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Red swamp crayfish *Procambarus clarkii* in the Atchafalaya River Basin: biotic and abiotic effects on population dynamics and physiological biomarkers of hypoxic stress

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RED SWAMP CRAYFISH *PROCAMBARUS CLARKII* IN THE ATCHAFALAYA RIVER
BASIN: BIOTIC AND ABIOTIC EFFECTS ON POPULATION DYNAMICS AND
PHYSIOLOGICAL BIOMARKERS OF HYPOXIC STRESS

A Dissertation

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
Doctor of Philosophy

in

The School of Renewable Natural Resources

by

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B.S., Nicholls State University, 2004
M.S., Nicholls State University, 2006
August 2012

DEDICATION

This dissertation is dedicated to my grandfather Sidney Kraemer Sr. (paw-paw Chackbay). He loved the swamps and wildlife of south Louisiana and enjoyed working in his swamp sanctuary, Malagay. My curiosity and passion for the outdoors is a piece of him that lives on in me. He would truly be amazed and delighted that his grandson was able to get a doctorate by working in swamps with gar, choupic, and crawfish.

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ABSTRACT

Crayfish harvested from the Atchafalaya River Basin (ARB) represent the majority of Louisiana wild crayfish landings. However, excluding water level influences, it is difficult to elucidate inter-annual harvest differences and intra-annual population variability among habitats. This research investigated ecological influences on population characteristics of red swamp crayfish *Procambarus clarkii* in the southeastern ARB as well as physiological biomarkers of hypoxic stress in *P. clarkii*. Biotic and abiotic effects on *P. clarkii* populations were examined throughout the 2008 and 2009 commercial crayfish seasons. *P. clarkii* catch per unit effort (CPUE) at sampling locations increased nearly 600% between sample years despite similar hydrologic and physicochemical conditions. The passage of Hurricane Gustav between sample years caused near anoxic conditions for several weeks throughout the lower ARB. Increased allochthonous inputs and reduced fish predation associated with Gustav likely contributed to the increased *P. clarkii* CPUE observed during 2009. During 2008, *P. clarkii* CPUE was highest at sample locations characterized by high dissolved oxygen (DO) concentrations and lowest at locations with relatively low DO levels. In addition, chronically hypoxic sampling locations yielded lower mean CPUE and carapace length during 2008. An increase in *P. clarkii* mean CPUE and a concomitant decrease in mean carapace length during the 2009 crayfish season indicated density-dependent growth. While abiotic factors undoubtedly influenced crayfish population characteristics, relative density appeared to have the largest effect on *P. clarkii* carapace length and may have depressed any physicochemical influences.

Lactate, glucose, and protein concentrations in *P. clarkii* hemolymph were examined in individuals from chronically hypoxic ARB habitats and laboratory simulated hypoxia experiments. *P. clarkii* from normoxic and hypoxic natural habitats did not display significantly

different hemolymph lactate or glucose concentrations, however, mean hemolymph protein concentration was significantly lower in crayfish from hypoxic areas. *P. clarkii* exposed to severe hypoxia in laboratory experiments had significantly higher hemolymph lactate and glucose concentrations, whereas large differences in protein concentrations were not observed. A hand-held lactate meter and refractometer proved to be reliable methods for determination of *P. clarkii* hemolymph lactate and protein concentrations, respectively.

CHAPTER 1

GENERAL INTRODUCTION

Crayfishes are a diverse group of decapod crustaceans with over 640 described species that are distributed on every continent except Antarctica (Crandall and Buhay 2008). North America contains the largest crayfish diversity within its two families, Astacidae and Cambaridae, which include 77% of the world's crayfish species (Taylor 2002), of which over two-thirds are endemic to the southeastern United States (Taylor et al. 2007). Currently, Louisiana has 39 species and subspecies of crayfish in six Cambarid genera (Walls 2009). Louisiana crayfish species are inhabitants in most freshwater ecosystems and play an important role ecologically, economically, and culturally.

Ecologically, crayfish are important components in freshwater ecosystems and food webs. As polytrophic consumers (omnivores), crayfish cannot be placed into one trophic level designation in aquatic communities. They are highly opportunistic feeders with the majority of their diets consisting of primary producers, invertebrates, and fishes (Momot et al. 1978; Hobbs 1993; Hill and Lodge 1994; Whitley and Rabeni 1997; Olsson et al. 2008), and they can fill the niche of primary consumers up to top predators in trophic food webs (Dorn and Wojdak 2004). Furthermore, crayfish are able to cycle nutrients and energy throughout the food web by converting detritus into available energy for higher trophic levels and by shredding material into finer particles that are more easily attacked by decomposers (Momot et al. 1978). This energy gets transferred to numerous organisms in freshwater ecosystems that utilize crayfish as a food resource. In Louisiana freshwater ecosystems such as the Atchafalaya River Basin (ARB), crayfish are a dietary component of terrestrial and semi-terrestrial animals, macroinvertebrates, fishes, and humans (Lambou 1961; Dugas et al. 1976; Chabreck et al. 1982; Snedden et al. 1999; Elsey 2006; Huner 2006; McClain et al. 2007; Gabrey 2010; Ianni 2011; Figure 1.1). Because of

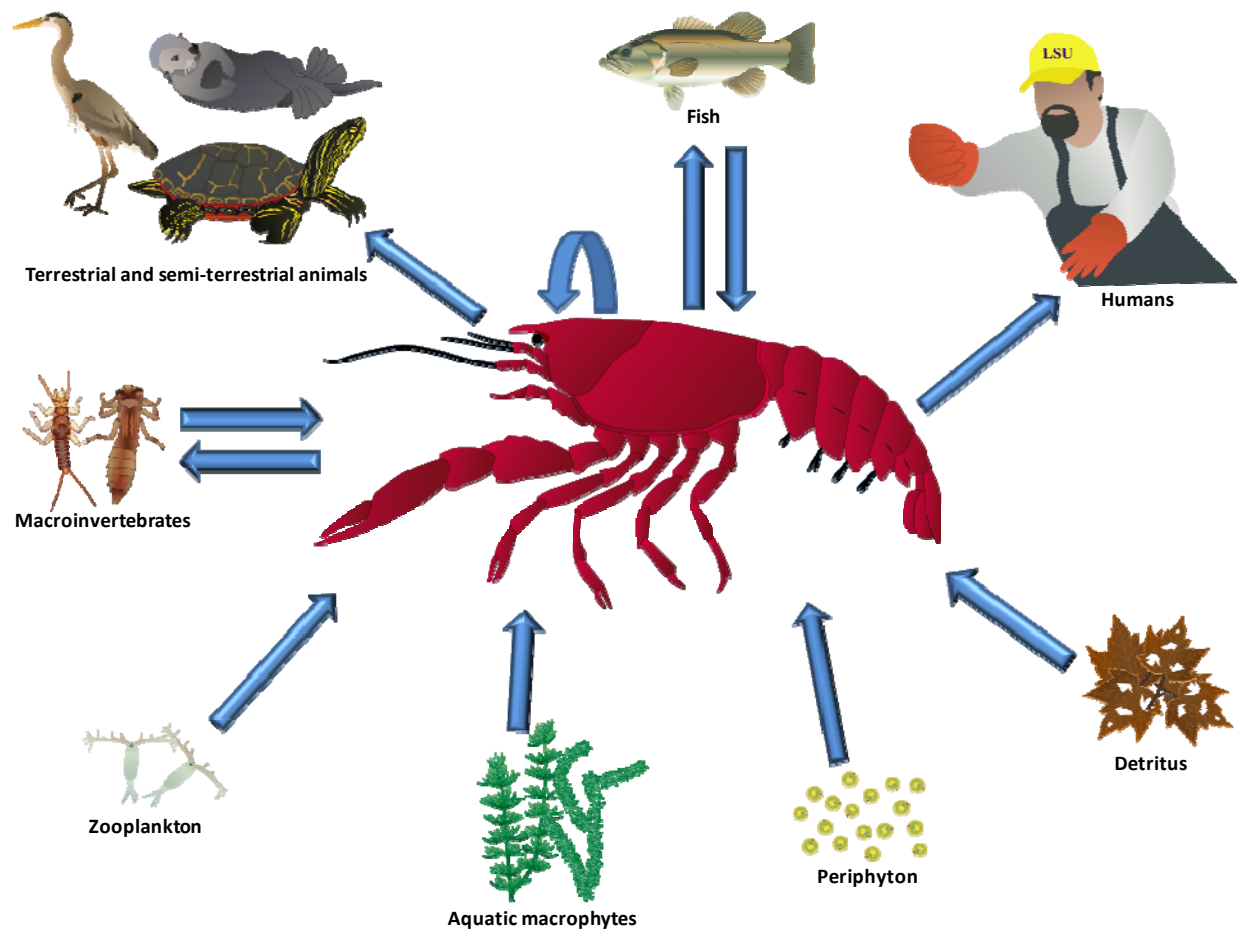


Figure 1.1. Energy flow through crayfish in the Atchafalaya River Basin ecosystem. Arrows point in the direction of energy flow (images courtesy K. Kraeer, T. Saxby, and L. Van Essen, Integration and Application Network, University of Maryland Center for Environmental Science).

their interactions with multiple levels in the trophic web, crayfish are considered to be a keystone species in many aquatic ecosystems (Momot et al. 1978; Crandall and Buhay 2008; Reynolds 2011).

As a multi-million dollar industry and a symbol that defines Louisiana, crayfish are one of the most culturally and economically important resources in the state. The red swamp crayfish *Procambarus clarkii* has become synonymous with Cajun culture and an inherent part of Louisiana. Louisiana is known as the crayfish capitol of the world and was the first state to designate it as the official state crustacean. Commercial crayfish harvests began in Louisiana in the late 19th century and have progressively increased since the mid 20th century (McClain et al. 2007; Walls 2009). Louisiana is the largest producer and consumer of crayfish in the U.S. (McClain et al. 2007) and commercial crayfish harvested from managed ponds (farmed) and natural systems (wild-caught) support over a thousand producers, two thousand fishermen, and numerous processors with an industry value of more than \$209 million, over one-third of Louisiana's total fisheries value (LSUAC 2012).

Commercial crayfish harvests are comprised of *P. clarkii* and the southern white river crayfish *P. zonangulus* with *P. clarkii* representing the majority of landings. Although extensive research has been conducted on various aspects of the biology, ecology, population structure, and optimal harvest characteristics of farmed crayfish in Louisiana, wild populations have received far less attention. Few studies have examined wild *P. clarkii* populations in Louisiana with the majority of research occurring more than three decades ago (e.g., Penn 1943; Penn 1956; Bryan et al. 1976; Konikoff 1977). Additionally, the effects of minimal harvest regulations and management on crayfish population dynamics is unclear. Currently in Louisiana, commercial wild crayfish harvesters are required to possess a valid freshwater fishing license in

addition to a commercial crayfish license. Traps cannot have flues exceeding 5.08 cm with a minimum hexagon mesh size of 1.905 cm by 1.746 cm, however, there are no size or creel limits or a state-regulated season. Localized reductions in crayfish abundance for extended periods have been reported in various harvest areas; nevertheless, natural crayfish populations for the most part have persisted.

Although *P. clarkii* are inhabitants in natural freshwater systems throughout Louisiana, the majority of the wild commercial landings (> 90%) are harvested from the ARB (Isaacs and Lavergne 2010). Harvests coincide with floodplain inundation from the annual Atchafalaya River flood pulse. However, flood pulse-associated physicochemical fluctuations, notably dissolved oxygen, and aquatic macrophyte density and composition are spatially and temporally variable among ARB habitats (Sabo et al. 1999a, b; Fontenot et al. 2001; Rutherford et al. 2001; Troutman et al. 2007; Walley 2007). Although *P. clarkii* are tolerant of relatively low dissolved oxygen concentrations (Nyström 2002), physiological stress from prolonged exposure (Bonvillain et al. 2012) may affect crayfish population dynamics. Physiological condition indices including hemolymph lactate, glucose, and protein concentrations can be used to examine the effects of sub-optimal environmental conditions on crayfish physiology and population health. Additionally, crayfish populations and harvests may be influenced by the occurrence of tropical weather systems, several of which have impacted the ARB in the past several decades. These storms influence ecosystem structure (Doyle 2009) and disrupt system-level physical processes and impact resident aquatic biota.

Study Area

The ARB in south-central Louisiana (Figure 1.2) is the largest remaining bottomland hardwood forest in North America, covering approximately 5,000 km² (Lambou 1990). High

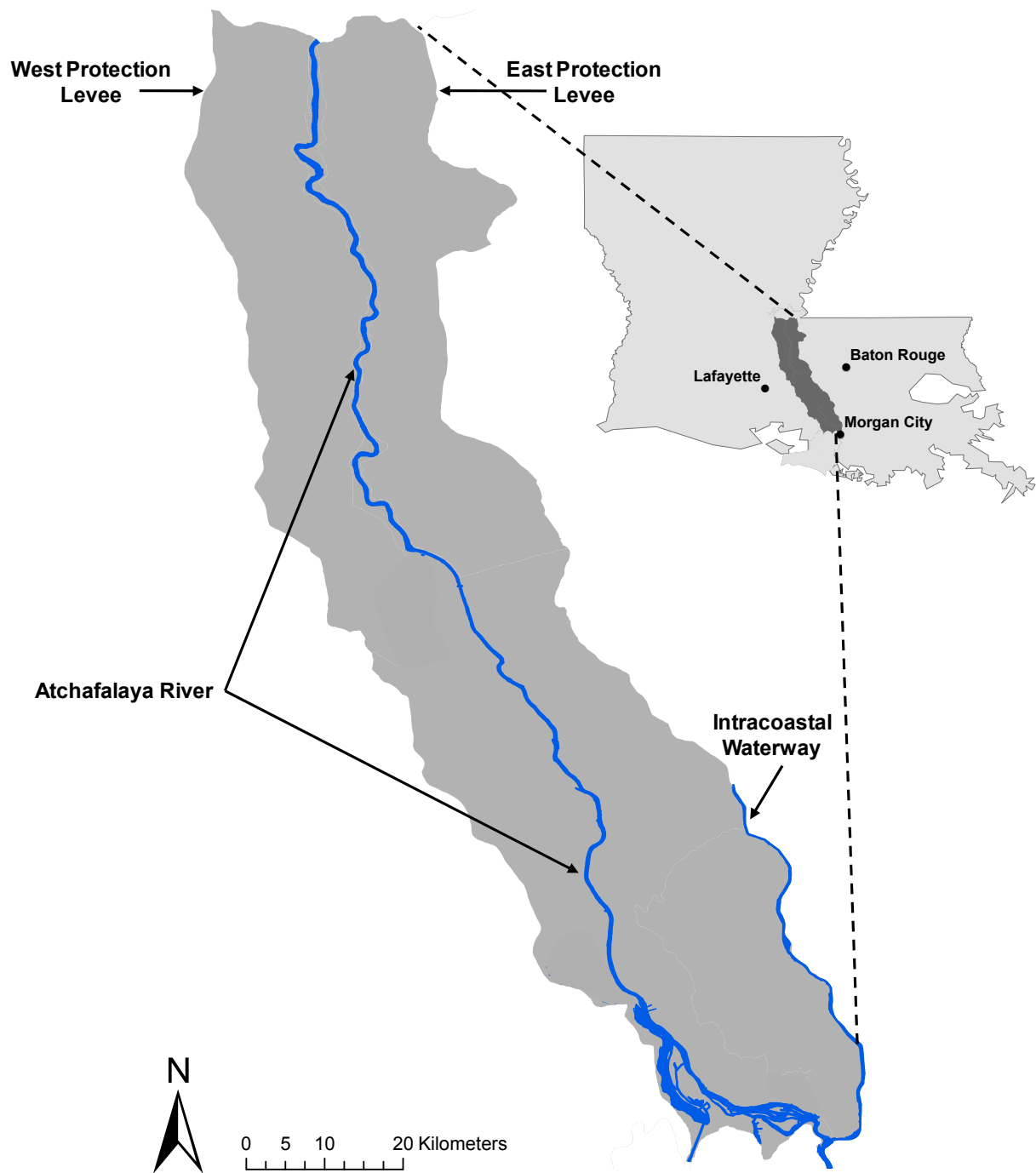


Figure 1.2. The Atchafalaya River Basin in south-central Louisiana.

biotic productivity in the ARB is attributed to the annual Atchafalaya River flood pulse which releases floodplain nutrients and transports additional mainstem river nutrients to floodplain habitats (e.g., Junk et al. 1989; Bayley 1995; Sparks 1995). The Atchafalaya River is a distributary of the Mississippi River and flows 220 km from the confluence of the Red and Mississippi Rivers and discharges into the Gulf of Mexico. In the 1950s, it was discovered that the Atchafalaya River was capturing an increasing amount of Mississippi River water and in the absence of any intervention the Mississippi River would eventually change its course and flow down the Atchafalaya River (Fisk 1952). In order to prevent the Mississippi River from changing its course, the Old River Control Structure was built in 1963 approximately 80 km northwest of Baton Rouge. Additionally, an auxiliary control structure and diversion channel was constructed in 1986 and the Murray Hydroelectric Power Plant was constructed in 1990. The Old River Control Structure complex now consists of three channels that direct Mississippi River water into the Atchafalaya River. The U. S. Army Corps of Engineers regulates the amount of water entering the Atchafalaya River and maintains flows at 30% of the combined volumes of the Mississippi and Red rivers, proportions that occurred in 1950 (van Beek et al. 1979).

The ARB is approximately 25-35 km wide (Ford and Nyman 2011) and is bounded by east and west protection levees, which can contain a flow rate of $42,735 \text{ m}^3 \text{ s}^{-1}$ to prevent downstream flooding in urban and agricultural areas (Jennings and Land 1977). Currently, the ARB floodplain consists of a mosaic of habitats including swamps, shallow headwater and backwater lakes, and numerous natural bayous and excavated canals. However, geomorphological changes associated with sedimentation are altering floodplain habitats and causing the ARB to fill. Since the 1950s, many lakes and swamps in the ARB have filled with

sediment (Tye and Coleman 1989; McManus 2002). Sedimentation reduces the total amount of water available during low-water periods, converts open waters to bald-cypress swamps, and bald-cypress swamps to bottomland hardwood forests (Hupp et al. 2008; Ford and Nyman 2011). Additionally, anthropogenic modifications associated with levee and canal construction, flood control, and navigation have altered the historic river-floodplain connection resulting in increased sedimentation (Tye and Coleman 1989; Hupp et al. 2008, 2009) and reduced water quality (Sabo et al. 1999a, b) throughout the ARB.

Crayfish vs. Crawfish

While both crayfish and crawfish are acceptable terms for members of Astacidea, crawfish is the commonly used term in the southern U.S. and crayfish is the most widely accepted term for the rest of the U.S. and the world. Penn (1943) and Walls (2009) both provide discussion and opinion on the crayfish versus crawfish debate. Crayfish is believed to have become popularized in T. H. Huxley's widely distributed college text book, "The Crayfish, An Introduction to the Study of Zoology" (1880). However, crawfish was the common name utilized by Say (1817) many years before Huxley's work. It is also believed that Constantine Rafinesque referred to the term crawfish in 1817 while studying specimens in the Ohio basin (Walls 2009). Furthermore, the term crawfish was used by Girard when he first described *P. clarkii* (then *Cambarus clarkii*) in 1852. Both crayfish and crawfish are utilized in scientific literature, however, most scientific publications, including the American Fisheries Society, prefer crayfish for common name usage. For this reason, the term crayfish is used throughout this dissertation.

Dissertation Synopsis

The research chapters in this dissertation were designed to examine ecosystem effects on various physical, physiological, and population attributes of *P. clarkii* in the ARB. Chapter 2 examines multiple biotic and abiotic influences on *P. clarkii* population dynamics through two sample years. Chapter 3 describes the physicochemical effects experienced in the ARB after a direct hit from Hurricane Gustav which occurred between *P. clarkii* sampling years. Chapter 4 investigates biomarkers of hypoxic stress in *P. clarkii* (hemolymph lactate, glucose, and protein concentrations) with laboratory and field hypoxia experiments. In chapter 5, I validate the use economical handheld devices for determination of *P. clarkii* hemolymph lactate and protein concentrations. Finally, Chapter 6 is a summary of the dissertation and its findings as well as recommendations for ARB crayfish and future research.

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CHAPTER 2

BIOTIC AND ABIOTIC EFFECTS ON RED SWAMP CRAYFISH *PROCAMBARUS CLARKII* POPULATION CHARACTERISTICS IN THE ATCHAFALAYA RIVER BASIN

Introduction

The crayfish industry in Louisiana is the largest in the United States (McClain et al. 2007) with a total value of more than \$209 million USD (LSUAC 2012). The red swamp crayfish *Procambarus clarkii* and the southern white river crayfish *Procambarus zonangulus* are harvested from managed ponds (farmed) and natural habitats (wild-caught) throughout Louisiana, with *P. clarkii* comprising the majority of landings. Farmed production of crayfish has greatly increased over the past several decades and extensive research has examined *P. clarkii* responses to environmental parameters in these controlled aquaculture environments. Conversely, there has been limited research into the ecological influences on *P. clarkii* populations from natural environments in Louisiana. Natural habitats often exhibit episodic water level fluctuations and extensive variability in biotic and abiotic factors that can affect crayfish harvest and population structure.

Over 90% of the wild crayfish harvest in Louisiana comes from the Atchafalaya River Basin (ARB) located in south-central Louisiana (Isaacs and Lavergne 2010; Figure 2.1). Crayfish harvesting in the ARB coincides with the annual Atchafalaya River flood pulse (Figure 2.2). Since 1980, Louisiana wild crayfish landings have averaged approximately 7 million kg annually. These landings are highly dependent on the annual water regime (Bryan et al. 1976; Konikoff 1977; Pollard et al. 1983; Alford and Walker 2011), which is the primary determinate of the magnitude of the wild crayfish harvest. However, the ARB hydroperiod varies substantially from year to year (Bonvillain et al. 2008). During the historic 1993 flood in the

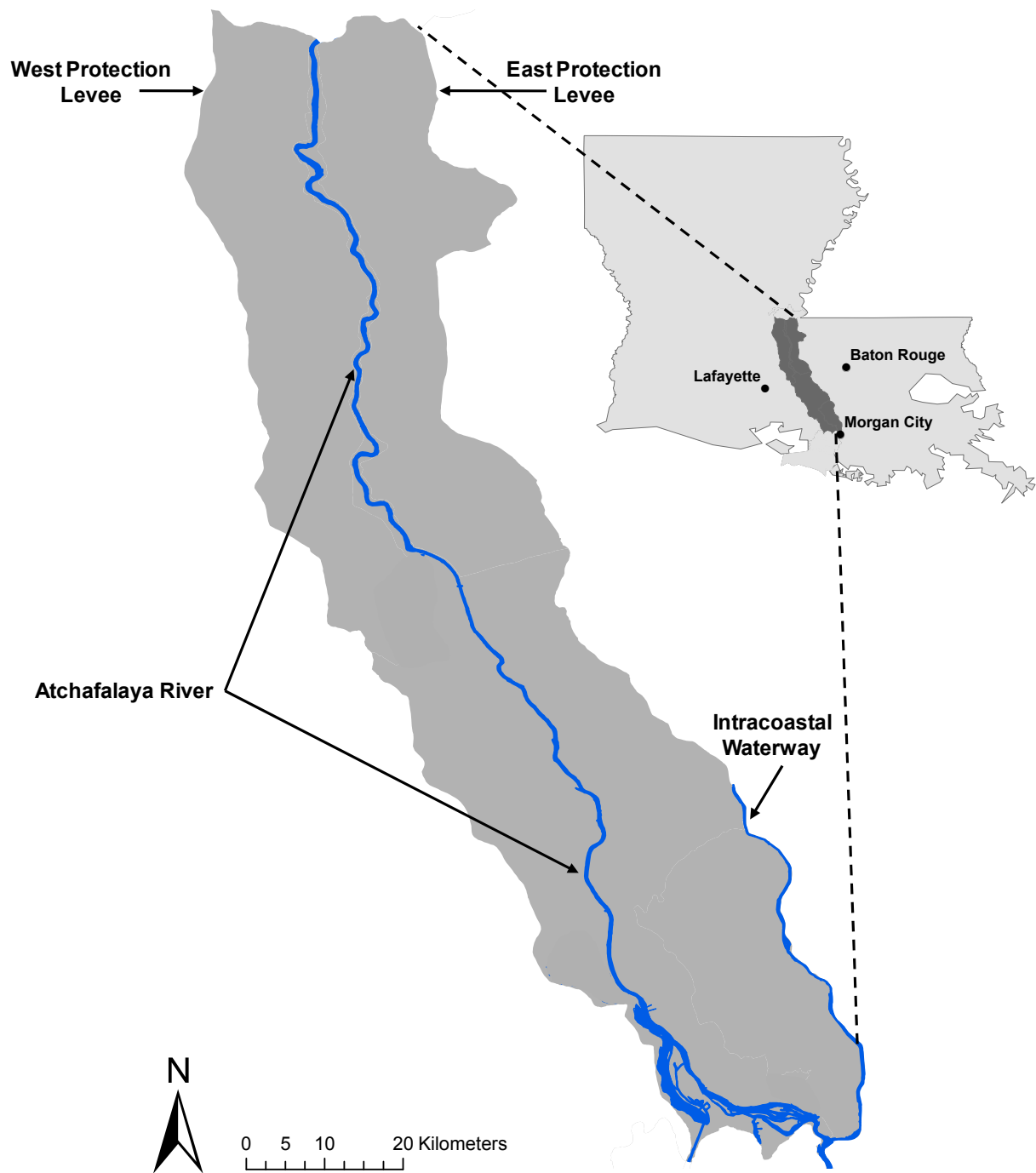


Figure 2.1. The Atchafalaya River Basin in south-central Louisiana.

Mississippi River, the annual crayfish yield was a record 22.5 million kg, whereas drought conditions during 2000 limited production to only 178,000 kg. This dependence on water level results in unpredictable annual harvests and fluctuations in market demands and prices (Dellenbarger and Luzar 1988). Furthermore, although there is no regulated crayfishing season, the harvesting period usually occurs when flood waters inundate floodplain habitats in the ARB. Rising floodplain waters are an environmental cue for crayfish to emerge from burrow habitats, with growth and mating typically occurring on the inundated floodplain. Conversely, when flood waters recede, crayfish will retreat to burrows where females fertilize their eggs with stored sperm and males and females persist during the dewatered period.

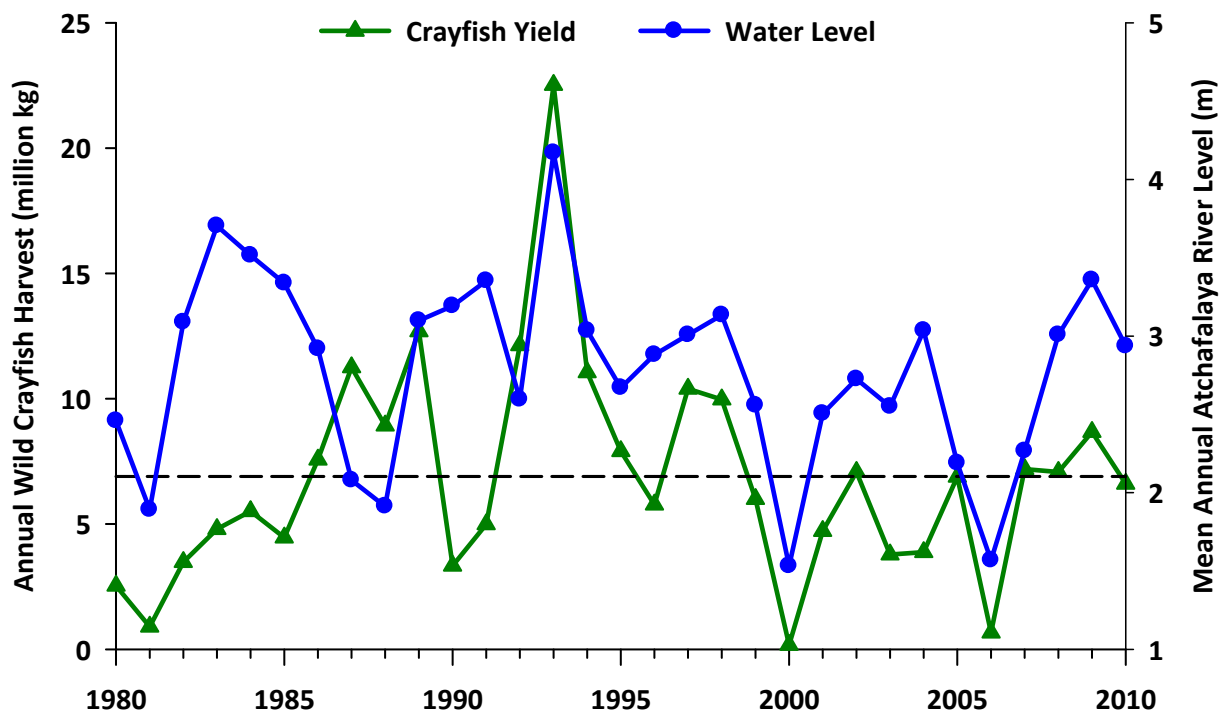


Figure 2.2. The Louisiana annual wild crayfish harvest (National Marine Fisheries Service) and mean annual Atchafalaya River level at Butte La Rose, Louisiana (U. S. Army Corps of Engineers recording gauge 03120) from 1980 to 2010. The dashed horizontal line represents the 30-year mean wild crayfish yield (6.9 million kg).

