality characteristics. Although the Houston River system was treated as a full drainage during sampling, it is actually a large tributary of the Calcasieu river.

component and 4 variables (dissolved oxygen, pH, temperature, and calcium hardness) with significant positive correlations to that component (>0.76 ; strict significance criteria were employed due to the small sample sizes of crayfish; Stevens 2002). Examination of site scores resulted in two distinct groups of sites corresponding to the Mermentau and Calcasieu drainages (Figure 2.3). In general, Mermentau sites scored more positively, indicating that these sites exhibited higher levels of calcium hardness, dissolved oxygen, temperature, and pH corresponding to greater impacts from surrounding agricultural lands. Conversely, Calcasieu sites scored more negatively and consisted of streams that were mostly adjacent to pine and mixed pine and hardwood forests (see table A.3 for physical profiles of sites). Sites within the Red, Vermillion-Teche, and Houston drainages showed considerable spread along this axis, reflecting substantial differences in water quality within these river systems.

Generalized linear mixed modelling found significant differences in mean values of the water quality variabilities (Table 2.1), although substantial within-drainage variation was evident. Overall, stream sites within the Mermentau drainage were higher in dissolved oxygen, temperature, pH, and calcium hardness than sites in any of the other drainages (see Table A.3). Following the Mermentau, Vermillion-Teche sites also had on average higher mean values of pH, dissolved oxygen, calcium hardness, and temperature compared to sites from the remaining three drainages. Sites in the Calcasieu, Red, and Houston showed no differences in mean values for any environmental factor with the exception of pH between the Red and Houston drainages.

Table 2.1: Means and standard deviations of environmental variables for drainages sampled (n=45, 9 sites per drainage) in summer 2013. Significant differences between drainages as determined by pair-wise estimates of differences in means resolved with a t-test. Means that do not share a difference letter are significantly different from each other. Abbreviations: Calc. = Calcasieu; Merm. = Mermentau; Red = Red River; V-Teche = Vermillion-Teche; S. H. = Sam Houston.

Drainage	Dissolved Oxygen (mg/L)		Temperature (°C)		pH		Calcium Hardness (mg/L)	
	Mean	Difference	Mean	Difference.	Mean	Difference	Mean	Difference
Calc.	3.70(2.42)	A	24.84(2.13)	A	6.94(0.55)	AC	34.24(19.14)	A
Merm.	6.84(2.38)	B	29.87(2.11)	B	7.69(0.20)	B	112.23(32.16)	B
Red	4.22(1.72)	A	27.31(1.76)	C	7.23(0.17)	ABC	45.65(28.39)	A
V-Teche	6.59(3.29)	B	28.80(2.27)	BCD	7.54(0.47)	BC	66.58(47.23)	A
S. H.	3.74(2.27)	A	26.33(1.77)	AC	6.86(0.49)	A	30.44(18.71)	A

Table 2.2: Tests of null-hypothesis that the canonical correlations in the current row and all that follow are zero. A significant result indicates that the variate pair and at least one of the following variate pairs contains a non-zero correlation and thus has meaningful correlations between variables in the variate pair.

Canonical Variate Pair	Canonical Correlation	Squared Canonical Correlation	Eigenvalue	F-Value	Pr > F
1	0.58	0.34	0.52	1.65	0.07
2	0.33	0.10	0.12	0.96	0.48
3	0.29	0.08	0.09	1.01	0.41
4	0.12	0.02	0.02	0.59	0.45

Table 2.3: Estimates of regression coefficients and fit statistics from generalized linear mixed models examining the relationship between *Procambarus pentastylus* and *P. natchitochae* catch per unit effort (CPUE) and water quality predictor variables at the drainage scale. Predictor variables included dissolved oxygen (mg/L), temperature (°C), pH, and calcium concentrations. *P. pentastylus* are endemic and restricted to streams of the Calcasieu drainage (n=9) whereas *P. natchitochae* are restricted to the Red River drainage (n=8). The fit statistic used to assess model fit at the drainage scale was the Pearson X^2 divided by the error degrees of freedom. A value close to 1 indicates good model fit. Both models were fit with a Poisson error distribution with a log canonical link function.

Predictor	<i>P. pentastylus</i>		<i>P. natchitochae</i>	
	Estimate	p	Estimate	p
Dissolved Oxygen	0.43	0.12	0.06	0.84
Temperature	-0.05	0.87	-0.27	0.52
pH	0.80	0.19	-0.55	0.94
Calcium	-0.13	0.09	-0.00	0.95
	X^2/DF		X^2/DF	
Model Fit	1.34		0.48	

Canonical correlation analysis constructed 4 sets of canonical variate pairs, but none yielded any significant canonical correlations (Table 2.2). Therefore no habitat or species gradients could be ascertained from this analysis for the broadly distributed species. Models of CPUE of *P. pentastylus* in the Calcasieu drainage found that none of the environmental variables significantly affected CPUE (Table 2.3). Similarly, no significant relationships were found with *P. natchitochae*, however, the model fit was poor compared to that for *P. pentastylus*.

2.4 DISCUSSION:

The goal of this project was to identify environmental and biotic variables that determine crayfish species distributions in central Louisiana coastal plain streams. We did not detect any significant habitat-species relationships for the broadly distributed species (*P. clarkii*, *P. acutus*, *C. puer*, and *O. lancifer*), which was expected given that

they were widely distributed amongst most sites and were prevalent within a wide variety of habitat conditions. Drainage-scale models for *P. pentastylus* and *P. natchitochae* found no significant relationships in CPUE as a function of the environmental predictors (pH, dissolved oxygen, calcium hardness, and temperature). We were unsuccessful in acquiring enough specimens of rare *Orconectes* species to model with drainage-scale modelling.

Soil geology is widely known to affect the geochemistry of surface and ground waters (e.g., hardness, alkalinity, pH), and such impacts may alter the utility and management of a land area for agricultural or conservation purposes (Wayland et al. 2003). We identified two distinct types of sites based on a combination water quality characteristics and the surrounding land use as well as the correlation between soil geology and land use. For example most sites that had clayey soils tended to be found in areas of intense agriculture (e.g., the Mermentau drainage) where such soils are useful for water retention and agricultural production, unlike sandy soils with high porosity and low rates of water retention (Newman 1984). Thus, the relationship between soil geology, water quality, and land use appears to be reflected in our PCA data and helped validate qualitative descriptions of the drainages as either agricultural (Mermentau) or forested (Calcasieu). Additionally, variability of site scores in each drainage corresponded to variability quantified in the generalized linear mixed models. Thus, we were successful in capturing and describing the variability of stream sites within each drainage.

Interestingly, the habitat variability was best described with water quality parameters, which has important implications because these same water quality parameters are also critical for crayfish physiology, e.g., calcium uptake is crucial for

proper crayfish carapace development (see Capelli and Magnusson 1983, Hammond et al. 2006, Edwards et al. 2015). However, the large within-drainage variability, as shown in our generalized linear mixed models, may limit interpretation of species-environmental relationships if few specimens from few sites are captured (e.g., the rare *Orconectes* species and *P. natchitochae*). Thus, future studies should either incorporate a larger sample size or increase collection efforts to try and discern these relationships.

Although it was not surprising that there were no significant environment-CPUE relationships amongst the broadly distributed species, the lack of these relationships in Calcasieu-endemic *P. pentastylus* was unexpected. Walls (2008) noted that *P. pentastylus* tend to be found in cool, higher oxygen streams, however, our collection efforts found that this species occurred abundantly in temperatures ranging from 13.54 to 25.4°C, as well as in dissolved oxygen concentrations ranging from 2.7 to 6.02 mg/L. These data suggest that *P. pentastylus* is a broadly-distributed species within the Calcasieu drainage. A similar interpretation for *P. natchitochae* is difficult to ascertain because of its presence at only few sites, small numbers captured at each site, and substantial between-site variability. On average, we captured one to four *P. natchitochae* per site, suggesting that either the species is quite uncommon or our sampling protocol did not effectively target the species; regardless *P. natchitochae* abundance was not associated with the measured environmental predictors.

None of the rare *Orconectes* species could be modelled effectively with any of the techniques employed in this study because of their low abundance and restricted distributions. These species are considered uncommon relative to other species and, in some cases, their documented distributions are severely restricted to a narrow band of

streams (e.g. *O. maletae*; Walls 1972, 2009, but see also Penn 1952, 1959). In this study, we did not find any rare *Orconectes* species outside their narrow historical ranges and, therefore, found no evidence of any range expansion. Additionally, when collected, *Orconectes* species were never abundant, suggesting that more effort may be required to collect these species and that collection efforts and subsequent modelling for these species should probably target streams peripheral to historical collection sites.

We did not collect several species in our study that were previously documented to occur within the study area. Given the wide variety of conditions and survival strategies crayfish use, such as burrowing (Grow and Merchant 1980) and migration (Flint 1977), missing species may not be as vulnerable to the gears and protocol used in this study. Studies of crayfish sampling gear efficacy in the southeastern U.S. (e.g. Dorn et al. 2005; Price and Welch 2009) have indicated that habitat factors may bias CPUE for certain species given a specific gear type. This study employed a combined-gear protocol to reduce individual gear effects on CPUE, although it still may not have been effective for some species we attempted to collect. Therefore, apparent absence of species, or lower CPUE, may be the result of limitations in spatial coverage within the drainages (9 sites in each of 5 drainages). The decision to exclude specimens captured with traps did not result in missing species at a site, as all species in traps were also captured by electrofishing/netting.

In our study, within-drainage variability of the environmental factors was consistently large across all drainages, implying that we sampled a variety of habitat conditions (e.g., low oxygen and high oxygen streams). Thus, each drainage can be reasonably expected to contain a substantial range of water qualities, and within-drainage

variability was not small enough for any drainage to contain streams of a narrow range of water qualities). However, because we focused on drainage-scale species-habitat relationships, additional data will be needed to model both within-drainage and within-stream variability. Although such multi-scale approaches may better quantify finer and less obvious relationships than examinations of relationships at a single scale (e.g. Poizat and Pont 1996), in some cases, it may be difficult to generate distributional models for localized species, or to generalize patterns from smaller scales to larger scales, especially for more broadly distributed species (Hernandez et al. 2006).

Based on our results, a small-scale approach (e.g., examination of the inhabited streams and immediate tributaries/distributaries) to understanding abundance and distribution will be needed for the three severely range-restricted *Orconectes* species found in this study. Larger-scale modelling may not be effective for these geographically isolated species relative to the more cosmopolitan *P. clarkii*, *P. acutus*, *C. puer*, and *O. lancifer*. *Procambarus pentastylus* are geographically isolated within the Calcasieu, yet broadly distributed and abundant, thus can be modelled with drainage-scale models. However, such an approach did not appear to be applicable for *P. natchitochae*.

2.4.1: SUMMARY:

This study quantified environmental-species relationships and among-drainage differences in environmental parameters to improve our understanding of factors influencing crayfish distributions that may be useful for modeling. All stream sites in this study could be accurately characterized as either being adjacent to agricultural or forest land based on water quality characteristics alone. Drainages differed from each other in pH, temperature, dissolved oxygen and calcium concentrations, but within-

drainage variability was large enough to indicate a large range of stream conditions that were sampled. Cosmopolitan species were not found to have significant species-environmental relationships, which was expected given their broad distribution across all sites in all drainages. No drainage-scale associations between abundance and environmental factors were found with endemic *P. pentastylus*, although this modelling scale appeared to be suitable for this species. *P. natchitochae* were poorly modelled with this approach due to low abundance among sites, even though it is a common species in the Red River Drainage. No rare *Orconectes* species were detected outside their historical ranges in this study, thus modelling, of these species, if possible, must be focused at local scales.

2.5 REFERENCES:

- Arujo, M. B., and M. Luoto. 2007. The importance of biotic interactions for modelling species distributions under climate change. *Global Ecology and Biogeography*. 16(6): 743-753.
- Austin, M. P. and K. P. van Niel. 2010. Improving species distribution models for climate change studies variable selection and scale. *Journal of Biogeography: Guest Editorial*.
- Calabrese, J. M., G. Certain, C. Kraan, and C. F. Dormann. 2013. Stacking species distribution models and adjusting bias by linking them to macroecological models. *Global Ecology and Biogeography*. 23(1): 99-112.
- Capelli, G. M. and J. J. Magnuson. 1983. Morphoedaphic and biogeographic analysis of crayfish distribution in northwest Wisconsin. *Journal of Crustacean Biology*. 3(4): 548-564.
- Cayuela, L., D. J. Golicher, A. C. Newton, M. Kolb, F. S. de Albuquerque, E. J. M. M. Arets, J. R. M. Alkemade, and A. M. Perez. 2009. Species distribution modeling in the tropics: problems, potentialities, and the role of biological data for effective species conservation. *Tropical Conservation Science*. 2(3): 319-352.
- Chapman, K. A. and P. B. Reich. 2010. Land use and habitat gradients determine bird community diversity and abundance in suburban, rural, and reserve landscapes of Minnesota, USA. *Biological Conservation*. 135(4): 527-541.

- Crandall, K. A. and J. E. Buhay. 2008. Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae – Decapoda) in freshwater. *Hydrobiologia*. 595(1): 295-301.
- Daigle, J. J., G. E. Griffith, J. M. Omernik, P. L. Faulkner, R. P. McCulloh, L. R. Handley, L. M. Smith, and S. S. Chapman. 2006. Ecoregions of Louisiana (color poster with map, descriptive text, summary tables, and photographs): Reston, Virginia, U.S. Geological Survey (map scale 1:1,000,000).
- Edwards, B. A., D. A. Jackson, and K. M. Somers. 2015. Evaluating the effect of lake calcium concentration on the acquisition of carapace calcium by freshwater crayfish. *Hydrobiologia*. 744: 91-100.
- Elith, J. and J. R. Leathwick. 2009. Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systematics*. 40: 677-697.
- Flint, R. W. 1977. Seasonal activity, migration, and distribution of the crayfish, *Pacifastacus leniusculus*, in Lake Tahoe. *American Midland Naturalist*. 97(2): 280-292.
- Frey, S. J. K., A. M. Strong, K. P. McFarland. 2011. The relative contribution of local habitat and landscape context to metapopulation processes: a dynamic occupancy modelling approach. *Ecography*. 35: 581-589.
- Godsoe, W. and L. J. Harmon. 2012. How do species interactions affect species distribution models? *Ecography*. 35: 811-820.
- Grow, L. and H. Merchant. 1980. The burrowing habitat of the crayfish *Cambarus diogenes diogenes* (Girard). *American Midland Naturalist*. 103(2): 231-237.
- Guisan, A. and W. Thuiller. 2005. Predicting species distribution: offering more than simple habitat models. *Ecology Letters*. 8: 993-1009.
- Hammond, K. S., J. W. Hollows, C. R. Townsend, and P. M. Lokman. 2006. Effects of temperature and water calcium concentration on growth, survival and moulting of freshwater crayfish, *Paranephrops zealandicus*. *Aquaculture*. 251(2-4): 271-279.
- Hernandez, P. A., C. H. Graham, L. L. Master, and D. L. Albert. 2006. The effect of sample size and species characteristics on performance of different species distribution modelling methods. *Ecography*. 29(5): 773-785.
- Hirst, C.N., and Jackson, D.A. 2007. Reconstructing community relationships: the impact of sampling error, ordination, approach, and gradient length. *Diversity and Distributions*. 13: 361-371.

- Hobbs Jr, H. H. 1974. A checklist of the North and Middle American Crayfishes (Decapada: Astacidae and Cambaridae). *Smithsonian Contributions to Zoology*. 166.
- Jackson, D.A. 1993. Stopping rules in principle component analysis: a comparison of heuristic and statistical approaches. *Ecology*. 74: 2204-2214.
- Jongman, R. H. G., C. J. F. Ter Braak, and O. F. R van Tongerern. 1995. Data analysis in community and landscape ecology. Cambridge University Press. Cambridge, UK.
- Juliano, S. A. 2010. Species introduction and replacement among mosquitos: interspecific resource competition or apparent competition? *Ecology*. 79: 255-268.
- Kaller, M. D., C. E. Murphy, W. E. Kelso. 2013. Drainagess for fish and ecoregions for macroinvertebrates: different spatial scales are needed to assess Louisiana wadable streams. *Transactions of the American Fisheries Society*. 142(3): 767-782.
- Kearney, M. and W. Porter. 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species ranges. *Ecology Letters*. 12: 1-17.
- Lep s, J. and P. Smilauer. 2003. Multivariate Analysis of Ecological Data using CANOCO. New York: Cambridge University Press.
- Louisiana Summary Agricultural and Natural Resources. 2013. State totals. Louisiana State University - Ag Center. <http://www.lsuagcenter.com/agsummary/archive/2013/-State-Totals/2013StateTotals.pdf>
- Louisiana Natural Heritage Program. 2014. Louisiana Department of Wildlife and Fisheries. <http://www.wlf.louisiana.gov/wildlife/louisiana-natural-heritage-program>
- Louiselle, B. A., C. A. Howell, C. H. Graham, J. M. Goerck, T. Brooks, K. G. Smith, and P. H. Williams. 2003. Avoiding pitfalls of using species distribution models in conservation planning. *Conservation Biology*. 17(6): 1591-1600.
- MacArthur, R. H. 1972. Geographical ecology: patterns in the distribution of species. Princeton University Press.
- Newman, A. C. D. 1984. The significance of clays in agriculture and soils. *Philosophical Transactions of the Royal Society A*. 311(1517): 375-389.