As floodplain water levels stabilize and temperatures increase in the ARB, high decomposition rates of floodplain organic matter reduces water column dissolved oxygen (DO) concentrations. Furthermore, anthropogenic modifications (levee and canal construction, distributary closures, and flood control structures) have altered the historic river-floodplain connectivity in the ARB. These modifications have increased sedimentation (Tye and Coleman 1989; Hupp et al. 2008) and reduced water circulation and flow patterns, often prompting the formation of hypoxic conditions ($\text{DO} \leq 2.0 \text{ mg/L}$) that can persist for several weeks to months across much of the floodplain (Sabo et al. 1999).

P. clarkii are tolerant of lower oxygen concentrations compared to other crayfishes (Nyström 2002) and have developed several behavioral and physiological adaptations such as utilization of atmospheric air, hypometabolism, bradycardia, and hyperventilation to cope with periods of sub-optimal DO concentrations (McMahon 1986; Reiber 1995). Although compensatory mechanisms afford *P. clarkii* the ability to tolerate hypoxic conditions, prolonged exposure could lead to detrimental population effects such as reduced survival (Avault et al. 1975; Melancon and Avault 1977; McClain 1999; Sladkova and Kholodkevich 2011), growth (Jussila and Evans 1997; McClain 1999; Reynolds 2002; McClain et al. 2007), and minimum size at maturity (Huner and Romaine 1978). Additionally, aquatic macrophyte density and composition may also influence crayfish population characteristics. Hydrophytes that enhance ARB hypoxia by reducing water circulation and increasing benthic decomposition rates may serve as daytime local DO refugia (Miranda et al. 2000; Troutman et al. 2007; Bunch et al. 2010) for crayfish inhabiting hypoxic areas. Crayfish density has been shown to have a positive correlation with macrophyte density (Jordan et al. 1996a) and plant beds provide crayfish with

beneficial forage and shelter (Jordan et al. 1996b; Cronin et al. 2002; Harper et al. 2002; Garvey et al. 2003; Foster and Harper 2006).

Minimal harvest regulations and management coupled with limited research have limited our understanding of the environmental factors that structure ARB crayfish populations. Currently, excluding water level influences, it is difficult to elucidate inter-annual harvest differences and intra-annual population variability among habitats. Furthermore, without adequate empirical knowledge of environmental influences on crayfish assemblages, it is difficult to assess the effects of federal and state restoration and management projects aimed at improving ecosystem function in the ARB on crayfish populations and harvests. Therefore, the purpose of this research was to examine the effects of biotic and abiotic parameters on population characteristics of *P. clarkii* in the ARB.

Study Area

The 5,000 km² ARB is the largest bottomland hardwood river-floodplain system in North America (Lambou 1990) and is comprised of shallow headwater and backwater lakes, numerous natural bayous and excavated petrochemical canals, and seasonally flooded swamps. The Atchafalaya River, the dominant feature of the ARB and the major tributary of the Mississippi River, receives 30% of the combined volumes of the Mississippi and Red Rivers. The U.S. Army Corps of Engineers regulates the amount of water entering the system through the Atchafalaya River with various water control structures. Although the annual timing, magnitude, and duration of the flood pulse varies, typically the ARB is inundated in the spring with dewatering occurring throughout the summer to early fall (Denes and Bayley 1983; Lambou 1990; Fontenot et al. 2001; Bonvillain et al. 2008). Development of spatially extensive and temporally persistent hypoxia (Sabo et al. 1999; Rutherford et al. 2001) is associated with

floodplain inundation and rising water temperatures (Kaller et al. 2011). Additionally, invasive aquatic macrophytes such as water hyacinth *Eichhornia crassipes*, hydrilla *Hydrilla verticillata*, alligator weed *Alternanthera philoxeroides*, and salvinia *Salvinia* spp. dominate the ARB aquatic macrophyte community and contribute to water quality impairment (Walley 2007).

Methods

Sample Locations

Sixteen locations within a 40 km² area of the southeastern ARB were selected for intensive crayfish sampling (Figure 2.3). Sample locations included habitats that are typically found throughout the lower ARB and exhibit variable morphological characteristics, hydrologic connectivity, physicochemistry, and macrophyte density and composition. Extensive sampling locations ($n = 11$ in 2008 and $n = 14$ in 2009) were sampled throughout the southeastern ARB during the 2008 and 2009 crayfish seasons (Figure 2.4) and exhibited habitat diversity similar to that of the intensive locations. Extensive sites were located south of Grand River, north of Duck Lake, and east of Grand Lake.

Field Collections

Crayfish were sampled semimonthly at the 16 intensive sites from 6 March to 11 November 2008 and from 13 February to 10 August 2009. However, I limited the data analyses to sample dates during the crayfish “season” which were from 6 March to 12 August 2008 and from 2 March to 13 July 2009. The crayfish “season” was established when the Atchafalaya River stage at Butte La Rose was greater than 2.5 m. At this level, floodplain habitats in the study area began to experience overbank flooding, similar to observations documented by Hupp et al. (2008) in the ARB (2.8 m). Five pillow design traps per site were baited with 150 g (Beecher and Romaine 2010) of Purina Cajun World™ manufactured bait and allowed to fish

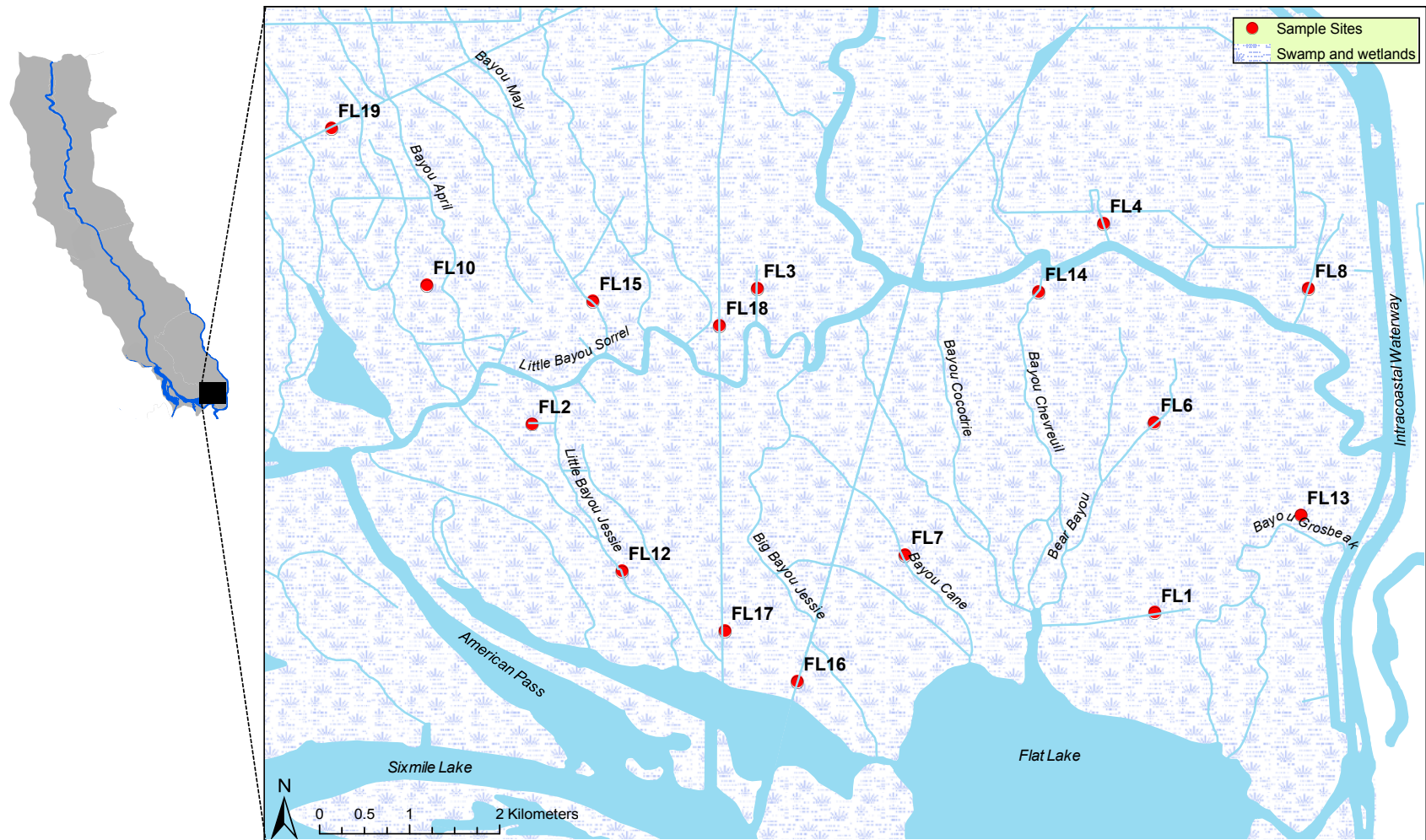


Figure 2.3. Intensive crayfish sampling locations ($n = 16$) in the southeastern Atchafalaya River Basin.

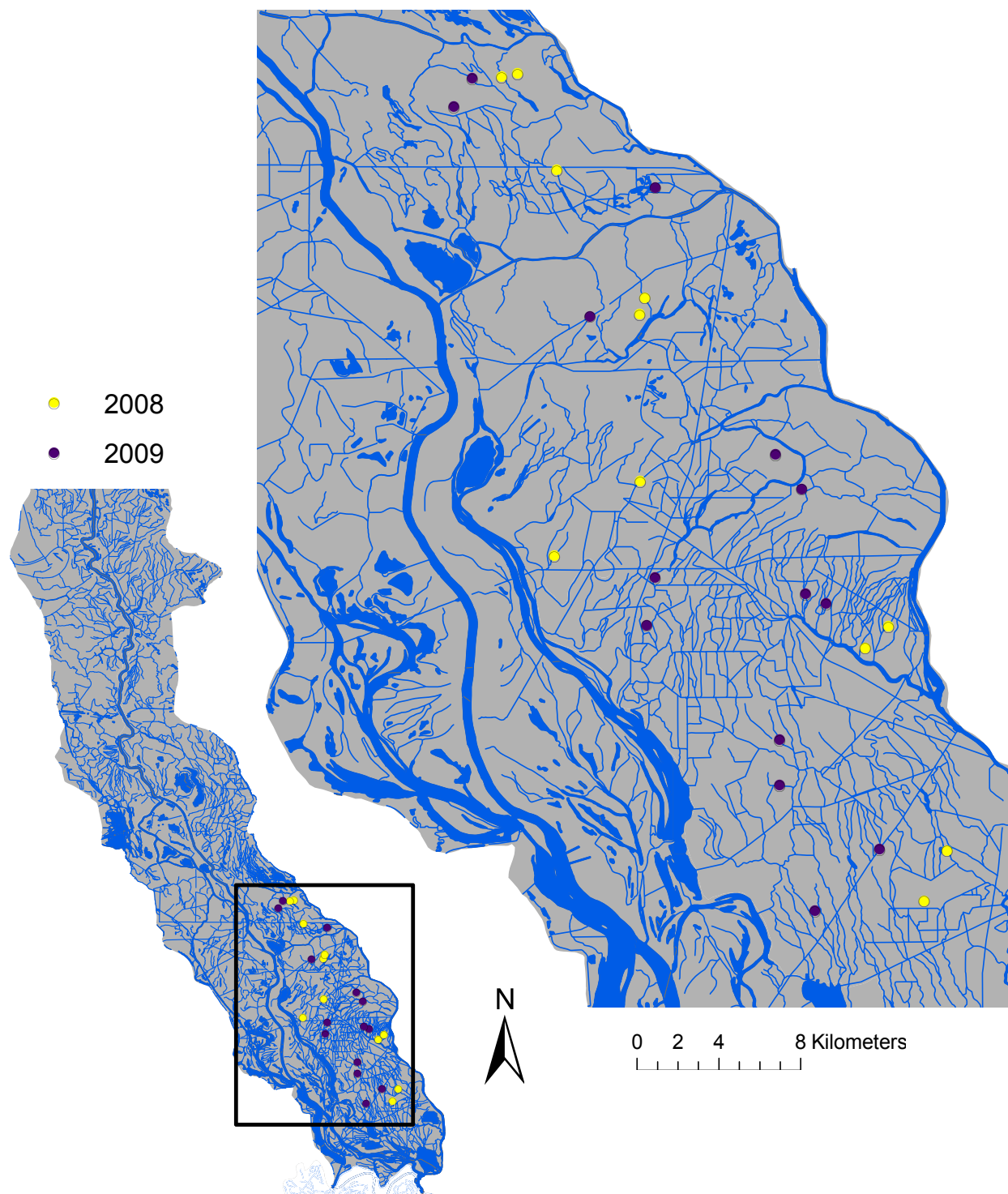


Figure 2.4. Extensive crayfish sampling locations in the southeastern Atchafalaya River Basin during the 2008 ($n = 11$) and 2009 ($n = 14$) crayfish seasons.

overnight. Traps were constructed from 0.772-cm galvanized square wire mesh and were 122 cm in height with two 5-cm funnel openings on the bottom (Figure 2.5). Commercial crayfish traps (1.905 cm mesh) generally retain market size individuals that have carapace lengths ≥ 37 mm, but I used a smaller mesh size in order to sample a greater number of juvenile crayfish. Traps were deployed at least 20 m apart on the floodplain and were secured to the surrounding trees or suitable vegetation. All captured crayfish were identified to species and I recorded sex, reproductive form (males only), and standard carapace length (mm) for all individuals. Standard carapace length is approximately 50% of total length (Romaine et al. 1977) and was measured with digital calipers from the tip of the rostrum to the posterior edge of the carapace. Cambaridae crayfishes exhibit cyclic dimorphism and sexually mature Form I males were identified by calcified tips on the first pair of gonopods and distinct copulatory hooks on the ischia of the third and fourth pair of pleopods (see Scholtz 2002; Walls 2009). Physical changes associated with reproductive form are not as apparent in female crayfish, so determination of reproductive form was limited to males.

On each sampling date, I measured surface DO (mg/L), temperature ($^{\circ}\text{C}$), pH, specific conductance (mS/cm), and turbidity (NTU) at every sample site with a handheld multiparameter water quality sonde (YSI model 6820, Yellow Springs, Ohio). Bottom and middle water column physicochemical measurements were taken when water depth ≥ 1 m. Additionally, in 2010 a continuous recording multiparameter water quality sonde (YSI model 6600, Yellow Springs, Ohio) was deployed at a normoxic location (site 12; $29^{\circ}46'$ N, $91^{\circ}14'$ W) from 12 March to 29 July and a chronically hypoxic location (site 3; $29^{\circ}48'$ N, $91^{\circ}14'$ W) from 13 April to 29 July to record DO fluctuations throughout the ARB flood pulse. Daily stage of the Atchafalaya River



Figure 2.5. Pillow design crayfish trap used during sampling. Traps were constructed from 0.772-cm galvanized wire mesh and were 122 cm in height with two 5-cm funnel openings on the bottom.

was obtained from the U.S. Army Corps of Engineers recording gauge located at Butte La Rose, Louisiana (gauge 03120, 30°16'57" N, 91°41'17" W). Aquatic macrophyte relative density within a 1 m radius of every trap was recorded on each sample date. Relative densities recorded at all traps within a site were then averaged to obtain a mean macrophyte density per site.

Water samples for metals analysis were collected at all sample locations on every sample date. Water samples were filtered through a 0.45 µm filter into 20 mL glass scintillation vials that had been washed in a dilute acid bath and rinsed with deionized water. Water samples were acidified with 4-5 drops of 8N trace metal grade nitric acid (HNO₃) in order to reduce the pH level below 2.0 to keep the metals in solution until analysis. Water samples were taken to the Wetland Biogeochemistry Institute at Louisiana State University for metals analysis. Digested samples were diluted to volume and analyzed with inductively coupled argon plasma emission spectrometry (Vista-MPX, CCD Simultaneous ICP-OES).

Data Analysis

Daily Atchafalaya River stage was obtained from the U. S. Army Corps of Engineers recording gauge located at Butte La Rose, Louisiana. Analysis of variance (ANOVA; PROC MIXED) was used to evaluate differences in the historic monthly mean Atchafalaya River stage from 1959 to 2010 with monthly stage during the 2008 and 2009 crayfish season. I used ANOVA with a Tukey-Kramer *post hoc* adjustment to compare 2008 and 2009 monthly stages during the crayfish season.

For all analyses, DO was square root transformed, turbidity was $\log_{(e)}(\text{turbidity} + 1)$ transformed, and catch per unit effort (CPUE) was $\log_{(e)}(\text{CPUE} + 1)$ transformed to more closely conform to the normal distribution. To evaluate potential differences in water column physicochemistry, I compared surface and bottom DO and temperature ($\log_{(e)}$ transformed)

measurements in separate mixed model ANOVAs. An inter-annual difference in DO concentration between sample years was evaluated with ANOVA. All statistical analyses were performed with SAS 9.2 and significance for all tests was determined using a 0.05 Type I error rate.

In order to obtain a predictive model that best approximated biotic and abiotic influences on *P. clarkii* carapace length and CPUE during the 2008 and 2009 crayfish seasons, I used the ALLMIXED2 SAS macro (Fernandez 2007) to determine an appropriate covariance structure and perform general linear mixed model selection. To improve the formulation of candidate models and minimize the inclusion of extraneous variables, explanatory variables were selected *a priori* such that models included only well-founded biological and ecological predictors (Burnham and Anderson 2002; Burnham et al. 2011). Explanatory variables included DO, temperature, turbidity, surface macrophyte relative density, and subsurface macrophyte relative density. Surface macrophytes included both floating and emergent species whereas subsurface macrophytes comprised all submersed species. A compound symmetry covariance structure was used for all models. In addition to statistical fit, ecological relevance and parsimony were considered when selecting the covariance structure (Gutzwiller and Riffell 2007). Compound symmetry fit the data adequately and assumed responses measured from the same site were equally correlated regardless of the time between samples, requiring estimation of only two additional model parameters. Akaike's information criterion corrected for small sample sizes (AIC_c) was used to rank and select the best candidate models. Delta AIC_c (ΔAIC_c), Akaike weights (w_i), and evidence ratios were used to compare models and to determine the best fit model. The ΔAIC_c is calculated as the difference between the AIC_c values for the selected model and the model with the best fit. Models with $\Delta AIC_c < 2$ have substantial support (Burnham and

Anderson 2002), therefore, models with $\Delta AIC_c > 2$ were excluded from consideration. Akaike weight is the relative likelihood of the model given the data and is interpreted as the probability that a particular model is the best approximating model in the candidate set (Burnham and Anderson 2002; Symonds and Moussalli 2011). Akaike weights are normalized to sum to one such that the data clearly support models with weights close to 1 (Johnson and Omland 2004). Evidence ratios provide a measure of how much more likely the best model is than the selected model. The independent variables that best predicted 2008 and 2009 carapace length and CPUE were then used to fit mixed multiple regression models (PROC MIXED) with restricted maximum likelihood and to compare parameter estimates among the models.

Inter-annual differences in mean *P. clarkii* carapace length and CPUE were also examined with separate ANOVAs. Simple linear regression (PROC REG) was used to examine the relationship of DO concentrations with *P. clarkii* carapace length and CPUE for both sample years. To examine variability in water quality among intensive sampling locations, I conducted a principal component analysis (PCA) on DO, temperature, pH, specific conductance, and turbidity for each sample year. I retained principal components (PC) with eigenvalues greater than one and physicochemical variables with eigenvectors greater than 0.4 (Hardle and Simar 2007). Site scores on PC1 and PC2 were plotted for each year to illustrate clustering of sites with similar physicochemical characteristics. *P. clarkii* carapace length and CPUE for total catch were compared among four apparent physicochemical site groupings for each sample year with separate ANOVAs and Tukey-Kramer *post hoc* adjustments.

Additionally, I wanted to investigate the effects of chronic hypoxia exposure and macrophyte density on *P. clarkii* population characteristics in the ARB. Intensive sample locations were classified as either normoxic or chronically hypoxic based on the number of

sample dates that hypoxia was detected at each site during the crayfish season. Normoxic sites exhibited hypoxic conditions on ≤ 3 sample dates whereas hypoxic sites exhibited hypoxia on ≥ 6 sample dates. To investigate the effect of macrophyte density, sites were separated into three groups based on relative macrophyte density: low (0 – 33%), medium (34 – 66%), and high (67 – 100%). Separate ANOVAs with Tukey-Kramer *post hoc* adjustments were performed to compare differences in carapace length and CPUE among physicochemical groups and macrophyte density groups for both sample years.

In order to investigate possible intraspecific density-dependent growth in sampled *P. clarkii* populations, I compared carapace length with CPUE for each sample year. However, because *P. clarkii* movement and growth increase as temperatures warm, I grouped individuals into either early or late season groups. The early groups consisted of individuals captured on the first five sample dates of the crayfish season (i.e., 6 March to 22 May 2008 and 2 March to 28 April 2009) while the late groups comprised the final five sample dates (i.e., 10 June to 12 August 2008 and 14 May to 13 July 2009). During both sample years, the early group was characterized by increasing crayfish CPUE whereas the late group demonstrated declining CPUE values after the peak season yield. Zero CPUE sites were omitted from analysis and linear regression was used to examine the relationship between carapace length and CPUE for each season group in 2008 and 2009. Additionally, I compared differences in carapace length and CPUE among sites for both sample years with separate ANOVAs and *post hoc* Tukey-Kramer adjustments.

Differences in carapace length and CPUE for market size individuals (≥ 37 mm carapace length) between sample years, between hypoxic and normoxic site groups, and among physicochemical site groups for 2008 and 2009 were examined with ANOVAs and Tukey-

Kramer *post hoc* adjustments. Additionally, the same analyses were performed on the carapace length of Form I males to determine effects on minimum size at maturity.

Intra-annual differences in *P. clarkii* CPUE and carapace length between intensive and extensive locations in 2008 and 2009 were determined with separate ANOVAs. Finally, to examine possible latitudinal variability in *P. zonangulus* distribution, I regressed the relative abundance of *P. zonangulus* in the total catch on decimal degree latitude from the extensive survey sites.

Results

Water Level and Physicochemistry

The 2008 Atchafalaya River flood pulse inundated floodplain habitats for over six months, reaching its peak in late April with slowly declining levels lasting to August (Figure 2.6). The 2009 flood pulse exhibited a lower magnitude and duration compared to 2008, however, water levels were sufficiently high enough to inundate floodplain areas for approximately five months. Peak water levels occurred in early June with a rapid drawdown through July (Figure 2.6). Comparisons of the 2008 hydrograph during the crayfish season (March – August) and the 52-year mean monthly Atchafalaya River stage indicated higher monthly stages in April ($P = 0.0090$) and July ($P = 0.0039$; Figure 2.6). Atchafalaya River stages during the 2009 crayfish season (March – July) were lower in March ($P < 0.0001$) and higher in June ($P = 0.0344$; Figure 2.6). Monthly comparisons between the two sample years demonstrated higher March ($P < 0.0001$), April ($P < 0.0001$), and July ($P < 0.0001$) stages in 2008.

Analysis of surface and bottom temperature ($F_{1,202} = 0.07$, $P = 0.7856$) and DO ($F_{1,202} = 0.12$, $P = 0.7302$) concentrations indicated homogeneous water column physicochemistry, thus

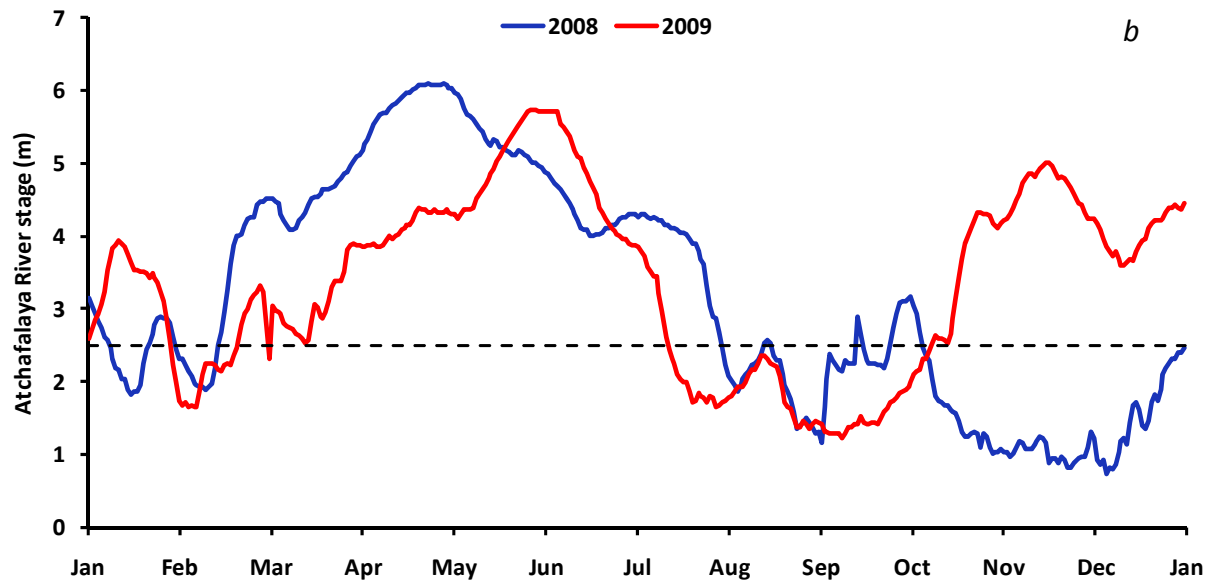
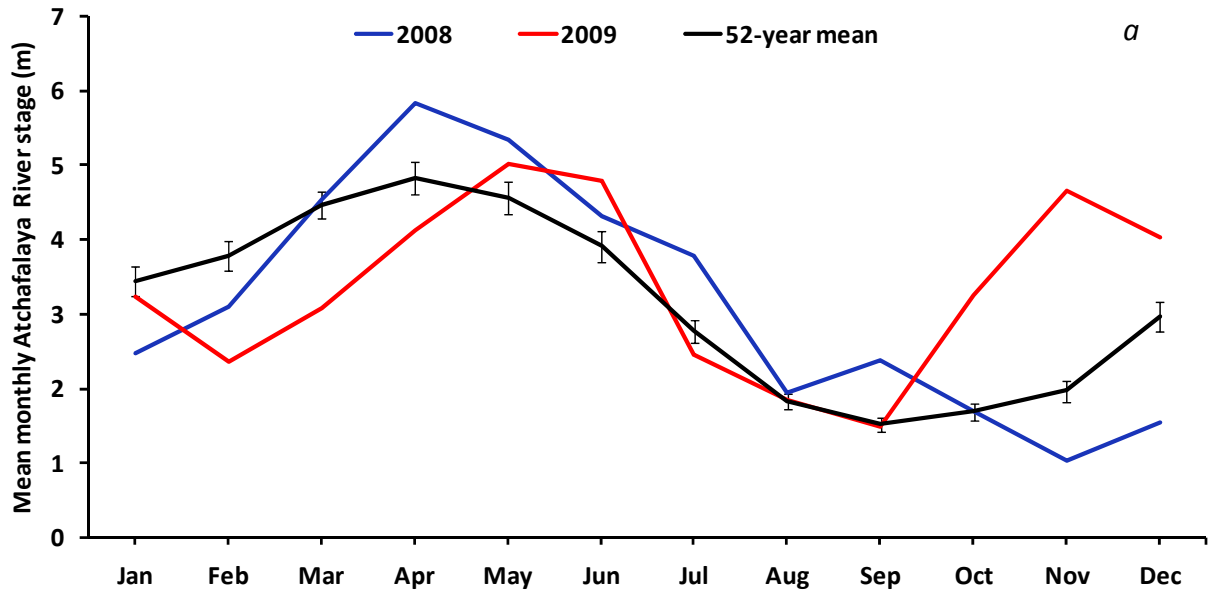


Figure 2.6. Mean monthly (a) and daily (b) Atchafalaya River stage at Butte La Rose, Louisiana (U.S. Army Corps of Engineers gauge 03120) during the 2008 and 2009 sample years. The black line on the mean monthly graph (a) represents the 52-year mean stage (\pm SE) from 1959-2010. The horizontal dashed line on the daily stage graph (b) represents floodplain inundation level (2.5 m) in the study area.

only surface water quality observations were used for all analyses. Comparison of inter-annual DO concentrations at intensive sample locations did not reveal a significant difference between 2008 (1.70 ± 0.05 mg/L) and 2009 (1.70 ± 0.05 mg/L; $F_{1,314} = 0.00$, $P = 0.9567$). Slight peaks in DO occurred at the end of the 2009 season (Figure 2.7). The response of DO concentrations in the ARB to fluctuating Atchafalaya River stages is evident in Figure 2.8. During the 2010 flood pulse, DO concentrations at site 12, a typically normoxic location, decreased with falling river stages as the site received an influx of hypoxic water from the dewatering floodplain and increased with rising stages that brought in cooler, oxygenated mainstem waters (Figure 2.8). Conversely, DO concentrations at site 3, a chronically hypoxic location, increased with receding water levels as hypoxic waters drained and the site received higher inputs from surrounding bayous and canals (Figure 2.8). Metal concentrations in water samples from intensive sampling locations did not display any discernible trends, and ordination techniques failed to demonstrate any spatial or temporal groupings, although most metals increased slightly in concentration as Atchafalaya River levels declined. A large spike in metal concentrations was observed on 10 September 2008, nine days after the passage of Hurricane Gustav over the ARB on 1 September.

Crayfishes

I collected 20,612 crayfishes representing six species from intensive and extensive sampling in 2008 and 2009 (Table 2.1). *P. clarkii* comprised 98% of the total crayfish catch, *Orconectes lancifer* 1.4%, *P. zonangulus* 0.4%, and the remaining 0.2% consisted of *Orconectes palmeri* ssp., *Cambarellus* spp., and *Cambarus ludovicianus*. *O. palmeri* were only collected on two sample dates (10 and 24 September) at two intensive study sites (12 and 13) outside of the crayfish season in 2008. Unfortunately, no Form I males were collected; however, specimens had red abdominal bands and were either *O. p. palmeri* or *O. p. creolanus*. One female *Cambarus ludovicianus* was collected each year at intensive sites on 9 July 2008 (site 17) and 25

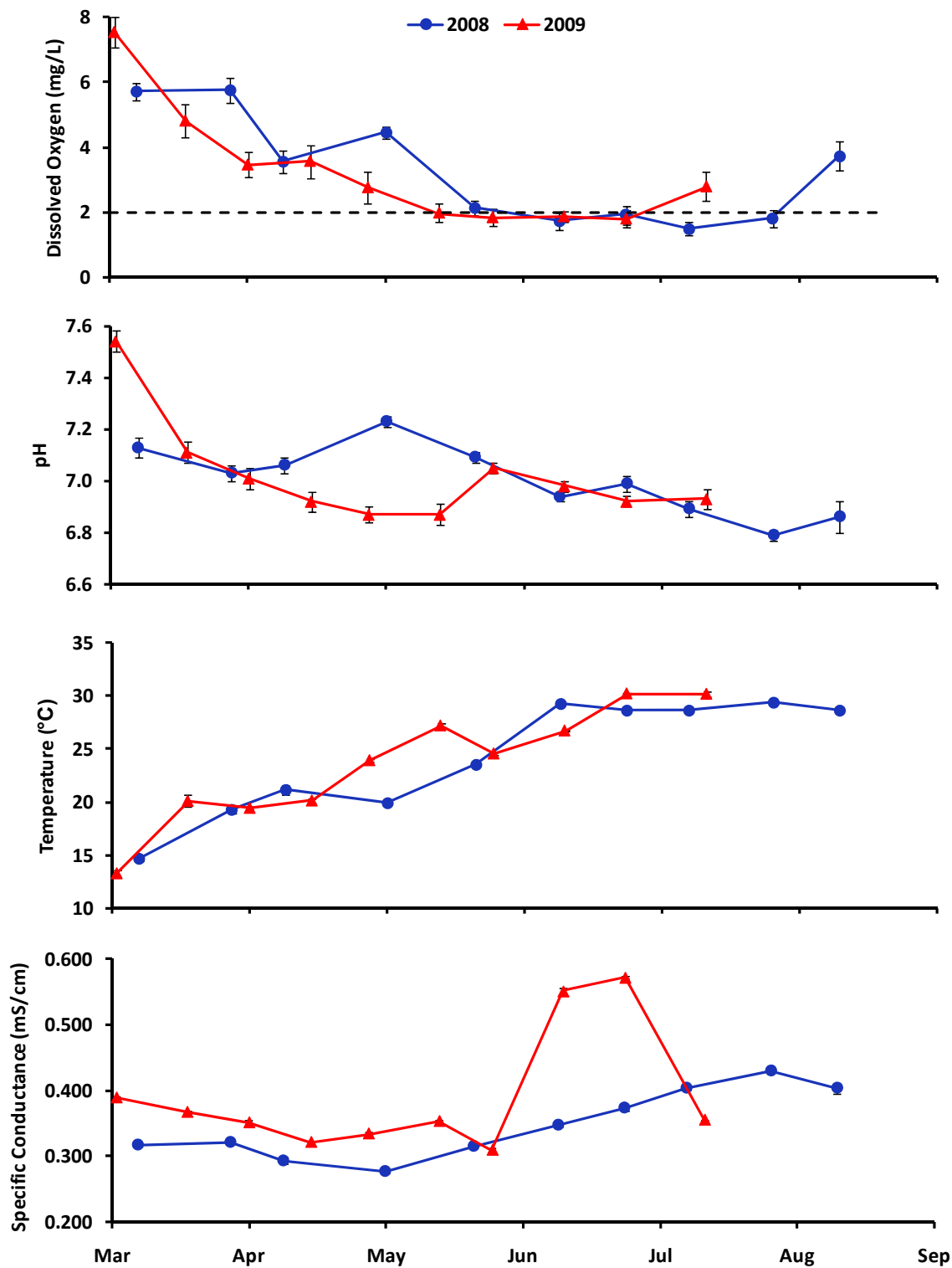


Figure 2.7. Mean (\pm SE) physicochemical measurements from intensive sample locations ($n = 16$) in the lower Atchafalaya River Basin during the 2008 and 2009 crayfish seasons. The dashed horizontal line on the dissolved oxygen graph indicates hypoxic level (dissolved oxygen ≤ 2 mg/L).

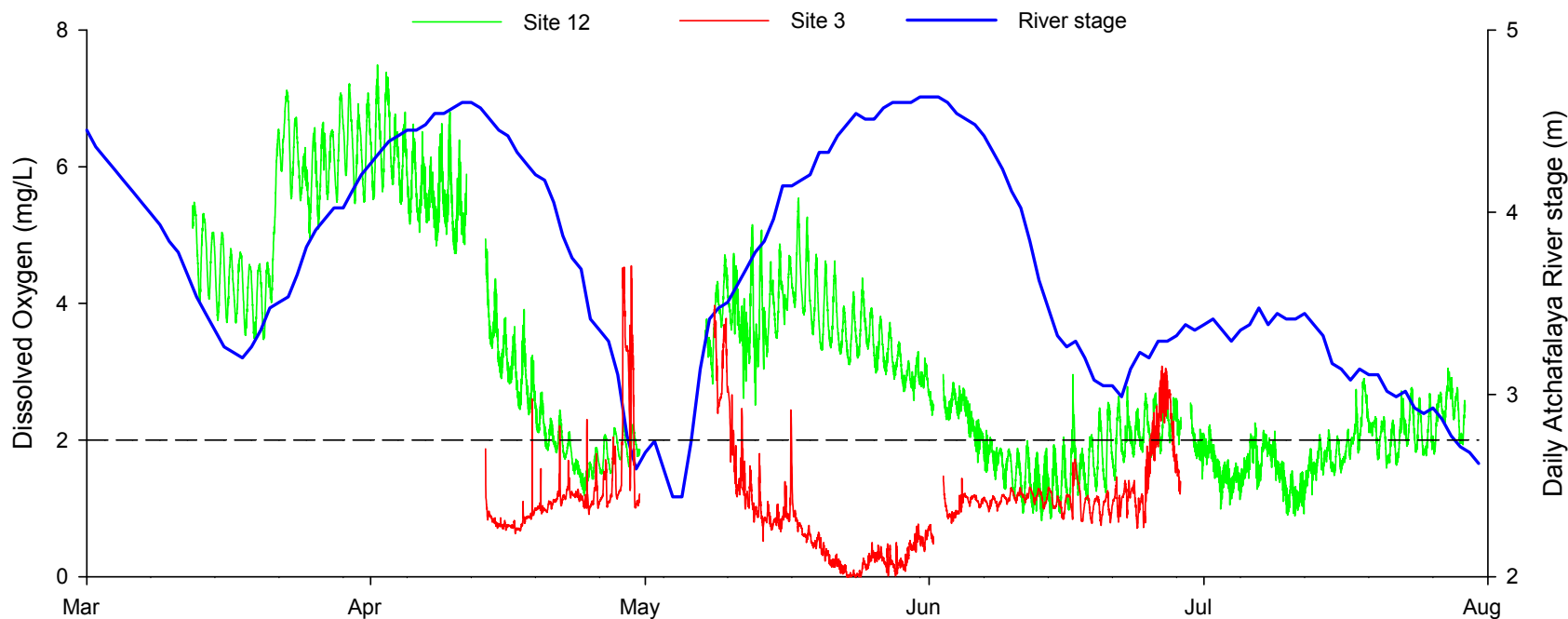


Figure 2.8. Quarter-hourly dissolved oxygen measurements at two aquatic habitats in the southeastern Atchafalaya River Basin and daily Atchafalaya River stage (blue) at Butte La Rose, Louisiana (U.S. Army Corps of Engineers gauge 03120) during the 2010 flood pulse. Site 12 (green) is a frequently normoxic habitat whereas site 3 (red) is a chronically hypoxic habitat. The dashed horizontal line indicates hypoxic level (dissolved oxygen ≤ 2 mg/L).

Table 2.1. Relative abundance of crayfish species collected during the crayfish season from 6 March to 12 August 2008 and 2 March to 13 July 2009 at intensive and extensive sampling locations. Numbers in parenthesis are crayfish collected outside of crayfish season (intensive survey only).

Species	2008	2009	Total
<i>Procambarus clarkii</i>			
Intensive	2,221 (154)	15,324 (462)	17,545 (616)
Extensive	908	1,139	2,047
<i>Orconectes lancifer</i>			
Intensive	33 (60)	72 (99)	105 (159)
Extensive	0	28	28
<i>Procambarus zonangulus</i>			
Intensive	0	0	0
Extensive	63	25	88
<i>Orconectes palmeri</i> ssp. ¹			
Intensive	0 (14)	0	0 (14)
Extensive	0	0	0
<i>Cambarellus</i> spp. ²			
Intensive	0	4 (3)	4 (3)
Extensive	0	1	1
<i>Cambarus ludovicianus</i>			
Intensive	1	1	2
Extensive	0	0	0

¹*O. palmeri* collected had red abdominal bands and are either *O. p. palmeri* or *O. p. creolanus*.

²Only two species of *Cambarellus* are found in Louisiana, *C. puer* and *C. shufeldtii*.

June 2009 (site 16). *P. zonangulus* was never collected during intensive sampling and was only collected at eight extensive sample locations north of 30°02'97" N latitude. The relative percent abundance of *P. zonangulus* in the total crayfish catch at these sites was positively related to latitude ($P = 0.0210$, $r^2 = 0.6161$; Figure 2.9).

Population Characteristics

During the 2008 crayfish season, 2,221 *P. clarkii* were collected at intensive sampling locations with 55% of the population male and 45% female. In 2009, 15,324 individuals were collected comprised of 51% male and 49% female. Three candidate models emerged that were most likely to explain 2008 *P. clarkii* CPUE ($\Delta AIC_c < 2$; Table 2.2). All three models included turbidity and surface macrophytes. Although the first model (turbidity, surface and subsurface macrophytes) had the lowest AIC_c and ΔAIC_c values, the third model, which included temperature, turbidity, and surface macrophytes, described the 2008 CPUE data adequately ($\Delta AIC_c = 0.70$, $w_i = 0.22$, evidence ratio = 1.42) and was chosen based on personal observations during data collection. The predictive model for 2008 CPUE is:

$$\log_{(e)}(CPUE+1) = 2.5530 + 0.0236(temperature) - 0.2692(\log_{(e)}[turbidity+1]) - 0.4687(surface)$$

The only approximating model for 2008 *P. clarkii* carapace length with a $\Delta AIC_c < 2$ was a positive relationship with temperature (Table 2.3). The predictive model for 2008 carapace length is:

$$carapace\ length = 25.9023 + 0.8112(temperature)$$

The top five approximating models for 2009 CPUE all included an association with DO (Table 2.4). Although the one candidate model with a sufficiently small AIC_c value included DO only,

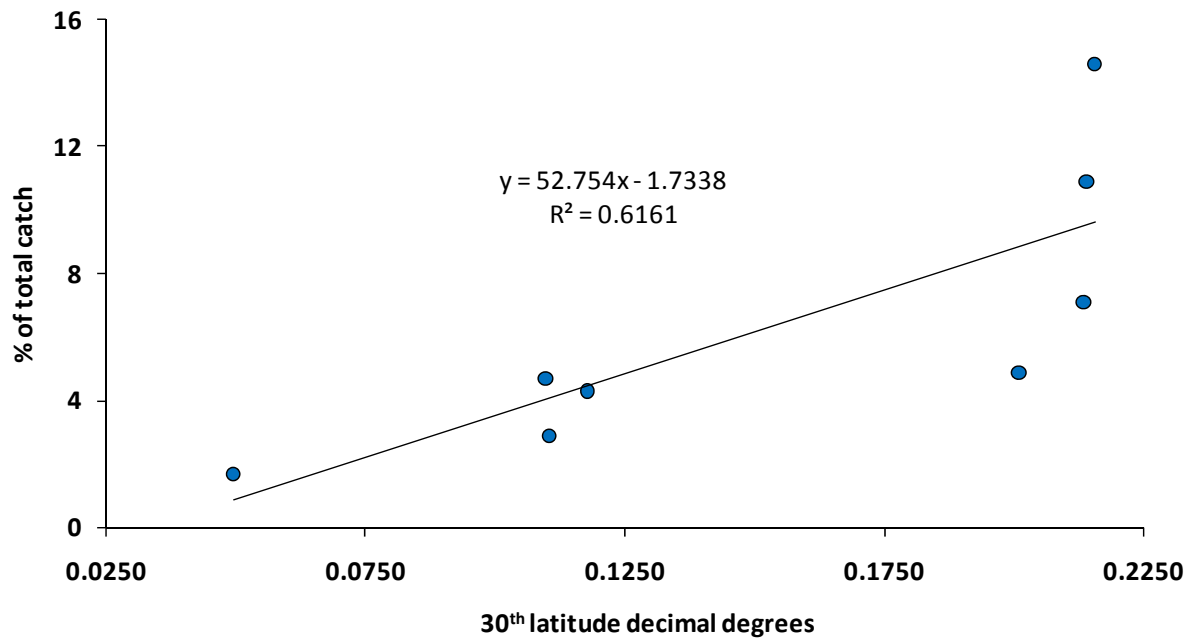


Figure 2.9. The relationship between latitude and the percent of *Procambarus zonangulus* in the total crayfish catch at extensive sampling sites in the Atchafalaya River Basin during 2008 and 2009.

Table 2.2. The five highest ranked, full, and null models using AIC-based model selection to explain *Procambarus clarkii* catch per unit effort at intensive sampling locations in the lower Atchafalaya River Basin during the 2008 crayfish season. AIC for small sample sizes (AIC_c), AIC_c differences (ΔAIC_c), Akaike weights (w_i), and evidence ratios for each model are also provided.

Model	AIC_c	ΔAIC_c	w_i	Evidence ratio
logturbid + surface + subsurface	261.51	0	0.32	1.0
logturbid + surface	262.14	0.63	0.23	1.37
temp + logturbid + surface	262.21	0.70	0.22	1.42
logturbid + subsurface	264.91	3.40	0.06	5.48
temp + logturbid + surface + subsurface	265.63	4.13	0.04	7.87
Full	273.98	12.47	0	> 10
Null	299.45	37.94	0	> 10

Note: Model parameters include the square root of dissolved oxygen (\sqrt{DO}), temperature (temp), $\log_e(\text{turbidity}+1)$ (logturbid), surface macrophytes (surface), and subsurface macrophytes (subsurface). The full model is fitted with all parameters while the null model includes only the intercept.

Table 2.3. The five highest ranked, full, and null models using AIC-based model selection to explain *Procambarus clarkii* size (carapace length) at intensive sampling locations in the lower Atchafalaya River Basin during the 2008 crayfish season. AIC for small sample sizes (AIC_c), AIC_c differences (ΔAIC_c), Akaike weights (w_i), and evidence ratios for each model are also provided.

Model	AIC_c	ΔAIC_c	w_i	Evidence ratio
temp	931.59	0	0.58	1.0
temp + subsurface	934.74	3.15	0.12	4.82
\sqrt{DO} + temp	934.88	3.29	0.11	5.18
temp + logturbid	935.93	4.34	0.07	8.74
temp + surface	935.95	4.36	0.07	8.84
Full	954.35	22.76	0	> 10
Null	1019.75	88.16	0	> 10

Note: Model parameters include the square root of dissolved oxygen (\sqrt{DO}), temperature (temp), $\log_e(\text{turbidity}+1)$ (logturbid), surface macrophytes (surface), and subsurface macrophytes (subsurface). The full model is fitted with all parameters while the null model includes only the intercept.

Table 2.4. The five highest ranked, full, and null models using AIC-based model selection to explain *Procambarus clarkii* catch per unit effort at intensive sampling locations in the lower Atchafalaya River Basin during the 2009 crayfish season. AIC for small sample sizes (AIC_c), AIC_c differences (ΔAIC_c), Akaike weights (w_i), and evidence ratios for each model are also provided.

Model	AIC_c	ΔAIC_c	w_i	Evidence ratio
\sqrt{DO}	424.47	0	0.59	1.0
$\sqrt{DO} + \text{logturbid}$	427.42	2.96	0.14	4.38
$\sqrt{DO} + \text{subsurface}$	428.34	3.88	0.09	6.94
$\sqrt{DO} + \text{temp}$	428.70	4.23	0.07	8.31
$\sqrt{DO} + \text{surface}$	428.83	4.36	0.07	8.86
Full	447.68	23.22	0	> 10
Null	453.27	28.80	0	> 10

Note: Model parameters include the square root of dissolved oxygen (\sqrt{DO}), temperature (temp), $\log_{(e)}(\text{turbidity}+1)$ (logturbid), surface macrophytes (surface), and subsurface macrophytes (subsurface). The full model is fitted with all parameters while the null model includes only the intercept.

the model exhibited a reasonably high Akaike weight ($w_i = 0.59$). The predictive model for 2009 CPUE is:

$$\log_{(e)}(CPUE+1) = 1.6204 - 0.2591(\sqrt{DO})$$

Similar to 2009 CPUE, the top estimated models of 2009 carapace length displayed an association with DO (Table 2.5). The only model to have a $\Delta AIC_c < 2$ was comprised of DO, temperature, and turbidity and displayed a high Akaike weight ($w_i = 0.85$). The predictive model for 2009 carapace length is:

$$carapace\ length = 34.0343 - 3.7414(\sqrt{DO}) + 0.4868(temperature) + 1.4330(\log_{(e)}[turbidity+1])$$

The parameter estimates and their associated t-tests for multiple regression models are given in Table 2.6.

Comparisons of inter-annual *P. clarkii* CPUE at intensive locations revealed a significantly higher CPUE in 2009 (21.09 ± 1.88) than in 2008 (3.14 ± 0.26 ; $F_{1,312} = 149.13$, $P < 0.0001$; Figure 2.10). However, *P. clarkii* in 2008 were significantly larger (46.50 ± 0.18 mm) than individuals during the 2009 season (42.02 ± 0.05 mm; $F_{1,17543} = 805.99$, $P < 0.0001$; Figure 2.10). Intra-annual comparisons of *P. clarkii* CPUE during the 2008 crayfish season revealed a significantly higher CPUE at extensive locations (16.51 ± 5.47) than intensive sites (3.14 ± 0.26 ; $F_{1,167} = 27.87$, $P < 0.0001$; Figure 2.10), although carapace lengths at extensive (46.45 ± 0.23 mm) and intensive locations (46.50 ± 0.18 mm) were similar ($F_{1,3127} = 0.03$, $P = 0.8717$; Figure 2.10). During the 2009 crayfish season, a difference in CPUE was not detected between extensive (16.38 ± 3.21) and intensive locations (21.09 ± 1.88 ; $F_{1,168} = 0.02$, $P = 0.8982$), however, *P. clarkii* carapace length was significantly smaller at extensive locations (41.32 ± 0.22 mm) compared to intensive locations (42.02 ± 0.05 mm; $F_{1,16461} = 11.35$, $P = 0.0008$; Figure 2.10). Inter-annual comparisons of CPUE at extensive sites revealed that locations were not dissimilar ($F_{1,23} = 0.27$, $P = 0.6066$). Although minor variations in intra-annual physicochemical

Table 2.5. The five highest ranked, full, and null models using AIC-based model selection to explain *Procambarus clarkii* size (carapace length) at intensive sampling locations in the lower Atchafalaya River Basin during the 2009 crayfish season. AIC for small sample sizes (AIC_c), AIC_c differences (ΔAIC_c), Akaike weights (w_i), and evidence ratios for each model are also provided.

Model	AIC_c	ΔAIC_c	w_i	Evidence ratio
\sqrt{DO} + temp + logturbid	858.15	0	0.85	1.0
\sqrt{DO} + temp	863.54	5.39	0.06	14.83
\sqrt{DO} + temp + logturbid + surface	864.65	6.50	0.03	25.77
\sqrt{DO} + temp + logturbid + subsurface	864.67	6.52	0.03	26.03
temp	866.48	8.33	0	64.52
Full	873.12	14.97	0	> 70
Null	933.34	75.19	0	> 70

Note: Model parameters include the square root of dissolved oxygen (\sqrt{DO}), temperature (temp), $\log_{(e)}(\text{turbidity}+1)$ (logturbid), surface macrophytes (surface), and subsurface macrophytes (subsurface). The full model is fitted with all parameters while the null model includes only the intercept.

Table 2.6. Multiple regression parameter estimates and associated t-tests for *Procambarus clarkii* catch per unit effort (CPUE) and carapace length (CL) at intensive sample locations during the 2008 and 2009 crayfish seasons.

Model	Regressor	Parameter estimate	t value	P
2008 CPUE	temperature	0.0236	2.36	0.0194
	$\log_{(e)}(\text{turbidity}+1)$	-0.2692	-7.00	< 0.0001
	surface macrophytes	-0.4687	-2.94	0.0038
2008 CL	temperature	0.8112	11.37	< 0.0001
2009 CPUE	$\sqrt{\text{DO}}$	-0.2591	-3.16	0.0019
2009 CL	$\sqrt{\text{DO}}$	-3.7414	-3.97	0.0001
	temperature	0.4868	0.0863	< 0.0001
	$\log_{(e)}(\text{turbidity}+1)$	1.4330	3.32	0.0011

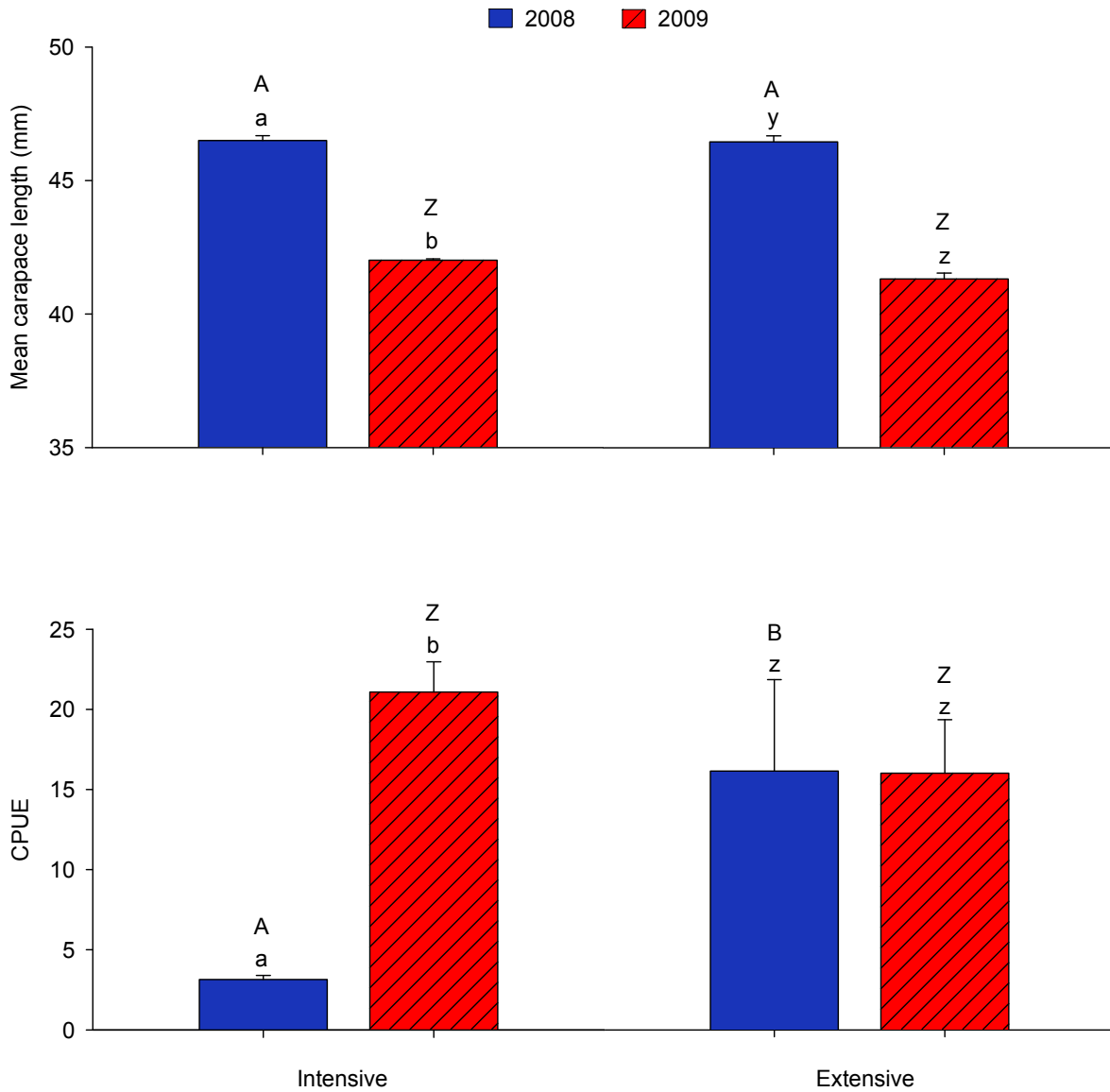


Figure 2.10. Mean (\pm SE) carapace length and catch per unit effort (CPUE) of *Procambarus clarkii* at intensive and extensive sample locations in the southeastern Atchafalaya River Basin during the 2008 and 2009 crayfish seasons. CPUE values were $\log_{(e)}(\text{CPUE}+1)$ transformed for analysis. Different lower case letters indicate a significant inter-annual difference ($P < 0.05$) within a population metric while different upper case letters indicate a significant intra-annual difference between intensive and extensive locations within a population metric for each sample year.

parameters between extensive and intensive locations were observed, these locations exhibited similar annual water quality responses (Figure 2.11). Inter-annual comparisons of extensive locations did not detect a difference in CPUE or carapace length between years.