- Penn, G. H. 1952. The genus *Orconectes* in Louisiana (Decapoda, Astacidae). *American Midland Naturalist*. 47(3): 743-748.
- Penn, G.H. 1959. An illustrated key to the crayfishes of Louisiana with a summary of their distribution in the state (Decapoda, Astacidae). *Tulane Studies*. 7:3-20
- Pickett, S. T. A. and J. N. Thompson. 1978. Patch dynamics and the design of nature reserves. *Biological Conservation*. 13: 27-37.
- Price, J. E., and S. M. Welch. 2009. Semi-quantitative methods for crayfish sampling: sex, size, and habitat bias. *Journal of Crustacean Biology*. 29(2): 208-216.
- Poizat, G. and D. Pont. 1996. Multi-scale approach to species-habitat relationships juvenile fish in a large river section. *Freshwater Biology*. 36(3): 611-622.
- Rabeni, C. F., K. J. Collier, S. M. Parkyn, B. J. Hicks. 1997. Evaluating techniques for sampling stream crayfish (*Paranephrops planifrons*). *New Zealand Journal of Marine and Freshwater Research*. 31: 693-700.
- Rahel, F. J. and W. A. Hubert. 1991. Fish assemblages and habitat gradients in Rocky Mountain-Great Plain streams: biotic zonation and additive patterns of community change. *Transactions of the American Fisheries Society*. 120(3): 319-332.
- Stevens, J.P. 2002. Applied multivariate statistics for the social sciences, 4th edition. Mahwah: Lawrence Erlbaum Publishers.
- Strahler, A. N. 1957. Quantitative analysis of watershed geomorphology. *Eos*. 38(6): 913-920.
- Taylor, C.A., M.L. Warren, Jr., J.F. Fitzpatrick, H.H. Hobbs III, R.F. Jezerinac, W.L. Pflieger, and H.W. Robison. 1996. Conservation status of crayfishes of the United States and Canada. *Fisheries*. 21:25-37.
- Taylor, C.A., G.A. Schuster, J.E. Cooper, R.J. DiStefano, A.G. Eversole, P. Hamr, H.H. Hobbs III, H.W. Robison, C.E. Skelton, and R.F. Thoma. 2007. A reassessment of the conservation status of crayfishes of the United States and Canada after 10+ years of increased awareness. *Fisheries*. 32: 372-389
- ter Braak, C.J.F. 1995. Ordination. In: Jongman ,R.H.G., ter Braak, C.J.F.,& Van Tongeren, O.F.R., eds. Data analysis in community and landscape ecology. New York: Cambridge University Press.
- Walls, J. G. 2009. The crawfishes of Louisiana. Louisiana State University Press. Baton Rouge, Louisiana.

- Walls, J. G., and J. B. Black. 2008. A new crayfish, *Procambarus (Pennides) pentasylus*, from southwestern Louisiana (Crustacea: Decapoda: Cambaridae) with a key to western species of the subgenus. *Proceedings of the Biological Society of Washington*. 121(1): 49-61.
- Walls, J. G. 1972. Three new crawfishes related to *Orconectes difcilis* (Faxon) (Decapoda: Astacidae). *Proceedings of the Biological Society of Washington*. 84(53): 449-458.
- Wayland, K. G., D. T. Long, D. W. Hyndman, B. C. Pijanowski, S. M Woodhams, and S. K. Haack. 2003. Identifying relationships between geochemistry and land use with synoptic sampling and R-mode factor analysis. *Journal of Environmental Quality*. 32(1): 180-190.

CHAPTER 3: SAMPLING CRAYFISH ASSEMBLAGES IN LOUISIANA COASTAL PLAIN STREAMS: GEAR, PROTOCOL, AND TIMING

3.1 INTRODUCTION:

Warmwater streams in the southeastern United States are globally unique in that they contain the highest diversities of freshwater fishes, mussels, and crayfishes north of the tropics (Neves et al. 1997, Warren et al. 2000, Thorp and Covich 2009). These community assemblages are characterized by high levels of endemism, which can be attributed to climatic and geological events of the Pliocene and Pleistocene eras (Avisé 1992, Felley 1992, Brown and Matthews 2006). Conservation of freshwater biota in this region is challenged by inadequate, antiquated distributional data, compounded by alteration of riparian and aquatic habitats and consequent landscape homogenization for agriculture and urbanization (Rahel 2002, Allan 2004, Martinuzzi et al. 2013, Haag and Williams 2014). Among the southeastern U.S. aquatic biota, basic distributional and ecological data on crayfish is lacking, which is especially problematic given that many populations are imperiled from anthropogenic habitat and water quality alterations (e.g., Crandall and Buhay 2008, Kilburn et al. 2014, Richman et al. 2014). Justification for the conservation and management of crayfish can be attributed, in part, to their multi-faceted roles as prey items, shredders, primary consumers, predators, and habitat modifiers in lotic and lentic aquatic systems (e.g., Lodge et al. 1994, Statzner 2000, Harvey et al. 2014). Thus, recent attention has focused on delineating factors that influence rates of decline in crayfish populations and diversity worldwide (Kozák et al. 2011).

Analysis of distributional and diversity data has revealed approximately 383 crayfish species in the U.S., with the highest diversity in the Gulf of Mexico coastal States (Taylor et al. 2007). These data were collected with methods ranging from digging

holes by hand to electrofishing, depending on project objectives and habitat type (Rabeni et al. 1997, DiStefano et al. 2003). The diversity of sampling techniques is due, in part, to crayfish using numerous habitat types, ranging from open sand and mud to more structurally complex substrates composed of rocks and woody debris, the latter often a response to predation and other density-dependent interactions (Olsson and Nystrom 2008, Adams 2014). Trapping (e.g., fyke nets, pots, or mesh traps) is a popular crayfish sampling method because the gear is inexpensive, simple to use, and yields quantitative data (Dorn et al. 2005, but see also Price and Welch 2009). Although large numbers of traps can be easily deployed in many habitat types, choice of trap location and bait type can significantly influence catch rates, and vulnerability to trapping may be dependent on species and/or life stage (e.g., Collins et al. 1983, Rach and Bills 1989). Although expense and effort may be higher, active sampling gears (e.g., seines, dropped or thrown quadrat samplers, or electrofishing equipment) have the advantage of increased areal coverage and reduced species specificity, and are becoming more popular for sampling stream-dwelling crayfishes (Parkyn and Collier 2004, Dorn and Volin 2009).

Gear choice will introduce bias into the sampling design (whether through size-selection, operator error, etc.), but it may be possible to reduce sampling bias by employing multiple gear-types to account for different habitat features and crayfish species' behaviors/tendencies, such as swimming ability, burrowing activity, and diel activity patterns (Clifford and Casey 1992, Knight and Bain 1996, Pallison et al. 2011). Employing multiple gears simultaneously can sample a wider variety of habitat conditions; however, logistics and personnel often dictate gear-type and effort. Louisiana stream habitats are difficult to sample with single gears because streams may abruptly

change character from woody, low-specific conductance streams with riffles and pools to deep, homogeneous, higher-specific conductance agricultural canals (Felley and Daniels 1992, Kaller et al. 2013). Comparison of crayfish catch per unit effort (CPUE) or other abundance estimates among such heterogeneous collections of sites presents statistical challenges that may be ameliorated through integration of data generated with multiple gear types (see Weaver et al. 1993). Such an approach may also overcome diel assemblage changes due to species differences in diurnal/nocturnal movements (Hill and Lodge 1994, Gherardi et al. 2000, Martin and Moore 2007). Depending on the species present, there may be a significant risk of underestimation of abundance and failure to detect rare species if time of day is not taken into account in the development of a crayfish sampling protocol.

This study tested the efficacy of a protocol for sampling crayfishes in streams located in central Louisiana. The streams sampled generally had relatively low levels of specific conductance and a diversity of depths and habitat types (e.g., high wood-densities, homogenous channels, gravel or clay bottoms), all of which can affect sampling efficiency. We hypothesized that crayfish CPUE would be greatest when data from both gear-types (backpack electrofisher and the dipnet) were combined and when streams were sampled at night. We also hypothesized that dipnets would be more biased towards smaller crayfish than electrofishers, and thus should yield smaller crayfishes on average regardless of time of day. Additionally, because crayfish are assumed to be more active during nocturnal hours, we assumed we would capture more large crayfish at night than during the day. Lastly, we hypothesized that different combinations of gears and times of day would yield different estimates of crayfish species richness on both per site

and per individual bases. Results of this study provide useful information regarding the design of sampling protocols determining lotic crayfish species presence and absence and/or abundances in the Gulf Coastal Plain

3.2 METHODS:

The study was conducted June-August 2014 in 20 central Louisiana streams within the Calcasieu, Houston, Vermillion-Teche, and Red River drainages. Sites in the Calcasieu and Houston drainages contained abundant wood and had predominantly sand substrate with some clay, whereas most of the Vermillion-Teche streams were agriculturally impacted, with homogeneous clay substrates (Felley 1992, Brown and Matthews 2006, Daigle et al. 2006). Red River sites were intermediate in habitat characteristics between the Vermillion-Teche and Calcasieu drainages. After assessing 40 potential sample stream sites for habitat characteristics and access, we randomly selected 20 sites (five from each drainage) in permanent streams that were wadeable (less than 1 m deep) and were greater than 3 m average width. Each sample site was divided into two, 120-meter-long sample reaches separated by 20 m, with the upper reach sampled at midday and the lower reach sampled 10-30 minutes after sunset. With two exceptions, both sample reaches were sampled moving upstream from the access point.

Prior to sampling, specific conductance (mS/cm) and depth (cm) were recorded with a YSI® Sonde (Model No. 9130). Water samples were collected for determination of calcium and alkalinity concentrations (mg/l) by chemical titration (HACH Calcium Hardness Category No. 1457-00; Alkalinity Category No. 20637-00). For safety reasons, the lower night site was always nearest to the bridge access point, but was far enough upstream so that we were confident no features of the road crossing were present in the

stream (e.g. road gravel, rip-rap, fallen timbers, usually about 30-40m away from bridge). Within each sample site, we recorded stream velocity, (mid-channel at 75% depth; FlowTracker[®], Sontek), depth, and stream habitat (substrate type, bank height, bank vegetation, woody debris density) along 6 transects spaced at 20-m intervals throughout the 120-m site.

Crayfishes were collected by two people, one with a backpack electrofisher (Halltech HT200B) and a dipnet (6.35mm mesh) who collected stunned crayfish, and one with only a dipnet that was used to scoop through vegetation, knock over rocks and debris, and sweep across the streambed. Each 20-m subreach (between successive transects) was sampled with two passes of both gear-types simultaneously (opposite banks for electrofishing and dipnetting to maintain independence for gear comparisons) for a total of 20 person-minutes (2 person-hours per reach). We assigned the same technician to a single gear type across all samples to minimize operator bias. Voltage and pulse frequency were regulated to produce 1.2-1.6 amps for all streams to reduce crayfish limb-loss associated with higher amperages (see Rabeni et al. 1997, Price and Welch 2009). Crayfishes collected from each sub-reach, gear type, and time of day were bagged separately and stored on ice for subsequent processing. Species and sex were identified, and carapace (tip of rostrum to caudal edge of cephalothorax) and abdominal lengths were measured to the nearest millimeter with calipers to obtain total length. Wet weight (mg) was determined with a scale balance, and all specimens were preserved in 95% ethanol and placed in a voucher collection at the Louisiana State University School of Renewable Natural Resources.

3.2.1 STATISTICAL ANALYSIS:

Mean differences in CPUE based on gear type and time of day were analyzed with analysis of covariance (ANCOVA; depth and specific conductance as covariates) with generalized linear mixed models including planned comparisons between time of day (night and day), gear type (backpack electrofisher, dipnet, and both gear-types), and interactions between time of day and gear type. Severe overdispersion was a characteristic of the dataset, so the model was fit with a negative binomial rather than Poisson distribution. Analysis of deviances indicated the best fitting ANCOVA for CPUE was a model containing all of the variables and their interactions. Differences in total length of crayfish as a function of gear type and time of day were modeled with a two-way mixed model analysis of variance (ANOVA) with the sampling site set as a blocking effect. Mixed-ANOVA was used to reduce the error in crayfish total length caused by differences in conditions between sites, which would include the effects of covariables used in the ANCOVA. Propensity of a gear type or time of day to detect male or female crayfish was examined with a mixed-effect logistic model with sampling site as a random term.

We calculated two sets of 9 species accumulation curves to determine whether it may be more prudent to maximize the number of sites or number of individuals sampled based on gear type used and time of day with expected species richness as an indicator (i.e., the expected diversity of a sample based on the size of the sample). One set of curves predicted diversity of crayfish with rarefaction, pooling individuals across all sites and randomly sampling without replacement, and the other predicted diversity as a function of the number of sites randomly sampled (100 permutations.) Both sets of

curves examined the following: 1) differences in expected richness among gears and the combined gear method with data pooled from both times of day, 2) differences in expected richness between day and night with data pooled from both gear types, and 3) differences in expected richness from data collected with a single gear type at a specific time of day. Significance differences among curves was determined by inspection of 95% confidence intervals (i.e., when confidence intervals did not overlap adjacent curves, following the more conservative criterion in Colwell et al. 2012).

Table 3.1: Crayfish species composition from the Summer 2014 stream samples. All specimens were collected from 20 sites in central Louisiana. Sex ratio of the cumulative total was roughly even ($n_{\text{female}} = 342$, $n_{\text{male}} = 339$). Asterices indicate species restricted to a single drainage.

Species	# Captured	Proportion of Sample
<i>Cambarellus puer</i>	14	0.021
<i>Orconectes hathawayi blacki</i> *	16	0.023
<i>Orconectes lancifer</i>	153	0.225
<i>Orconectes palmeri longimanus</i>	6	0.009
<i>Orconectes palmeri palmeri</i>	70	0.103
<i>Orconectes maletae</i> *	16	0.023
<i>Procambarus acutus</i>	26	0.038
<i>Procambarus clarkii</i>	22	0.032
<i>Procambarus natchitochae</i> *	176	0.258
<i>Procambarus pentastylus</i> *	208	0.305
<i>Procambarus zonangulus</i>	1	0.001
Grand Total	681	

3.3 RESULTS:

Sampling produced 681 crayfish representing 11 species, of which 4 (*Orconectes blacki*, *O. maletae*, *P. pentastylus*, and *P. natchitochae*) were restricted to a specific drainage (Table 3.1). These 4 species accounted for 60% of the total, with 56% being either *P. pentastylus* or *P. natchitochae*. *P. natchitochae* is not endemic to Louisiana,

but is considered distributionally isolated within the Red River drainage. All of these geographically restricted species were collected at two or more sites, with the exception of *O. maletae*, which was found at only one. Sexes were evenly represented in the collections ($n_{\text{male}} = 339$, $n_{\text{female}} = 342$). Non-reproductive form males totaled 292 individuals whereas reproductive form males numbered 47 individuals.

Table 3.2: Results of analysis of covariance analyzed with a negative binomial generalized linear model testing for effects of gear type (backpack electrofisher, dipnet, and both) and time of day (day and night) on crayfish catch per unit effort (CPUE). Coefficients presented are log-transformed and represent the proportional change in the log CPUE relative to a baseline category. In this analysis, the baselines were “day” for time of day and “both gears” for gear type. Depth (m) and specific conductance (mS/cm) were treated as continuous covariates whereas time of day and method were treated as categorical predictors with two levels each (day versus night and backpack shocker versus dipnet, respectively). An equal-slopes model was fit because of a lack of interactions with covariates. Overdispersion was slightly present in the data ($X^2/\text{df} = 1.22$) but within reasonable bounds.

Variable	Mean	Std. Error	Z-statistic	p-value
Intercept	3.283	0.337	9.731	<0.001
Night	0.125	0.288	0.433	0.655
Dipnet	-1.724	0.312	-5.521	<0.001
Electrofisher	-0.196	0.289	-0.679	0.497
Depth	0.007	0.004	-1.757	0.078
Specific Conductance	-0.738	0.637	-1.160	0.246
Night X Dipnet	-0.435	0.435	-1.000	0.318
Night X Electrofisher	-0.104	0.409	-0.254	0.800

Results of ANCOVA indicated that either a combined gear approach or electrofishing alone yielded greater CPUE compared to dipnets (Table 3.2). Electrofishing and electrofishing/dipnetting yielded higher mean CPUE and greater sample variability than dipnetting, with no CPUE differences between electrofishing and the combined gears (Figures 3.1A and 3.1B). No significant interactions were found among any of the treatment effects and covariates, confirming homogeneity of

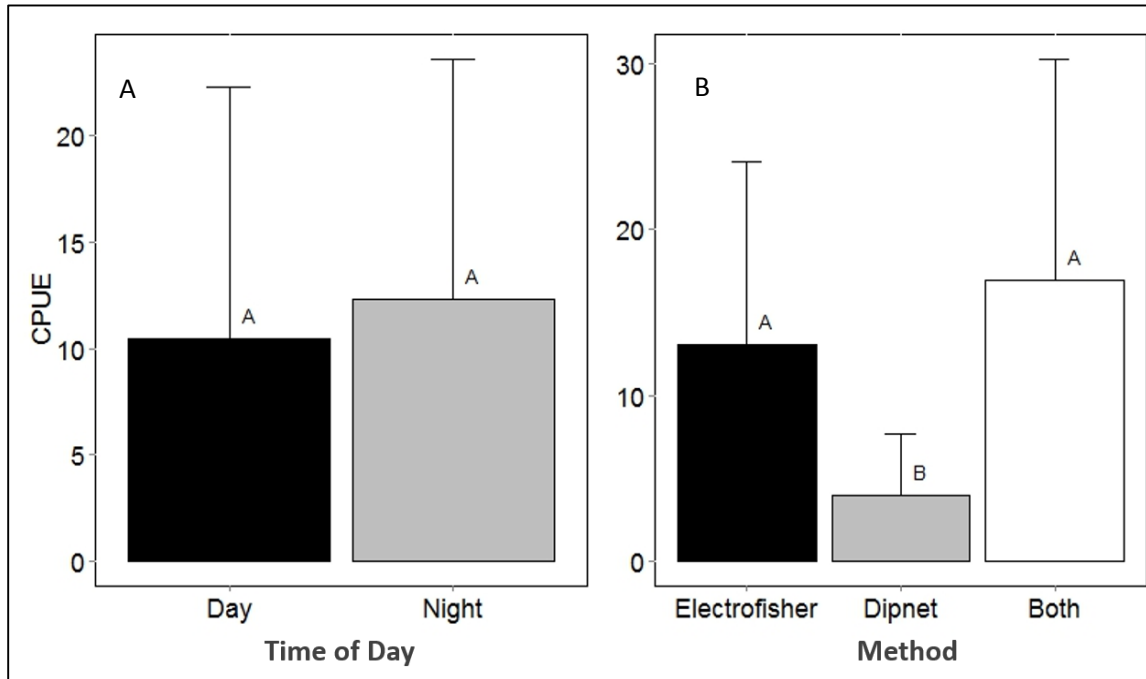


Figure 3.1: Barplots of mean crayfish catch per unit effort (CPUE) and standard deviation (error bars) for different levels of time of day (A) and sampling method (B). Means presented are raw means. Significant differences, indicated by letters, were determined with pre-planned contrasts of group means obtained through negative binomial analysis of covariance controlling for specific conductance and depth.

regression slopes and valid interpretation of a separate slopes model. Neither time of day nor the depth and specific conductance covariates were found to significantly affect CPUE.