The PCA of water quality parameters at intensive study sites in 2008 yielded two components with eigenvalues greater than 1.0 that explained 71% of the physicochemical variation among sample locations (Table 2.7). Turbidity, DO, and pH were positively correlated with PC1, whereas temperature was positively and specific conductance was negatively correlated with PC2. Plots of site scores on PC1 and PC2 demonstrated that physicochemical characteristics tracked spatial groupings closely (Figure 2.12). American Pass sites (2, 12, 16, and 17) were located in the southwest portion of the study area and received an influx of well-oxygenated mainstem water resulting in higher DO, pH, and turbidity. Flat Lake sites (1, 6, and 7) displayed higher temperatures and lower specific conductance measurements than other locations. Backwater sites (3, 10, 15, 18, and 19) were characterized by high specific conductance and low turbidity, pH and DO concentrations. Low DO water from the surrounding floodplain caused chronic hypoxia at these sites for the majority of the crayfish season. Intermediate sites (4, 8, 13, and 14) functioned as a transition zone between backwater areas and Flat Lake locations. These sites tended to have lower pH and DO concentrations than American Pass and Flat Lake sites but not as chronically low as backwater locations. In addition, DO concentrations at intermediate sites decreased from east to west with distance from Intracoastal Waterway influences. Comparisons of *P. clarkii* carapace length among the four physicochemical groups revealed significantly larger individuals from Flat Lake sites with the smallest individuals captured at backwater locations (Figure 2.13). Backwater sites also produced the lowest CPUE values while American Pass and Flat Lake sites yielded the highest

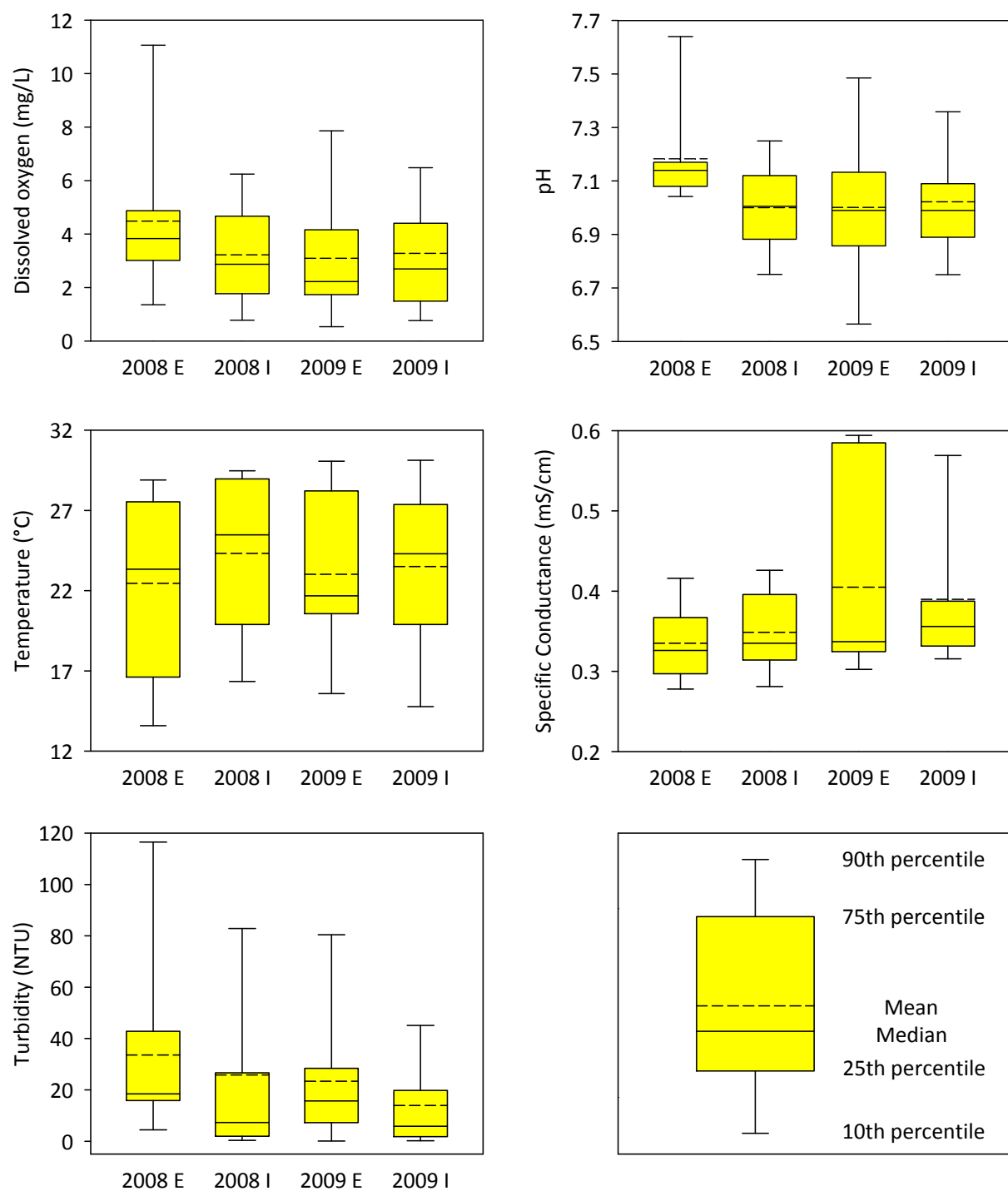


Figure 2.11. Box plots of physicochemical parameters during 2008 and 2009 intensive (I) and extensive (E) sampling in the Atchafalaya River Basin. The lower right plot provides a summary of the information provided by the box plots. Sample sizes: 2008 extensive ($n = 11$), 2008 intensive ($n = 160$), 2009 extensive ($n = 14$), and 2009 intensive ($n = 156$).

Table 2.7. Loadings of physicochemical variables from intensive sampling locations ($n = 16$) in the lower Atchafalaya River Basin during the 2008 and 2009 crayfish seasons. Principal component (PC) 1 explained 39% of the physicochemical variation among sample sites in 2008 and 54% in 2009. PC 2 explained 32% of the physicochemical variation among sample sites in 2008 and 31% in 2009. Physicochemical variables with loadings greater than 0.4 were retained for analysis.

Physicochemical variable	2008		2009	
	PC1	PC2	PC1	PC2
Dissolved oxygen	0.54		0.59	
pH	0.51		0.56	
Turbidity	0.44		0.55	
Temperature		0.57		0.65
Specific conductance		-0.63		-0.68

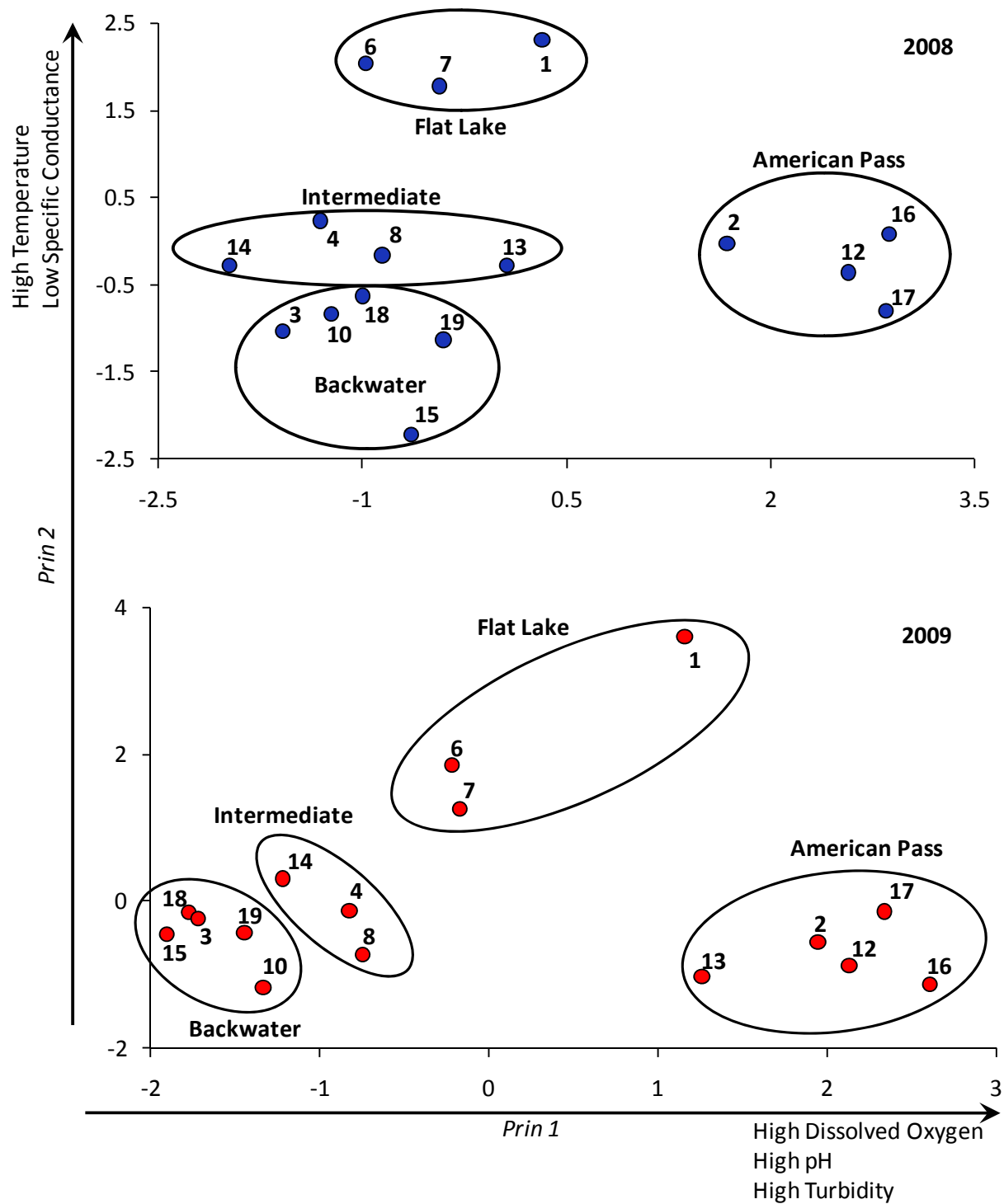


Figure 2.12. Biplots of principal components analysis of physicochemical parameters collected at intensive crayfish sampling locations ($n = 16$) in the lower Atchafalaya River Basin during the 2008 and 2009 crayfish seasons. Sample dates in 2008 range from 6 March to 12 August ($n = 10$) and 2009 samples dates range from 2 March to 13 July ($n = 10$).

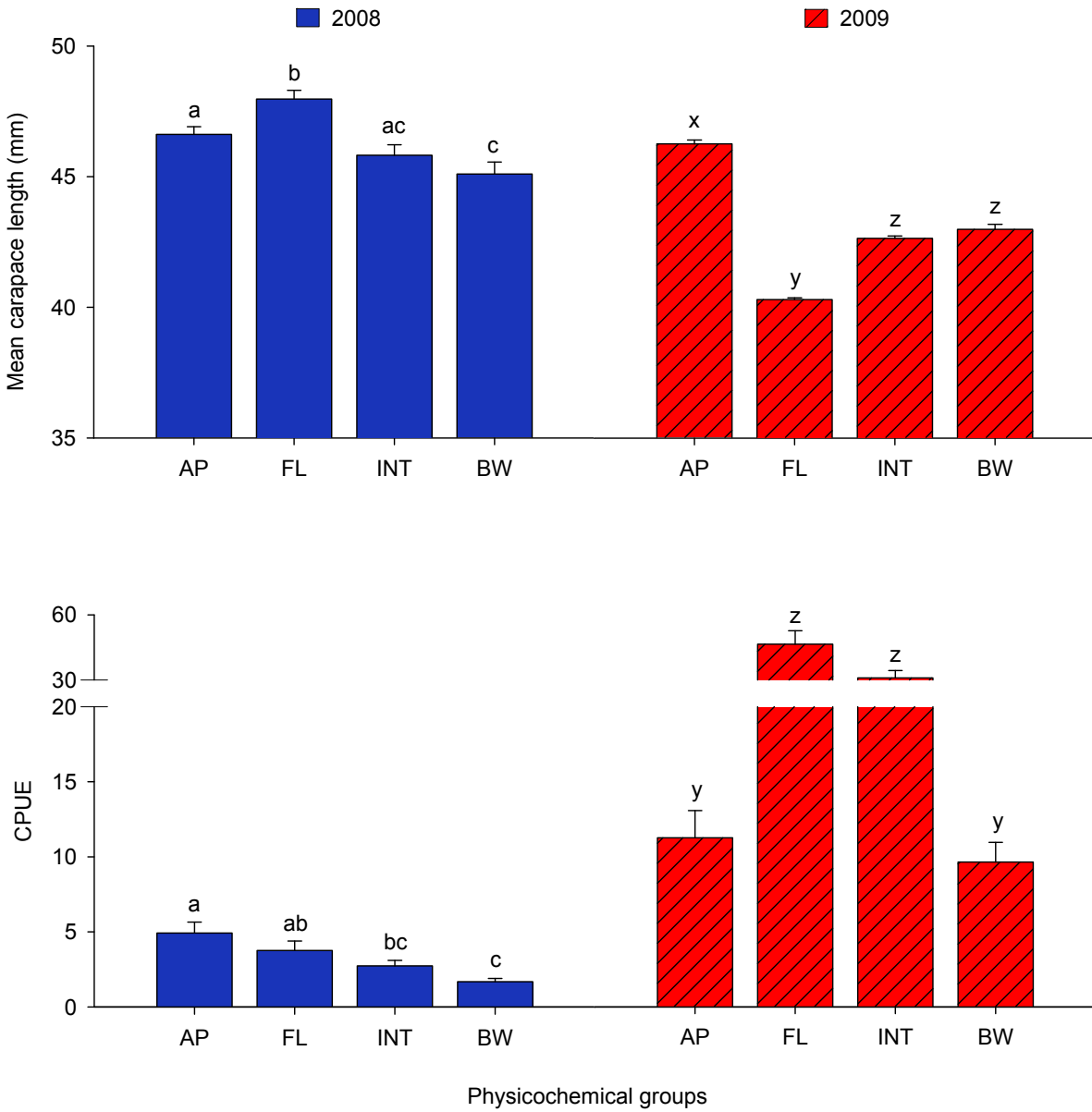


Figure 2.13. Mean (\pm SE) carapace length and catch per unit effort (CPUE) of *Procambarus clarkii* from physicochemical classifications of intensive sample locations during the 2008 and 2009 crayfish seasons. CPUE values were $\log_e(\text{CPUE}+1)$ transformed for analysis. Groups with different letters indicate a significant difference ($P < 0.05$) within a population metric for each year. Figure abbreviations include: AP (American Pass), FL (Flat Lake), INT (intermediate), and BW (backwater).

CPUE values during 2008. CPUE for market size *P. clarkii* demonstrated similar trends to those of total CPUE but no significant differences in carapace length among physicochemical groups were detected (Figure 2.14).

The 2009 PCA yielded PCs with the same variable correlations as 2008, which explained 85% of the physicochemical variation among sample locations (Table 2.7, Figure 2.12). The only sample location to switch physicochemical groups was site 13, which moved from the intermediate group in 2008 to the American Pass group in 2009 (Figure 2.12). During the 2009 crayfish season, American Pass locations produced significantly larger *P. clarkii* while Flat Lake sites exhibited significantly smaller individuals (Figure 2.13). Conversely, Flat Lake and intermediate locations had significantly higher CPUE values than American Pass and backwater sites (Figure 2.13). Market size individuals displayed similar trends in mean carapace length and CPUE across physicochemical groups (Figure 2.14). Mean carapace length of Form I males was lowest at intermediate sites in 2008 (Figure 2.15). Form I male carapace length in 2009 was dissimilar among all physicochemical groups with the smallest individuals captured at Flat Lake sites and the largest individuals taken from American Pass locations (Figure 2.15).

In 2008, sites with low relative densities of aquatic macrophyte (sites 1, 2, 3, 4, and 17) had significantly smaller *P. clarkii* compared to medium (sites 6, 7, 12, 14, and 16) and high (sites 8, 10, 13, 15, 18, and 19) density locations (Figure 2.16). Conversely, 2008 CPUE was significantly lower at high macrophyte density locations (Figure 2.16). ANOVA comparisons of 2009 macrophyte density groups did not detect any differences in CPUE among low (1, 2, 4, 8, 13, and 17), medium (3, 6, 7, 12, and 16), and high (10, 14, 15, 18, and 19) density locations (Figure 2.16). However, *P. clarkii* carapace length was dissimilar among all three macrophyte

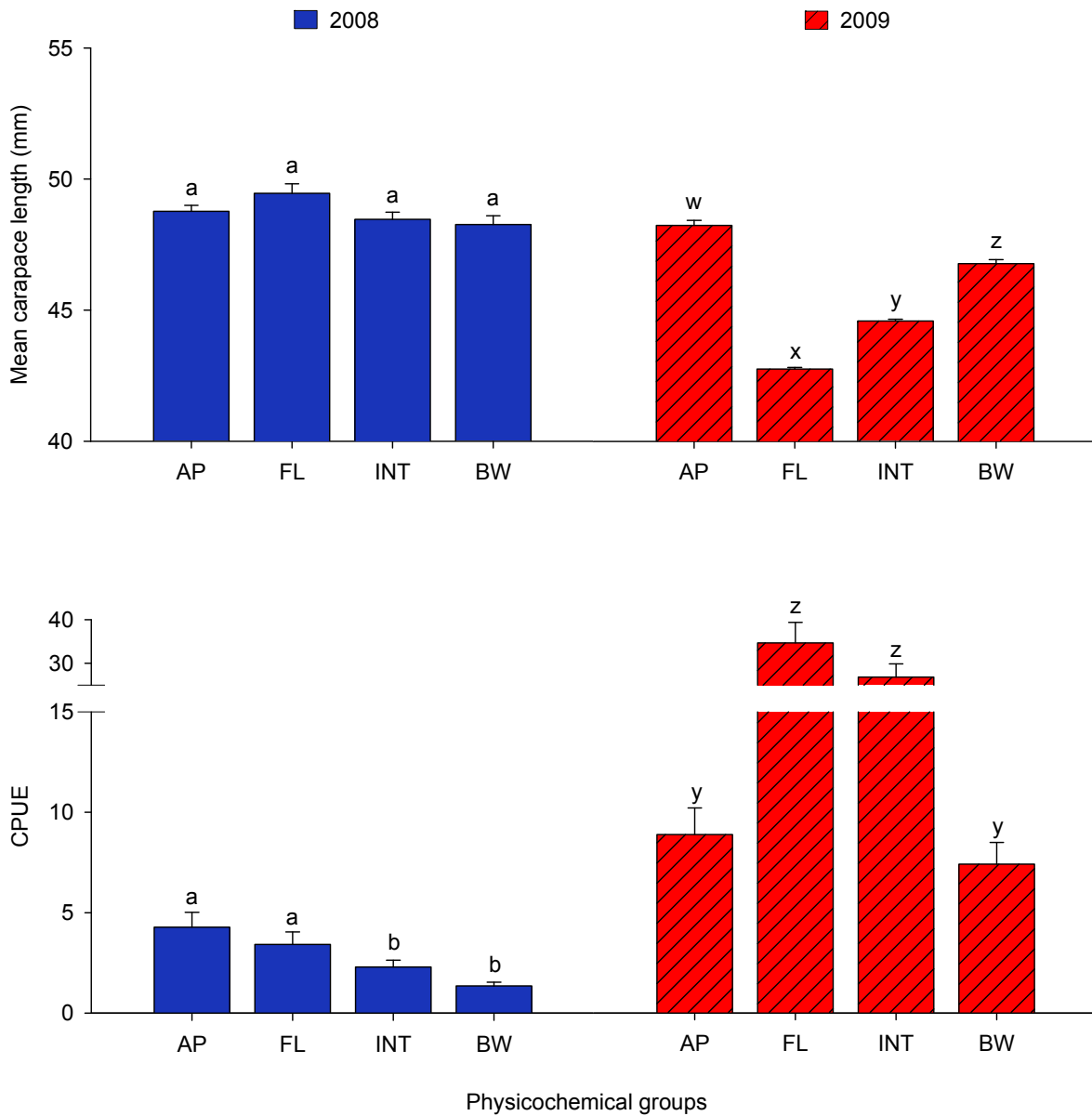


Figure 2.14. Mean (\pm SE) carapace length and catch per unit effort (CPUE) of market size *Procambarus clarkii* (carapace length ≥ 37 mm) from physicochemical classifications of intensive sample locations during the 2008 and 2009 crayfish seasons. CPUE values were $\log_e(\text{CPUE}+1)$ transformed for analysis. Groups with different letters indicate a significant difference ($P < 0.05$) within a population metric for each year. Figure abbreviations include: AP (American Pass), FL (Flat Lake), INT (intermediate), and BW (backwater).

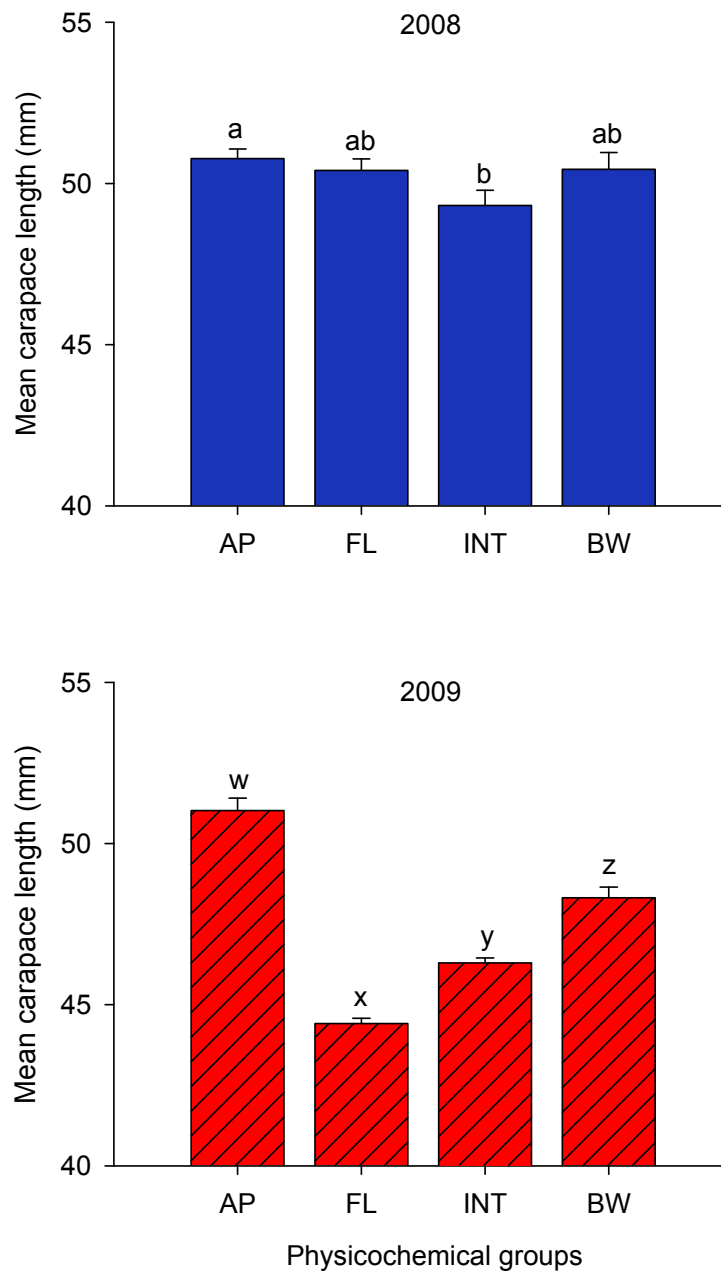


Figure 2.15. Mean (\pm SE) carapace length of Form I male *Procambarus clarkii* from physicochemical classifications of intensive sample locations during the 2008 and 2009 crayfish seasons. Groups with different letters indicate a significant difference ($P < 0.05$) within each year. Figure abbreviations include: AP (American Pass), FL (Flat Lake), INT (intermediate), and BW (backwater).

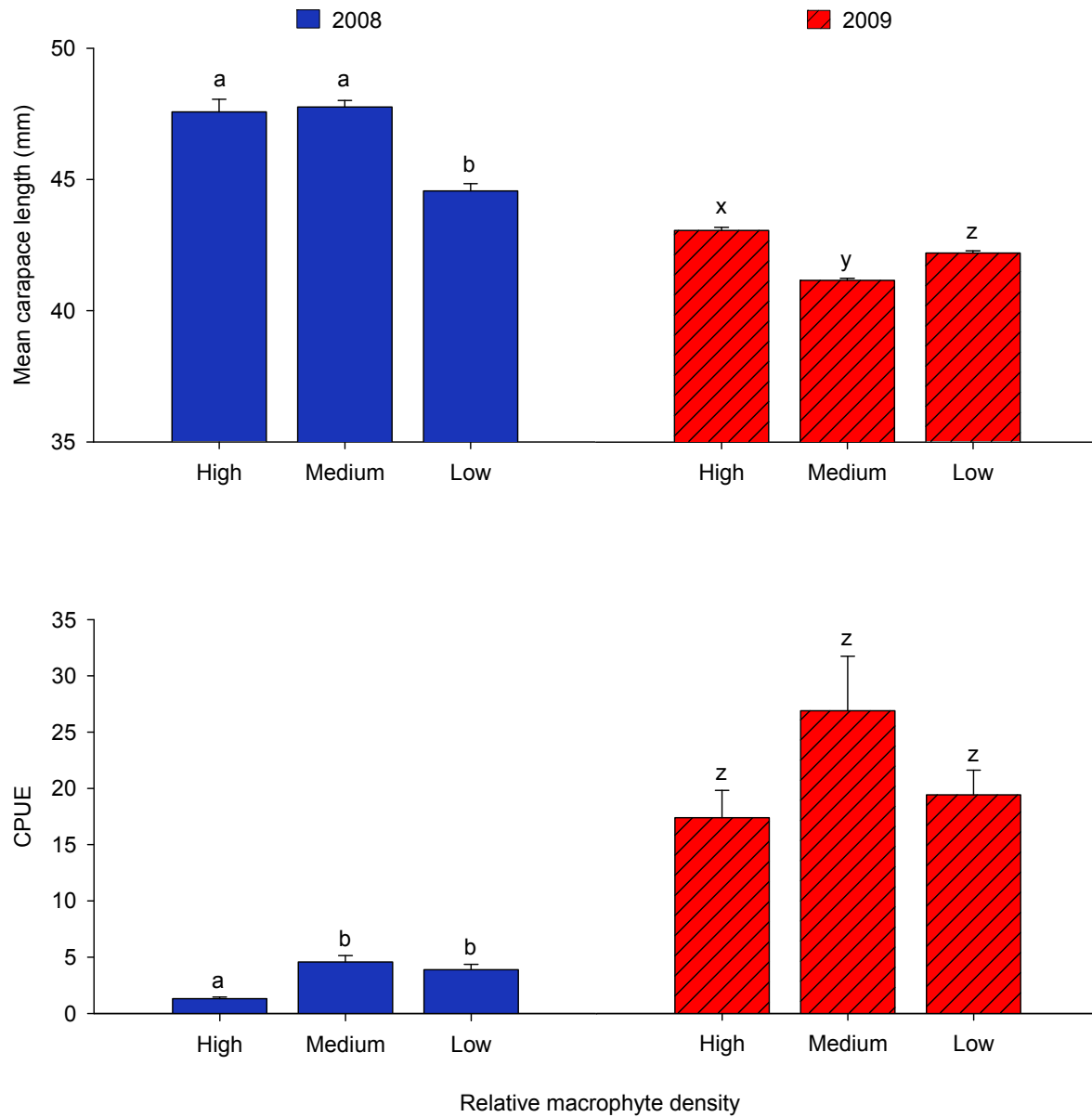


Figure 2.16. Mean (\pm SE) carapace length and catch per unit effort (CPUE) of *Procambarus clarkii* from low (0 – 33%), medium (34 – 66%), and high (67 – 100%) aquatic macrophyte relative density classifications at intensive sample locations during the 2008 and 2009 crayfish seasons. CPUE values were $\log_{(e)}(\text{CPUE}+1)$ transformed for analysis. Relative densities with different letters indicate a significant difference ($P < 0.05$) within a population metric for each year.

groups, with smaller individuals at medium density sites and larger individuals at high density locations.

Sample sites classified as normoxic or chronically hypoxic were the same for 2008 and 2009. Normoxic locations included sites 1, 2, 4, 6, 7, 8, 12, 13, 16, and 17 while chronically hypoxic locations included sites 3, 10, 14, 15, 18, and 19. In 2008, *P. clarkii* CPUE was significantly lower at hypoxic (2.25 ± 0.26) sites than at normoxic sites (3.68 ± 0.38 ; $F_{1,156} = 6.31$, $P = 0.0130$). Furthermore, hypoxic sites had significantly smaller individuals (45.82 ± 0.35 mm) than normoxic sites (46.75 ± 0.21 mm; $F_{1,2219} = 5.49$, $P = 0.0193$). Market size crayfish also displayed significantly lower CPUE (1.91 ± 0.26) in hypoxic sites compared to normoxic locations (3.17 ± 0.38 ; $F_{1,156} = 6.75$, $P = 0.0103$), however, carapace length at hypoxic (48.37 ± 0.28 mm) and normoxic (48.95 ± 0.17 mm) locations were not statistically dissimilar ($F_{1,1919} = 3.25$, $P = 0.0716$). In 2009, *P. clarkii* were significantly larger at hypoxic locations (42.99 ± 0.12 mm) compared to normoxic sites (41.67 ± 0.06 mm; $F_{1,15322} = 115.61$, $P < 0.0001$) and CPUE was significantly lower at hypoxic sites (15.20 ± 2.12) than normoxic areas (24.68 ± 2.68 ; $F_{1,154} = 5.19$, $P = 0.0241$). Market size individuals were also significantly larger at hypoxic locations (45.87 ± 0.09 mm) than normoxic locations (43.99 ± 0.05 mm; $F_{1,12076} = 340.92$, $P < 0.0001$) and CPUE was significantly lower in hypoxic areas (12.16 ± 1.82 ; normoxic = 19.40 ± 2.05 ; $F_{1,154} = 5.50$, $P = 0.0203$). Carapace length of Form I males from hypoxic (49.97 ± 0.39 mm) and normoxic (50.41 ± 0.23 mm) areas was similar in 2008 ($F_{1,940} = 0.93$, $P = 0.3347$). Conversely, carapace length of Form I male crayfish in hypoxic areas (47.23 ± 0.20 mm) were significantly larger than normoxic locations (46.21 ± 0.14 mm) in 2009 ($F_{1,2312} = 18.18$, $P < 0.0001$). The smallest Form I male recorded during this study was 29.9 mm (carapace length) captured on 24 September 2008 at site 14. Overall, DO displayed a negative relationship with *P.*

clarkii carapace length in both 2008 ($\beta = -0.10$, $P < 0.0001$, $r^2 = 0.2080$) and 2009 ($\beta = -0.46$, $P < 0.0001$, $r^2 = 0.1547$). Although a relationship between DO and CPUE was not detected during 2008, a negative relationship was found for 2009 ($\beta = -5.23$, $P < 0.0019$, $r^2 = 0.0611$).