The two-way mixed model ANOVA indicated a significant difference in mean total length between the gear-types, but not time of day (Table 3). The electrofisher captured larger individuals on average than the dipnet (although variability was high with the electrofisher), but there were no time of day differences in mean length nor any significant interactions (Day = 40mm, Night = 41mm, Figures 3.2A and 3.2B).

Table 3.3: Summary statistics of a two-way mixed ANOVA to test differences in mean total length of crayfish between times of day (day versus night transects), and gear types (backpack electrofishers versus dipnets). Total lengths of crayfish (n=681) were log transformed to homogenize variance. Site was treated as a blocking factor for this analysis. A test for significant block by predictor interactions is provided. A significant block interaction would indicate that there is a dependence of a treatment mean on the identity of the blocking factor (i.e., the site).

Treatment Differences			Block by Treatment interactions		
Variable	F-Value	p-value	Variable	F-Value	p-value
Gear Type	11.448	0.004	Method	1.871	0.172
Time of Day	0.174	0.682	Time of Day	7.244	0.007
Time of Day X Method	0.553	0.468	Time of Day X Method	1.725	0.190

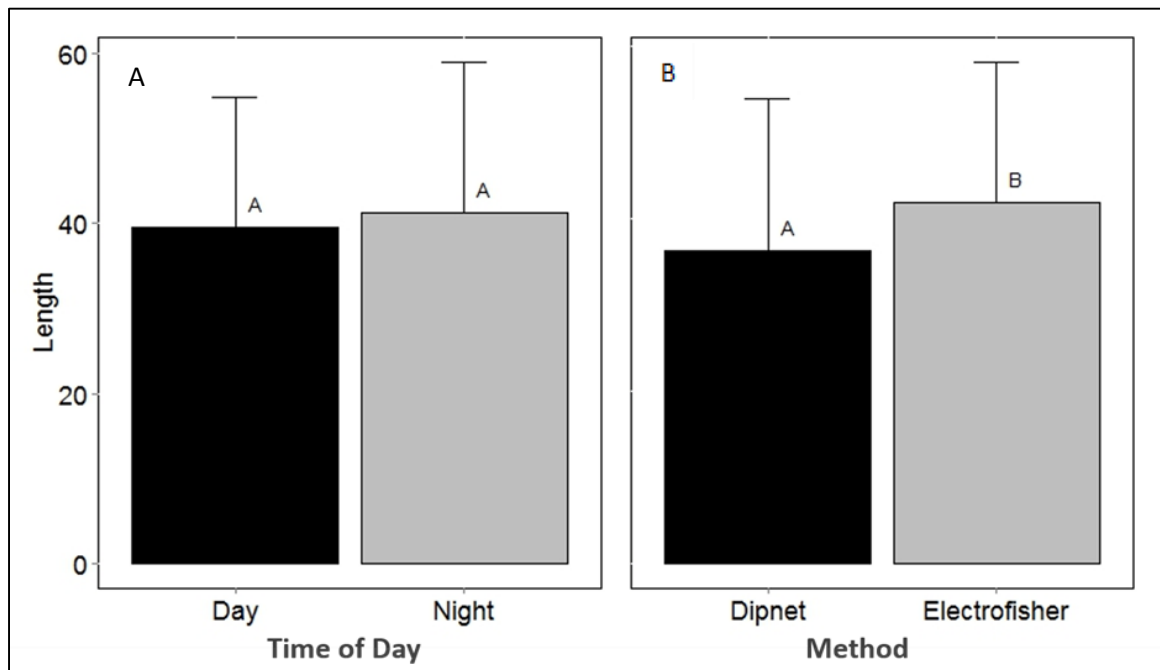


Figure 3.2: Barplots of mean total length (mm) and standard deviations (error bars) of crayfishes sampled at different times of day (A) and with different sampling methods (B). Means presented are raw means. Significant differences, as indicated by letters above bars, were determined with mixed-effect ANOVA setting site as a block effect and pre-planned contrasts of group means.

However, a significant block by treatment effect was detected for the time of day predictor, which implied that the time of day effect on total length depended on site identity; therefore care must be taken to interpret a true lack of difference between the sites. Logistic regression indicated that neither gear type nor time of day influenced the probability of collecting males or females (Table 3.4).

Table 3.4: Logistic regression ran with a generalized linear mixed model to determine the odds of sampling an individual sex with a given gear type (backpack shocker or dipnet) and time of day (day or night). Species was treated as a random effect for modelling purposes, (n=681). Reproductive and non-reproductive form males were treated as a single level because of insufficient representation of reproductive males. The high odds ratios imply that each sex has an equal probability (1) of being sampled with a gear type at any time of day.

Parameter	Odds Ratios*	Std. Error	z-statistic	p-value
Intercept	146.4	228.9	0.640	0.522
Method	230.0	228.9	1.005	0.315
Time of Day	105.9	218.2	-0.482	0.627
Method X Time of Day	264.8	242.5	-1.092	0.275

Species accumulation curves showed minimal differences in estimated maximum richness among gears and times of day (at most a difference in one species, Figures 3 and 4). However, rates of increase in expected species richness, and thus efficiency of our sampling to document species diversity, were different depending on whether the data were examined on a per site or per individual basis. On a per site basis, all curves graphing different combinations of gear types and times of day generally showed a pattern of constantly increasing species richness and significant separation of confidence intervals at around 15 sites (Figures 3.3a, 3.3b, and 3.3c). Electrofishers and combined gear methods required fewer sites to predict higher richness in contrast to the dipnet

(Figures 3.3a and 3.3c). However, sampling sites during the day with both gear types yielded significantly greater richness than sampling at night (Figure 3.3b).

When examining expected richness on a per individual basis, levelling off occurred in almost all curves at around 100-140 individuals regardless of combination of gear-type and the point of separation of confidence intervals between curves generally depended on the gear type (Figures 3.4a, 3.4b, and 3.4c). The dipnet by itself was less variable and better at estimating species richness than either the electrofisher and combined gear approaches regardless of time of day (Figures 4a and 4c). However, a combined gear approach employed during the day was shown to have higher expected species richness than during the night (4b). Additionally, the accumulation curve for combined gears sampling at night reached an asymptote of 10 species whereas the curve for combined gears sampling during the day had reached maximum richness in the data set, but had not yet reached an obvious asymptote.

3.4 DISCUSSION:

The goal of this study was to determine if gear type and time of day affects estimates of crayfish CPUE, body length, sex ratios, and diversity. We found support for our hypotheses that a combination of electrofishing and dipnetting captured the greatest number of individuals, the greatest range of sizes, and equally sampled the sexes, although we also found that electrofishing alone was on average as effective as a combined gear approach for CPUE. Individually, electrofishing sampling was characterized by a higher CPUE and larger mean crayfish size relative to dipnetting. However, richness captured with both gears was similar in that both caught a maximum of 11 species. In addition, we found support for our hypothesis that there were

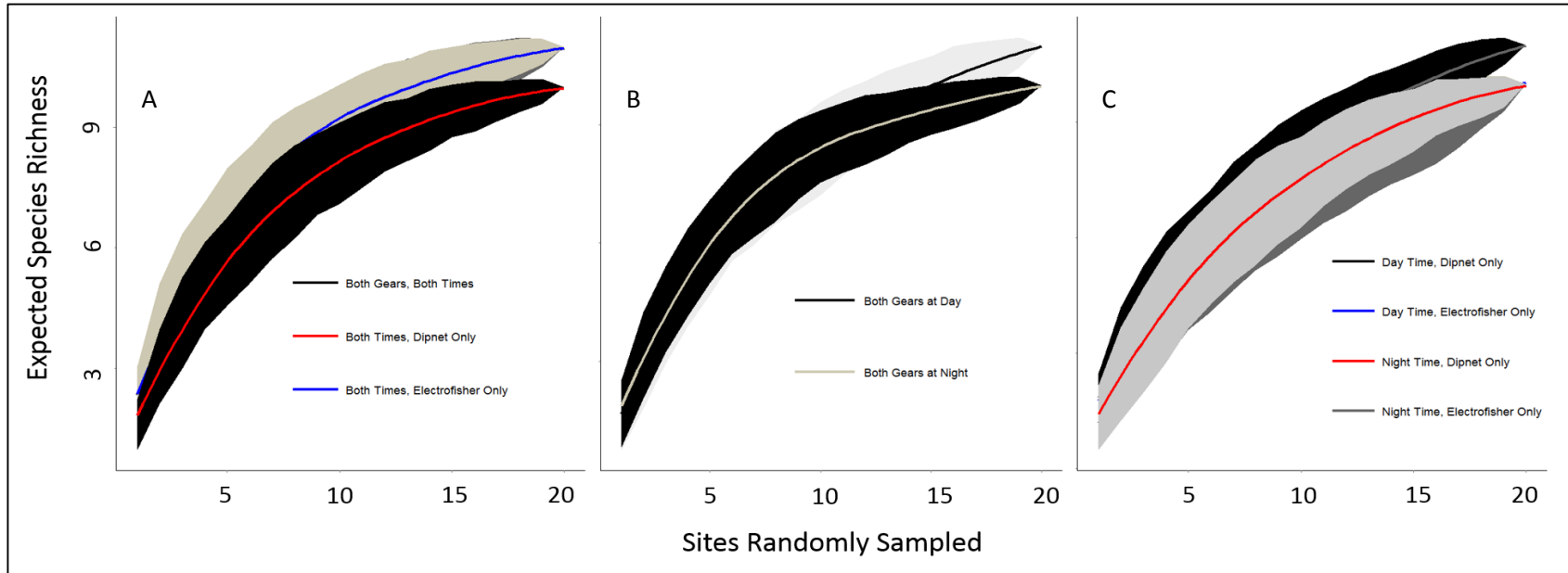


Figure 3.3: Species accumulation curves showing expected crayfish richness as a function of sites randomly sampled (for 100 permutations) with different combinations of gear types and times of day. A) curves represent diversity data obtained by sampling with an indicated gear type at both times of day. B) curves represent diversity data obtained by sampling with both gear types during the day or night. C) curves represent diversity data obtained by sampling with a single gear type at a specific time of day. Bands around lines are 95% confidence bands. The point where the confidence curve of one line no longer overlaps another line is the divergence point between the curves (i.e., when there would be reasonable differences in expected richness between the curves. Some curves may be stacked on top of each and thus hidden behind other curves, indicating no significant divergence between curves.

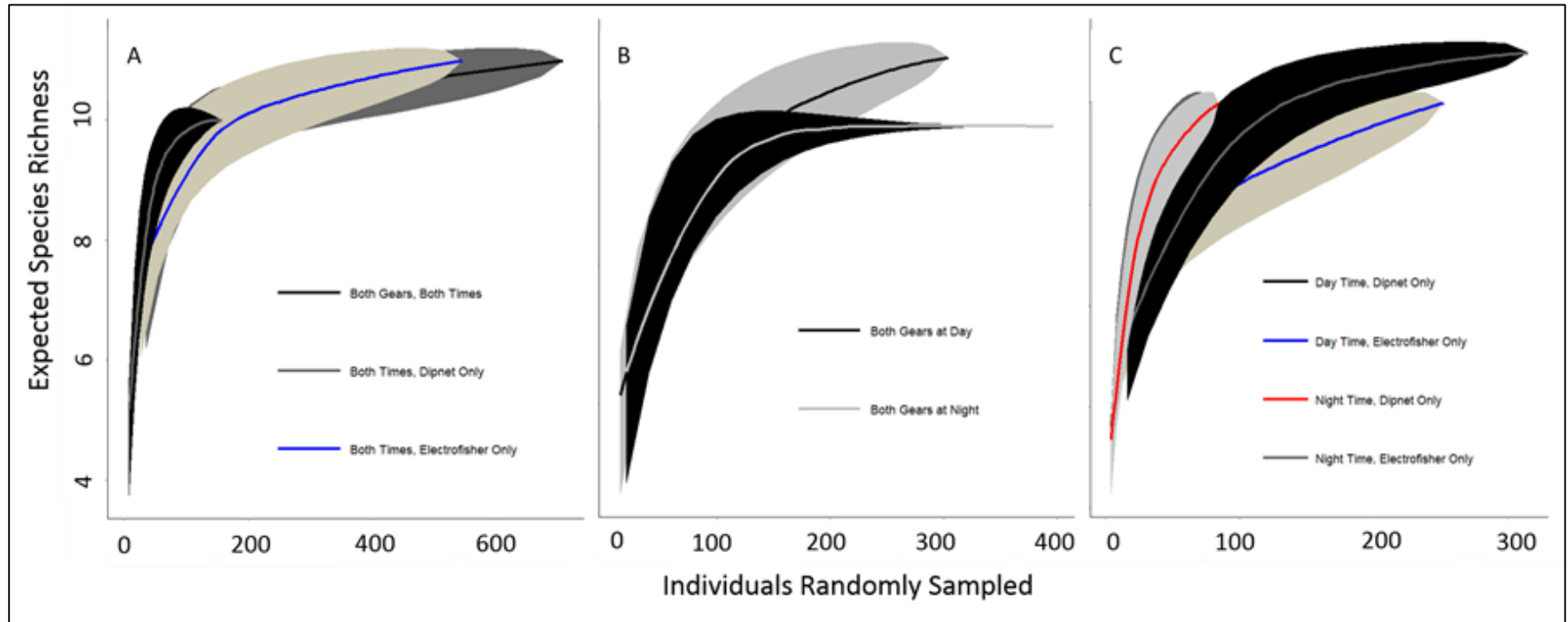


Figure 3.4: Species accumulation curves showing expected crayfish richness as a function of individuals randomly sampled (without replacement; rarefaction) with different combinations of gear types and times of day. A) curves represent diversity data obtained by sampling with an indicated gear type at both times of day. B) curves represent diversity data obtained by sampling with both gear types during the day or night. C) curves represent diversity data obtained by sampling with a single gear type at a specific time of day. Bands around lines are 95% confidence bands. The point where the confidence curve of one line no longer overlaps another line is the divergence point between the curves (i.e., when there would be reasonable differences in expected richness between the curves). Some curves may be stacked on top of each and thus hidden behind other curves, indicating no significant divergence between curves.

differences in expected sample expected richness by gear type; however the differences depended on whether or not we examined diversity data on a per site or per individual basis. Electrofishers and a combined gear approach usually yielded higher expected species richness estimates on a per site basis than dipnets, while the opposite was true on a per individual basis. Differences in expected species richness between times of day were only found when the data from both gear types was pooled. Overall, these results suggested that combined gears is an effective sampling approach if obtaining a representative crayfish size range is important; however, electrofishing alone also appeared to be sufficient to estimate CPUE, sex ratios, and expected species richness.

Substantial variability in mean CPUE among sites occurred in the electrofisher and the combined gear samples, which is to be expected given the variability in habitat among and within sample sites. Importantly, low specific conductance (0.012-0.057 mS/cm in the study streams) and habitat characteristics (e.g., large wood debris piles, see Kaller et al. 2013) can limit the effectiveness of backpack electrofishers and may have reduced sampling efficiency at some sites. Although dipnet samples exhibited low variability, dipnets typically yielded only a few crayfish per site. Surprisingly, analyses did not detect a significant influence of time of day or the interaction of time of day and gear type on CPUE.

The importance of gear-related size selection has been widely documented for various aquatic organisms (e.g., fishes, see Gwinn et al. 2010) and is especially important because the species in our study ranged in size from 9 mm (juvenile *P. natchitochae* and *Cambarellus puer*) to 88 mm (*P. clarkii*). Analyses of the crayfish

size data indicated that dipnets caught smaller individuals (mean 36 mm total length) than the electrofishing unit (mean 41 mm) regardless of time of day. Price and Welch (2009) also found that dipnets often caught smaller crayfish relative to electrofishing, although the 5-mm difference in mean lengths may not be important in distributional and presence/absence studies. Arguably more important, however, was the amount of variation in sizes captured with both gears, and the data indicated that neither gear type sampled narrow length ranges and variation during either time of day. Thus, the gears did not appear to be size selective in this study. It should be noted, however, that although we did not find a difference in CPUE or total length by time of day, we sampled our night reaches shortly after complete sunset. Thus, it is possible that nocturnal activity may be better observed at later hours (midnight, pre-dawn) than during our sampling times. Future studies are needed to compare multiple time periods during the day and night and re-examine differences in CPUE and body size.

One of the main drawbacks of many community diversity assessments is that diversity indices at a site reflect sample composition, which may or may not be representative of the actual in-stream assemblage, if the sampling methodology is biased (McIntosh 1967). Consequently, different combinations of gear type and time of sampling produced different predictions of species richness as was shown from the divergences of the species accumulation curves, which we expected. However, a substantial difference was observed in the asymptotic behavior of the curves calculated on per individual and per site bases, which supported our initial hypothesis of different richness predictions. The species accumulation curves calculated with rarefaction of individuals exhibited clear asymptotes and showed clear divergence between curves of

different combinations of gear types and times of day. In these curves, almost all the crayfish diversity in our samples was captured with about 75-125 individuals regardless of time of day and gear type.

However, because no clear asymptote was reached, divergence of accumulation curves calculated on a per site basis usually did not occur until about 15 sites were sampled regardless of the combination of gear type and time of day. Additionally, when divergence occurred, there was no clear asymptote reached even at the end of the sampling effort. Colwell et al. (2012) noted that estimates of species richness are derived from curves that exhibit asymptotic behavior, and when no asymptote is reached, the sample size is probably too small. Thus estimates of species richness obtained from different combinations of gears and times of day will be overestimated in part due to conservative estimates of variance (i.e., the confidence bands are wider, making clear divergence of curves more difficult to ascertain). In this study, lack of clear asymptotes suggests that an insufficient number of sites may have been sampled. Therefore, our data support our hypothesis that diversity estimates will differ among the gear types and times of day on a per individual basis, but we could not necessarily reject the hypothesis when examining on a per site basis.