During the 2008 crayfish season, mean *P. clarkii* carapace length at the 16 intensive sampling locations ranged from 40.20 – 52.72 mm while CPUE values ranged from 0.56 – 7.23. Site 8 had the lowest CPUE (0.56 ± 0.18) but the largest mean carapace length (52.72 ± 1.08 mm) among the 16 sample locations (Table 2.8). Furthermore, the top three sample locations with largest mean carapace length values (8, 13, and 10) were three of four sites with the lowest CPUE values. The largest mean CPUE value (7.23 ± 2.16) was recorded at site 12 which also produced 12.9% of the total *P. clarkii* captured during the 2008 season at intensive locations. In the 2009 crayfish season, mean *P. clarkii* carapace length at the 16 intensive sampling locations ranged from 38.68 – 47.59 mm and CPUE values ranged from 4.41 – 76.58. Site 6 produced 22.7% of the total *P. clarkii* captured during intensive sampling and the highest CPUE (76.58 ± 10.80), however, mean carapace length (39.89 ± 0.09 mm) was the second lowest among intensive locations (Table 2.9). Additionally, the five sites with the largest mean carapace lengths (2, 12, 10, 16, and 17) were in the bottom half of intensive sampling CPUE values. Mean CPUE values at all intensive locations increased from 2008 to 2009 and mean carapace length values decreased at all sites between the two sample years except at sites 2 and 4. Sites 6, 8, and 13 had the largest decreases in mean carapace length between 2008 and 2009 (9.05, 9.10, and 11.39 mm respectively) and three of the five largest increases in mean CPUE (72.14, 33.84, and 23.92 respectively). Length frequencies of *P. clarkii* during the 2008 (Figure 2.17) and 2009 (Figure 2.18) crayfish seasons demonstrate a larger percentage of individuals in the 50-59 and > 60 mm carapace length size classes in the total catch during 2008. Furthermore, comparisons of

Table 2.8. Mean (\pm SE) catch per unit effort (CPUE), transformed CPUE ($\log_{(e)}[\text{CPUE}+1]$), and carapace length of *Procambarus clarkii* at 16 intensive sampling locations in the lower Atchafalaya River Basin during the 2008 crayfish season. Different letters indicate a significant difference ($P < 0.05$) within a column.

Sample site	<i>n</i> CPUE; carapace length	CPUE ¹	$\log_{(e)} (\text{CPUE}+1)$	Carapace length (mm)
1	9; 213	4.89 \pm 1.45	1.47 \pm 0.29 ^{ad}	46.65 \pm 0.49 ^{aef}
2	10; 225	4.84 \pm 1.33	1.53 \pm 0.24 ^{ad}	45.62 \pm 0.53 ^{ae}
3	10; 124	2.78 \pm 0.61	1.22 \pm 0.16 ^{abcd}	43.72 \pm 0.75 ^{de}
4	10; 158	3.40 \pm 0.57	1.40 \pm 0.13 ^{ad}	40.20 \pm 0.62 ^c
6	10; 207	4.44 \pm 1.08	1.52 \pm 0.19 ^{ad}	48.94 \pm 0.42 ^{bf}
7	10; 99	2.06 \pm 0.32	1.07 \pm 0.11 ^{abcd}	48.74 \pm 1.03 ^{ab}
8	10; 28	0.56 \pm 0.18	0.39 \pm 0.11 ^c	52.72 \pm 1.08 ^{bg}
10	10; 37	0.74 \pm 0.14	0.52 \pm 0.08 ^{bc}	50.14 \pm 1.44 ^{ab}
12	10; 286	7.23 \pm 2.16	1.80 \pm 0.27 ^d	47.71 \pm 0.50 ^{afg}
13	10; 77	1.71 \pm 0.41	0.90 \pm 0.16 ^{abc}	51.37 \pm 0.89 ^b
14	10; 244	5.15 \pm 0.68	1.76 \pm 0.12 ^{ad}	46.92 \pm 0.52 ^{af}
15	10; 34	0.83 \pm 0.32	0.51 \pm 0.13 ^{bc}	48.16 \pm 1.65 ^{abe}
16	10; 178	3.96 \pm 0.96	1.43 \pm 0.20 ^{ad}	47.08 \pm 0.54 ^{af}
17	10; 136	3.64 \pm 0.98	1.32 \pm 0.23 ^{abd}	45.35 \pm 0.78 ^{ae}
18	10; 101	2.37 \pm 0.50	1.12 \pm 0.14 ^{abcd}	41.60 \pm 0.81 ^{cd}
19	10; 74	1.67 \pm 0.28	0.92 \pm 0.13 ^{abc}	48.27 \pm 0.95 ^{ab}

¹The reported untransformed CPUE mean values are the arithmetic means and not the back-transformed values from the $\log_{(e)}(\text{CPUE}+1)$, which yield geometric means.

Table 2.9. Mean (\pm SE) catch per unit effort (CPUE), transformed CPUE ($\log_{(e)}[\text{CPUE}+1]$), and carapace length of *Procambarus clarkii* at 16 intensive sampling locations in the lower Atchafalaya River Basin during the 2009 crayfish season. Different letters indicate a significant difference ($P < 0.05$) within a column.

Sample site	<i>n</i> CPUE; carapace length	CPUE ¹	$\log_{(e)} (\text{CPUE}+1)$	Carapace length (mm)
1	9; 1,040	25.58 \pm 6.23	2.75 \pm 0.47 ^{abcf}	38.68 \pm 0.18 ^k
2	10; 303	6.41 \pm 1.75	1.65 \pm 0.31 ^{ac}	47.59 \pm 0.45 ⁱ
3	10; 171	4.41 \pm 1.35	1.38 \pm 0.27 ^c	41.36 \pm 0.70 ^{abgh}
4	10; 803	16.06 \pm 2.96	2.61 \pm 0.27 ^{abc}	43.91 \pm 0.24 ^{ej}
6	10; 3,481	76.58 \pm 10.80	4.20 \pm 0.22 ^f	39.89 \pm 0.09 ^h
7	10; 1,731	35.22 \pm 6.72	3.36 \pm 0.26 ^{bef}	42.08 \pm 0.13 ^{bg}
8	10; 1,720	34.40 \pm 5.80	3.33 \pm 0.28 ^{bdf}	43.62 \pm 0.13 ^{dj}
10	9; 363	8.10 \pm 2.60	1.70 \pm 0.40 ^{ac}	45.93 \pm 0.42 ^{cfi}
12	10; 333	7.81 \pm 1.71	1.92 \pm 0.27 ^{acd}	47.01 \pm 0.41 ^{fi}
13	10; 1,272	25.63 \pm 6.15	3.04 \pm 0.23 ^{abf}	39.98 \pm 0.16 ^h
14	10; 2,006	42.36 \pm 5.24	3.63 \pm 0.22 ^{bf}	42.98 \pm 0.14 ^{ad}
15	10; 507	10.32 \pm 2.49	2.12 \pm 0.29 ^{acde}	41.25 \pm 0.38 ^g
16	8; 205	6.45 \pm 2.60	1.60 \pm 0.35 ^{ac}	45.38 \pm 0.54 ^{cef}
17	10; 374	9.08 \pm 2.13	2.08 \pm 0.24 ^{acde}	45.00 \pm 0.44 ^{ce}
18	10; 264	6.53 \pm 1.24	1.84 \pm 0.22 ^{ac}	44.32 \pm 0.53 ^{cde}
19	10; 751	18.77 \pm 4.07	2.68 \pm 0.29 ^{abc}	42.65 \pm 0.31 ^{ab}

¹The reported untransformed CPUE mean values are the arithmetic means and not the back-transformed values from the $\log_{(e)}(\text{CPUE}+1)$, which yield geometric means.

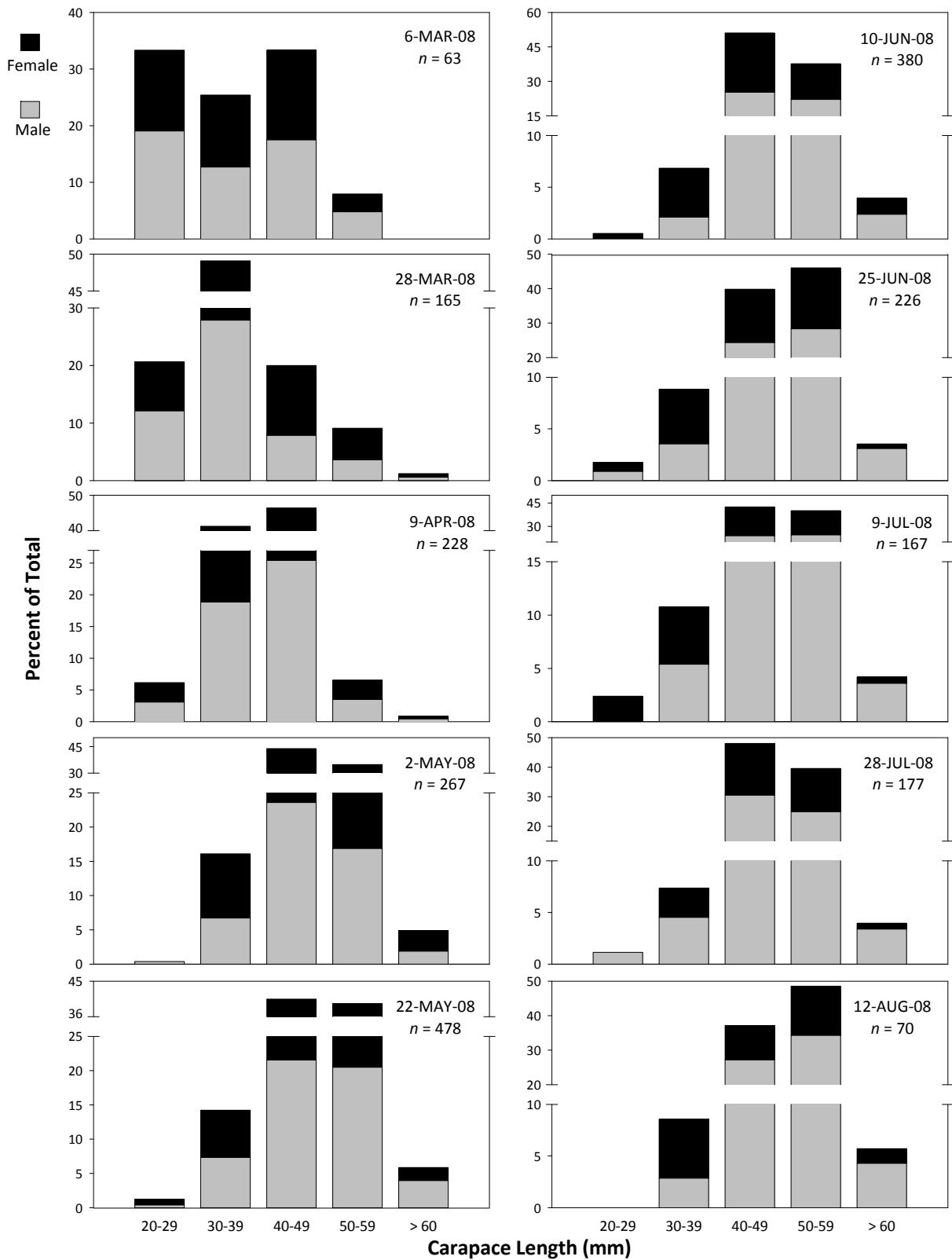


Figure 2.17. Length frequencies for *Procambarus clarkii* collected at intensive sample locations in the lower Atchafalaya River Basin during the 2008 crayfish season.

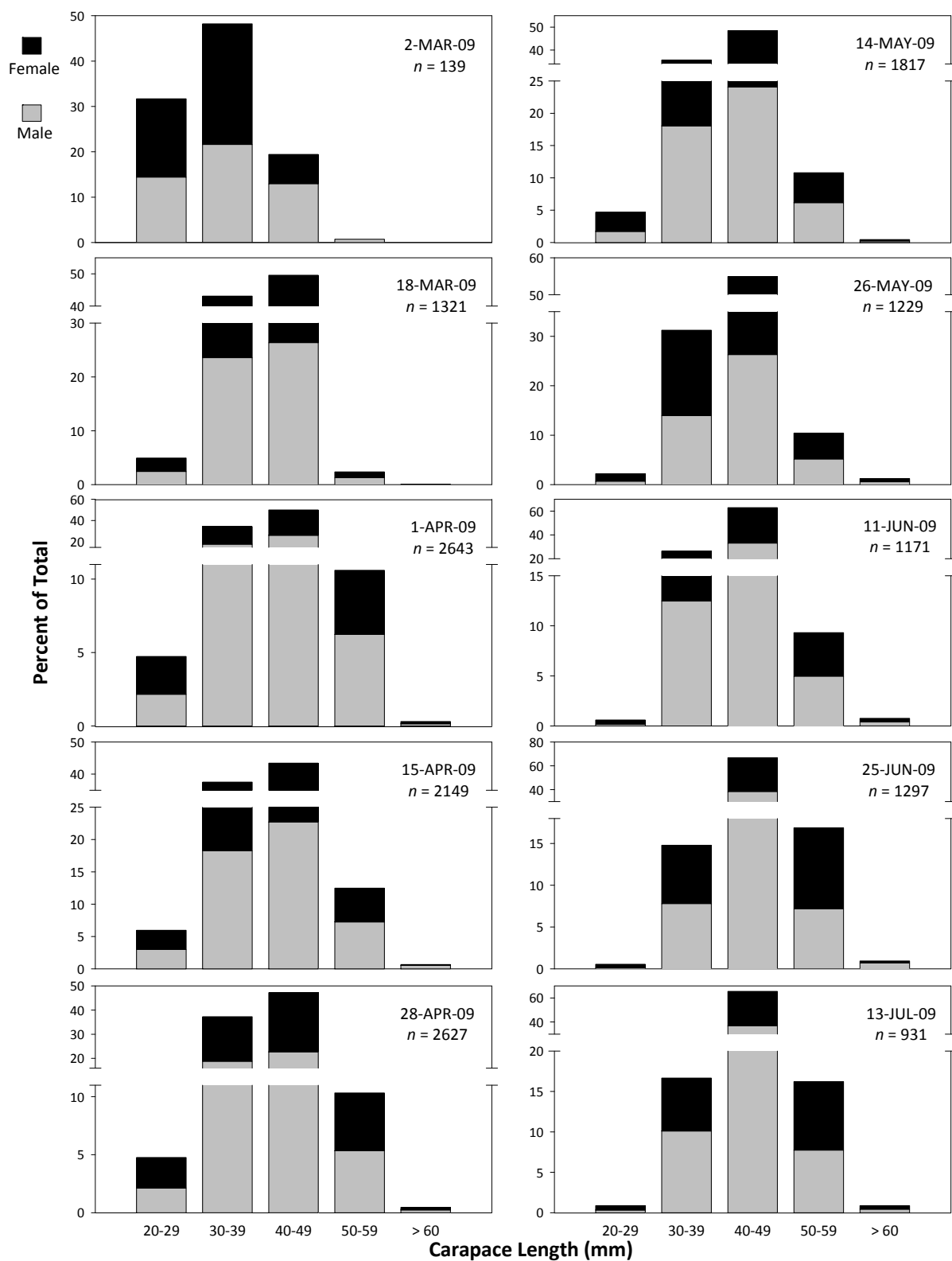


Figure 2.18. Length frequencies for *Procambarus clarkii* collected at intensive sample locations in the lower Atchafalaya River Basin during the 2009 crayfish season.

P. clarkii mean carapace length and CPUE during the early half of the crayfish season revealed a significant relationship in both 2008 ($P = 0.0005$, $r^2 = 0.1505$) and 2009 ($P < 0.0001$, $r^2 = 0.3208$). However, while carapace length and CPUE were not correlated in the latter half of 2008 ($P = 0.9495$, $r^2 = 0.0001$), a significant relationship was detected in 2009 ($P = 0.0364$, $r^2 = 0.0578$).

Discussion

Although environmental variables may structure local *P. clarkii* populations on a site specific scale, the hydrologic cycle of the Atchafalaya River has a basin-wide effect on the ARB crayfish assemblage and is the ultimate determinate of the magnitude of the annual crayfish harvest. The timing, magnitude, and duration of the flood pulse are all important hydrologic components in structuring the annual ARB crayfish harvest (Alford and Walker 2011). Essentially, poor floodplain inundation results in below average crayfish harvests (see 2000 and 2006 in Figure 2.2). Both sample years in this study, 2008 and 2009, experienced flood pulses conducive to above average crayfish yields. From March to August, 2008 there were 144 days of Atchafalaya River stages at Butte La Rose at or above 3.7 m, whereas 2009 experienced 100 days above this benchmark, defined by Hupp et al. (2008) as the level when the majority of ARB floodplain habitats are inundated. The 2008 flood was characterized by high water levels and prolonged floodplain inundation, which protracted the crayfish “season” until mid to late August. Although the 2009 flood pulse was a lower magnitude and duration event than 2008, floodplain inundation was more than sufficient to provide a favorable crayfish season that lasted until mid to late July.

Despite similar crayfish “seasons” in terms of timing and hydrologic conditions that are favorable for crayfish harvests, *P. clarkii* population characteristics at intensive sampling

locations differed substantially between sample years. While the annual commercial crayfish yield in the ARB increased 22% from 2008 to 2009, total abundance and CPUE at intensive sampling locations both increased almost 600% between years. The fact that CPUE for intensive and extensive locations in 2009 were similar coupled with similar 2008 and 2009 extensive CPUE values demonstrates that intensive locations in 2009 are representative of southeastern ARB *P. clarkii* populations. However, *P. clarkii* CPUE at intensive locations was significantly lower than extensive locations in 2008, suggesting that population responses at intensive locations were not representative of ARB populations, and perhaps were experiencing a localized population reduction. Anecdotal evidence from personal communications with local crayfish harvesters suggests that crayfish yields in the area of the intensive sample locations were low in 2008 and the preceding few years. This was also apparent in a reduced commercial crayfish harvest in the area, as evidenced by few observed crayfish traps compared to 2009. Causes of localized crayfish reductions and duration of these phenomena remain unclear. One possible explanation is high commercial crayfishing pressure over several years may temporarily reduce the abundance of crayfish in these areas. Bryan et al. (1976) observed lower *P. clarkii* abundance in areas of the ARB with high commercial crayfishing pressure than areas that experienced low pressure.

The passage of Hurricane Gustav over the ARB on 1 September 2008 may have indirectly influenced crayfish populations and contributed to increased abundance at the local and basin scale. Hurricane Gustav was a strong category 2 hurricane on the Saffir-Simpson scale with measured wind speeds in excess of 166 km/h (Beven and Kimberlain 2009) which produced extensive defoliation throughout the ARB. DO concentrations in the lower ARB began to decline two days post-landfall and reached near anoxic levels (Bonvillain et al. 2011) resulting in

an extensive fish kill estimated at 128 million individuals (LDWF 2008). *P. clarkii* and other aquatic detritivores may benefit from hurricane associated extensive defoliation and low DO levels that threaten other aquatic fauna. Because of increased allochthonous inputs and massive fish kills in the ARB caused by Hurricane Gustav, 2009 crayfish populations experienced decreased predatory pressure and an abundant food supply which may have contributed to increased *P. clarkii* abundance (e.g., Seiler and Turner 2004; Birnbaum et al. 2007; Dorn 2008). Similar increases in shrimp landings have been observed after hurricane passage in estuarine systems (Burkholder et al. 2004; Stevens et al. 2006). Although the passage of Hurricane Andrew over the ARB in 1992 resulted in extensive fish kills similar to Hurricane Gustav, because river stages in the ARB were extremely high in 1993, it is not possible to gauge the storm-related indirect effects on ARB crayfish populations for this storm event.

P. clarkii size (carapace length) also demonstrated marked differences between sample years, with smaller individuals at intensive and extensive locations in 2009. Increased CPUE resulted in smaller crayfish sizes as observed in *P. clarkii* length frequencies throughout the 2008 and 2009 crayfish seasons (Figures 2.17 and 2.18). Although 2009 had much higher CPUE values, 2008 yields produced a higher percentage of large size class individuals (50-59 and > 60 mm). Furthermore, 2009 *P. clarkii* carapace length demonstrated a significant negative relationship with CPUE during the latter half of the crayfish season, when density effects on growth were more pronounced (McClain 1995a, b). The increase in crayfish abundance and decrease in *P. clarkii* size during the 2009 crayfish season indicates density-dependent growth at both the basin and local scales. Density-dependent growth in *P. clarkii* has been demonstrated in numerous environments (Jarboe and Romaine 1995; McClain 1995a, b; Alcorlo et al. 2008; Ramalho et al. 2008; Anastácio et al. 2009; McClain 2010; Romaine and Villágran 2010) and is

of particular interest among commercial crayfish operations because of the higher market price for larger individuals (McClain and Romaine 1995; McClain et al. 2007). Decreased growth at high densities can be the result of increased social interaction and antagonism or decreased food and habitat availability (Flint and Goldman 1977; Goyert and Avault 1978; Jones and Ruscoe 2000; Reynolds 2002; Karplus and Barki 2004). However, high densities can inhibit crayfish growth in the absence of nutritional limitations (McClain and Romaine 1995). In addition to reduced overall carapace length in 2009, *P. clarkii* density-dependent growth is evident at intensive sampling locations, and site 6 best illustrates this relationship. Although site 6 had a considerably higher mean CPUE (76.58) than other sample locations (Table 2.9), mean carapace length of *P. clarkii* was less than 40 mm, not much larger than minimum market size individuals at 37 mm. Additionally, intensive locations during 2008 also exhibited some indication of density-dependence growth (e.g., site 8 had the highest mean carapace length and the lowest mean CPUE), albeit not as obvious as 2009.

The observed increase in the number of crayfish harvesters and traps at intensive locations in 2009 suggests an increase in harvest pressure in the area compared to 2008. Although the possibility that harvesters may bias *P. clarkii* carapace length values by removing larger individuals from the population exists, it is not believed to have influenced crayfish size at intensive locations for two reasons. First, crayfish growth in high density areas is likely to remain stunted even when individuals are removed from the population (Jarboe and Romaine 1995). Secondly, mean *P. clarkii* carapace length at intensive and extensive locations during 2009 was similar, indicating that the increased harvest pressure at intensive locations did not influence the observed carapace length measurements.

Poikilothermic animals such as crayfish demonstrate temperature dependent growth and activity (Reynolds 2002; Alcorlo et al. 2008; McClain 2010), and this was reflected in three of the four predictive models that included positive associations with water temperature. Increasing water temperatures stimulate microbial respiration associated with decomposition of floodplain organic matter, in turn reducing DO concentrations in inundated floodplain habitats (Junk et al. 1989; Bayley 1995; Sparks 1995). Even typically well-oxygenated water bodies experience DO reductions as they receive hypoxic floodplain waters during periods of dewatering (Meyer 1992; see figure 2.8). The negative associations of *P. clarkii* carapace length and CPUE with DO in the predictive models are likely an artifact of the temporal DO declines experienced in the ARB throughout the inundation period.

Abiotic variables undoubtedly influence crayfish population characteristics, however, relative crayfish density appears to be a more influential effect on ARB crayfish population structure than any physicochemical effects, which may be more influential during periods of low crayfish density (e.g., 2008 sampling season). The effects of reduced DO environments on *P. clarkii* populations were observed at backwater locations, which typically experienced the lowest DO concentrations and produced the lowest CPUE values during both sample years and the smallest individuals during 2008, although mean carapace length differences among 2008 physicochemical groups (e.g., Flat Lake, American Pass, Intermediate, and Backwater) were not more than 3 mm. Moreover, CPUE was higher at normoxic sites than chronically hypoxic locations during both sample years. *P. clarkii* populations inhabiting chronically hypoxic environments likely experienced increased mortality rates (Avault et al. 1975; McClain 1999; Sladkova and Kholodkevich 2011), particularly during juvenile stages (Melancon and Avault 1977), resulting in reduced population sizes (Hobbs and Hall 1974). Although reduced fish

densities in hypoxic areas (Rutherford et al. 2001) may have diminished aquatic predation pressure on crayfish, predation by terrestrial and semi-terrestrial animals (e.g., birds, raccoons, turtles, etc.) may have increased as crayfish moved to the air-water interface (Huner 2006) to supplement their oxygen demands with atmospheric oxygen (Taylor and Wheatly 1981; McMahon and Wilkes 1983; McMahon and Hankinson 1993; McMahon and Stuart 1999). Carapace length differences attributable to reduced DO concentrations during 2009 may be somewhat distorted due to density-dependent effects. The smaller mean carapace length observed at normoxic sites is likely a consequence of density-dependent growth rather than an association of larger individuals in hypoxic locations. However, responses of *P. clarkii* population attributes in backwater locations suggest possible sub-optimal water quality effects. Backwater and sufficiently-oxygenated American Pass locations exhibited similar mean CPUE values, but backwater locations had significantly smaller individuals in terms of carapace length (Figures 2.14, 2.15). Crayfish exposed to higher DO concentrations generally have shorter intermolt periods and faster growth (Jussila and Evans 1997), as observed at American Pass locations, while prolonged hypoxic exposure, especially during juvenile stages, has been shown to suppress growth (McClain 1999). Furthermore, active foraging and food consumption may be diminished in hypoxic waters (Bailey et al. 1985; Das and Stickle 1993; Paschke et al. 2010), which would in turn reduce protein concentrations and subsequent growth in starved individuals (Wen et al. 2007).

Chronically hypoxic conditions did not appear to affect the size of Form I male *P. clarkii* at intensive locations. The Form I stage of maturity in Cambarid crayfishes represents the sexually active and non-growth stage. Therefore, examination of relative sizes of Form I crayfish can serve as a biomarker of environmental stressors as individuals from stressed

environments mature at smaller sizes (Romaine and Lutz 1989). Form I male *P. clarkii* exhibit smaller mean carapace lengths at high density locations which mirrors total population responses during 2009. These results agree with other studies which describe smaller Form I males from high density areas (Jarboe and Romaine 1995; Alcorlo et al. 2008).

The fact that smaller Form I male *P. clarkii* were not observed at chronically hypoxic habitats, particularly in 2008 when density-dependent growth did not appear to influence crayfish populations, may be attributed to physiological, biological, and environmental influences. *P. clarkii* are tolerant of relatively low DO concentrations (Nyström 2002) and have developed multiple physiological mechanisms in order to exploit sub-optimal oxygen habitats (see McMahon 1986; Reiber 1995). The 2.0 mg/L DO concentration criterion used to classify water bodies as hypoxic may not be the correct standard for evaluating stressful environments to *P. clarkii*. Although juvenile *P. clarkii* may be more susceptible to DO concentrations below 2 mg/L (Melancon and Avault 1977), oxidative stress thresholds for adults appear to be lower. Several studies have suggested stress and lethal DO concentrations for *P. clarkii* at or near 1.0 mg/L (Avault et al. 1975; Konikoff 1977; McClain et al. 2007; Bonvillain et al. 2012). Bonvillain et al. (2012) demonstrated that *P. clarkii* exposed to 2.0 mg/L DO concentrations were not physiologically stressed while individuals subjected to 1.0 mg/L DO levels displayed marked evidence of hypoxic stress. These authors suggest a critical oxygen level for *P. clarkii* somewhere between 1 and 2 mg/L, and recorded mortalities of *P. clarkii* in traps during the present study corroborate this threshold. Dead *P. clarkii* were only observed in completely submerged traps, restricting crayfish access to the air-water interface, and with site DO concentrations less than 1.2 mg/L. In addition to the ability of *P. clarkii* to utilize atmospheric oxygen in hypoxic environments, diurnal oxygen fluctuations (Bonvillain et al. 2012) and

increased DO concentrations within macrophyte beds (Fontenot et al. 2001) in hypoxic ARB habitats may mitigate physiological stress for crayfish inhabiting these areas.

Aquatic macrophyte density did not appear to considerably affect *P. clarkii* population characteristics, and is likely less influential than density and physicochemical factors. Carapace length differences observed during 2009 can be attributed to density-dependent growth and not necessarily macrophyte densities. Additionally, backwater locations were generally associated with high macrophyte densities, which contribute to lower DO concentrations in these areas by reducing water circulation and increasing benthic decomposition rates (Mwabvu and Sasa 2009). However, dense aquatic macrophytes stands in hypoxic areas may provide crayfish with daytime DO refugia (Miranda et al. 2000; Fontenot et al. 2001; Troutman et al. 2007; Bunch et al. 2010) and provide structure to support air-water interface access (Avault et al. 1975; McClain et al. 2007). Increased habitat complexity provided by macrophytes may also reduce predation pressure on crayfish populations (Jordan et al. 1996a, b; Harper et al. 2002; Garvey et al. 2003; Foster and Harper 2006; Agostinho et al. 2007). Although these and other studies have documented increases in crayfish abundance associated with increasing macrophyte densities, the present study did not reveal the same trend. However, while not statistically significant, medium macrophyte densities did produce the highest CPUE values during both sample years and the largest individuals during 2008 (2009 carapace length were density influenced). Perhaps medium macrophyte densities provide sufficient habitat for *P. clarkii* while avoiding the negative consequences associated with high density macrophyte stands such as reduced water circulation, high benthic decomposition rates, and water column hypoxia.