The sex of collected crayfishes may be of particular importance because positive identification in some species is limited to sexually mature males (Hobbs 1974). Mature males can be quite small in some species (<2cm in total length, such as in *Cambarellus puer*), and might be less susceptible to electrofishing than dipnet sweeping, particularly if they inhabit leaf litter or other structurally complex habitats. Our data did not reflect sex selectivity as a function of time of day or gear type. Although we did not examine

sex ratios by species, the interaction of sampling gear type and season could be important for some crayfish species that exhibit changes in habitat use throughout the year associated with reproductive molting of females and males, egg incubation, and mate seeking (Hobbs 1981). These concerns become particularly important in the case of less-common taxa (e.g., *O. hathawayi blacki*, *O. maletae*), which often yield insufficient data to accurately assess population characteristics.

Crayfish species presence and abundance in a given stream are dependent on biotic and abiotic relationships, as well as temporal influences on spatial distribution and habitat use, and choice of sampling method can significantly bias inferences of these population parameters (e.g. Byrne et al. 1999, McManamay et al. 2014, Mojica et al. 2014). For example, although our study documented 11 species, current distribution records indicate that 19 species should inhabit the study region. However, at least 5 *Cambarus* and *Fallicambarus* species are primary burrowers (Walls 2009), and probably would not have been encountered in our samples. Their absence highlights the importance of also taking into account survival strategies of individual species, such as burrowing propensity, which may increase in some traditionally stream-dwelling species in response to stream dewatering or adverse water qualities (Taylor 1983). Stream dwelling species we expected to, but did not, collect included *Orconectes hathawayi*, *Faxonella clypeata*, *Procambarus kensleyi*, and *Cambarellus shufeldtii*. Thus, an established protocol may require additional specialized methods to accurately document diversity (e.g., excavation). Lack of these species in our collections suggests that 1) these species may be restricted in their distribution (similar to *Astacus*

leptodactylus, Pöckl 1999), 2) that they were not vulnerable to capture with our sampling methods, or 3) that they may be much rarer than previously reported.

3.4.1 SUMMARY:

This study found that backpack electrofishing or electrofishing combined with of dipnetting yielded higher crayfish CPUE than dipnetting alone in soft-substrate streams in central Louisiana. With limited personnel, electrofishing alone yielded a comparable CPUE, species richness and sex ratio to that of a combined gear approach. Although electrofishing collected larger crayfish than dipnetting, differences in mean length were not substantial (5 mm), and there was high variability among samples. Contrary to our hypothesis, we found little evidence that time of day was an important factor influencing the richness, abundance, or length of collected crayfishes. Estimates of species richness on a per site basis with accumulation curves failed to reach an asymptote, indicating that the predicted richness was accompanied by substantial variance and therefore we could not interpret any meaningful differences among the curves for different combinations of gear type and time of day. However, on a per individual basis, the curves reached an interpretable asymptote and showed clear divergences. We found that more individuals were needed to estimate maximum richness with the backpack electrofisher than with the dipnet. Individual-based curves for the dipnet tended to diverge sharply and early from the curves for the backpack electrofisher regardless of time of day. Dipnets proportionally captured most of the species richness in our samples with fewer individuals compared to the backpack electrofisher, which required many more individuals to reach a similar level expected species richness.

3.5 REFERENCES

- Adams, S. B., 2014. Crayfish use of trash versus natural cover in incised, sand bed streams. *Environmental Management*. 53(2): 382-392.
- Allan, J. D. 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Review of Ecological Systems*. 35: 257-284.
- Awise, J. C. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos*. 63: 62-76.
- Brown, A. V. and W. J. Matthews. 2006. Stream ecosystems of the United States. River and Stream Ecosystems of the World. 2nd printing. University of California Berkley Press. 89-116.
- Byrne, C. F, J. M. Lynch, and J. T. Bracken. 1999. A sampling strategy for stream populations of white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet) (Crustacea: Astacidea). *Biology and Environment: Proceedings of the Royal Irish Academy*. 99B(2): 89-94.
- Capelli, G. M. and J. J. Magnuson. 1983. Morphoedaphic and biogeographic analysis of crayfish distribution in northwest Wisconsin. *Journal of Crustacean Biology*. 3(4): 548-564.
- Clifford, H. F. and R. J. Casey. 1992. Differences between operators in collecting quantitative samples of stream macroinvertebrates. *Journal of Freshwater Ecology*. 7(3): 271-276.
- Crandall, K. A. and J. E. Buhay. 2008. Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae – Decapoda) in freshwater. *Hydrobiologia*. 595: 295-301.
- Collins, N. C., H. H. Harvey, A. J. Tierney, and D. W. Dunham. 1983. Influence of predatory fish density on trapability of crayfish in Ontario Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. 40(10): 1820-1828.
- Colwell, R. K., A. Chao, N. J. Gotelli, S. Lin, C. X. Mao, R. L. Chazdon, and J. T. Longino. 2012. Models and estimators linking individual-based and sample-based rarefaction, extrapolation, and comparison of assemblages. *Journal of Plant Ecology*. 5(1): 3-21.
- DiStefano, R. J., C. M. Gale, B. A. Wagner, and R. D. Zweifel. 2003. A sampling method to assess lotic crayfish communities. *Journal of Crustacean Biology*. 23(3): 678-690.

- Dorn, N. J., R. Urgelles, and J. C. Trexler. 2005. Evaluating active and passive sampling methods to quantify crayfish density in a freshwater wetland. *Journal of the North American Benthological Society*. 24(2): 346-356.
- Dorn, N. J. and J. C. Volin. 2009. Resistance of crayfish (*Procambarus* spp) populations to wetland drying depends on species and substrate. *Journal of the North American Benthological Society*. 28(4): 766-777.
- Felley, J. D. and G. L. Daniels. 1992. Life history of the sailfin molly (*Poecilia latipinna*) in two degraded waterways of southwestern Louisiana. *The Southwestern Naturalist*. 37(1): 16-21.
- Gherardi, F., S. Barbaresi, and G. Salvi. 2000. Spatial and temporal patterns in the movement of *Procambarus clarkii*, an invasive crayfish. *Aquatic Sciences*. 62(2): 179-193.
- Gwinn, D. C., M. S. Allen, and M. W. Rogers. 2010. Evaluation of procedures to reduce bias in fish growth parameter estimates resulting from size-selective sampling. *Fisheries Research*. 105: 75-79.
- Haag, W. R. and J. D. Williams. 2014. Biodiversity on the brink: an assessment of conservation strategies for North American freshwater mussels. *Hydrobiologia*. 735: 45-60.
- Harvey, G. L., A. J. Henshaw, T. P. Moorhouse, N. J. Clifford, H. Holah, J. Grey, and D. W. Macdonald. 2014. Invasive crayfish as drivers of fine sediment dynamics in rivers: field and laboratory evidence. *Earth Surface Processes and Landforms*. 39(2): 259-271.
- Hill, A. M. and D. M. Lodge. 1994. Diel changes in resource demand: competition and predation in species replacement among crayfishes. *Ecology*. 75(7): 2118-2126.
- Hobbs JR, H. H. 1974. A checklist of the North and Middle American Crayfishes (Decapada: Astacidae and Cambaridae). *Smithsonian Contributions to Zoology*. 166.
- Hobbs JR, H. H. 1981. The crayfishes of Georgia. *Smithsonian Contributions to Zoology*. 318.
- Kaller, M. D., C. E. Murphy, and W. E. Kelso. 2013. Drainage for fish and ecoregions for macroinvertebrates: different spatial scales are needed to assess Louisiana wadable streams. *Transactions of the American Fisheries Society*. 142(3): 767-782.

- Kilburn, S. L., C. A. Taylor, G. A. Shuster. 2014. Conservation assessment and habitat notes for three rare Alabama crayfishes: *Cambarus cracens*, *Cambarus scotti*, *Cambarus unestami*. *Southeastern Naturalist*. 13(1): 108-118.
- Knight, J. G. and M. B. Bain. 2006. Sampling fish assemblages in forested floodplain wetlands. *Ecology of Freshwater Fishes*. 5(2): 76-85.
- Kozák, P., L. Füreder, A. Kouba, J. Reynolds, and C. Souty-Grosset. 2011. Current conservation strategies for European crayfish. *Knowledge and Management of Aquatic Ecosystems*. 401.
- Lodge, D. M., M. W. Kershner, and J. E. Aloï. 1994. Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. *Ecology*. 75(5): 1265-1281.
- Martin III, A. L and P. A. Moore. 2007. Field observations of agonism in the crayfish, *Orconectes rusticus*: shelter use in a natural environment. *Ethology*. 113(12): 1192-1201.
- Martinuzzi, S., S. R. Januchowski-Hartley, B. M. Pracheil, P. B. McIntyre, A. J. Planting A., D. J. Lewis, and V. C. Radeloff. 2013. Threats and opportunities for freshwater conservation under future land use change scenarios in the United States. *Global Change Biology*. 20(1): 113-124.
- McIntosh, R. P. 1967. An index of diversity and the relation of certain concepts to diversity. *Ecology*. 48(3): 392-404.
- McManamay, R. A, D. J. Orth, and H. I. Jager. 2014. Accounting for variation in species detection in fish community monitoring. *Fisheries Management and Ecology*. 21(2): 96-112.
- Mojica, J. I., J. Lobon-Cervia, and C. Castellanos. 2014. Quantifying species richness and abundance in Amazonian streams: assessment of a multiple gear method suitable for *Terra firme* stream fish assemblages. *Fisheries Management and Ecology*. 21(3): 220-233
- Neves, R. J., A. E. Bogan, J. D. Williams, S. A. Ahlstedt, and P. W. Hartfield. 1997. Status of the aquatic mollusks in the southeastern United States: a downward spiral of diversity. *Aquatic Fauna in Peril: The Southeastern Perspective*. Special Publication 1: 43-86.
- Ollson, K. and P. Nystrom. 2008. Non-interactive effects of habitat complexity and adult crayfish on survival and growth of juvenile crayfish (*Pacifastacus leniusculus*). *Freshwater Biology*. 54(1): 35-46.

- Paillison, J.-M., A. Sodieux, and J.-P. Damien. 2011. Capture efficiency and size selectivity of sampling gears targeting red-swamp crayfish in several freshwater habitats. *Knowledge and Management of Aquatic Ecosystems*. 401(6).
- Parkyn, S. M. and K. J. Collier. Interaction of press and pulse disturbance on crayfish populations: flood impacts in pasture and forest streams. *Hydrobiologia*. 527(1): 113-124.
- Price, J. E. and S. M. Welch. 2009. Semi-quantitative methods for crayfish sampling: sex, size, and habitat bias. *Journal of Crustacean Biology*. 29(2): 208-216.
- Pöckl, M. 1999. Distribution of crayfish species in Austria with special reference to introduced species. *Freshwater Crayfish*. 12: 733-770.
- Rabeni, C. F., K. J. Collier, S. M. Parkyn, B. J. Hicks. 1997. Evaluating techniques for sampling stream crayfish (*Paranephrops planifrons*). *New Zealand Journal of Marine and Freshwater Research*. 31: 693-700.
- Rach, J. J. and T. D. Bills. 1989. Crayfish control with traps and largemouth bass. *The Progressive Fish Culturist*. 51(3): 157-160.
- Rahel, F. J. 2002. Homogenization of freshwater faunas. *Annual Review of Ecological Systems*. 33: 291-315.
- Richman, N. I., M. Bohm, S. B. Adams, F. Alvarez, E. A. Bergey, J. J. S. Bunn, Q. Burnham, J. Cordeiro, J. Coughran, K. A. Crandall, K. L. Dawkins, R. J. DiStefano, N. E. Doran, L. Edsman, A. G. Eversole, L. Füreder, J. M. Furse, F. Gherardi, P. Hamar, D. M. Holdich, P. Horowitz, K. Johnston, C. M. Jones, J. P. G. Jones, R. L. Jones, T. G. Jones, T. Kawai, S. Lawler, M. Lopez-Meija, C. Pedraza-Lara, J. D. Reynolds, A. M. M. Richardson, M. B. Shultz, G. A. Schuster, P. J. Sibley, C. Souty-Grosset, C. A. Taylor, R. F. Thoma, J. Walls, T. S. Walsh, and B. Collen. 2015. Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). *Philosophical Transactions of the Royal Society B*. 370(1662).
- Statzner, B., E. Fievet, J.-Y. Champagne, R. Morel, and E. Herouin. 2000. Crayfish as geomorphic agents and ecosystem engineers: biological behavior affects sand and gravel erosion in experimental streams. *Limnology and Oceanography*. 45(5): 1030-1040.
- Taylor, C.A., G.A. Schuster, J.E. Cooper, R.J. DiStefano, A.G. Eversole, P. Hamr, H.H. Hobbs III, H.W. Robison, C.E. Skelton, and R.F. Thomas. 2007. A reassessment of the conservation status of crayfishes of the United States and Canada after 10+ years of increased awareness. *Fisheries* 32: 372-389.
- Taylor, R. C. 1983. Drought-induced changes in crayfish populations along a stream continuum. *American Midland Naturalist*. 110(2): 286-298.

Thorpe, J. H. and A. P. Covich. 2009. Ecology and classification of North American Freshwater Invertebrates, Third Edition. Academic Press.

Warren JR, M. L., B. M. Burr, S. J. Walsh. H. L. Bart JR, R. C. Cashner, D. A. Etnier, B. J. Freeman, B. R. Kuhajda, R. L. Mayden, H. W. Robison, S. T. Ross, W. C. Starnes. 2000. Diversity, distribution, and conservation status of the native freshwater fishes of the southeastern United States. *Fisheries*. 25(10): 7-31.

Weaver, M. J., J. J. Magnuson, and M. K. Clayton. 1993. Analyses for differentiating littoral fish assemblages with catch data from multiple sampling gears. *Transactions of the American Fisheries Society*. 122(6): 1111-1119.

CHAPTER 4: THE INFLUENCE OF BIOLOGICAL FACTORS IN THE FORMATION OF INTRASPECIFIC DOMINANCE HIERARCHIES IN THE CRAYFISHES, *ORCONECTES HATHAWAYI BLACKI* AND *PROCAMBARUS PENTASTYLUS* (ASTACIDEA; CAMBARIDAE)

4.1 INTRODUCTION:

Crayfish are particularly well known for intraspecific and interspecific agonism that usually results in the formation of dominant-subordinate social hierarchies (Bovberg 1953, Copp 1986, Duffy and Thiel 2003). Experiments examining agonism have shown that subordinate individuals may be restricted to lower quality habitat patches and less-protective refugia, which in the case of interspecific interactions may ultimately displace them from native habitats (e.g., Bovberg 1970, Fero et al. 2008, Hill and Lodge 1999). Examples of agonistic displacement have been reported for *Cambaroides japonicus* (displaced by *Pacifastacus leniusculus*) in Japan as well as *Orconectes propinquus* (displaced by *Orconectes rusticus*) in the United States (Capelli and Munjal 1982, Usio et al. 2001). Although potentially exacerbated or ameliorated by other factors, such as food availability, it is clear that agonism is an important ecological factor that can influence crayfish spatial ecology, and, thus, should be considered in the course of modelling species distributions (Capelli and Munjal 1982).

In central Louisiana, *Orconectes hathawayi blacki* and *Procambarus pentastylus* are commonly found in the Houston River/West Fork Calcasieu system, although *P. pentastylus* is much more widely distributed and usually much more abundant in these streams. Although of similar size, *O. blacki* has much larger chelae, and we were particularly interested in the consequences of the different chelae morphologies on social interactions and dominance hierarchies within these two species given limited shelter availability (e.g. Klar and Crowley 2012). These morphological characteristics, along

with the innate propensity of the species to engage in aggressive displays with conspecifics, can affect the frequency, magnitude, and duration of intraspecific interactions (Thorp and Ammermen 1978). All of which can be affected by chemical signatures of conspecifics and predators, which can alter the behavior of interacting individuals and ameliorate or exacerbate hierarchical structure (e.g., Garvey et al 1994). Acquistapace et al. (2004) demonstrated that the presence of small amounts of stress chemicals produced by conspecific *P. clarkii* could influence behavioral activities of other crayfish under controlled conditions. Similarly, Martin (2014) noted decreased movement and increased shelter occupancy of *P. clarkii* with addition of a chemical cue by a familiar co-occurring predator (*Micropterus salmoides*).

In this study, we used laboratory tanks to investigate intraspecific dominance patterns in *O. blacki* and *P. pentastylus* to identify important morphological and biological factors that affect agonistic behavior. We tested several hypotheses with these experiments, including whether crayfish sex, body size, and chela size strongly influenced dominance, as well as whether the presence of a predator cue would alter the frequency or probability of winning of agonistic encounters.

4.2 METHODS

All experiments were performed in indoor tanks at Louisiana State University. Specimens of *O. blacki* (n=78) and *P. pentastylus* (n=80) were collected from Hickory Branch, a tributary of the Sam Houston River, with a backpack electrofisher (Halltech Model No. HTB2600), dipnets (6.35mm mesh), and seines (60 mm mesh), removing individuals less than 32 mm total length and those with serious injury (e.g., cracked carapace). To reduce stress after collection and subsequent transportation back to the

laboratory, all individuals were kept overnight in an aerated ice chest filled with water from the stream site and stocked with PVC shelters. Transport water temperature was allowed to slowly equilibrate to the laboratory temperature.

On the day following collection, total length, claw size (width of the palm at its widest), weight, sex and male form were recorded. Each measured individual was then placed into an isolated acclimation chamber for 10 days prior to experimentation under a 12:12 photoperiod such that darkness extended from 12:00AM to 12:00PM, which conveniently allowed observations under dark conditions. Although crayfish behavior under normal and altered photoperiod conditions was not investigated, the 10-day acclimation period appeared to provide sufficient time for the crayfish to adjust behaviorally. The holding chamber for each crayfish was a transparent, covered, square 946-mL plastic container with a single hole in the lid for an aeration tube and vent. Three, 1-cm holes in the bottom of each container drained water from the holding chamber into a larger, opaque 1.9L-container that could be emptied and refilled during water changes without disturbing crayfish in the holding chambers. All chambers were arranged on shelf racks in groups of four, with each shelf illuminated by a single nightlight (4-watt bulb). Shelf racks were completely covered with an opaque tarp to block out ambient light from the holding chambers. Water used during both acclimation and experiments was aerated and dechlorinated tap water, with water quality maintained by not feeding crayfish during the acclimation period and changing the water in each holding chamber every other day. On days with no water changes, we recorded dissolved oxygen and temperature with a dissolved oxygen meter (ThermoOrion® Dissolved Oxygen Meter, Model No. 850; ThermoScientific) and measured ammonia, hardness,

alkalinity, pH, chlorine, nitrite, and nitrate with aquarium test strips (Tetra® Easy Strips: Ammonia; 6-in-1).

Competition trials were performed in 2 rectangular raceway-style tanks (described in Daniel and Brown 2014) that were modified to consist of two arenas per tank (Figure 4.1). Dimensions of each arena were approximately 40x90x25 cm separated by pressure-treated plywood with all edges sealed with silicone sealant (GE® Silicone II; White). Prior to the start of this study, we repeatedly filled and drained the arenas over a span of two days for 2 weeks to test structural integrity, as well as to leach any residual preservative chemicals from the plywood. Five numbered shelters haphazardly scattered

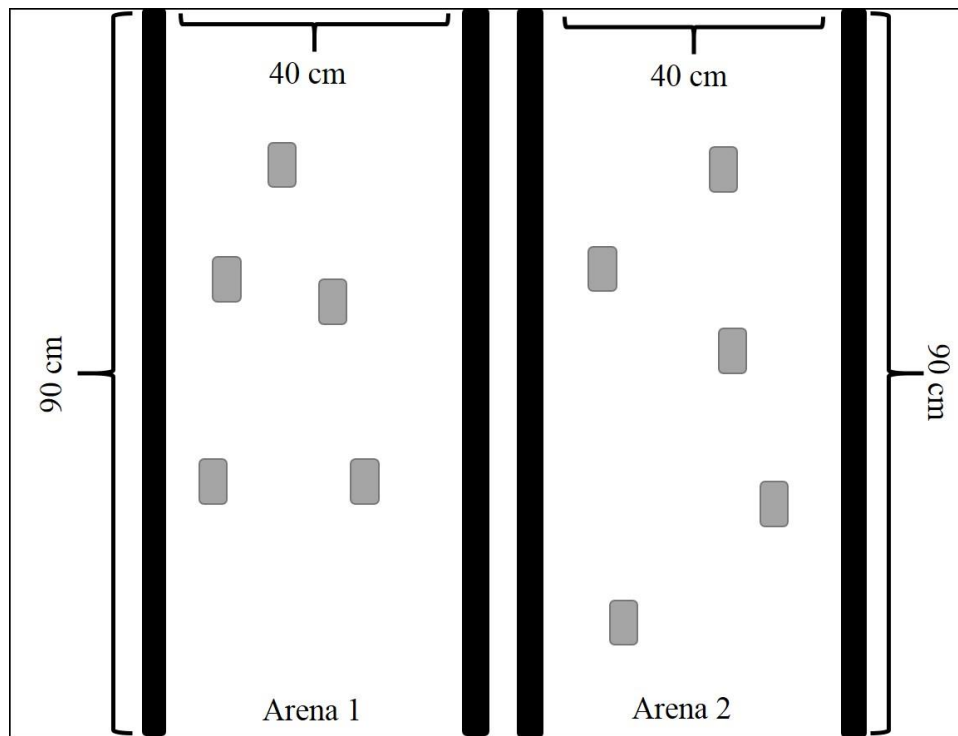


Figure 4.1: Schematic of the crayfish competition arenas. Gray boxes represent locations of capped PVC pipes that were haphazardly placed and used for shelter during the experiments. Dark bars are the wooden partitions that separate the arenas from each other. PVC shelters were randomly distributed around the tank before each trial, and thus, this schematic is not meant to dictate actual locations of the PVC shelters.

throughout each arena were made with 10-cm long PVC tubes (4-cm diameter) that were capped on one end so as to provide a single entrance and exit. All shelters were cleaned with soap and water and air-dried between replicate trials. On the morning of the experiments, arenas were filled with dechlorinated water to a depth of 15cm (approximately 54L), and water quality was tested to ensure consistent conditions between the acclimation chambers and the experimental arenas. If chlorine was present, 5mg sodium thiosulfate was added and subsequent dechlorination confirmed with water quality test strips.

Sixteen experimental trials total were carried out examining intraspecific agonism under the presence (8 trials, 4 for both species) or absence of a predator cue (also 8 trials total, 4 for both species). Ten conspecific crayfish were randomly assigned to a trial in which each group had a mixture of sexes, sizes, and abnormalities (e.g., a missing chela). The predator cue consisted of 5L of water drawn from a separate 50-L tank that held a single adult Largemouth Bass (*Micropterus salmoides*, 424g; a common predator in habitats where these crayfishes occur). We conducted the 8 trials without the predator cue first in order to prevent any possible contamination. Water quality in the Largemouth Bass tank was maintained with bi-daily 25% water changes, although no water changes for crayfish or the Largemouth Bass occurred the day preceding an experiment.

Prior to the commencement of each trial, the dorsal side of the carapace for each crayfish was painted with dots or stripes with red nail polish under red light for identity purposes. After the nail polish dried for 5 minutes, all 10 crayfish were added at the same time to the arena and allowed 3 minutes to adjust to the tank conditions. Two observers then visually observed crayfish interactions and shelter occupancies for 2

hours, with one recording interactions between individuals, and the other recording shelter times and occupancies. All arenas were illuminated with an overhead red light to allow for easy observations of behavior. The spectral sensitivity to red light wavelengths

Table 4.1: Ethogram for use in scoring dominance interactions between individual crayfishes as adapted from Duffy and Thiel (2007). Each score indicates the type of interaction between crayfish. Winners of dominance bouts were noted while recording the type of interaction. Negative numbers correspond to retreats and usually indicated the loss of a dominance bout.

Score	Description of interaction
-2	Tailflipping and rapid backswimming away from the opponent.
-1	Walking backwards, always away from the opponent
0	No response
1	Threat displays (raising chelae and approaching opponent)
2	Secondary antennae whipping
3	Pushing opponent with chelae, chelae may be open but no grappling seen, boxing behavior.
4	Grasping and grappling of opponent, especially apparent attempts to flip opponent over
5	Maiming, or attempting to seriously maim, the opponent. Legs may be torn off or individual killed during bout.

in crayfish tends to be very low (see Goldsmith and Hernandez 1968), so red light helped maintain the illusion of darkness. Interactions between crayfish were scored according to an ethogram summarized by Duffy and Thiel (2007, Table 4.1) and adapted from work by Zelandt Schneider et al. (1999, 2001) and Rubenstein and Hazlett (1974). The ethogram scores from Duffy and Thiel (2007) helped categorize the type of interaction experienced by each individual for each dominance bout and helped determine the victor. To reduce interpretation bias, all individuals were used only once, and the same observer was used to score the interactions for all trials.

Bouts typically consisted of two belligerents, and the identity of both was recorded as well as the type of interaction and the result of each interaction (i.e. win, loss, draw). Losses were identified by an individual backwards swimming or backing away from the opponent. A draw was defined as two individuals who retreated or stopped interacting with each other after a prolonged bout. Individuals that occupied shelters were recorded, their shelter occupancy timed (in whole minutes) and the frequency of shelter-based bouts counted (evictions, voluntary abandonment, failures to evict, successful defense, and shelter fights initiated).

4.2.1: STATISTICAL ANALYSIS

Because few Form I (reproductive stage) males of either species were collected, all males (Form I and non-reproductive Form II) were treated as a single sex category. Individual crayfish were also separated into two categories depending on the presence or absence of a physical abnormality, defined in our experiments as missing or damaged anatomy that may have affected the outcome of a dominance interaction. The most common physical abnormality was a missing chelae; other abnormalities included missing pereopods, antennae, and uropods, and damaged eyestalks. To treat each type of physical abnormality separately for analysis would have unfairly weighted cross-treatment comparisons because of the relatively small observed frequency of other types of abnormalities compared to a missing chelae; therefore, we grouped all abnormalities together and treated them as a single dichotomous variable.

Data were analyzed with a mix of qualitative and quantitative techniques in order to take into account the lower sample sizes associated with less-frequent dominance interactions. For the qualitative analyses, crayfish size data were binned into three size

classes (class 1: 32-45 mm, class 2: 45 – 59 mm and class 3: 60-94mm). The experimental design had the potential for correlated outcomes, because an individual could interact sequentially with more than one opponent. Consequently, analytical approaches were selected that allowed for specification of covariance structures through implementation of generalized linear models (Faraway 2006; Bolker et al. 2009). The overall strategy for these analyses was to construct candidate generalized linear models and generalized linear mixed models with canonical link functions (e.g., identity, log, or logit) and error distributions (e.g., normal, Poisson, or binomial) that incorporated fixed and/or random predictor variables. Best-fitting models were selected from among candidates by chi-square/degree of freedom fit statistic closest to 1. These models were used to describe: 1) the number of dominance bouts as a function of total length, chelae size, sex, and, presence of morphological abnormality, with the experimental tank and presence of predator cue as covariables; and 2) the probability of a win in a dominance bout between asymmetrically-sized and matched-sized ($\leq 2\text{mm}$ total length difference) individuals as a function of a total length, chelae size, sex, and morphological abnormality, with experimental tank and presence of predator cue as covariables. These models did not fit well for comparisons of frequencies of interactions with or without predator cue, so these data were analyzed with a chi-square test of independence. All analyses were performed separately for each species in SAS/STAT (vers. 9.4).

For both species, the best-fitting models for mean counts of dominance bouts was a log link function and negative binomial error distribution model that included presence of morphological abnormality, total length, chelae size, and sex. Additionally, we also tested for correlation between crayfish total length and chelae size. In cases where the

two were strongly correlated, we removed chelae size and retained the total length covariate for the models. *Post-hoc* comparisons of means were performed with pairwise tests based on least square means with a Tukey-Kramer experiment-wise error adjustment. Additionally, the probability of a crayfish winning a bout with a same-sized individual was modelled for each species with a logit link and binomial error distribution with sex, and morphological abnormality as predictors and chelae size as a continuous covariate and presence of predator cue and tank identity as blocking factors.

Analyses of shelter-based bouts were qualitative mainly because of too small a sample size obtained from all trials. Although there was frequent ingress and egress of individuals from shelters throughout all experiments, shelter-based interactions constituted only 6% of all interactions in all experiments. To examine time-per-occupancy, quantiles were calculated across each level of the predictors for both species under both the predator cue treatment and the no cue treatment. Additionally, the proportions of interactions that were shelter-based (in essence, the intensity of shelter-based dominance interactions) were calculated across each level of the biological predictors (size class, sex, and presence of abnormality).

4.3 RESULTS:

Field collections of crayfish successfully procured 80 individuals of both species for use in the intraspecific dominance trials, however 2 *O. blacki* died during acclimation before they could be used in the experiments. Total lengths of crayfish used in the experiments ranged from 35 to 75 mm for *O. blacki* and from 32 to 94 mm for *P. pentastylus* (Table 4.2). Correlation analyses found that chelae size was significantly correlated with body size in both species; however, the correlation was strong in *P.*

Table 4.2: Morphological and biological summary data of *Orconectes blacki* and *Procambarus pentastylus* used in all 16 intraspecific dominance trials. Abnormalities were defined as physical morphological conditions that could negatively affect an individual's odds of achieving dominance in an agonistic interaction (i.e., missing claws, legs, damaged carapace, etc).

Orconectes blacki (n=78)

Size Classes	Total Individuals	Mean Total Length (mm \pm SD)	Mean Weight (g \pm SD)	Mean Chelae Width (mm \pm SD)	Number of Males	Number of Females	Number With Abnormalities
1	25	41.1 \pm 2.9	2.1 \pm 0.7	4.9 \pm 0.9	12	13	12
2	37	51.2 \pm 3.8	4.0 \pm 1.2	6.5 \pm 1.2	22	15	18
3	16	67.8 \pm 4.6	9.0 \pm 2.8	8.0 \pm 2.7	9	7	9

Procambarus pentastylus (n=80)

Size Classes	Total Individuals	Mean Total Length (mm \pm SD)	Mean Weight (g \pm SD)	Mean Chelae Width (mm \pm SD)	Number of Males	Number of Females	Number With Abnormalities
1	16	42.1 \pm 2.8	1.8 \pm 0.4	2.4 \pm 0.6	10	7	7
2	42	52.0 \pm 3.2	3.4 \pm 0.9	3.4 \pm 0.8	19	23	15
3	22	68.9 \pm 8.9*	7.5 \pm 3.4*	5.7 \pm 2.0	4	17	8

*Large standard deviation due to two individuals with extremely large body sizes (94mm and 91mm total length).

Mean length, weight, and chelae width of crayfishes excluding extreme sizes:

Length = 66.3 \pm 4.5mm; Weight = 6.8 \pm 1.8g; Chelae = 5.2 \pm 1.0mm

pentastylus compared to *O. blacki* (*P. pentastylus*: $r=0.91$, $p < 0.01$; *O. blacki*: $r=0.59$, $p < 0.01$). Therefore, in models for *P. pentastylus*, chelae size was not used if total length was a predictor in order to reduce the confounding effect of either variable on the other.

Regarding the total number of dominance bouts, total length and sex were the most important predictors for *O. blacki*; however, no factors were significant for *P. pentastylus* (Table 4.3). Subsequent analysis of the total length and sex factors for *O. blacki* with least square means found that the incident rate for dominance bouts increased by approximately 4% for every 1mm-increase in total length. Females had on average 29% more dominance bouts than males. Regarding the probability of a crayfish being dominant in any given interaction, total length was the only significant predictor for both *O. blacki* and *P. pentastylus*, with a positive trend indicating that larger total lengths increased the odds of dominance (Table 4.4). In size-matched bouts, no biological factors were found to be significant for *O. blacki*, however in *P. pentastylus* chelae size, sex, and the interaction of sex and presence of abnormality were significant (Table 4.5). The relationship of chelae size and odds of dominance was found to be strongly positive with an increase in the log odds by 0.87 for each 1-mm difference.

However, the interpretation of the sex variable is confounded by the interaction with presence of physical abnormality. Further interpretation of this interaction with odds ratios found that females without abnormalities and males with abnormalities were 8.6 times more likely ($p= 0.05$) and 74 times more likely ($p < 0.01$), respectively, to be dominant than females with abnormalities. Additionally, males with abnormalities were

Table 4.3: Summary statistics and incident rate ratios (IRR) obtained through negative binomial regression modelling of the counts of intraspecific dominance bouts between conspecific crayfish. Chelae size was not examined in the *P. pentastylus* because it was strongly correlated with total length as determined by correlation tests. IRRs are given if p-value is significant (bolded) and are defined as the percent change in the expected log count of dominance bouts for every unit increase (in the case of chelae size or total length) or compared to the baseline category (female for the sex predictor and presence of abnormality for the abnormality predictor).

Summary Statistics	<i>Orconectes blacki</i>			<i>Procambarus pentastylus</i>		
	F-value	p	IRR	F-value	p	IRR
Total Length	27.51	<0.01	0.04	1.68	0.20	--
Chelae Size	0.36	0.55	--	--	--	--
Sex	5.77	0.02	0.29	0.38	0.54	--
Abnormality	0.03	0.86	--	0.42	0.52	--
Sex*Abnormality	3.66	0.06	--	0.41	0.52	--

Table 4.4: Summary statistics and regression coefficients (change in log odds) obtained through logistic regression modelling factors affecting the odds of being dominant in a dominance bout between conspecific crayfish. Chelae size was not examined in the *P. pentastylus* because it was strongly correlated with total length as determined by correlation tests. Expected changes in the log odds are given if p-value is significant (bolded) and are defined as the change of the log odds of dominance for every unit increase in the covariate predictor (in the case of chelae size or total length) or compared to the baseline category (female for the sex predictor and presence of abnormality for the abnormality predictor).

Summary Statistics	<i>Orconectes blacki</i>			<i>Procambarus pentastylus</i>		
	F-value	p	Change in Log Odds	F-value	p	Change in Log Odds
Total Length	24.72	<0.01	0.03	8.02	<0.01	0.04
Chelae Size	1.14	0.29	--	--	--	--
Sex	0.54	0.46	--	1.62	0.20	--
Abnormality	1.00	0.32	--	0.08	0.77	--
Sex*Abnormality	0.09	0.76	--	1.57	0.21	--

Table 4.5: Summary statistics and odds ratios obtained through logistic regression modelling factors affecting the odds of being dominant in a dominance bout between match-sized (within 2mm) conspecific crayfish. Data are grouped by species. Expected changes in the log odds are given if p-value is significant (bolded) and are defined as the change of the log odds of dominance for every unit increase in the covariate predictor (in the case of chelae size or total length) or compared to the baseline category (female for the sex predictor and presence of abnormality for the abnormality predictor).

Summary Statistics	<i>Orconectes blacki</i>			<i>Procambarus pentastylus</i>		
	F-value	p	Change in Log Odds	F-value	p	Change in Log Odds
Chelae Size	0.00	0.98	--	9.48	<0.01	0.87
Sex	0.00	0.99	--	11.77	<0.01	4.31
Abnormality	0.00	0.99	--	0.17	0.68	--
Sex X Abnormality	0.00	0.99	--	1.57	<0.01	--

8.8 times more likely to be dominant than females without abnormalities ($p = 0.03$) and 14 times more likely to be dominant than males without abnormalities ($p = 0.02$).