Lastly, an interesting observation documented during this study was the catch (or lack of) of *P. zonangulus*. The fact that over 18,000 crayfishes were sampled from 2008 to 2009 at

intensive locations and no *P. zonangulus* were observed is convincing evidence that this species is absent from the crayfish community in the in these areas of the ARB. Additionally, *P. zonangulus* was only detected at extensive locations north of latitude 30°02'97", and the percent of *P. zonangulus* in the total crayfish catch displayed an increasing trend with latitude (Figure 2.9). *P. zonangulus* locations exhibited similar physical and chemical characteristics as intensive and non-*P. zonangulus* extensive locations. However, a closer examination of water temperatures revealed subtle differences along a latitudinal gradient in the ARB. Mean water temperatures at intensive locations on sample dates immediately before and after *P. zonangulus* sample dates were 2-6° C higher than *P. zonangulus* locations. The trend of increasing water temperatures with decreasing latitude in the eastern ARB is also present in data collected from 2001-2009 at five southeastern ARB sampling locations (A. R. Harlan, Louisiana State University Agricultural Center, unpublished data). Although temperature variations between northern and southern sample locations may appear insignificant, these differences may be sufficient to exclude *P. zonangulus* from southern ARB locations. The natural range of *P. zonangulus* extends to higher latitudes than *P. clarkii* and *P. zonangulus* are better adapted for growth in cool waters and are more abundant in flowing, riverine systems. Thermal limits have not been established for *P. zonangulus*, but evidence suggests that they achieve higher growth rates than *P. clarkii* at cooler temperatures. Croll and Watts (2004) demonstrated that *P. zonangulus* acquired food more effectively at lower temperatures than *P. clarkii*; however, food consumption by *P. zonangulus* decreased at higher temperatures while *P. clarkii* fed maximally. Furthermore, *P. zonangulus* exhibit higher growth rates at lower water temperatures than *P. clarkii* in farmed (Romaine and Lutz 1989) and wild populations (O'Brien 1977). The slightly warmer water temperatures experienced in lower latitude areas of the southeastern ARB may be

above the optimal thermal limits for *P. zonangulus* and effectively exclude this species from these habitats.

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CHAPTER 3

ACUTE PHYSICOCHEMICAL EFFECTS IN A LARGE RIVER-FLOODPLAIN SYSTEM ASSOCIATED WITH THE PASSAGE OF HURRICANE GUSTAV¹

Introduction

Hurricane Gustav made landfall near Cocodrie, Louisiana on 1 September 2008, as a strong category 2 hurricane on the Saffir-Simpson scale with measured wind speeds in excess of 166 km/h and tropical storm force winds extending approximately 321 km from the eye wall (Beven and Kimberlain 2009). As the hurricane passed over the lower Atchafalaya River Basin (ARB) in south-central Louisiana (Figure 3.1), it deposited up to 250 mm of rainfall (Grumm 2008) and produced extensive defoliation. Within two weeks of Hurricane Gustav, Hurricane Ike made landfall on 13 September 2008 approximately 483 km west of the ARB in Galveston, Texas as a category 2 storm with 175 km/h winds (Berg 2009). Although the ARB was not in Hurricane Ike's direct path, the immense size and intensity of the storm generated significant precipitation and hurricane force winds throughout southern Louisiana (Berg 2009).

Researchers have documented various environmental characteristics of the ARB (e.g., rainfall and runoff patterns, Denes and Bayley 1983; circulation, thermal, and chemical cycles, Sabo et al. 1999a; seasonal hypoxia, Sabo et al. 1999b; and nitrogen budgets, Xu 2006), but there is limited information about the physicochemical changes associated with storms such as Hurricane Gustav that are not infrequent phenomena along the Gulf coast. Because of the difficulty in predicting the landfall of a hurricane, researchers often rely on variations in long-term organismal monitoring programs (Stevens et al. 2006) or inter-annual changes in biogeochemical cycles (Paerl et al. 2001) to assess both temporary and longer-term impacts

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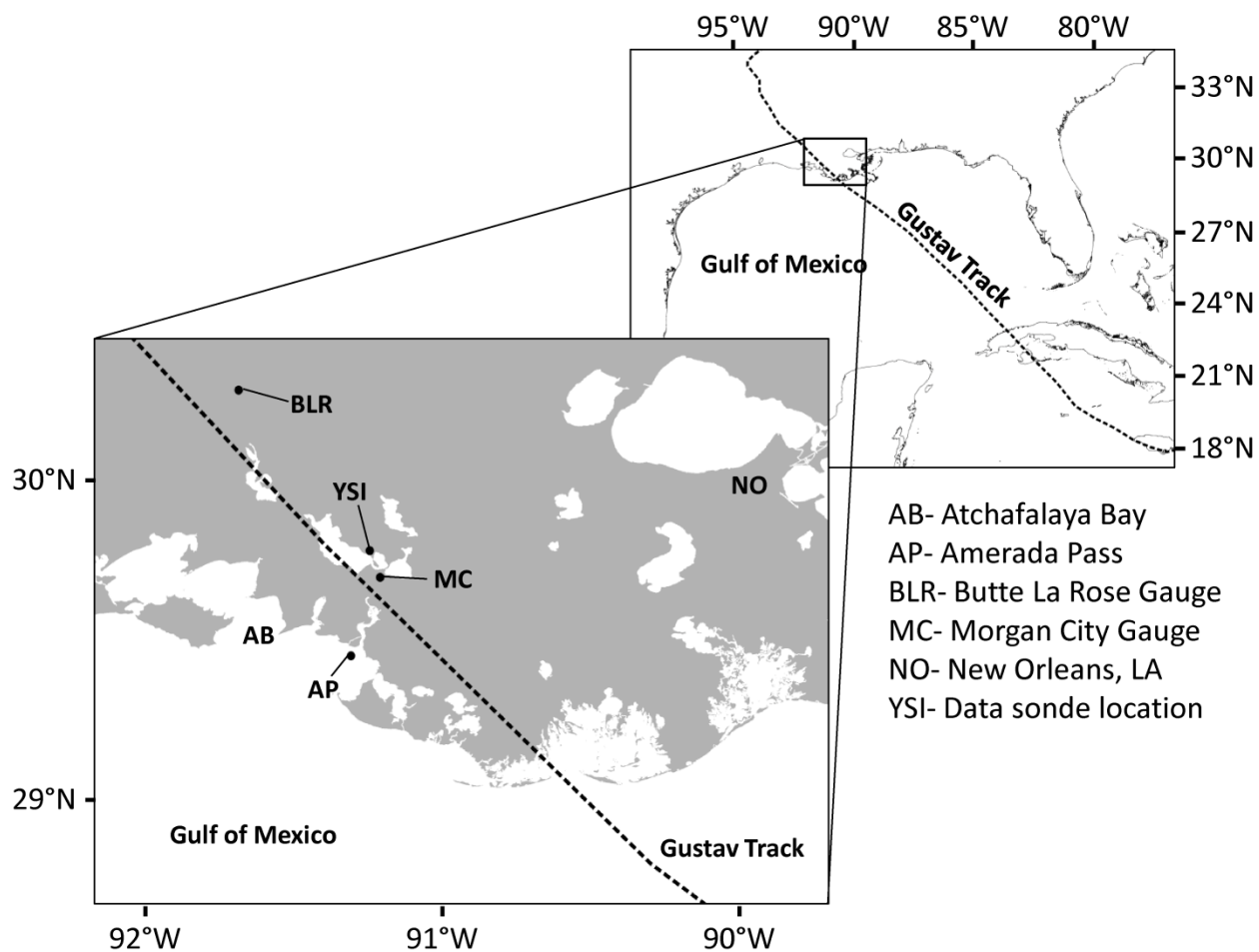


Figure 3.1. Hurricane Gustav's track across south-central Louisiana and the lower Atchafalaya River Basin on 1 September 2008.

associated with large-scale storms. Although multiple studies have documented storm-related shifts in the density, distribution, and composition of various aquatic biota, such as benthic macrofauna (Balthis et al. 2006; Poirrier et al. 2008), decapods (Knott and Martore 1991), fishes (Greenwood et al. 2006; Paperno et al. 2006; Stevens et al. 2006; Van Vrancken and O'Connell 2010), and aquatic plants (Havens et al. 2001; James et al. 2008; Jin et al. 2011), most studies do not have real-time environmental data directly associated with passage of the storm. Further, although considerable research has addressed storm-related impacts on ecosystem-level physical processes such as deleterious nutrient cascades (Paerl et al. 2001) and episodic hypoxia events (Stevens et al. 2006), most hurricane related studies have chronicled conditions in brackish systems. As a result, post-hurricane conditions in freshwater ecosystems remain largely unknown and to our knowledge, no study has collected continuous water quality data or examined short-term physicochemical variability following storm landfall. In this paper, I present quarter-hourly physicochemical data in the days before, during, and for over a week after the passage of Hurricane Gustav, examine water quality fluctuations over a three month period prior to and following the storm from periodic water quality assessments, and characterize changes in river stage in the aftermath of sequential hurricanes in this large river-floodplain ecosystem.

Study Area

The ARB in south-central Louisiana is the largest remaining bottomland hardwood river-floodplain system in North America (Lambou 1990) and is comprised of shallow headwater and backwater lakes, numerous bayous and excavated petrochemical canals, and seasonally flooded swamps (Rutherford et al. 2001). The Atchafalaya River, the dominant feature of the ARB and a major tributary of the Mississippi River, receives 30% of the combined volumes of the

Mississippi and Red Rivers. The U. S. Army Corps of Engineers regulates the amount of water entering the system through the Atchafalaya River with various water control structures. Although the annual timing, magnitude, and duration of the flood pulse varies, typically, the ARB is inundated in the spring followed by a pronounced drawdown period lasting from late summer to early fall (Denes and Bayley 1983; Lambou 1990; Fontenot et al. 2001; Bonvillain et al. 2008). During periods of inundation, the ARB forms a nearly continuous water body. However, anthropogenic modifications (levee and canal construction and flood control structures) have altered the historic river-floodplain connectivity resulting in increased sedimentation (Hupp et al. 2008) and reduced water circulation and flow patterns that often prompts the formation of hypoxic conditions (dissolved oxygen $[DO] \leq 2$ mg/L; Sabo et al. 1999b).

Methods

On 29 August 2008, three days prior to Hurricane Gustav's landfall, a continuous recording multiparameter water quality sonde (YSI model 6600, Yellow Springs, Ohio) was secured to the base of a tree approximately 0.75 m below the surface in Little Bayou Jessie (29°46' N, 91°14' W), a bayou located in the southeastern ARB (Figure 3.1). Because Little Bayou Jessie consistently had the highest DO concentrations in our study area and hypoxia was not recorded during 2008 sampling prior to the passage of Hurricane Gustav, I selected this location for sonde deployment to observe hurricane associated physicochemical fluctuations. DO (mg/L), temperature (°C), specific conductance (mS/cm), pH, turbidity (NTU), and relative depth (m; the change in water level relative to the deployment depth of the sonde) were recorded at 15 minute intervals ($n = 96$ observations per day) until sonde retrieval on 10 September 2008. Because of OBS sensor malfunction, turbidity measurements were only obtained through 5

September 2008. Wind velocity (m s^{-1}) and barometric pressure (mbar) observations were obtained from the nearby Amerada Pass Meteorological Station (Figure 3.1; National Oceanic and Atmospheric Administration, gauge 876422).

From 7 March 2008 – 2 March 2009 I collected surface DO, temperature, specific conductance, and pH every two to four weeks with a handheld multiparameter water quality sonde (YSI model 6820, Yellow Springs, Ohio) at 16 sample locations located in a 40 km^2 area of the southeastern ARB. Sample locations included habitats that are typically found throughout the lower ARB (e.g., bayous and canals) and exhibit variable morphological characteristics, flow velocities, river-floodplain connectivity, and macrophyte density and composition. Daily stage of the Atchafalaya River was obtained from the U. S. Army Corps of Engineers recording gauges located at Morgan City (gauge 03780, $29^\circ 41' 47'' \text{ N}$, $91^\circ 12' 39'' \text{ W}$) and Butte La Rose (gauge 03120, $30^\circ 16' 57'' \text{ N}$, $91^\circ 41' 17'' \text{ W}$), Louisiana (Figure 3.1).

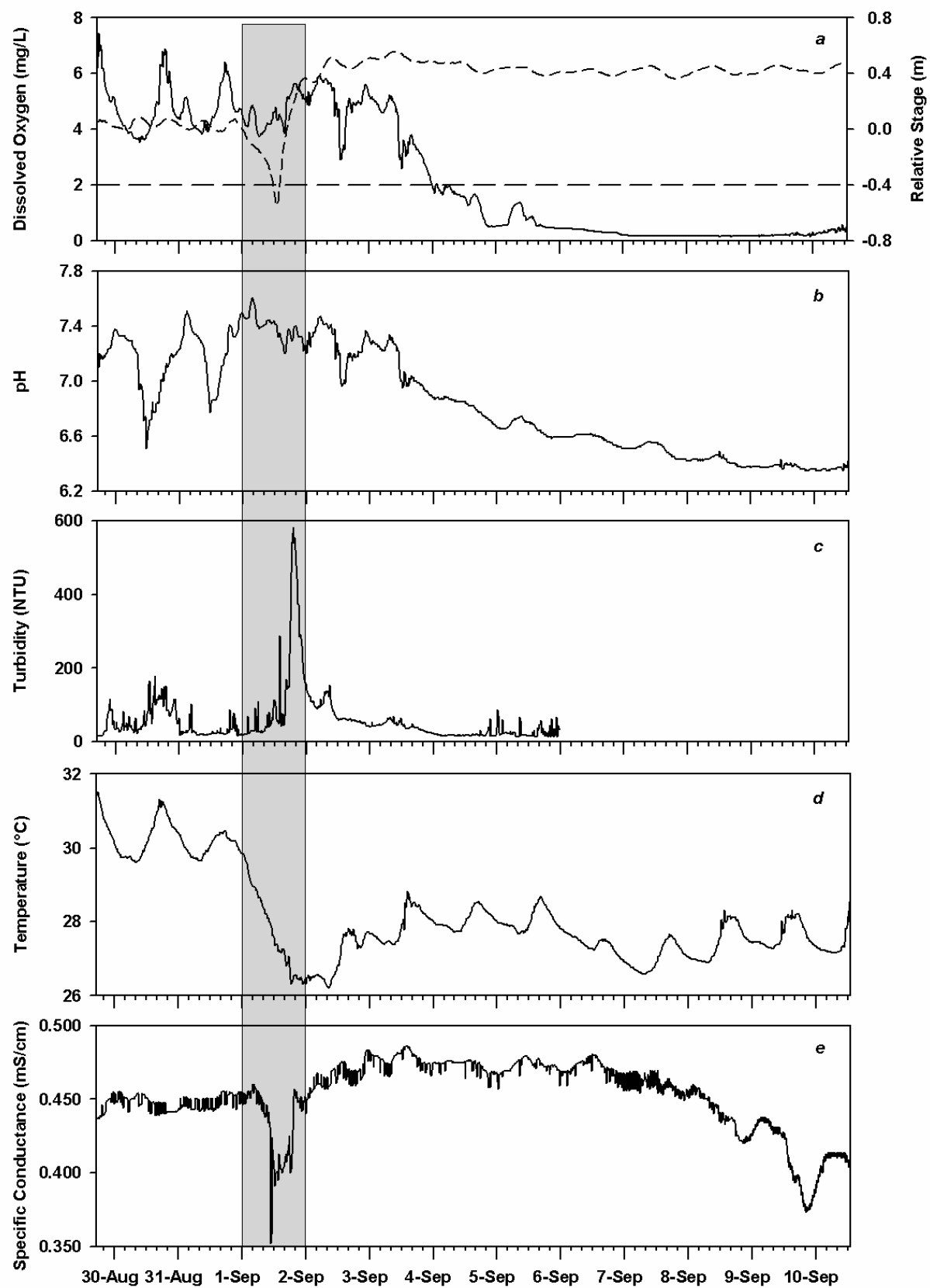
To assess the physicochemical effects of Hurricane Gustav, I grouped and then contrasted water quality observations at the 16 sampling locations for three consecutive sampling dates preceding (pre-Gustav, 54-day period prior to the arrival of the hurricane) and following (post-Gustav, 45-day period after hurricane landfall) storm passage. Pre-Gustav sample dates included 9 July, 28 July, and 12 August, whereas post-Gustav sample dates included 9 September, 24 September, and 16 October. The temporal periods selected for analysis include both the seasonal low DO concentration and DO recovery after storm passage. Principal components analysis (PCA) with a varimax rotation was used to examine pre- and post-Gustav physicochemical differences in DO, temperature, specific conductance, and pH from each period, and I retained principal components (PC) with eigenvalues greater than 1 (PC1 and PC2) and physicochemical variables with correlations greater than 0.4 for subsequent analysis (Hardle and Simar 2007).

Pre- and post-Gustav PC scores were plotted on PC1 and PC2 to graphically examine hurricane-related differences among sites. Multivariate analysis of variance (MANOVA) with a Tukey-Kramer *post hoc* adjustment was used to examine statistically significant physicochemical differences between pre- and post-Gustav groups and between individual sample dates (SAS 9.1.3 2003). Physicochemical univariate results from MANOVA analysis were interpreted when models were significant. Significance for all statistical tests was determined at an $\alpha = 0.05$ level.

Results

During the two days prior to the arrival of Hurricane Gustav, Little Bayou Jessie daily water quality from sonde observations were relatively predictable (Figure 3.2). Once the hurricane made landfall, however, daily mean water temperature dropped over 2°C and considerable variation was measured in both DO and water level (Figure 3.2). The large water level oscillations observed in Little Bayou Jessie as well as at Amerada Pass coincide with increased wind velocities and a pronounced drop in barometric pressure associated with the passage of the hurricane (Figure 3.3). Barometric pressure lows recorded at Amerada Pass during Hurricane Gustav have the potential to introduce relative depth error up to 0.29 m in Little Bayou Jessie sonde measurements. Although DO concentration degraded substantially 48 hours after hurricane passage, the daily mean DO level (3.91 ± 0.10 mg/L) did not become hypoxic until 4 September, three days post-Gustav landfall (1.43 ± 0.05 mg/L, daily maximum = 2.04 mg/L; Figure 3.2). By 7 September, six days post-Gustav landfall, daily mean DO concentration was near anoxia (0.17 ± 0.00 mg/L; Figure 3.2), where it remained until sonde retrieval on 10 September. DO declined at a rate of 0.18 mg/L/hr until it reached hypoxia where the declination rate then slowed to 0.02 mg/L/hr as levels approached anoxia (0.16 mg/L). The decline in post-storm DO was accompanied by a steady drop in pH (Figure 3.2). Additionally,

Figure 3.2. Quarter-hourly sonde physicochemical measurements from 29 August to 10 September 2008 in Little Bayou Jessie located in the lower Atchafalaya River Basin. Hurricane Gustav made landfall on 1 September 2008. (a) Dissolved oxygen (solid line) and relative stage (short broken line), (b) pH, (c) turbidity, (d) temperature, and (e) specific conductance. The dashed horizontal reference line on the dissolved oxygen graph (a) indicates hypoxic level (dissolved oxygen ≤ 2 mg/L). The grey box is a representative 24 hour temporal domain that captures hurricane passage over the sample area.



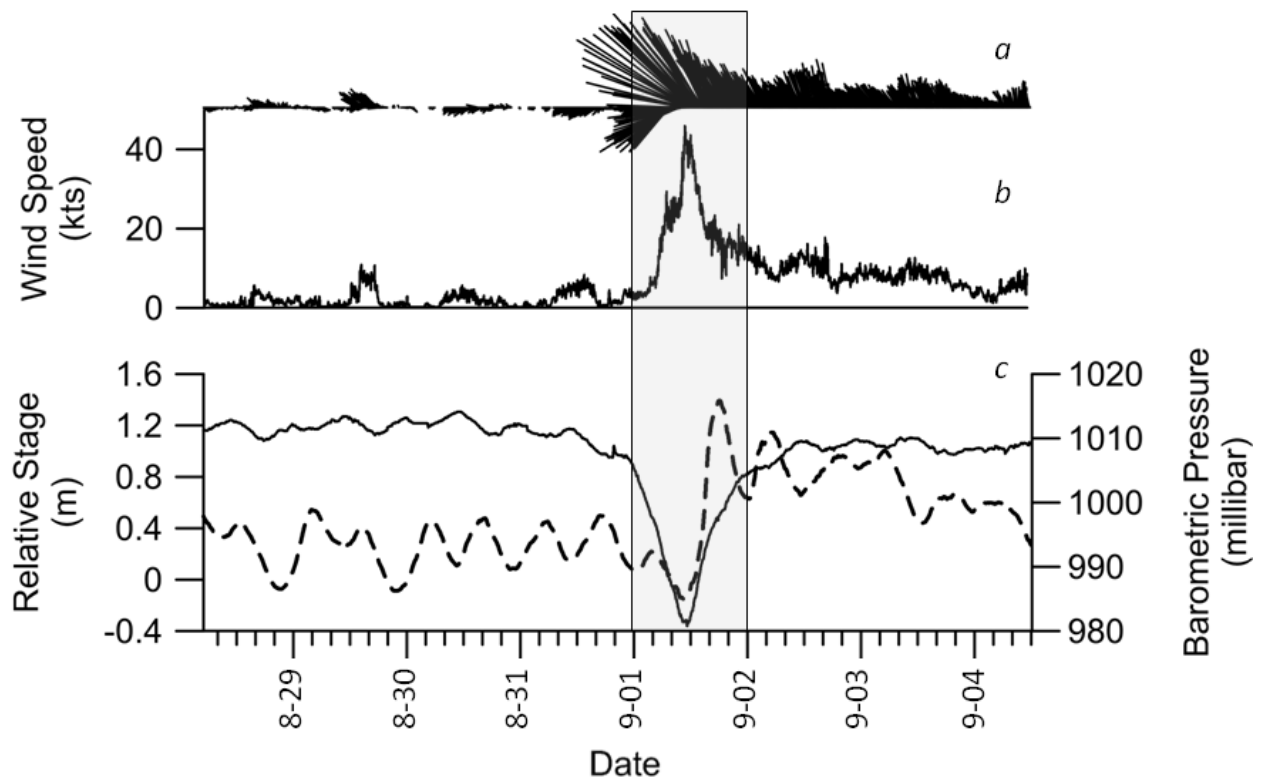
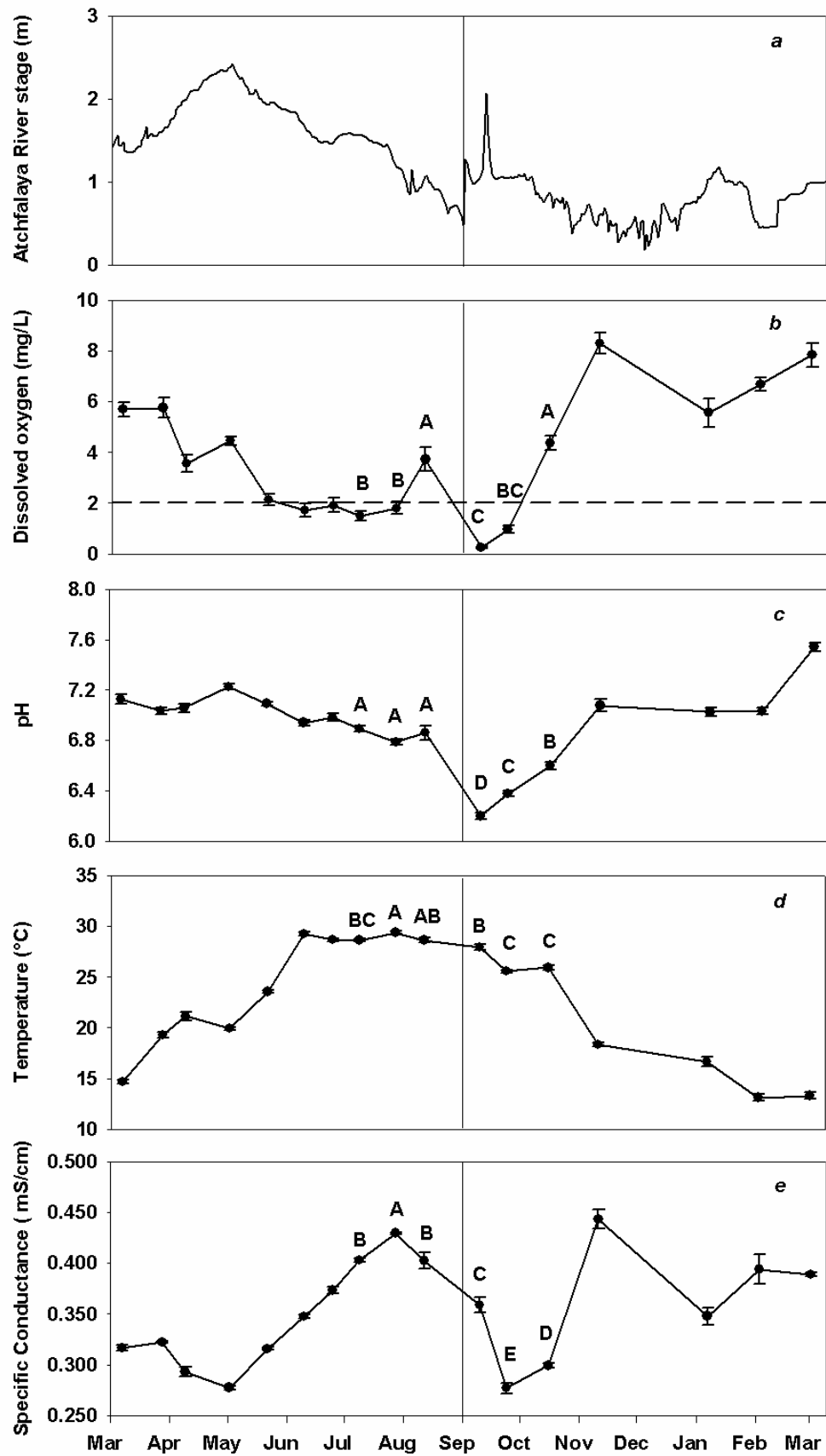


Figure 3.3. Meteorological and water data obtained from Amerada Pass (National Oceanic and Atmospheric Administration, gauge 876422) associated with passage of Hurricane Gustav. (a) Wind velocity vectors (vectors point in the direction of the wind and indicate wind magnitude), (b) wind speed measurements, and (c) relative stage (broken line) and barometric pressure (solid line) from 28 August to 4 September 2008. The grey box is a representative 24 hour temporal domain that captures hurricane passage over the sample area.

peak turbidity measurements were recorded during hurricane passage (Figure 3.2). Hurricane Gustav effects were also evident with an Atchafalaya River stage increase at Morgan City and perceptible declines in mean DO, pH, and specific conductance levels, each reaching an annual low immediately following storm passage at the 16 sample locations (Figure 3.4).

The PCA yielded two PCs (PC1 and PC2) with eigenvalues greater than 1.0 that explained 76% of the physicochemical variation between pre- and post-Hurricane Gustav observations. Temperature and specific conductance were positively correlated with PC1 (0.95 and 0.93, respectively), whereas DO and pH were positively correlated with PC2 (0.97 and 0.44). Plots of site scores on PC1 and PC2 revealed a pre-Gustav (9 July, 28 July, and 12 August) group overlapping across sample dates (Figure 3.5), with most sites characterized by higher water temperatures and elevated specific conductance. Substantial variation occurs on 12 August as sites were recovering from seasonal DO and pH lows at various rates. Conversely, post-Gustav observations (10 September, 24 September, and 16 October) tended to separate by individual sample date and displayed a trend of increasing DO and pH and decreasing temperature as date increased. Although decreased temperatures and specific conductance largely influence separation of post-Gustav sample dates, all sample sites display similar temporal physicochemical recovery following Hurricane Gustav. Overall differences in physicochemistry between pre- and post-Gustav sample dates were evident (MANOVA, Wilks' $\Lambda = 0.10$, $F_{4,91} = 209.29$, $P < 0.0001$), which were also reflected by each of the physicochemical parameters between the two periods ($P < 0.0001$). Additionally, physicochemical comparisons among sample dates ($n = 6$) revealed that pH and specific conductivity for post-Gustav sample dates were dissimilar from each other and from all pre-Gustav dates (Figure 3.4). Initially, post-Gustav DO concentrations were lower than pre-Gustav

Figure 3.4. Mean (\pm SE) physicochemical measurements from sample locations ($n = 16$) in the lower Atchafalaya River Basin from 7 March 2008 to 2 March 2009. The vertical line on each graph represents Hurricane Gustav landfall on 1 September 2008. Results of the *post hoc* (Tukey-Kramer) comparisons of physicochemical variables among sample dates are given; different letters within a physicochemical parameter indicate a significant difference by date ($P < 0.05$). (a) Daily Atchafalaya River stage at Morgan City, Louisiana (U. S. Army Corps of Engineers gauge 03780), (b) dissolved oxygen, (c) pH, (d) temperature, and (e) specific conductance. The dashed horizontal line on the dissolved oxygen graph (b) indicates hypoxic level (dissolved oxygen ≤ 2 mg/L).