Presence of a predator cue influenced the frequency of dominance bouts within the size classes and sexes of *O. blacki* (Size Class $X^2 = 44.8$, $p < 0.001$; Sex $X^2 = 15.9$, $p < 0.001$) and *P. pentastylus* (Size Class $X^2 = 15.0$, $p < 0.001$; Sex $X^2 = 15.1$, $p < 0.001$). Class 1 and 2 individuals in both species generally decreased frequencies of dominance bouts in the presence of the predator cue, whereas interactions increased in class 3. Male crayfish in both species and female *P. pentastylus* decreased the frequency of dominance bouts (all $p < 0.001$) in the presence of a predator cue, whereas the female *O. blacki* increased the frequency of dominance bouts ($p < 0.001$). *P. pentastylus* with physical abnormalities interacted about 4 times less often when a predator cue was present than when it was absent ($X^2 = 78.6$, $p < 0.001$). Crayfish with no abnormalities increased the frequency of dominance bouts when the predator cue was present.

Shelter-based bouts constituted only about 3% of all dominance interactions in the experiments under a predator cue and 5% in experiments without a predator cue for *O.*

blacki and about 10% and 4%, respectively, for *P. pentastylus*, which precluded quantitative analyses of these data (Table B.2). We did observe greater proportions of shelter bouts in class 1 individuals under a predator cue compared to the other size classes in both species, (9% of the total bouts in *O. blacki* and 21% in *P. pentastylus*), and proportionally more shelter bouts for male *O. blacki* compared to *P. pentastylus*. Females of both species generally had proportionally higher shelter bouts than males when exposed to predator cue. Presence or absence of an abnormality had little effect on the level of shelter interactions for either species. Median time per occupancy for *O. blacki* increased for all size classes, sexes, and levels of abnormality in the presence of the predator cue (Table 4.6). Although class-1 *P. pentastylus* and those with abnormalities also increased occupancy times when exposed to the predator cue, larger individuals generally showed decreased median times per occupancy.

4.4 DISCUSSION:

Taken together, results suggested that there are species differences in the frequencies and intensities of dominance bouts between *O. blacki* and *P. pentastylus*, especially in response to a predator cue. For both species, larger crayfish engaged in more frequent interactions with a higher probability of winning. However, chelae size was found only to be important in size-matched pairings of *P. pentastylus*, and no biological variables were found to be important for *O. blacki*. Thus, these results appear to both agree and disagree with studies reported by Garvey and Stein (1993) and Fero and Moore (2008) on other *Orconectes* species (i.e., increased body and chela size were both positively related to successful dominance). Interestingly, smaller *O. blacki* and *P. pentastylus* did not engage in more interactions than larger individuals, which was

Table 4.6: Shelter occupancy time quantiles, in seconds, for crayfishes of *Orconectes blacki* and *Procambarus pentastylus* during dominance trials under the presence or absence of a predator cue. The predator cue was 5L of water from a 50L tank holding a largemouth bass (*Micropterus salmoides*).

<i>Orconectes blacki</i> (n=78)		<u>Predator Cue</u>		<u>No Predator Cue</u>		
<u>Grouping Factor</u>	<u>25% Quantile</u>	<u>Median</u>	<u>75% Quantile</u>	<u>25% Quantile</u>	<u>Median</u>	<u>75% Quantile</u>
Size Class 1	345	802.5	1320	60	100	240
Size Class 2	60	420	1380	80	200	780
Size Class 3	1260	1410	1560	150	740	3150
Male	120	802.5	1320	60	150	720
Female	585	1020	1380	135	230	760
Abnormality Present	345	585	1280	60	180	780
Abnormality Absent	60	1320	4560	80	195	690

<i>Procambarus pentastylus</i> (n=80)		<u>Predator Cue</u>		<u>No Predator Cue</u>		
<u>Grouping Factor</u>	<u>25% Quantile</u>	<u>Median</u>	<u>75% Quantile</u>	<u>25% Quantile</u>	<u>Median</u>	<u>75% Quantile</u>
Size Class 1	1380	3030	6660	60	210	1350
Size Class 2	40	120	5160	450	689	1110
Size Class 3	15	66	1860	278.6	766	982.5
Male	60	1380	5160	320	810	1320
Female	40	96	2610	60	450	990
Abnormality Present	120	1380	6660	60	759	990
Abnormality Absent	40	216	2610	345	578.6	1320

reported for *O. rusticus* (Schroeder and Huber 2001) and *P. clarkii* (Figler et al. 1995). Additionally, we also noted in one trial the importance of presence of a physical abnormality coupled with sex. While it is possible that there may be aggressive differences between the sexes in response to a physical handicap such as a missing chelae, the result may be an artefact of the random assignments that usually allowed for encounters with at least 2 or 3 other individuals with abnormalities. Notably, some individuals without chelae won dominance bouts, especially within match-sized pairings.

Surprisingly, sex was a significant predictor of the number of bouts but not the outcome for *O. blacki*, which contrasts with findings by Rabeni (1985) who found that sex was a significant factor in the outcome of dominance fights of similar sized *Orconectes luteus* and *O. punctimanus*. However, the lack of a sex effect, along with the lack of other factors being significant in determining a dominance outcome among same-sized *O. blacki*, implies other factors probably dictate social dominance, such as overall size, aggressiveness, and remembered social learning (e.g., Shroeder and Huber 2001, Zulant-Schneider 2001). However, only about 90 dominance bouts out of 545 total could be considered size-matched bouts. Thus, it is possible that either *O. blacki* tended to avoid similar-sized individuals or the result is an artefact of the random assignment.

Interestingly, results of size-matched bouts for *O. blacki* contrast with *P. pentastylus*, i.e., for *P. pentastylus*, chelae size, sex, and the interaction of the sex and presence of physical abnormality appeared to strongly impact the odds of dominance. Indeed, male *P. pentastylus* with abnormalities were substantially more likely to be dominant in dominance bouts than other males and females, and especially males that were not morphologically handicapped. This result needs to be further explored by taking

into account differences in the type of abnormality because, due to the wide range of categories in the abnormality factor, the identity of the abnormality may be more important. For example, an individual with both chelae missing may not escalate as many fights as individuals with one chelae and therefore not win as many fights.

The small sample size for the shelter fights can be attributed to few individuals interacting with crayfish in occupied shelters, which was surprising considering the general importance of shelter resources to crayfish. Although we expected to observe increased shelter-based interactions when a predator cue was added, that was not the case in this study, and behaviors of the two species with respect to shelter were inconsistent. Differences in shelter occupancy times between the species potentially highlight behavioral differences that may strongly influence outcomes of intra- and interspecific interactions within and between these species, as well as the susceptibility to predatory mortality (e.g., Blank and Figler 1996).

Although the size classes of both species responded similarly to the predator cue in terms of a general decrease in the frequency of interactions, differences in the effects of sex and morphological condition on the agonistic behavior of the two species were not anticipated. Our results agree with previous observations that smaller crayfish avoid predators by reducing activity (Roth and Kitchell 2005, Hazlett and Schoolmaster 1998). During our trials, smaller crayfish generally interacted far less frequently than larger crayfish, although we did not measure locomotory activity *per se*. Other authors have attributed the reduced activity of smaller individuals to their greater susceptibility to predation by fish and larger conspecifics (DiDonato and Lodge 1993). Stein and Magnusson (1976) noted that crayfish tended to suppress motion and increase defensive

posturing when an active predator was present. It is probable that larger crayfish can better defend themselves and can thus engage in more frequent dominance fights than smaller individuals when predators are present (see also Mather and Stein, 2011, Garvey et al. 1993). As a consequence, behavior of dominant individuals may influence spatial distributions and predator avoidance strategies of subordinates (e.g., avoiding conflict all together, decreasing movement, or increasing spacing in aquatic systems where crayfish predators such as black bass (*Micropterus* sp.) are common (Carver 1975; Douglas 1974; Ross et al. 1987; Douglas and Jordan 2002).

One unexpected outcome of these experiments was the diametrically opposed patterns of dominance in the two species with regard to size, sex, and physical condition in the presence of the predator cue. For example, male *O. blacki* typically interacted more than females in the absence of a predator cue, but this pattern switched when a predator cue was added and females increased their rates of agonistic interactions. These results present interesting challenges for development of additional studies on competition effects on susceptibility to predation (i.e., whether sex or physical condition of an individual would increase the likelihood that the individual would partake in risky behaviors, such as engaging in dominance interactions, under the presence of a predator cue). Pintor et al. (2008) found a similar trend of behavioral change in *Pacifastacus leniusculus*, which exhibited more frequent aggressive bouts and foraging behavior when exposed to a predator cue. Of particular interest are the interspecific differences in responses to predator cues between sexes and between injured and uninjured individuals. Presumably, these behaviors represent innate responses that convey a survival advantage in the presence of predators. Many predatory fishes have been introduced throughout the

U.S., and behavioral responses that may have been advantageous with other predators may not be advantageous with the new suite of predators.

Biogeographically, distributions of both of our focal species are interesting in that they both occur in the same drainage and thus there is great opportunity for interspecific interactions and agonism between them (Walls 2007, Walls 1976). However, *O. blacki* are mostly restricted to streams in the Houston River/West Fork Calcasieu river system, whereas *P. pentastylus* are broadly distributed throughout the entire Calcasieu river drainage. Previous collection data showed that abundances of *O. blacki* were low relative to *P. pentastylus*, in some cases by an order of a magnitude, and our data analyses indicated that abundances of the two species were negatively correlated. Thus, it is possible that *O. blacki* are not able to expand their current range boundary in part because of interactions with *P. pentastylus*. In this particular study, we were not able to examine this intriguing question in part due to the difficulty in collecting additional *O. blacki*. Future studies are planned to replicate these trials and examine interspecific interactions between the two species.

4.4.1: SUMMARY

The results supported our expectations about the role of body size in agonistic interactions for both species. The species differed in the role of sex and morphological abnormality in dominance interaction and highly unexpectedly, both species exhibited changes in the influence of body size, abnormality, and sex on the frequency of interactions when a predator cue was present versus absent. Taken together, these results may have important implications for understanding the intra- and interspecific interactions that may be limiting the distribution and abundance of *O. blacki*.

4.5 REFERENCES:

- Acquistapace, P., W. H. Daniels, and F. Gherardi. 2004. Behavioral responses to 'alarm odors' in potentially invasive and non-invasive crayfish species from aquaculture ponds. *Behavior*. 141(6): 691-702.
- Blank, G. S. and M. H. Figler. 1996. Interspecific shelter competition between the sympatric crayfish species *Procambarus clarkii* (Girard) and *Procambarus zonangulus* (Hobbs and Hobbs). *Journal of Crustacean Biology*. 16(2): 300-309.
- Bolker, B. M., M. E. Brooks, C. J. Clark, S.W Geange, J. R. Poulsen, M. H. H. Stevens, and J-S.S. White, 2008. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution*. 24:127-135.
- Bovbjerg R. V. 1953. Dominance order in the crayfish *Orconectes virilis* (Hagen). *Physiological Zoology*. 26: 173-178.
- Bovbjerg R. V. 1970. Ecological isolation and competitive exclusion in two crayfish (*Orconectes virilis* and *Orconectes immunis*). *Ecology* 51: 225-236.
- Capelli, G. M. and B. L. Munjal. 1982. Aggressive interactions and resource competition in relation to species displacement among crayfishes of the genus *Orconectes*. *Journal of Crustacean Biology*. 2(4): 486-492.
- Carver, D.C. 1975. Life history study of the spotted bass (*Micropterus punctulatus*) (Rafinesque) in Six-Mile Creek, Louisiana. Fisheries Bulletin 15. Baton Rouge: Louisiana Department of Wildlife and Fisheries.
- Copp, N. H. 1986. Dominance hierarchies in the crayfish *Procambarus clarkii* (Girard, 1852) and the question of learned individual recognition (Decapoda: Astacidea). *Crustaceana*. 51(1): 9-24
- Daniel, W.M. and K.M. Brown. 2014. The role of life history and behavior in explaining unionid mussel distributions. *Hydrobiologia* 734:57-68
- DiDonato, G. T. and D. M. Lodge. 1993. Species replacement among *Orconectes* crayfishes in Wisconsin Lakes: The role of fish predation. *Canadian Journal of Fisheries and Aquatic Sciences*. 50(7): 1484-1488.
- Douglas, N. H. 1974. Freshwater Fishes of Louisiana. Claitor's Publishing Division, Baton Rouge, Louisiana.
- Douglas, N. H. and R. J., Jordan. 2002. Louisiana's inland fishes: A quarter century of change. *Southeastern Fishes Council Proceedings*. 43: 1-10.

- Duffy, J. E. and M. Thiel. 2007. Evolutionary ecology of social and sexual systems: crustaceans as model organisms. Oxford University Press.
- Faraway, J.J. 2006. Extending the Linear Model with R: Generalized Linear, Mixed Effect, and Nonparametric Regression Models. Chapman and Hall/CRC Press, Boca Raton, FL.
- Fero, K. and P. A. Moore. 2008. Social spacing of crayfish in natural habitats: what role does dominance play? *Behavioral Ecology and Sociobiology*. 62: 1119–1125.
- Figler, M. H., J. E. Finklestein, M. Twum, and H. V. S. Peeke. 1995. Intruding male red swamp crayfish *Procambarus clarkii*, immediately dominate members of established communities of smaller, mixed-sex conspecifics. *Aggressive Behavior*. 21(3): 225-236.
- Garvey, J. E. and R. A. Stein. 1993. Evaluating how chelae size influences the invasion potential of an introduced crayfish (*Orconectes rusticus*). *American Midland Naturalist*. 129: 172-181.
- Garvey, J. E., R. A. Stein, and H. M. Thomas. 1994. Assessing how fish predation and interspecific prey competition influence a crayfish assemblage. *Ecology*. 75(2): 532-547.
- Goldsmith, T. H. and H. R. Hernandez. 1968. Comparative studies of crustacean spectral sensitivity. *Zeitschrift fur Vergleichende Physiologie*. 60: 156-175.
- Hazlett, B. A. and D. R. Schoolmaster. 1998. Responses of Cambarid crayfish to predator odor. *Journal of chemical ecology*. 24(11): 1757-1770.
- Hill, A. M. and D. M. Lodge. 1999. Replacement of resident crayfishes by an exotic crayfish: the roles of competition and predation. *Ecological Applications*. 9(2): 678-690.
- Klar, N. M. and P. H. Crowley. 2012. Shelter availability, occupancy and residency in size-assymmetric contests between rusty crayfish, *Orconectes rusticus*. *Ethology*. 118: 118-126.
- Martin, C. W. 2014. Naïve prey exhibit reduced antipredator behavior and survivorship. *PeerJ*. e665.
- Mather, M. E. and R. A. Stein. 1993. Direct and indirect effects of fish predation on the replacement of a native crayfish by an invading congener. *Canadian Journal of Fisheries and Aquatic Sciences*. 50(6): 1279-1288.

- Pintor, L. M., A. Sih, and M. L. Bauer. 2008. Differences in aggression and boldness between native and introduced populations of an invasive crayfish. *Oikos*. 117(11): 1629-1636.
- Rabeni, C. F. 1985. Resource partitioning by stream dwelling crayfish: The influence of body size. *American Midland Naturalist*. 113(1): 20-29
- Ross, S. T., J. A. Baker, and K. E. Clark. 1987. Microhabitat partitioning of southeastern stream fishes: Temporal and spatial predictability. In: Matthews, W. J. and D. C. Heins (eds.). *Symposium on the Evolutionary and Community Ecology of North American Stream Fishes*. University of Oklahoma Press, p. 4251.
- Roth, B. M. and J. F. Kitchell. 2005. The role of size-selective predation in the displacement of *Orconectes* crayfishes following rusty crayfish invasion. *Crustaceana*. 78(3): 297-310
- Rubenstein, D.I., and B. A. Hazlett. 1974. Examination of the agonistic behavior of the crayfish *Orconectes virilis* by character analysis. *Behaviour*. 50: 193-216.
- Thorp, J. H. and K. S. Ammerman. 1978. Chemical communication and agonism in the crayfish, *Procambarus acutus acutus*. *American Midland Naturalist*. 100(2): 471-474.
- Schroeder, L. and R. Huber. 2001. Fight strategies differ with size and allometric growth of claws in crayfish, *Orconectes rusticus*. *Behavior*. 138: 1437-1449.
- Stein, R. A. and J. J. Magnusson. 1976. Behavioral response of crayfish to a fish predator. *Ecology*. 57(4): 751-761.
- Usio, N., M. Konishi, and S. Nakano. 2001. Species displacement between an introduced and a 'vulnerable' crayfish: the role of aggressive interactions and shelter competition. *Biological Invasions*. 3:179-185.
- Zulandt Schneider, R. A., R. W. S. Schneider, and P. A. Moore. 1999. Recognition of dominance status by chemoreception in the red swamp crayfish, *Procambarus clarkii*. *Journal of Chemical Ecology*. 25: 781-794.
- Zulandt Schneider, R. A., R. Huber, and P. A. Moore. 2001. Individual and status recognition in the crayfish *Orconectes rusticus*: the effects of urine release on fight dynamics. *Behavior*. 138(2): 137-153.

CHAPTER 5: CONCLUSION

5.1 DISCUSSION

In this work, I successfully investigated some key ecological aspects of the crayfish communities of central Louisiana. First, I found that, as expected, broadly distributed species found in various habitat types exhibit little correlations and no significant habitat or species gradients could be ascertained at the regional scale for those species. Drainage-scale modelling of broadly distributed, but drainage-isolated *Procambarus pentastylus* showed no significant relationships, similarly to the regionally distributed species. However, such models poorly fit *P. natchitochae*, *Orconectes blacki*, *O. hathawayi*, and *O. maletae* mainly due to their rarity and poor detection in the samples although *P. natchitochae* are documented to be widely distributed throughout the Red River Drainage. Thus, local scale collection efforts and modelling may be more appropriate for rare *Orconectes* species in this region. I then examined how effective my sampling was between the sites in terms of the amount of effort I employed with the gear used. I found that time of day considerations may potentially not be important for sampling crayfish in this area, a combine gear protocol results in excellent efficiency in terms of number of crayfish captured for the amount of effort employed, and that both gear types (backpack electrofishers and dipnets) had substantial variation in size of individuals sampled. Finally, I examined intraspecific agonism in experimental trials between conspecifics of *Orconectes blacki* and *Procambarus pentastylus* and established patterns and trends of dominance within species. I also note that in both species, body size in term of total length is an important factor and that larger chelae sizes increase the

odds of dominance in same-size-class pairings. Also, I found that there were general reductions in the frequency of dominance interactions and increases in median shelter occupancy time when dominance interactions were observed under a predator cue as opposed to no cue present.