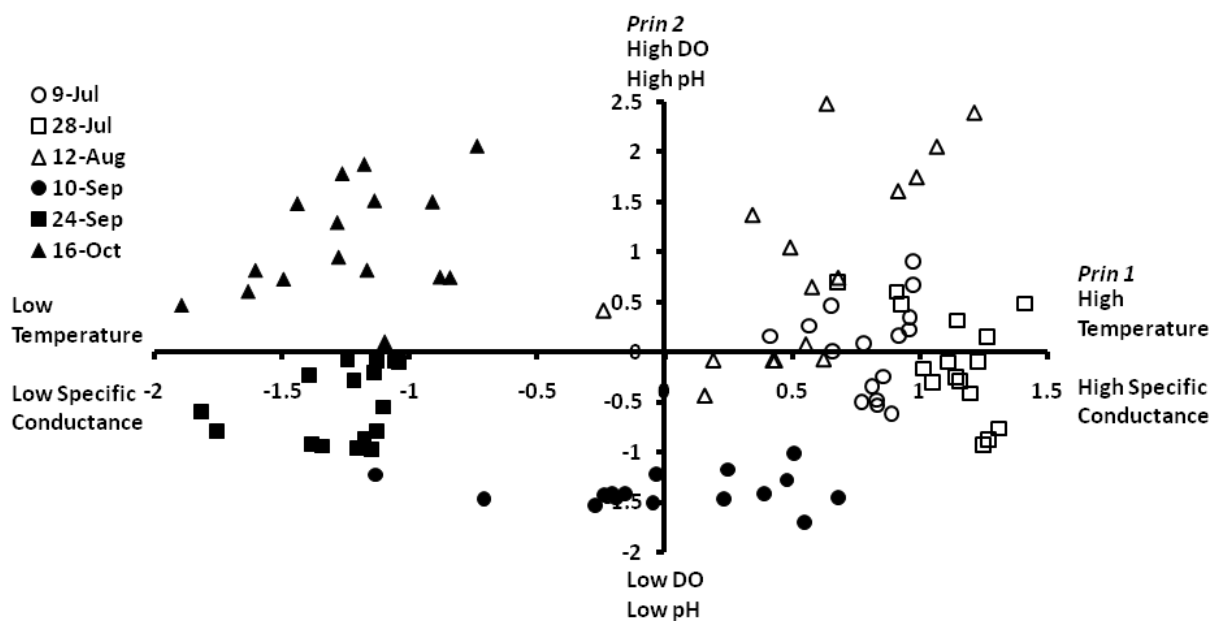


Figure 3.5. A bi-plot of principal components analysis of pre- and post-Gustav physicochemical parameters (dissolved oxygen, pH, temperature, and specific conductance) collected at multiple sample locations ($n = 16$) in the lower Atchafalaya River Basin during 2008. Open shapes are pre-Gustav sample dates (9 July, 28 July, and 12 August) and filled shapes are post-Gustav sample dates (10 September, 24 September, and 16 October). Multivariate analysis of variance detected a significant difference between pre- and post-Gustav physicochemistry (Wilks' Lambda = 0.10, $F_{4,91} = 209.29$, $P < 0.0001$).

DO observations in the weeks prior to the storm, but concentrations had already surpassed 12 August levels by 16 October (Figure 3.4). Although there was a clear physicochemical shift following the passage of both storms, water quality observations suggested that conditions were returning to 12 August pre-storm levels throughout the sample area within six weeks of the passage of hurricanes Gustav and Ike (Figure 3.4). Water quality changes were accompanied by a substantial increase in discharge from the Atchafalaya River (Figure 3.6).

Discussion

As Hurricane Gustav made landfall on the morning of 1 September, water depth in Little Bayou Jessie fell 0.54 meters (Figure 3.2) as northwest winds (Figure 3.3) transported water south and out of the bayou. Following eyewall passage, winds shifted to the southeast (Figure 3.3), pushing water northward, which resulted in a Little Bayou Jessie depth increase greater than 1 m (Figure 3.2). The relatively consistent specific conductance measurements as the storm passed over the system suggest that the storm surge in Little Bayou Jessie was not coastal or estuarine in origin (Figure 3.2). Given that Little Bayou Jessie drains into the northwest portion of Flat Lake, a shallow, freshwater lake (~ 3 m deep) in the southern portion of the study area and ARB, the observed depth oscillations were probably produced by a combination of Flat Lake surge coupled with variable flows and intermittent connectivity across much of the floodplain (Figure 3.2). As the high-intensity, unidirectional winds generated by Hurricane Gustav subsided, Little Bayou Jessie depth began to return to pre-storm levels, coincident with a rapid turbidity spike (Figure 3.2). This phenomenon is likely attributable to the mixing of different water masses as well as an increased suspended sediment load entering Little Bayou Jessie as the system drained.

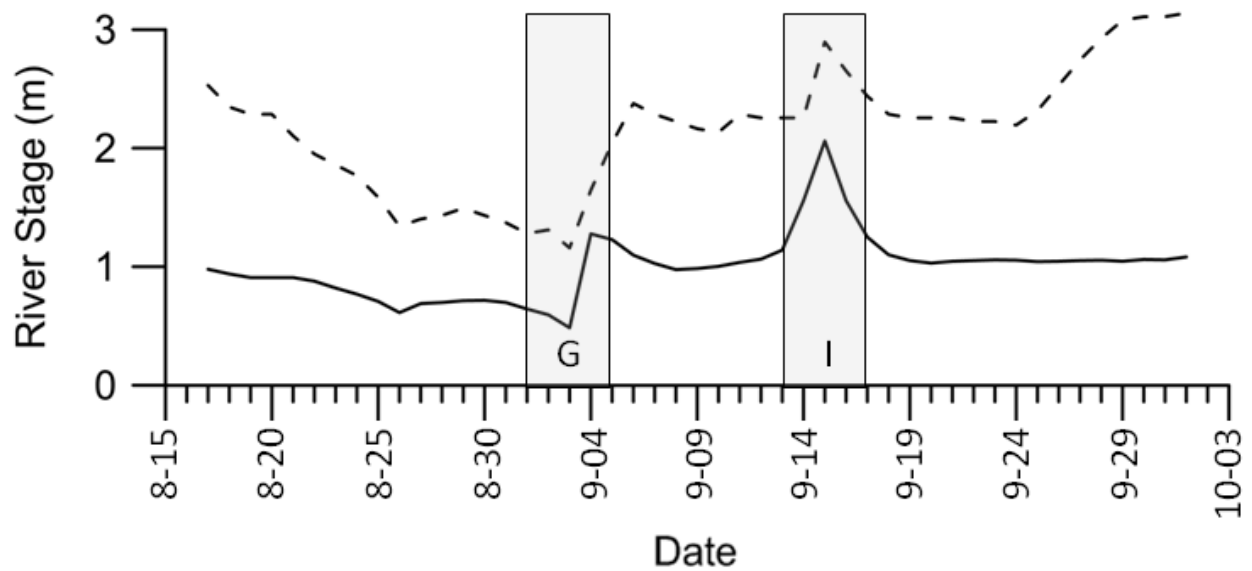


Figure 3.6. Atchafalaya River stage at Butte La Rose (broken line) and Morgan City (solid line; U. S. Army Corps of Engineers gauges 03120 and 03780 respectively), Louisiana from 15 August to 3 October 2008. Hurricane Gustav (G) made landfall on 1 September and Hurricane Ike (I) made landfall on 13 September during 2008. The grey box represents a 96 hour period that includes the passage of each storm.

Pervasive hypoxia is a common result following hurricane passage in coastal and near-coastal ecosystems (Tilmant et al. 1994; Mallin et al. 1999; Paerl et al. 2001; Mallin et al. 2002; Burkholder et al. 2004; Balthis et al. 2006; Stevens et al. 2006; Tomasko et al. 2006; Poirrier et al. 2008). In Little Bayou Jessie, DO concentrations became hypoxic less than 72 hours after Hurricane Gustav made landfall, and reached near anoxic levels five days post-storm (Figure 3.2). Prior to the passage of Hurricane Gustav, DO concentrations in Little Bayou Jessie never reached hypoxic levels during 2008 daytime sampling. Potential mechanisms for hurricane associated DO declines can be attributed to increased allochthonous inputs and subsequent decomposition (Van Dolah and Anderson 1991; Balthis et al. 2006), elevated biochemical oxygen demand levels (Mallin et al. 1999; Mallin et al. 2002; Tomasko et al. 2006), and wind-driven resuspension of benthic sediments (Jin et al. 2011) that can increase sediment oxygen demand rates and reduce water column DO concentrations (Wainright and Hopkinson 1997; Hickey 1998; Matlock et al. 2003; Waterman et al. 2011). Additionally, elevated riverine discharge (Figure 3.6) and increased turbidity likely reduced the euphotic zone, leading to decreased phytoplankton production (Tilmant et al. 1994; Mallin et al. 1999; Mallin et al. 2002; Burkholder et al. 2004; James et al. 2008), temporally shifting the productivity of the system towards heterotrophy. Daily sonde pH measurements in Little Bayou Jessie began to decline on 4 September and coincide with DO decreases that suggest the presence of elevated carbon dioxide and hydrogen ions associated with the onset of anaerobic conditions throughout the water column (see Makkaveev 2009). Interestingly, DO returned to, and pH was near 12 August pre-Gustav levels within six weeks (Figure 3.4) despite the passage of Hurricane Ike on 13 September. Although Ike made landfall almost 500 km west of the ARB, strong winds and rain associated with the storm triggered rapid, sustained fluctuations in the Atchafalaya River. These

fluctuations lasted for three weeks (Figure 3.6) and are likely the reason for the delayed return of specific conductance to pre-Gustav level (Figure 3.4). While it is unclear if Hurricane Ike prolonged post-Gustav DO and pH recovery in the ARB, it does not appear to have significantly hindered improvement.

In the ARB, hypoxic conditions generated by the arrival of the flood pulse tend to occur gradually, allowing aquatic organisms the opportunity to acclimate to reduced oxygen levels or relocate to more favorable environments (Rutherford et al. 2001). Mean rate of oxygen decline in the lower ARB during a typical flood pulse is approximately 0.06 mg/L/day (C. P. Bonvillain unpublished data). Consequently, large non-storm related fish kills are typically not experienced during the ARB flood pulse. Conversely, acute hurricane-associated reductions in DO can result in extensive fish mortality (Tabb and Jones 1962; Knott and Martore 1991; Tilmant et al. 1994; Mallin et al. 1999; Mallin et al. 2002; Burkholder et al. 2004) and lead to observed changes in fish community abundance and composition (Greenwood et al. 2006; Paperno et al. 2006; Stevens et al. 2006; Van Vrancken and O'Connell 2010). Because of rapid oxygen decline rates (4.32 mg/L/day to hypoxia then 0.48 mg/L/day to near anoxia), I observed near anoxic conditions at all 16 sample stations less than a week (0.23 mg/L mean DO concentration, Figure 3.4) after Hurricane Gustav. Fishes probably had limited opportunity to find suitable oxygen refugia which resulted in an extensive fish kill estimated at 128 million individuals throughout the ARB (LDWF 2008).

It is difficult to predict how environmental conditions in coastal ecosystems (both freshwater and brackish) oscillate in response to the passage of a hurricane because of variability in storm severity and point of landfall. In the ARB, spatially extensive and temporally persistent hypoxia (Sabo et al. 1999b; Rutherford et al. 2001) is associated with widespread overbank

flooding and rising water temperatures in late spring (Kaller et al. 2011). At the end of the flood pulse, hypoxic water on the inundated floodplain moves to canals, bayous, and lakes as Atchafalaya River stages decline (Sabo et al. 1999b; Fontenot et al. 2001), with DO recovery typically occurring after floodplain disconnection when water levels stabilize and primary production increases (Rutherford et al. 2001), often over a period of months. Our observations indicate that DO concentrations were already past seasonal lows by late summer in 2008, so the recovery time for ARB water quality after Hurricane Gustav was rapid relative to recovery from the flood pulse (Figure 3.4). An earlier strike might have overlapped with floodplain dewatering and could have prolonged sub-optimal oxygen conditions at the end of the flood pulse, whereas arrival of a storm later in the year would usually coincide with higher DO concentrations and lower water temperatures, perhaps weakening the severity and duration of post-storm hypoxia.

The increased understanding from this study of physicochemical changes following hurricane passage has implications for water management decisions in the ARB prior to and after storm landfall. I suggest that flood management practices in the ARB be evaluated regarding water releases and flooding potential after major storms, because if releases are feasible, an opportunity may exist to reduce storm-related impacts to the ARB, a situation that is not common among northern Gulf coast ecosystems. Because flows into the Atchafalaya River can be controlled, increased releases of oxygenated Mississippi River water into the ARB following a hurricane could help mitigate declines in physicochemical conditions, shorten recovery times, and perhaps reduce the probability of storm-related fish kills. However, sustained post-storm discharge in the Atchafalaya River could compromise levees damaged in the initial strike of a powerful storm and exacerbate flooding threats to Morgan City and other coastal areas.

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CHAPTER 4

PHYSIOLOGICAL BIOMARKERS OF HYPOXIC STRESS IN RED SWAMP CRAYFISH *PROCAMBARUS CLARKII* FROM FIELD AND LABORATORY EXPERIMENTS²

Introduction

Crayfish are ecologically important taxa in the United States, and economically important to the southeastern US region. Conservation status of the 363 crayfish species listed by the American Fisheries Society Endangered Species Committee recently reported 174 taxa (47.9%) as Vulnerable (54), Threatened (52), Endangered (66), or possibly Extinct (2; Taylor et al. 2007). Populations can be assessed regarding their overall health and risk to extirpation by use of physiologically-based conditional indices and biomarkers of acute and chronic stressors. However, such indices of condition and information on how these parameters relate to individual health are not yet established for crayfish. Crayfish physiology is an important component of health due to issues of water quality, habitat alteration, and presence of diseases. Anthropogenic stressors put populations at risk and could also threaten an important agricultural industry with a total value of more than \$181 million USD in Louisiana (LSUAC 2011).

Over 90% of the wild crayfish harvest in Louisiana comes from the Atchafalaya River Basin (ARB) located in south-central Louisiana (Isaacs and Lavergne 2010). The ARB is the largest bottomland hardwood river-floodplain system in North America (Lambou 1990) and its mosaic of bayous, petrochemical canals, lakes, and swamps, all affected by the annual Atchafalaya River flood pulse, provide a diversity of ideal habitats and conditions for a variety of crayfish species. However, anthropogenic modifications (levee and canal construction) have altered water circulation and flow patterns that facilitate the formation of hypoxic waters

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(dissolved oxygen [DO] ≤ 2 mg/L) that can persist for several weeks to months throughout extensive areas of the ARB during the annual flood pulse (Sabo et al. 1999).

Environmental hypoxia is not uncommon in many aquatic habitats and the biota occupying these environments must adapt and acclimate to temporary or extended periods of reduced oxygen concentrations in order to survive. Crayfish routinely encounter hypoxic conditions in aquatic or burrow habitats and have developed several physiological adaptations to cope with periods of sub-optimal oxygen concentrations (Mauro and Thompson 1984; McMahon 1986; Reiber 1995; Morris and Callaghan 1998; Reiber and McMahon 1998; Fujimori and Abe 2002; McMahon 2002; Silva-Castiglioni et al. 2010). Behaviorally, crayfish often move to the air-water interface in hypoxic areas to take advantage of higher DO concentrations, and may move out of the water completely (Taylor and Wheatly 1981; McMahon and Wilkes 1983; McMahon and Hankinson 1993; McMahon and Stuart 1999). Physiologically, crayfish have developed compensatory mechanisms such as hypometabolism, bradycardia, hyperventilation, and modulation of hemocyanin oxygen affinity that allow them to maintain homeostatic balance in hypoxic water. Although physiological adaptations and tolerance thresholds to hypoxia vary among crayfish species, the red swamp crayfish *Procambarus clarkii* is able to tolerate lower oxygen concentrations compared to other species (Nyström 2002) and can maintain high survival rates in oxygen poor environments (Powell and Watts 2006). Although compensatory mechanisms afford *P. clarkii* and other crayfishes the opportunity to tolerate hypoxic conditions, prolonged exposure could lead to detrimental population effects such as reduced survival (Avault et al. 1975; Melancon and Avault 1977; McClain 1999; Sladkova and Kholodkevich 2011) and growth (Jussila and Evans 1997; McClain 1999; Reynolds 2002; McClain et al. 2007).

Hypoxic stress elicits multiple physiological responses in crayfish. Hemolymph lactate, glucose, and protein concentrations in crayfish have been shown to fluctuate in response to hypoxia (Gäde 1984; Mauro and Thompson 1984; Morris and Callaghan 1998; Fujimori and Abe 2002; Silva-Castiglioni et al. 2010) and quantifying these biomarkers allows for examination of physiological responses during hypoxic stress. However, most of this research has been conducted in the laboratory in the absence of confounding biotic and abiotic factors which can significantly influence physiological responses to hypoxia in natural systems. Therefore, the purpose of the present study was to examine hemolymph lactate, glucose, and protein concentrations in *P. clarkii* from normoxic ($\text{DO} > 2 \text{ mg/L}$) and chronically hypoxic natural habitats in the ARB, as well as in individuals exposed to simulated hypoxia in the laboratory.

Methods

Field Collections

Adult intermolt *P. clarkii* were collected from two hypoxic and two normoxic sites in the southeastern ARB during floodplain inundation with five pillow traps per site baited with 150 g of Purina[®] manufactured bait that were fished overnight. I tried to minimize handling stress and possible hemolymph lactate and glucose increases associated with aerial exposure and processing time by sampling no more than two individuals per trap. Crayfish hemolymph was collected by pericardial cavity puncture (20-gauge needle) and transferred via capillary tube into a microcentrifuge tube and placed immediately on ice, allowed to clot, and stored at -4°C in the laboratory. I recorded carapace length (mm), weight (g), and sex of sampled crayfish. Five hemolymph samples per site ($n = 10$ normoxic, $n = 10$ hypoxic) were collected on 12 April, 7 May, 1 June, and 1 July 2010. I was unable to attain *P. clarkii* hemolymph samples after 1 July due to receding floodplain water levels and a retreat of crayfish into burrows. Temperature ($^{\circ}\text{C}$),

DO (mg/L), and pH were recorded with a handheld multiparameter water quality sonde (YSI model 6820, Yellow Springs, Ohio) at each sample site. Additionally, a continuous recording water quality sonde (YSI model 6600, Yellow Springs, Ohio) was deployed from 21-27 April 2010 at one of the sampled hypoxic sites to record DO concentrations at 15 minute intervals ($n = 96$ observations per day).

Laboratory Experiments

P. clarkii were collected from the ARB and acclimated to laboratory conditions in a recirculating raceway for a minimum of four weeks prior to experimentation. While in raceways, crayfish were fed algal wafers *ad libitum* semi-weekly. I recorded the carapace length, weight, and sex of adult intermolt crayfish then placed them into individual 1-L polyethylene amber bottles with 0.635-cm holes over the entire bottle to allow uninhibited water circulation. Bottles restricted access to atmospheric oxygen at the air-water interface and eliminated conspecific interactions. Individual crayfish in bottles were placed into nine 30-L aquaria filled with ARB water and equipped with a standpipe and under gravel filter powered by an air stone. Each aquarium was aerated with atmospheric air ($DO > 7.5$ mg/L) and contained six crayfish (3 males and 3 females), which were allowed to acclimate in the aquaria for 24 h before the beginning of experimental trials. Three DO concentrations were randomly assigned to the nine aquaria: control ($DO > 7.5$ mg/L), moderate hypoxia ($DO \sim 2$ mg/L), and severe hypoxia ($DO \sim 1$ mg/L). Control aquaria were kept at full DO saturation by bubbling air through the under gravel filter. Moderate and severe hypoxia conditions were created by bubbling in nitrogen or a nitrogen/air mixture. Because bubbling nitrogen can decrease water temperatures, heaters (Stealth 50, Marineland Aquarium Products, Cincinnati, Ohio) were added to all aquaria to maintain consistent temperatures among treatments (Table 4.1).

Table 4.1. Mean (\pm SE) of water quality parameters in aquaria ($n = 3$ per treatment) during experimental trials. Asterisks denote a significant difference ($P < 0.05$) within experiments.

Experiment	Treatment	n	Dissolved Oxygen (mg/L)	Temperature (°C)	pH
12 h	Control	18	8.45 ± 0.06	25.07 ± 0.15	7.44 ± 0.01
	Moderate	18	1.93 ± 0.04	24.67 ± 0.06	$7.33 \pm 0.02^*$
	Severe	18	1.03 ± 0.02	25.04 ± 0.15	7.50 ± 0.03
24 h	Control	24	8.01 ± 0.02	24.83 ± 0.09	7.40 ± 0.01
	Moderate	24	2.05 ± 0.03	24.59 ± 0.08	$7.28 \pm 0.03^*$
	Severe	24	1.10 ± 0.02	24.40 ± 0.07	7.38 ± 0.02
48 h	Control	18	8.62 ± 0.05	24.28 ± 0.21	7.40 ± 0.01
	Moderate	18	2.09 ± 0.03	23.88 ± 0.06	$7.22 \pm 0.02^*$
	Severe	18	0.99 ± 0.05	24.15 ± 0.06	7.42 ± 0.02

Three separate experiments were performed in which *P. clarkii* were exposed to aforementioned experimental DO concentrations for 12, 24, and 48 h ($n = 54$ crayfish per time trial). DO levels in moderate and severe hypoxia aquaria were decreased at similar rates and a time trial began when the desired DO concentrations were attained (usually within 2.5 h). Temperature, DO, and pH were recorded with a handheld multiparameter water quality sonde. Crayfish hemolymph was extracted from individuals at the conclusion of each time trial with the same methods used in the field, with the clotted sample stored at -4°C .

Hemolymph Processing

Hemolymph clots were broken up, the sample was centrifuged ($2415g$ for 15 min at 4°C), and the supernatant serum was extracted. Spectrophotometric assays were performed in triplicate and read with a BioTek[®] Synergy 2 Multi-Purpose Microplate Reader (BioTek[®], Winooski, Vermont) and interpreted with Gen5[™] analysis software (BioTek[®], Winooski, Vermont).

Hemolymph lactate concentration was enzymatically determined with an L-lactate assay kit (Eton Bioscience Inc., San Diego, California). Each sample was diluted fifty fold with deionized water. Twenty μL of diluted samples and lactate standards were added to 96-well microplates. Fifty μL of Lactate Assay Solution was then added to each well, mixed by gentle agitation, and incubated at 37°C for 30 minutes. The reaction was then stopped by adding 50 μL of 0.5-M acetic acid to each well. Standard curves were prepared at concentrations of 0, 39, 78, 156, 312.5, 625, 1250, and 2500 μM L-lactate per manufacturer's instructions. Standard curves for lactate assays had R^2 values of 0.99 or greater. Absorbance was measured at 490 nm and reported as mmol/L.

Total protein concentration of crayfish serum was determined with the Pierce Coomassie Plus assay (Thermo Fisher Scientific Inc., Rockford, Illinois) modified from the Bradford method (Bradford 1976). Each sample was diluted one hundred fold with deionized water. Standard curves were prepared at concentrations of 0, 25, 125, 250, 500, 750, 1000, 1500, and 2000 $\mu\text{g/mL}$ bovine serum albumin per manufacturer's instructions. Standard curves for protein assays had R^2 values of 0.99 or greater. Absorbance was measured at 595 nM and reported as mg/mL.

Hemolymph glucose concentration was determined with a colorimetric QuantiChromTM Glucose Assay Kit as per manufacturer instructions (BioAssay Systems, Hayward, California). Standard curves were prepared at concentrations of 0, 50, 100, 200, and 300 mg/dL $\text{C}_6\text{H}_{12}\text{O}_6$ per manufacturer's instructions. Standard curves for glucose assays had R^2 values of 0.97 or greater. Absorbance was measured at 630 nM and reported as mmol/L.

Data Analysis

All statistical tests were performed with SAS 9.1.3 and significance for all statistical tests was determined with a Type I error rate of $\alpha = 0.05$.

Analysis of variance (ANOVA) with a Tukey-Kramer *post hoc* adjustment was used to test for differences in DO concentrations between hypoxic sites as well as between normoxic sites. Additionally, ANOVA with a Tukey-Kramer *post hoc* adjustment was performed to test for differences in carapace length, weight, and DO between hypoxic and normoxic treatments. Lactate and glucose concentrations from *P. clarkii* hemolymph collected at field sites were $\log_{(e)}$ transformed to improve normality. Analysis of covariance (ANCOVA; PROC MIXED) with a Tukey-Kramer *post hoc* adjustment was used to examine significance of Julian date (covariate) and to test for differences in hemolymph protein and glucose concentrations between hypoxic

and normoxic treatments. Hemolymph lactate concentrations from hypoxic and normoxic sites were not influenced by sampling date (i.e., ANCOVA slopes equal to zero), so an ANOVA with a Tukey-Kramer *post hoc* adjustment was performed to test for a difference between treatments.

ANOVA with a Tukey-Kramer *post hoc* adjustment was used to test for differences in temperature and pH among treatments for each time trial experiment. Variances of hemolymph lactate, glucose, and protein concentrations from laboratory experiments were homogeneous (Levene's test). Lactate concentrations were inverse transformed and glucose concentrations were $\log_{(e)}$ transformed to improve normality. A two-level hierarchical ANOVA (PROC MIXED) with a Tukey-Kramer *post hoc* adjustment was used for each physiological biomarker to examine statistically significant differences among DO treatments for each time trial experiment. Additionally, a two-way ANOVA (PROC GLM) was used to test for differences in physiological responses between sexes.

Results

Field Collections

Crayfish size (carapace length) and weight did not differ significantly between hypoxic (48.76 ± 0.86 mm, 21.96 ± 1.35 g) and normoxic (50.19 ± 0.86 mm, 24.25 ± 1.40 g) treatments. Mean DO concentrations did not differ between hypoxic sites and were below 2.0 mg/L on every sample date. Similarly, mean DO concentrations did not differ between normoxic sites and were above hypoxic level on every sample date. However, diurnal mean DO concentrations from hypoxic sites (1.21 ± 0.07 mg/L) were significantly lower than normoxic sites (3.64 ± 0.60 mg/L; $F_{1,14} = 32.22$, $P < 0.0001$). Quarter-hourly sonde measurements taken over a one week period at the hypoxic site revealed elevated DO concentrations in the afternoon with concentrations above 1.5 mg/L recorded on six days and 2.0 mg/L on three days (Figure 4.1).

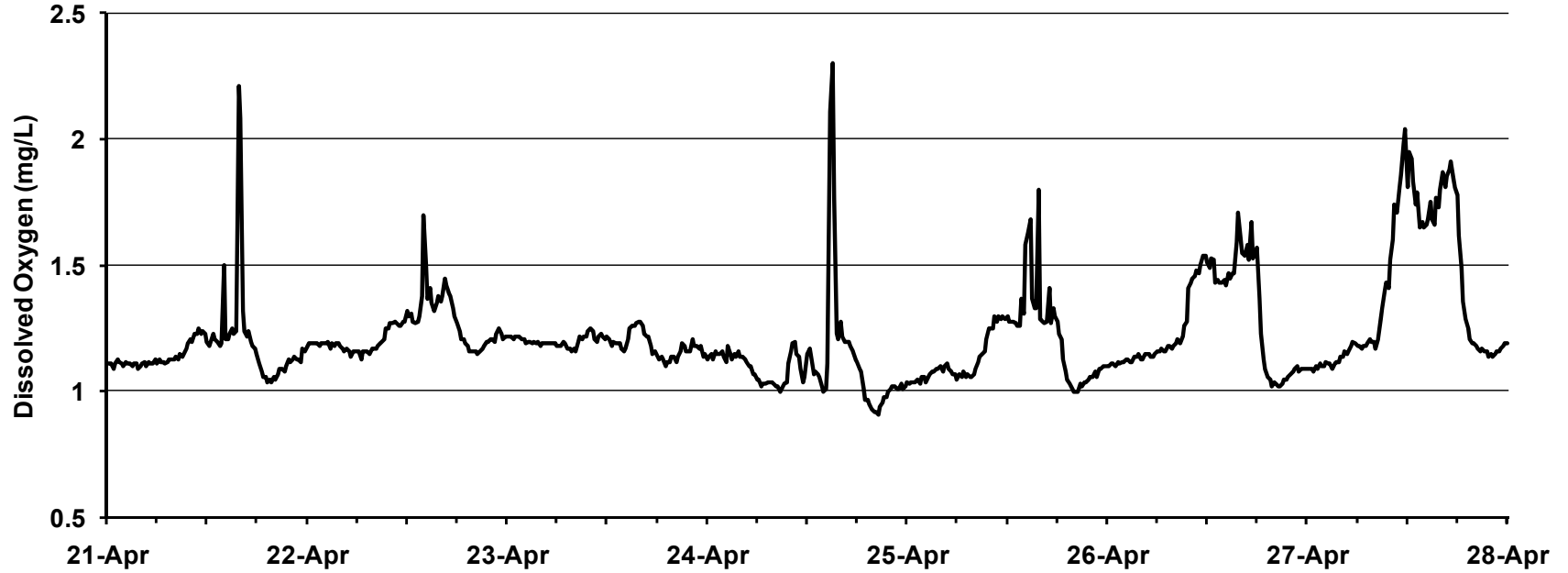


Figure 4.1. Quarter-hourly dissolved oxygen sonde measurements from 21-27 April 2010 at a chronically hypoxic site in the lower Atchafalaya River Basin.

These DO concentrations represent total water column DO concentrations based on previous sampling in the ARB which demonstrated that DO levels are homogeneous throughout the floodplain water column (Halloran 2010).