This thesis, therefore, provides evidence for environmental and biological factors structuring crayfish communities within these central Louisiana river drainages and offers some indirect insight as to the metacommunity dynamics of the crayfishes in the region. However, the context of this thesis demonstrates an example of a globally concerning problem affecting the ecology of wild crayfishes: a lack of general research interest and concern for the conservation and biology of these animals. Fortunately, concern for the population, genetic, and ecological integrity of crayfish species appears to be on the rise and more research is beginning to provide much needed data as to the current state of many native populations (e.g. Kozak et al. 2011, Owen et al. 2015, Richman et al. 2015). Unfortunately, Louisiana lags behind severely in the ability to conduct such surveys of crayfish communities and populations because the interest and available literature is simply not as prevalent outside of studies for the management and production of *P. clarkii* and *P. zonangulus*. As a result, the data presented herein represent some of the most up-to-date information on distributions of native species in the state even though the data focus solely on one region of the state.

Further research on the ecology of crayfishes will be needed as Louisiana is projected to make shifts from forested to largely agricultural lands and/or urban environment in the coming decades (Wear and David 2011). Land-use impacts on aquatic species have been widely documented and the effects of changes in land use on

wild crayfish populations, namely from natural forest to other land use types, are not an exception (Harding et al. 1999, Schulz et al. 2002, Allan 2004). As the state's human population grows and expands, encroachment on aquatic habitats may endanger crayfish as well as other range-restricted species and therefore population assessments will be required, especially if a species becomes federally listed for protection. It is the hope of this writing that the data contained will be successful in assisting conservation and ecological efforts to model and protect the various crayfish species of the state.

5.2 REFERENCES:

- Allan, J. D. 2004. Landscapes and riverscapes: The influence of land use on stream ecosystems. *Annual Review of Ecological and Evolutionary Systems*. 35: 257-284.
- Harding, J. S., E. F. Benfield, P. V. Bolstad, G. S. Helfman, and E. B. D. Jones. Stream biodiversity: The ghost of land use past. *Proceedings of the National Academy of Sciences*. 95(25): 14843-14847.
- Kozák, P., L. Füreder, A. Kouba, J. Reynolds, and C. Souty-Grosset. 2011. Current conservation strategies for European crayfish. *Knowledge and Management of Freshwater Ecosystems*. 401(1).
- Owen, C. L., H. Bracken-Grissom, D. Stern, and K. A. Crandall. 2015. A synthetic phylogeny of freshwater crayfish: insights for conservation. *Philosophical Transactions of the Royal Society B*. 370(1662).
- Richman, N. I., M. Bohm, S. B. Adams, F. Alvarez, E. A. Bergey, J. J. S. Bunn, Q. Burnham, J. Cordeiro, J. Coughran, K. A. Crandall, K. L. Dawkins, R. J. DiStefano, N. E. Doran, L. Edsman, A. G. Eversole, L. Füreder, J. M. Furse, F. Gherardi, P. Hamar, D. M. Holdich, P. Horowitz, K. Johnston, C. M. Jones, J. P. G. Jones, R. L. Jones, T. G. Jones, T. Kawai, S. Lawler, M. Lopez-Meija, C. Pedraza-Lara, J. D. Reynolds, A. M. M. Richardson, M. B. Shultz, G. A. Schuster, P. J. Sibley, C. Souty-Grosset, C. A. Taylor, R. F. Thoma, J. Walls, T. S. Walsh, and B. Collen. 2015. Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). *Philosophical Transactions of the Royal Society B*. 370(1662).
- Shulz, H. K., P. Smietana, and R. Shulz. 2002. Crayfish occurrence in relation to land use properties: Implementation of a geographic information system (GIS). *Knowledge and Management of Aquatic Ecosystems*. 367: 861-872.

Wear, D. N. and J. G. Griess. 2011. The southern forest futures project: technical report.
USDA-Forest Service. SRS-Technical Report No. 178.

APPENDIX A: CRAYFISH CAPTURE DATA AND ENVIRONMENTAL DATA

Table A.1: List of species expected to reside within the drainages sampled and capture notes based on collection experience and the literature. Drainage codes: H = Houston, C = Calcasieu, V = Vermillion-Teche, M = Mermentau, R = Red River. Asterices indicate a potential new occurrence in the indicated drainage

<u>Species</u>	<u>Common Name</u>	<u>Drainages</u>	<u>Notes</u>
<i>Orconectes hathawayi blacki</i>	Calcasieu Painted Crayfish	S, C*	
<i>O. h. hathawayi</i>	Teche Painted Crayfish	V, M, C*	No Form 1 Confirmation in this study
<i>O. maletae</i>	Kisatchie Painted Crayfish	R	Captured in only two sites
<i>O. palmeri palmeri</i>	Gray-Speckled Painted Crayfish	M* V*	An East of Mississippi River Species found in agriculturally impacted streams
<i>O. palmeri longimanus</i>	Western Painted Crayfish	C	Identification based off Form II and species distribution records
<i>O. lancifer</i>	Shrimp Crayfish	H, C, R, V, M	Usually abundant where found
<i>Faxonella clypeata</i>	Ditch Fencing Crayfish	H, C, R, V, M	Possibly found in side channels, not in main streams
<i>Cambarus diogenes</i>	Devil Crayfish	H, C, R, V, M	Burrowing crayfish; not expected in stream channels
<i>C. ludovicianus</i>	Painted Devil Crayfish	H, C, R, V, M	Burrowing crayfish; not expected in stream channels
<i>Procambarus hinei</i>	Marsh Crayfish	C, V, M	Not found in any streams during collection
<i>P. kensleyi</i>	Free State Chimney Crayfish	H, C, R, V, M	Not found in any streams during collection
<i>P. tulaneii</i>	Giant Bearded Crayfish	R	Not expected south of Red River
<i>P. acutus</i>	White River Crayfish	H, C, R, V, M	Abundant, easily confused with <i>P.</i> <i>zonangulus</i>

(Table A. 1 Continued)

Table A.1: List of species expected to reside within the drainages sampled and capture notes based on collection experience and the literature. Drainage codes: H = Houston, C = Calcasieu, V = Vermillion-Teche, M = Mermentau, R = Red River. Asterices indicate a potential new occurrence in the indicated drainage

<u>Species</u>	<u>Common Name</u>	<u>Drainages</u>	<u>Notes</u>
<i>P. zonangulus</i>	Southern White River Crayfish	H, C, R, V, M	Abundant, easily confused with <i>P. acutus</i>
<i>P. dupratzi</i>	Southwestern Creek Crayfish	C*	Sabine drainage; range may overlap at headwaters of Calcasieu River Drainage
<i>P. natchitochae</i>	Red River Creek Crayfish	R, V	
<i>P. pentastylus</i>	Calcasieu Creek Crayfish	H, C	Endemic to Calcasieu and Sam Houston streams
<i>P. vioscai</i>	Pinelands Creek Crayfish	R	May extend into areas below Red River
<i>P. clarkii</i>	Red Swamp Crayfish	H, C, R, V, M	
<i>Fallicambarus fodiens</i>	Digger Crayfish	H, C, R, V, M	Burrowing crayfish; not expected in stream channels
<i>F. dissitus</i>	Pine Hills Digger Crayfish	S	Burrowing crayfish; not expected in stream channels
<i>Cambarellus shufeldtii</i>	Cajun Dwarf Crayfish	R	Sympatric with <i>Cambarellus puer</i> , unknown if they co-occur in the same stream.
<i>C. puer</i>	Swamp Dwarf Crayfish	H, C, R, V, M	Sympatric with <i>Cambarellus shufeldtii</i> , unknown if they co-occur in the same stream.

Table A.2: Crayfish collection data for the 2013 and 2014 collection years in streams of Central Louisiana. Coordinates are in UTM, zone 15N. Asterices indicate that identity was not confirmed with a Form I male individual and instead established based on historical species distributions and morphology. Hyphens indicate no collection data available for that year.

<u>Stream Site</u>	<u>Coordinates</u>		<u>Drainage</u>	<u>Species</u>	2013	2014
	<u>Easting</u>	<u>Northing</u>			<u>Total</u>	<u>Total</u>
Bayou Arceneaux	516231	3417266	Calcasieu	<i>Orconectes lancifer</i>	17	17
				<i>Orconectes</i> sp.	0	1
				<i>Procambarus acutus</i> *	0	1
				<i>Procambarus pentastylus</i>	0	1
Calcasieu River Tributary	506984	3371830	Calcasieu	<i>O. lancifer</i>	43	56
				<i>Procambarus clarkii</i>	1	0
				<i>P. pentastylus</i>	8	4
Flat Creek	505913	3393453	Calcasieu	<i>Cambarellus puer</i>	2	--
				<i>O. lancifer</i>	1	--
				<i>P. zonangulus</i>	2	--
Little Marsh Bayou	489839	3367809	Calcasieu	None collected	0	--
Mill Creek	520200	3413033	Calcasieu	<i>C. puer</i>	7	--
				<i>P. clarkii</i>	10	--
Sandy Branch	528038	3433902	Calcasieu	<i>P. pentastylus</i>	22	--
Schoolhouse Creek	500522	3449777	Calcasieu	<i>O. palmeri longimanus</i>	5	0
				<i>P. pentastylus</i>	14	70
				<i>P. acutus</i> *	0	1
Six Mile Creek	506971	3408799	Calcasieu	<i>O. p. longimanus</i>	6	1

(Table A.2 Continued)

Table A.2: Crayfish collection data for the 2013 and 2014 collection years in streams of Central Louisiana. Coordinates are in UTM, zone 15N. Asterices indicate that identity was not confirmed with a Form I male individual and instead established based on historical species distributions and morphology. Hyphens indicate no collection data available for that year.						
<u>Stream Site</u>	<u>Coordinates</u>		<u>Drainage</u>	<u>Species</u>	<u>2013</u>	<u>2014</u>
	<u>Easting</u>	<u>Northing</u>			<u>Total</u>	<u>Total</u>
Six Mile Creek	506971	3408799	Calcasieu	<i>P. pentastylus</i>	52	30
Ten Mile Creek	516231	3417266	Calcasieu	<i>O. p. longimanus</i>	9	5
				<i>P. pentastylus</i>	13	44
Bayou Blanche	553499	3339647	Mermentau	<i>Cambarus ludovicianus</i>	1	--
				<i>O. lancifer</i>	1	--
				<i>P. clarkii</i>	1	--
Bayou Blanche Tributary	561436	3341234	Mermentau	<i>P. clarkii</i>	6	--
Bayou Mallet	556088	3374898	Mermentau	<i>O. lancifer</i>	7	--
Bayou Mallet Tributary	554610	3365047	Mermentau	<i>O. lancifer</i>	5	--
Grande Cooley	556088	3374898	Mermentau	<i>O. lancifer</i>	10	--
				<i>P. clarkii</i>	1	--
Jennings-Norwood Canal	533496	3339805	Mermentau	<i>C. puer</i>	5	--
				<i>P. clarkii</i>	12	--
				<i>P. clarkii</i>	14	--
Little Bayou	564611	3324089	Mermentau	<i>P. clarkii</i>	14	--
Lyons Point Gully	560166	3332979	Mermentau	<i>P. clarkii</i>	1	--

(Table A.2 Continued)

Table A.2: Crayfish collection data for the 2013 and 2014 collection years in streams of Central Louisiana. Coordinates are in UTM, zone 15N. Asterices indicate that identity was not confirmed with a Form I male individual and instead established based on historical species distributions and morphology. Hyphens indicate no collection data available for that year.

Stream Site	Coordinates		Drainage	Species	2013	2014
	Easting	Northing			Total	Total
Bayou Nezpique	533655	3372349	Mermentau	<i>O. lancifer</i>	3	--
				<i>P. clarkii</i>	1	--
Bayou Castor	520013	3474251	Red River	<i>C. ludovicianus</i>	1	0
				<i>O. maletae</i>	2	13
				<i>P. natchitochae</i>	1	24
Bayou Derbonne	504733	3489067	Red River	<i>O. lancifer</i>	1	--
				<i>O. maletae</i>	1	--
Bayou Maurice	511811	3482254	Red River	<i>P. acutus</i>	1	--
				<i>P. natchitochae</i>	2	--
Cherry Creek	522129	3480601	Red River	<i>P. clarkii</i>	1	6
				<i>P. natchitochae</i>	1	0
				<i>Procambarus</i> sp.	1	17
Old River Tributary	495340	3506530	Red River	<i>P. clarkii</i>	4	1
				<i>P. natchitochae</i>	1	0
				<i>Cambarus diogenes</i>	0	1
				<i>Orconectes lancifer</i>	0	1

(Table A.2 Continued)

Table A.2: Crayfish collection data for the 2013 and 2014 collection years in streams of Central Louisiana. Coordinates are in UTM, zone 15N. Asterices indicate that identity was not confirmed with a Form I male individual and instead established based on historical species distributions and morphology. Hyphens indicate no collection data available for that year.

Stream Site	Coordinates		Drainage	Species	2013	2014
	Easting	Northing			Total	Total
St. Pierre	505497	3477525	Red River	<i>O. lancifer</i>	16	--
Winn Creek	469644	3504342	Red River	<i>P. natchitocahe</i>	1	--
Youngs Bayou 1	481349	3508818	Red River	<i>C. ludovicianus</i>	1	0
				<i>P. natchitochae</i>	81	47
Youngs Bayou 2	479531	3508316	Red River	<i>P. natchitochae</i>	4	44
Beckwith Tributary	458195	3378604	Houston	None Collected	0	--
Bearhead Creek	450469	3377016	Houston	<i>C. puer</i>	6	0
				<i>O. hathawayi blacki*</i>	1	0
				<i>O. lancifer</i>	5	1
				<i>P. clarkii</i>	0	1
				<i>P. acutus*</i>	0	1
Bearhead Creek Tributary	437385	3361168	Houston	<i>P. clarkii</i>	9	--
Buckstone Marsh	451951	3384530	Houston	<i>C. puer</i>	6	--
				<i>C. ludovicianus</i>	1	--
Buxton Creek	459253	3365798	Houston	<i>O. lancifer</i>	3	7

(Table A.2 Continued)

Table A.2: Crayfish collection data for the 2013 and 2014 collection years in streams of Central Louisiana. Coordinates are in UTM, zone 15N. Asterices indicate that identity was not confirmed with a Form I male individual and instead established based on historical species distributions and morphology. Coordinates for Gum Gully were not collected. Hyphens indicate no collection data available for that year.

Stream Site	Coordinates		Drainage	Species	2013	2014
	Easting	Northing			Total	Total
Cowpen Creek	470895	3373418	Houston	<i>O. h. blacki</i> *	2	4
				<i>O. lancifer</i>	8	23
				<i>P. clarkii</i>	0	1
				<i>P. pentastylus</i>	0	2
Gum Gully	-----	-----	Houston	None Collected	0	--
Hickory Branch	473117	3374370	Houston	<i>O. h. blacki</i>	9	11
				<i>P. pentastylus</i>	19	7
				<i>O. lancifer</i>	0	1
Windham Creek	445177	3377228	Houston	<i>C. puer</i>	12	8
				<i>C. ludovicianus</i>	2	0
				<i>P. clarkii</i>	5	1
Bayou Boeuf Diversion Canal	569049	3424641	Vermillion-Teche	<i>C. ludovicianus</i>	1	0
				<i>O. lancifer</i>	31	1
				<i>O. palmeri</i> sp.	35	48
Bayou Joe Marcel	566568	3396251	Vermillion-Teche	<i>O. h. hathawayi</i> *	1	0
				<i>Orconectes</i> sp.	0	10
				<i>P. clarkii</i>	0	4

(Table A.2 Continued)

Table A.2: Crayfish collection data for the 2013 and 2014 collection years in streams of Central Louisiana. Coordinates are in UTM, zone 15N. Asterices indicate that identity was not confirmed with a Form I male individual and instead established based on historical species distributions and morphology. Hyphens indicate no collection data available for that year.

Stream Site	Coordinates		Drainage	Species	2013	2014
	Easting	Northing			Total	Total
Bayou Rouge	570901	3438664	Vermillion-Teche	<i>O. h. hathawayi</i> *	1	--
				<i>P. clarkii</i>	2	--
Beaver Creek	536340	3408270	Vermillion-Teche	<i>O. lancifer</i>	12	--
Cypress Creek Tributary	539535	3414488	Vermillion-Teche	<i>C. puer</i>	9	--
				<i>P. clarkii</i>	4	--
Caney Bayou	552645	3420408	Vermillion-Teche	None Collected	0	--
Clear Bayou			Vermillion-Teche	<i>O. lancifer.</i>	--	3
			<i>Orconectes</i> sp.	--	6	
			<i>P. clarkii</i>	--	5	
			<i>P. natchitochae</i>	--	60	
Coulee-Carrigue	583290	3387467	Vermillion-Teche	<i>O. lancifer</i>	58	45
Indian Creek	548411	3440516	Vermillion-Teche	<i>P. clarkii</i>	1	--
Plaquemine-Bruly	583396	3378154	Vermillion-Teche	<i>O. lancifer</i>	1	17
				<i>Orconectes</i> sp.	0	4

Table A.3: Stream water quality data for sites sampled for crayfish in summer 2013. Calcium and Alkalinity were determined with wet chemical titration methods.

Site	Drainage	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Conductivity (mS/cm)	Alkalinity (mg/L)	Calcium Hardness (mg/L)	Turbidity (NTUs)
Bayou Arceneaux	Calcasieu	2.78	7.12	29.36	0.367	60.1	68.48	852
Calcasieu River	Calcasieu	7.05	7.27	25.89	0.161	40	34.24	101.9
Tributary								
Flat Creek	Calcasieu	0.72	6.08	22.6	0.104	31.3	34.24	104.5
Little Marsh	Calcasieu	0.60	7.02	26.15	0.125	34	51.36	47
Bayou								
Mill Creek	Calcasieu	1.82	7.89	24.35	0.207	70.9	51.36	373
Sandy Branch	Calcasieu	4.52	7.35	23.42	0.054	11.5	17.12	50
Schoolhouse	Calcasieu	3.55	6.75	25.4	0.083	22	17.12	52.5
Creek								
Six Mile Creek	Calcasieu	6.02	6.63	23.02	0.035	20	17.12	21
Ten mile Creek	Calcasieu	6.30	6.39	23.39	0.052	12	17.12	11.9
Bayou Blanche	Mermentau	5.09	7.58	31.08	0.459	135.2	68.48	229.7
Bayou Blanche	Mermentau	6.11	7.69	30.27	0.012	153.4	102.72	93.3
Tributary								
Bayou Mallet	Mermentau	6.40	7.60	28.47	0.661	234.4	119.84	108.2
Bayou Mallet	Mermentau	4.72	7.57	27.8	0.741	245.6	136.96	140.2
Tributary								
Bayou Nezpique	Mermentau	11.51	8.11	33.35	0.297	112.6	68.48	144.5
Grande Coulee	Mermentau	5.96	7.63	31.31	0.795	262	136.96	60.5
Jennings-	Mermentau	4.49	7.44	27.77	0.135	307.2	136.96	216
Norwood Canal								

(Table A. 3 Continued)

Table A.3: Stream water quality data for sites sampled for crayfish in summer 2013. Calcium and Alkalinity were determined with wet chemical titration methods.

Site	Drainage	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Conductivity (mS/cm)	Alkalinity (mg/L)	Calcium Hardness (mg/L)	Turbidity (NTUs)
Little Bayou	Mermentau	7.58	7.67	27.32	0.771	216.4	154.08	141
Lyon's Point Gully	Mermentau	9.74	7.92	31.46	0.921	83.4	85.6	202.3
Bayou Castor	Red River	6.05	7.3	26.79	0.095	33.6	17.12	17.4
Bayou Derbonne	Red River	6.04	7.46	29.9	0.342	138.8	102.72	93.3
Bayou Maurice	Red River	5.82	7.06	26.96	0.081	27.2	17.12	17.4
Bayou St. Pierre	Red River	4.02	7.3	27.91	0.305	89.4	51.36	73
Cherry Creek	Red River	1.00	7.15	26.59	0.085	48	17.12	69.7
Old River	Red River	5.17	7.27	30.25	0.357	118.8	68.48	89.5
Tributary								
Winn Creek	Red River	3.38	7.3	24.83	0.248	62.2	51.36	45.7
Youngs Bayou 1	Red River	2.68	6.9	26.28	0.718	59.8	34.24	52
Youngs Bayou 2	Red River	3.81	7.35	26.31	0.21	71.6	51.36	45.7
Winn Creek	Red River	3.38	7.3	24.83	0.248	62.2	51.36	15
Bearhead Creek	Houston	2.74	7.02	25.91	0.079	41	17.12	87.5
Bearhead Creek	Houston	3.51	6.81	26.24	0.078	19.6	17.12	57.3
Tributary								
Beckwith Creek	Houston	2.68	6.98	25.72	0.121	33.6	17.12	145.2
Tributary								
Buckstone Marsh	Houston	5.46	5.93	23.9	0.044	37.5	17.12	490
Buxton Creek	Houston	8.22	7.63	30.11	0.373	84.9	68.48	60.2

Table A.3 Continued)

Table A.3: Stream water quality data for sites sampled for crayfish in summer 2013. Calcium and Alkalinity were determined with wet chemical titration methods.

Site	Drainage	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Specific Conductance (mS/cm)	Alkalinity (mg/L)	Calcium Hardness (mg/L)	Turbidity (NTUs)
Gum Gully	Houston	5.18	7.1	27.9	0.203	46.4	51.36	32.1
Hickory Branch	Houston	3.55	6.3	26.47	0.059	42.6	34.24	16.1
Windham Creek	Houston	1.76	6.84	25.61	0.051	15.2	17.12	68.2
Bayou Bouef	Vermillion-	5.13	7.65	28.43	0.333	127.2	85.6	40.1
Diversion Canal	Teche							
Bayou Joe	Vermillion-	12.73	8.34	34.08	0.44	100.5	102.72	69
Marcel	Teche							
Bayou Rouge	Vermillion-	10.52	7.91	28.76	0.564	225.6	154.08	21.2
	Teche							
Beaver Creek	Vermillion-	4.8	7.22	26.52	0.082	81.2	17.12	68
	Teche							
Caney Bayou	Vermillion-	5.7	7.3	29.71	0.128	35.2	34.24	582.4
	Teche							
Coulee Carrigue	Vermillion-	7.61	7.74	28.3	0.485	105.6	102.7	23.3
	Teche							
Cypress Creek	Vermillion-	3.55	7.3	26.7	0.126	50.7	34.24	470.3
Tributary	Teche							
Indian Creek	Vermillion-	2.46	6.7	27.33	0.081	25.2	17.12	189.5
	Teche							
Plaquemine-	Vermillion-	6.81	7.69	29.37	0.381	136.4	51.36	71
Bruly	Teche							

Table A.4: Stream physical habitat profile data for sites sampled for crayfish in summer 2013. Data shown are means and standard deviations of the habitat parameters. Wet width refers to the average wet channel width of the subreaches measured for the samples. Means of stream flow and depth are based off 24 measurements whereas wet width, canopy cover, and bank height are based off 6 measurements.

Site	Drainage	Stream Flow (m/s)	Stream Depth (cm)	Wet Width (m)	Canopy Cover (%)	Bank Height (m)
Bayou Arceneaux	Calcasieu	0.041 ± 0.025	48 ± 14.06	6.82 ± 2.10	70 ± 17.34	1.875 ± 0.644
Calcasieu River Tributary	Calcasieu	0.021 ± 0.021	50 ± 31.77	4.33 ± 1.60	90 ± 1.37	3.208 ± 0.450
Flat Creek	Calcasieu	0.004 ± 0.004	37 ± 12.03	3.20 ± 1.25	97 ± 3.76	1.625 ± 0.598
Little Marsh Bayou	Calcasieu	0.010 ± 0.011	48 ± 21.60	3.43 ± 0.48	97 ± 4.59	1.750 ± 0.337
Mill Creek	Calcasieu	0.010 ± 0.007	27 ± 12.84	3.18 ± 0.80	99 ± 3.57	1.520 ± 0.391
Sandy Branch	Calcasieu	0.007 ± 0.007	37 ± 13.24	2.50 ± 0.86	98 ± 4.08	2.583 ± 0.359
Schoolhouse Creek	Calcasieu	0.015 ± 0.017	68 ± 31.56	6.18 ± 1.02	98 ± 2.58	2.000 ± 0.477
Six Mile Creek	Calcasieu	0.109 ± 0.077	73 ± 28.95	10.28 ± 1.45	93 ± 9.22	1.833 ± 0.389
Ten mile Creek	Calcasieu	0.147 ± 0.071	54 ± 24.66	7.67 ± 1.46	91 ± 11.56	2.083 ± 0.597
Bayou Blanche	Mermentau	0.093 ± 0.069	47 ± 20.52	6.18 ± 0.69	61 ± 14.29	2.146 ± 0.757
Bayou Blanche Tributary	Mermentau	0.011 ± 0.008	27 ± 15.35	4.22 ± 1.36	12 ± 19.30	2.788 ± 1.192
Bayou Mallet	Mermentau	0.018 ± 0.011	27 ± 10.72	4.95 ± 0.89	83 ± 14.70	1.375 ± 0.345
Bayou Mallet Tributary	Mermentau	0.042 ± 0.068	59 ± 17.39	4.18 ± 0.30	100 ± 1.02	1.271 ± 0.271
Bayou Nezpique	Mermentau	0.002 ± 0.004	27 ± 12.69	2.43 ± 0.88	78 ± 20.40	1.604 ± 0.856
Grande Coulee	Mermentau	0.108 ± 0.084	40 ± 29.29	4.02 ± 1.75	75 ± 24.72	2.854 ± 0.856
Jennings-Norwood Canal	Mermentau	0.004 ± 0.006	16 ± 4.81	2.67 ± 0.52	43 ± 7.19	3.146 ± 1.068

(Table A. 4 Continued)

Table A.4: Stream habitat physical profile data for sites sampled for crayfish in summer 2013. Data shown are means and standard deviations of the habitat parameters. Wet width refers to the average wet channel width of the subreaches measured for the samples. Means of stream flow and depth are based off 24 measurements whereas wet width, canopy cover, and bank height are based off 6 measurements.

Site	Drainage	Stream Flow (m/s)	Stream Depth (m)	Wet Width (m)	Canopy Cover (%)	Bank Height (m)
Little Bayou	Mermentau	0.049 \pm 0.037	27 \pm 10.38	4.28 \pm 0.59	73 \pm 22.75	2.667 \pm 0.246
Lyon's Point Gully	Mermentau	0.005 \pm 0.010	64 \pm 10.34	5.93 \pm 1.75	25 \pm 17.32	1.958 \pm 1.322
Bayou Castor	Red River	0.013 \pm 0.011	44 \pm 28.74	5.90 \pm 1.12	57 \pm 27.84	2.917 \pm 0.469
Bayou Derbonne	Red River	0.014 \pm 0.015	39 \pm 25.20	3.70 \pm 1.13	88 \pm 15.53	2.833 \pm 0.326
Bayou Maurice	Red River	0.007 \pm 0.011	42 \pm 30.19	3.87 \pm 1.68	100 \pm 1.02	2.125 \pm 0.250
Bayou St. Pierre	Red River	0.006 \pm 0.010	62 \pm 30.65	7.23 \pm 0.45	69 \pm 26.25	1.125 \pm 0.311
Cherry Creek	Red River	0.001 \pm 0.001	38 \pm 16.54	1.97 \pm 0.36	98.8 \pm 3.06	2.667 \pm 0.749
Old River	Red River	0.0411 \pm 0.023	14 \pm 8.13	2.5 \pm 1.28	98 \pm 6.12	3.729 \pm 1.303
Tributary						
Youngs Bayou 1	Red River	0.002 \pm 0.005	21 \pm 19.52	1.82 \pm 0.89	98 \pm 4.01	2.833 \pm 0.326
Youngs Bayou 2	Red River	0.008 \pm 0.008	32 \pm 17.47	3.25 \pm 1.72	79 \pm 13.85	2.729 \pm 0.719
Winn Creek	Red River	0.006 \pm 0.006	35 \pm 20.89	4.77 \pm 1.09	98 \pm 2.47	2.958 \pm 0.396
Bearhead Creek	Houston	0.012 \pm 0.013	45 \pm 24.68	4.13 \pm 1.32	97 \pm 5.85	2.500 \pm 0.977
Bearhead Creek	Houston	0.012 \pm 0.010	66 \pm 22.33	5.27 \pm 0.59	64 \pm 21.54	1.250 \pm 0.261
Tributary						
Beckwith Creek	Houston	0.016 \pm 0.013	57 \pm 21.23	4.70 \pm 0.84	93 \pm 8.66	2.583 \pm 0.515
Tributary						
Buckstone Marsh	Houston	0.010 \pm 0.007	25 \pm 9.88	3.13 \pm 0.41	95 \pm 6.00	1.917 \pm 0.359
Buxton Creek	Houston	0.024 \pm 0.019	38 \pm 11.34	4.00 \pm 0.56	15 \pm 11.00	3.667 \pm 1.267

(Table A. 4 Continued)

Table A.4: Stream habitat physical profile data for sites sampled for crayfish in summer 2013. Data shown are means and standard deviations of the habitat parameters. Wet width refers to the average wet channel width of the subreaches measured for the samples. Means of stream flow and depth are based off 24 measurements whereas wet width, canopy cover, and bank height are based off 6 measurements.

Site	Drainage	Stream Flow (m/s)	Stream Depth (m)	Wet Width (m)	Canopy Cover (%)	Bank Height (m)
Gum Gully	Houston	0.014 ± 0.011	60 ± 19.16	4.23 ± 1.07	41 ± 29.64	2.292 ± 0.865
Hickory Branch	Houston	0.061 ± 0.053	54 ± 18.83	6.98 ± 1.58	56 ± 16.55	1.458 ± 0.437
Windham Creek	Houston	0.004 ± 0.007	27 ± 9.76	2.77 ± 0.60	100 ± 0.00	1.147 ± 0.515
Bayou Bouef	Vermillion-	0.040 ± 0.046	51 ± 25.89	8.50 ± 2.60	93 ± 7.01	3.083 ± 0.289
Diversion Canal	Teche					
Bayou Joe	Vermillion-	0.034 ± 0.017	28 ± 10.58	3.37 ± 0.75	39 ± 20.147	1.792 ± 0.541
Marcel	Teche					
Bayou Rouge	Vermillion-	0.124 ± 0.138	26 ± 9.42	4.76 ± 0.53	98 ± 3.26	1.250 ± 0.783
	Teche					
Beaver Creek	Vermillion-	0.011 ± 0.009	48 ± 13.82	4.83 ± 1.04	82 ± 10.48	2.958 ± 0.396
	Teche					
Caney Bayou	Vermillion-	0.016 ± 0.030	28 ± 8.15	3.55 ± 0.95	73 ± 8.51	1.500 ± 0.477
	Teche					
Coulee Carrigue	Vermillion-	0.071 ± 0.048	43 ± 11.11	4.35 ± 1.09	76 ± 14.11	2.333 ± 0.492
	Teche					
Cypress	Vermillion-	0.001 ± 0.003	26 ± 18.01	2.33 ± 0.39	99 ± 2.09	1.917 ± 0.417
Creek Tributary	Teche					
Indian Creek	Vermillion-	0.011 ± 0.012	68 ± 25.06	3.14 ± 0.74	66 ± 45.41	2.458 ± 0.811
	Teche					
Plaquemine-	Vermillion-	0.019 ± 0.011	46 ± 9.63	4.93 ± 0.71	14 ± 13.85	1.729 ± 0.779
Bruly	Teche					

APPENDIX B: EXTENDED COMPETITION TRIAL DATA

Table B.1: Contingency table tabulating the frequencies of intraspecific shelter interactions in two species of crayfish within the biological variables: size class (small, 32-45mm; medium, 46-59mm; and large, >60m), presence of physical abnormality, and sex. Chi-square tests of independence were performed to assess whether the frequency of shelter interactions was affected by the association of presence of predator (*Micropterus salmoides*) cue and the biological variables. Frequencies also include expected values for comparison. Significant p-values are bolded.

<i>Orconectes blacki</i>	Cue Present		Cue Absent		Chi Square Tests	
	<u>Total</u>		<u>Total</u>			
<u>Size Class</u>	<u>Interactions</u>	<u>Expected</u>	<u>Interactions</u>	<u>Expected</u>	<u>X²</u>	<u>p</u>
1	5	2.5	3	5.4	4.600	0.100
2	5	7.3	18	15.7		
3	2	2	5	4.8		
<u>Abnormality</u>						
Present	8	7	14	15	0.554	0.457
None	4	5	12	11		
<u>Sex</u>						
Male	7	4.7	8	10	*	0.157
Female	5	7	18	16		

* p-value calculated with Fisher's Exact Test; 25% of cells had expected values less than 5

(Table B. 1 Continued)

Table B.1: Contingency table tabulating the frequencies of intraspecific shelter interactions in two species of crayfish within the biological variables: size class (small, 32-45mm; medium, 46-59mm; and large, >60m), presence of physical abnormality, and sex. Chi-square tests of independence were performed to assess whether the frequency of shelter interactions was affected by the association of presence of predator cue (*Micropterus salmoides*) and the biological variables. Frequencies also include expected values for comparison. Significant p-values are bolded.

<i>Procambarus pentsatylus</i>	Cue Present		Cue Absent		Chi Square Tests	
<u>Size Class</u>	<u>Total</u>	<u>Expected</u>	<u>Total</u>	<u>Expected</u>	<u>X²</u>	<u>P</u>
	<u>Interactions</u>		<u>Interactions</u>			
1	11	10	4	5	13.8	0.001
2	12	18	15	9		
3	15	10	0	5		
<u>Abnormality</u>						
Present	9	11.3	8	6	2.054	0.152
None	29	27	11	13		
<u>Sex</u>						
Male	11	15	12	8	6.160	0.013
Female	27	22	7	11		

Table B.2: Comparisons between two species of crayfish, *Procambarus pentastylus* and *Orconectes blacki* of proportions of intraspecific dominance bouts that were shelter focused (i.e., crayfish attempted to evict a conspecific or defended against an eviction) under presence of predator cue. Shelter interactions accounted for about 7% of all interactions in all experiments and thus were generally rare.

<i>Procambarus pentastylus</i>	<u>Under Predator Cue</u>	<u>Under No Predator Cue</u>
<u>Proportion of Shelter Interactions:</u>	<u>Percentage</u>	<u>Percentage</u>
Of All Interactions	10.9	4.2
Among Size Class 1	21.6	3.6
Among Size Class 2	6.1	6.0
Among Size Class 3	15.0	0.0
Among Males	5.7	10.9
Among Females	2.9	11.3
Among Individuals with Abnormality	13.4	3.5
Among Individuals without Abnormality	10.3	4.8
<i>Orconectes blacki</i>	<u>Under Predator Cue</u>	<u>Under No Predator Cue</u>
<u>Proportion of Shelter Interactions:</u>	<u>Percentage</u>	<u>Percentage</u>
Of All Interactions	3.3	5.1
Among Size Class 1	8.6	2.9
Among Size Class 2	3.1	5.7
Among Size Class 3	1.4	5.2
Among Males	3.6	2.4
Among Females	2.9	9.9
Among Individuals with Abnormality	4.2	5.7
Among Individuals without Abnormality	2.2	2.2

VITA

William Robert Budnick, is a native of Seymour, Connecticut. He attended Auburn University in Auburn, Alabama from August 2008 to May 2012 where he earned his Baccalaureate in Fisheries and Allied Aquacultures (Summa Cum Laude). During his time in Auburn, he was involved with various research projects including an independent study funded by the university. After graduating, he promptly began work at Louisiana State University studying crayfish distributions throughout the state. He will receive his Masters of Science in May 2015 and will begin doctoral research at the University of Texas – Arlington starting in Fall 2015.