Sampling date did not influence *P. clarkii* hemolymph lactate concentrations. Mean hemolymph lactate concentrations from crayfish collected in hypoxic and normoxic areas were 20.26 ± 3.93 mmol/L and 9.99 ± 1.46 mmol/L respectively, however, there was not a significant difference between treatments. Mean lactate concentrations from hypoxic sites were higher than normoxic sites on every sample date except for 7 May 2010 (Figure 4.2A).

ANCOVA revealed that the slopes of the regressions for hypoxic and normoxic treatments with sampling date were not significantly different. Mean crayfish hemolymph glucose concentrations from hypoxic and normoxic areas were 1.22 ± 0.11 mmol/L and 1.34 ± 0.15 mmol/L respectively and were not significantly different (Figure 4.2B).

As with glucose, ANCOVA revealed that the slopes of the regressions for hypoxic and normoxic treatments with sampling date were not significantly different. However, crayfish hemolymph protein concentrations from hypoxic areas (49.47 ± 2.71 mg/mL) were significantly lower than those from crayfish collected in normoxic areas (61.61 ± 3.48 mg/mL; $F_{1,77} = 8.89$, $P = 0.0038$). Moreover, mean protein concentrations in crayfish collected from normoxic sites were higher than hypoxic sites on every sample date (Figure 4.2C).

Laboratory Experiments

The pH of moderate hypoxia treatment tanks was significantly lower in all three laboratory time trial experiments, although differences were not more than 0.2 pH units (Table 4.1). There was no significant difference in temperature among treatments in any of the time trials. Crayfish mortalities occurred in each time trial but were limited to only severe hypoxia

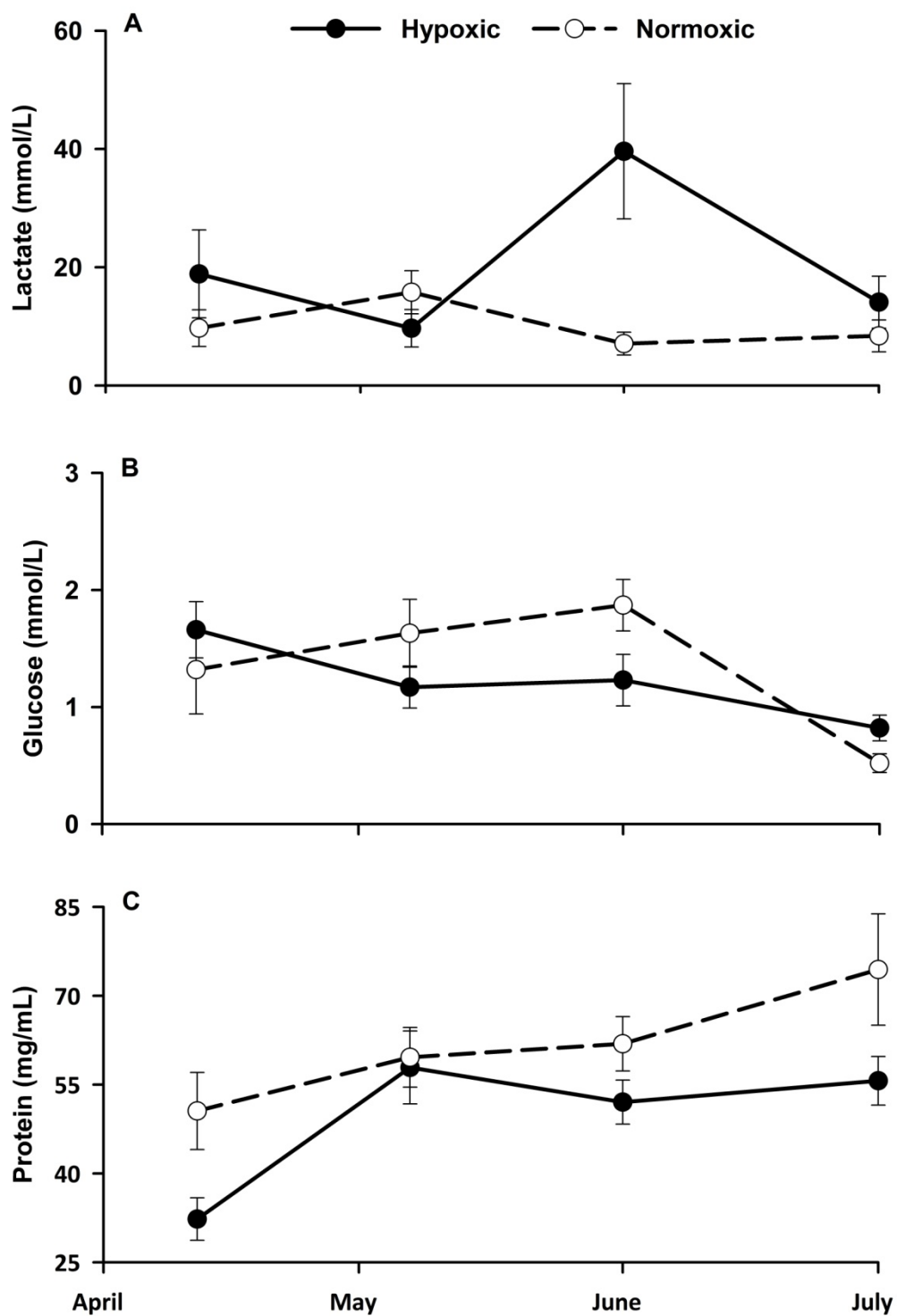


Figure 4.2. Mean (\pm SE) hemolymph (A) lactate, (B) glucose, and (C) protein concentrations in *P. clarkii* from hypoxic and normoxic sites in the Atchafalaya River Basin during 2010 sampling.

treatment tanks (12 h $n = 2$; 24 h $n = 1$; 48 h $n = 9$). Hemolymph lactate, protein, and glucose concentrations did not differ statistically between male and female *P. clarkii*.

P. clarkii mean hemolymph lactate concentrations were significantly higher in individuals subjected to the severe hypoxia treatment for the 12, 24, and 48 h experiments (Figure 4.3A). Moreover, mean lactate concentration in crayfish from severe hypoxia treatments exhibited an increasing trend with exposure time. Lactate concentrations for the moderate hypoxia and control treatments did not differ statistically in any of the time trials.

Mean hemolymph glucose concentration from the moderate hypoxia DO treatment was not dissimilar from severe hypoxia and control treatments but severe hypoxia-exposed individuals had significantly higher concentrations compared to control subjects after 12 h. Additional exposure time revealed significantly higher mean glucose concentrations in severe hypoxia treatments relative to moderate hypoxia and control treatments at both 24 and 48 h (Figure 4.3B). Mean hemolymph glucose concentrations from control and moderate hypoxia treatments were not dissimilar in the 24 and 48 h experiments.

Mean hemolymph protein concentration from the moderate hypoxia treatment was not significantly different from severe hypoxia and control treatments, however, severe hypoxia exposed individuals had significantly higher concentrations than control subjects in the 24 h experiment. Treatments did not differ statistically in the 12 and 48 h experiments (Figure 4.3C).

Discussion

Procambarus clarkii frequently inhabits environments that experience extended periods of aquatic hypoxia in the ARB, and have developed behavioral and physiological adaptations in order to successfully exploit these sub-optimal DO environments. I observed changes in hemolymph lactate, glucose, and protein concentrations from crayfish exposed to hypoxic water

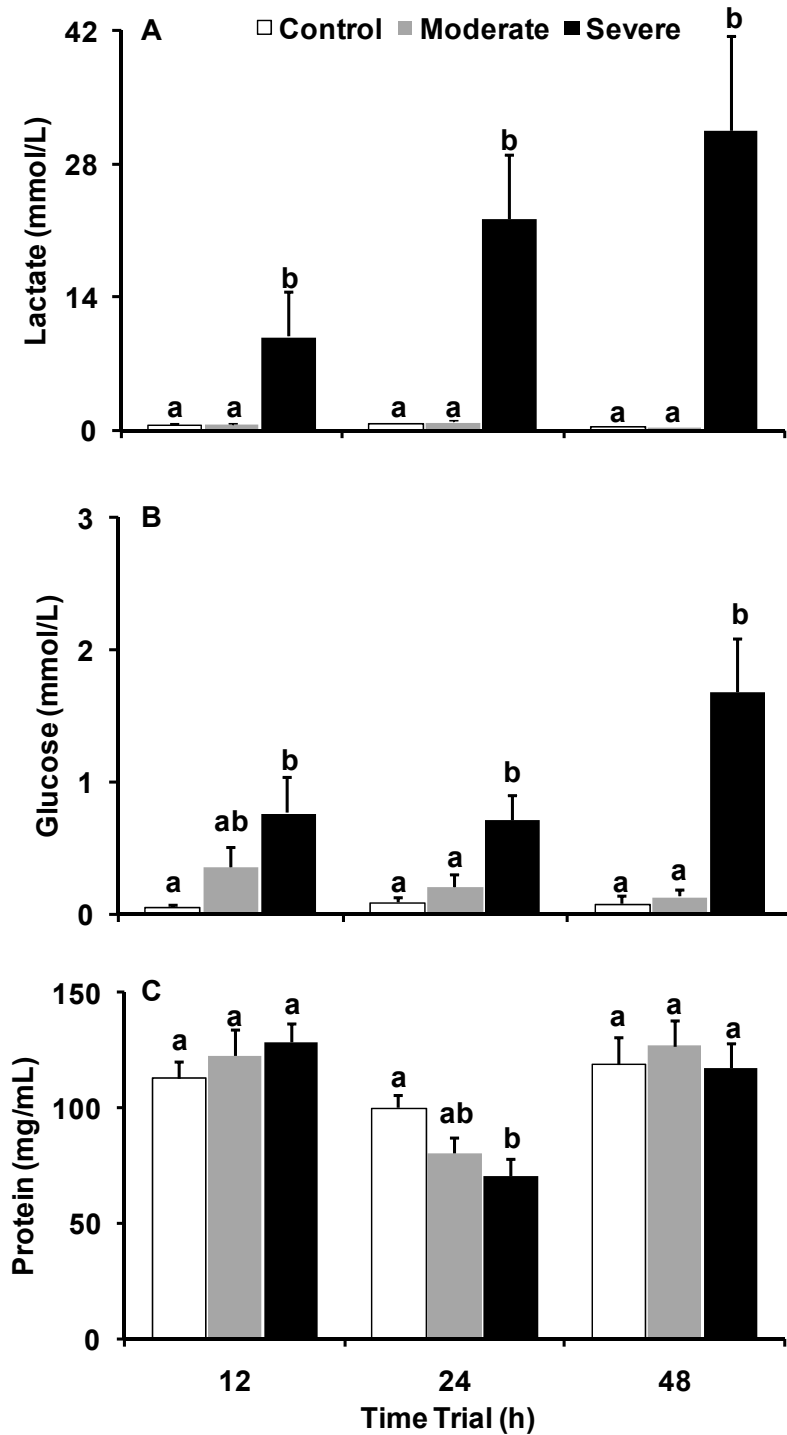


Figure 4.3. Mean (±SE) hemolymph (A) lactate, (B) glucose, and (C) protein concentrations in *P. clarkii* subjected to three laboratory simulated dissolved oxygen (DO) concentrations during three exposure time experiments. Control: DO > 7.5 mg/L, moderate hypoxia: DO ~ 2 mg/L, and severe hypoxia: DO ~ 1 mg/L. Different letters indicate a significant difference ($P < 0.05$) for each physiological parameter within a time trial experiment.

in both field and laboratory experiments. As environmental DO concentrations become unfavorable, some crayfish will switch to anaerobic metabolism in order to maintain homeostasis (Gäde 1984; Morris and Callaghan 1998; Fujimori and Abe 2002; Silva-Castiglioni et al. 2010). Lactate is the major end product of anaerobic metabolism in crustaceans while also enhancing hemocyanin O₂-binding affinity during hypoxia (Truchot 1980; Järvenpää et al. 1983; Bouchet and Trouhot 1985; McMahon 1986; Morris and Callaghan 1998; McMahon 2001; Qui et al. 2011). Hemolymph lactate accumulation is related to the duration and severity of hypoxia (Albert and Ellington 1985) and researchers have documented lactate increases from hypoxia-stressed crayfish (Gäde 1984; Mauro and Thompson 1984; Morris and Callaghan 1998; Jackson et al. 2001; Fujimori and Abe 2002; Silva-Castiglioni et al. 2010, 2011). Thus, elevated hemolymph lactate concentration appears to serve as a viable biomarker of hypoxic stress in crayfish. I observed significantly higher mean hemolymph lactate concentrations in *P. clarkii* from severe hypoxia treatments in all three laboratory time trial experiments and a 220% increase in lactate concentration from 12 h to 48 h, suggesting activation of anaerobic pathways. Because crayfish were kept in bottles during experimentation, individuals were restricted from using atmospheric air to supplement their oxygen uptake, affirming that the observed lactate concentrations are representative of the physiological response to treatment DO concentrations. Interestingly, hyperlactaemia was not observed in moderate hypoxia-treatment crayfish, with mean concentrations similar to control individuals. These results suggest that a DO concentration of 2 mg/L does not elicit anaerobic metabolism activation and therefore does not appear to be physiologically stressful to quiescent *P. clarkii*.

P. clarkii hemolymph glucose concentrations from severe hypoxia treatments were also significantly higher in all three laboratory time trials. Hyperglycemia is commonly recognized

as a stress response to hypoxia in decapod crustaceans (e.g., crab, Zou et al. 1996; shrimp, Racotta et al. 2002; lobster, Ocampo et al. 2003; and crayfish, Silva-Castiglioni et al. 2010). During hypoxia, glycogenolysis enhances hemolymph glucose concentrations in order to provide increased substrate for anaerobic glycolysis (Oliveira et al. 2001; Silva-Castiglioni et al. 2010, 2011; Marqueze et al. 2011). The somewhat elevated hemolymph glucose concentration observed in the 12 h moderate hypoxia treatment may be the result of a physiological preparation for impending anaerobic glycolytic demands (Taylor and Spicer 1987; Zou et al. 1996). Detection of declining DO concentrations by *P. clarkii* at the start of the experimental trial initiated glucose mobilization for use in a potentially stressful hypoxic environment. When DO concentrations stabilized at approximately 2 mg/L, superfluous glucose was presumably synthesized back into glycogen. Although glycogen concentrations were not measured in this study, increasing glycogen associated with decreasing glucose in crustaceans has been previously documented (Oliveira et al. 2001; Morris and Adamczewska 2002; Patterson et al. 2007; Silva-Castiglioni et al. 2010, 2011; Marqueze et al. 2011). Furthermore, there was a decline in *P. clarkii* mean hemolymph glucose concentration with exposure time in the moderate hypoxia treatment (Figure 4.3B).

P. clarkii from normoxic and hypoxic field sites showed no significant difference in mean glucose or lactate concentrations even though individuals from hypoxic sites demonstrated lactate concentrations of more than twice that of crayfish from normoxic sites. This was a result of high lactate concentrations from a few hypoxic exposed individuals on 1 June (Figure 4.2A). It is not surprising, however, that hemolymph glucose and lactate concentrations from individuals captured in hypoxic and normoxic areas were similar. Bimodal respiration in crayfish is possible because of trichobranchiate gills which do not collapse in air and allow for

utilization of atmospheric air during reduced DO periods. Total and partial emergence and aerial ventilation increases crayfish hemolymph oxygenation (Taylor and Wheatly 1981; McMahon and Wilkes 1983; McMahon and Hankinson 1993; McMahon and Stuart 1999) and reduces hemolymph lactate levels (Gäde 1984). This adaptive strategy could enable *P. clarkii* inhabiting low oxygen environments to avoid anaerobiosis and accumulation of concomitant metabolites.

Biotic and abiotic characteristics of natural habitats may also mitigate *P. clarkii* physiological stress in hypoxic environments. Hydrophytes that enhance ARB hypoxia by reducing water circulation and increasing benthic decomposition rates may serve as local DO refugia (Miranda et al. 2000; Bunch et al. 2010) for crayfish inhabiting these environments, at least during the day. Fontenot et al. (2001) observed normoxic conditions in littoral macrophyte beds adjacent to hypoxic limnetic areas in the ARB. Aquatic macrophyte stands could help offset environmental anaerobiosis that crayfish would experience in the surrounding hypoxic water.

Many crayfish are oxygen regulators and can maintain normal metabolic rates during reduced ambient oxygen concentrations until a critical oxygen level (P_c) is reached. At oxygen concentrations below P_c , oxyconformation reduces metabolic rates prompting anaerobic metabolism activation and subsequent lactate and glucose accumulation (Morris and Callaghan 1998). The observed increase in *P. clarkii* lactate and glucose hemolymph concentrations from severe hypoxia treatments in all three time trial experiments suggests that DO concentrations were below P_c and that anaerobic metabolism was initiated. Conversely, *P. clarkii* from moderate hypoxia treatment tanks did not display significantly increased hemolymph lactate and glucose concentrations relative to control individuals. These results suggest that P_c for quiescent *P. clarkii* under laboratory conditions is between 1 and 2 mg/L. This may prove beneficial to

crayfish inhabiting hypoxic areas of the ARB. Diurnal oxygen concentrations in hypoxic ARB waters regularly increased to near or above 2 mg/L over the week that I monitored (Figure 4.1). If the P_c of *P. clarkii* in the ARB is similar to that indicated by our laboratory results, then diel oxygen fluctuations in hypoxic areas in conjunction with the ability of crayfish to utilize atmospheric oxygen may allow crayfish to escape prolonged exposure to periods below P_c which would allow for increased metabolic activity and elimination or reduction of accumulated anaerobic metabolites. Although environmental parameters (Staples et al. 2000), activity (Crear and Forteach 2000), and feeding (Robertson et al. 2002) can all influence P_c , it can still be interpreted as a homeostatic balance point between oxyregulation and oxyconformation and a physiological response to reduced oxygen environments (Reiber 1995).

Although biotic and abiotic factors may offset hemolymph lactate and glucose concentrations in *P. clarkii* exposed to naturally hypoxic habitats, hemolymph protein concentrations demonstrate marked effects of chronic hypoxic exposure. Protein concentrations in ARB *P. clarkii* from normoxic areas were significantly and consistently higher than individuals sampled from hypoxic areas (Figure 4.2C). Although ANCOVA failed to demonstrate date as a significant covariate, the disparity in protein concentrations between normoxic and hypoxic areas appears to intensify through time and extended hypoxic exposure appears to slow increases in hemolymph protein concentrations. In the current study, *P. clarkii* hemolymph protein concentrations from normoxic areas increased throughout the spring and summer presumably due to increased photoperiod, temperatures, and food availability (Sladkova and Kholodkevich 2011). Conversely, lower protein concentrations in *P. clarkii* from hypoxic areas may be the result of both extrinsic and intrinsic factors. Active foraging and food consumption may be diminished in hypoxic waters (Bailey et al. 1985; Das and Stickle 1993;

Paschke et al. 2010), requiring hemolymph protein concentrations to be used as organic reserves (Oliver and MacDiarmid 2001) which would in turn reduce protein concentrations in starved individuals (Wen et al. 2007). Silva-Castiglioni et al. (2007) also documented decreased hemolymph protein concentrations in crayfish from natural habitats during reduced DO periods. Additionally, *P. clarkii* occupying hypoxic waters above P_c and utilizing these physiological compensation mechanisms, may fall below P_c as the result of increased oxygen consumption associated with activity, feeding, (Crear and Forteach 2000) and digestive processes (Du Preez et al. 1992).

Hemolymph protein concentration appears to be a biomarker of chronic rather than acute hypoxic stress in *P. clarkii* as demonstrated by our field and laboratory results. Although *P. clarkii* in the ARB are exposed to chronic hypoxia that can last for periods of weeks to months, laboratory experiments that subjected crayfish to acute hypoxic conditions for a maximum of two days failed to elicit consistent significant differences in hemolymph protein concentrations. Changes in hemolymph protein concentrations associated with laboratory hypoxia experiments were also not documented in *Parastacus defossus* after 8 h of exposure (Silva-Castiglioni et al. 2010), *Litopenaeus stylirostris* after 24 h of exposure (Mugnier et al. 2008), *Penaeus vannamei* after 2 weeks of exposure (Racotta et al. 2002), or *Orconectes rusticus* after 3.5 weeks of exposure (Wilkes and McMahon 1982).

P. clarkii in this study have demonstrated multiple physiological responses to environmental hypoxia. The high oxygen affinity of *P. clarkii* hemolymph (McMahon and Hankinson 1993; McMahon and Stuart 1999; McMahon 2001; Powell and Watts 2006) allows this species to maintain normal metabolic functions at relatively low DO concentrations. The short hypoxic exposure time and relatively rapid rate of oxygen decline in laboratory

experiments compared to the onset of naturally occurring hypoxia in the ARB (Bonvillain et al. 2011) suggests that hemolymph lactate and glucose concentrations are acute biomarkers of hypoxic stress in *P. clarkii* whereas protein levels are an indicator of chronic hypoxic stress. Furthermore, these results demonstrate that physiological responses by animals to environmental stressors under laboratory simulations can vary markedly from the actual responses experienced in natural environments.

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CHAPTER 5
VALIDATION AND USE OF HAND-HELD DEVICES FOR DETERMINATION OF
HEMOLYMPH PROTEIN AND LACTATE CONCENTRATIONS IN RED SWAMP
CRAYFISH *PROCAMBARUS CLARKII*

Introduction

The crayfish industry in Louisiana is the largest in the United States (McClain et al. 2007) with a total value of more than \$209 million (LSUAC 2012). Crayfish are harvested either from managed ponds (farmed) or natural habitats (wild) with the majority of landings consisting of the red swamp crayfish *Procambarus clarkii*. While water quality parameters fluctuate both spatially and temporally, hypoxia (dissolved oxygen ≤ 2.0 mg/L) is commonly observed in both farmed and wild crayfish habitats (Avault et al. 1975; McClain 1999; Sabo et al. 1999; Rutherford et al. 2001; Bonvillain et al. 2012). Although *P. clarkii* are able to tolerate relatively low dissolved oxygen concentrations (Nyström 2002), prolonged exposure to sub-optimal dissolved oxygen conditions can lead to detrimental population effects such as reduced survival (Avault et al. 1975; Melancon and Avault 1977; McClain 1999; Sladkova and Kholodkevich 2011), growth (Jussila and Evans 1997; McClain 1999; Reynolds 2002; McClain et al. 2007), and minimum size at maturity (Huner and Romaine 1978). Furthermore, physiologically stressed crayfish harvested from low dissolved oxygen waters may suffer increased mortality rates during the transport and storage processes associated with the crayfish industry. Crayfish are shipped and stored in 16 to 20 kg plastic mesh sacks without water for several days (McClain et al. 2007). Although these methods are the most economical and provide high survival rates, they still expose crayfish to stressful conditions which may be exacerbated in individuals removed from poor water quality habitats.

Physiologically-based conditional indices provide a means to determine an animal's physical condition. Biomarkers such as hemolymph lactate and protein concentrations have been

used to examine physiological health in crayfish (Jussila et al. 1999; Silva-Castiglioni et al. 2010; Sladkova and Kholodkevich 2011; Bonvillain et al. 2012). However, conventional analytical methods for determining hemolymph metabolite concentrations require expensive laboratory equipment and materials including laboratory analysis time. These methods are not economical or timely for most individuals in the crayfish industry or persons examining potential influences of environmental stressors on crayfish populations.

Hand-held devices provide a quick and economical alternative for determination of physiological parameters. Refractometric methods for examining hemolymph protein concentrations in crustaceans have been used to determine physiological health in lobsters (Leavitt and Bayer 1977; Oliver and MacDiarmid 2001; Ozbay and Riley 2002; Bolton et al. 2009; Chandrapavan et al. 2009), prawns (Smith and Dall 1982; Moore et al. 2000), and crab (Uhlmann et al. 2009). However, refractometric determination of hemolymph protein concentration has not been evaluated in crayfish. Additionally, the use of a hand-held lactate meter is a novel, economical method for determination of hemolymph lactate concentrations in crustaceans. The purpose of this study was to validate the accuracy of a hand-held refractometer and lactate meter for determination of *P. clarkii* hemolymph protein and lactate concentrations by comparing results from the hand-held devices against spectrophotometric measurements.

Methods

Hemolymph Collection and Processing

Adult intermolt *P. clarkii* were collected from five locations in the southeastern Atchafalaya River Basin during floodplain inundation with five pillow traps per site that were baited with 150 g of Purina[®] manufactured bait and fished overnight. Crayfish hemolymph was collected by pericardial cavity puncture (20-gauge needle) and transferred via capillary tube into

a microcentrifuge tube and placed immediately on ice, allowed to clot, and stored at -4°C in the laboratory. Five hemolymph samples per site were collected monthly from April to July 2010. Because of limited hemolymph volume in some samples, available quantities were used for either lactate ($n = 81$) or protein ($n = 85$) determinations.

Laboratory hemolymph samples were obtained from *P. clarkii* that were subjected to hypoxic exposure experiments conducted by Bonvillain et al. (2012). Following examination of spectrophotometric results of hemolymph lactate and protein concentrations from aforementioned *P. clarkii*, hemolymph samples were selected to obtain a broad range of lactate ($n = 45$) and protein ($n = 51$) concentrations. Hemolymph samples were extracted with the same methods used in the field, with the clotted sample stored at -4 °C.

Hemolymph Lactate and Protein Determinations with Spectrophotometry

Hemolymph clots were broken up, the sample was centrifuged (2415g for 15 min at 4°C), and the supernatant serum was extracted. Spectrophotometric assays were performed in triplicate and read with a BioTek[®] Synergy 2 Multi-Purpose Microplate Reader (BioTek[®], Winooski, Vermont) and interpreted with Gen5[™] analysis software (BioTek[®], Winooski, Vermont).

Hemolymph lactate concentration was enzymatically determined with an L-lactate assay kit (Eton Bioscience Inc., San Diego, California). Each sample was diluted fifty fold with deionized water. Twenty µL of diluted samples and lactate standards were added to 96-well microplates. Fifty µL of Lactate Assay Solution was then added to each well, mixed by gentle agitation, and incubated at 37°C for 30 minutes. The reaction was then stopped by adding 50 µL of 0.5-M acetic acid to each well. Standard curves were prepared at concentrations of 0, 39, 78, 156, 312.5, 625, 1250, and 2500 µM L-lactate per manufacturer's instructions. Standard curves

for lactate assays had R^2 values of 0.99 or greater. Absorbance was measured at 490 nM and reported as mmol/L.

Total protein concentration of crayfish serum was determined with the Pierce Coomassie Plus assay (Thermo Fisher Scientific Inc., Rockford, Illinois) modified from the Bradford method (Bradford 1976). Each sample was diluted one hundred fold with deionized water. Standard curves were prepared at concentrations of 0, 25, 125, 250, 500, 750, 1000, 1500, and 2000 $\mu\text{g/mL}$ bovine serum albumin per manufacturer's instructions. Standard curves for protein assays had R^2 values of 0.99 or greater. Absorbance was measured at 595 nM and reported as mg/mL.

Hemolymph Lactate and Protein Determinations with Hand-Held Devices

A 25 μL aliquot of hemolymph sample was applied to a Cobas[®] BM-Lactate test strip (Roche Diagnostics, Mannheim, Germany) and an Accutrend[®] Lactate meter (Roche Diagnostics GmbH, Mannheim, Germany) was then used to determine lactate concentrations in *P. clarkii* hemolymph samples. The measurement range for the lactate meter was between 0.7 – 26 mmol/L, thus lactate concentrations below 0.7 mmol/L resulted in non-quantified measurements and were excluded from further analysis. *P. clarkii* hemolymph protein concentrations were examined with a Reichert[®] Vet 360 temperature compensated hand-held refractometer (Reichert Inc., Depew, New York). A 50 μL aliquot of hemolymph sample was placed on the refractometer to determine protein concentration.

Data Analysis

All statistical analyses were performed using SAS 9.1.3. Simple linear regressions (PROC GLM) were used to examine the relationships between spectrophotometer and lactate meter lactate concentrations and spectrophotometer and refractometer protein concentrations.

Analysis of covariance (PROC MIXED) was used to test for significant differences between field and laboratory measurements.

Results

Slopes and intercepts for regressions of field and laboratory data were not statistically different for lactate or protein concentrations; therefore, a common slope was used to compare hemolymph spectrophotometer results with hand-held lactate and protein device concentrations. *P. clarkii* hemolymph lactate concentrations from spectrophotometer and lactate meter measurements demonstrated a strong positive relationship ($P < 0.0001$; $r^2 = 0.9257$; Figure 5.1). Additionally, spectrophotometer and refractometer hemolymph protein measurements demonstrated a significant positive relationship ($P < 0.0001$; $r^2 = 0.8299$; Figure 5.2).

Discussion

Both the lactate meter and refractometer hemolymph lactate and protein measurements show a strong correlation with results obtained via spectrophotometric analyses. These results demonstrate that the hand-held lactate meter and refractometer are reliable methods for determining *P. clarkii* hemolymph lactate and protein concentrations, respectively. Furthermore, these hand-held instruments can be used to examine crayfish physiological stress and health in individuals exposed to transport and handling conditions from commercial operations, as well as hypoxia-induced stress in natural populations.

Hand-held instruments provide a rapid and economical means for examining crayfish nutritional and physiological condition. Analytical methods require thousands of dollars in laboratory equipment (e.g., spectrophotometer and associated supplies) in addition to the hundreds of dollars for metabolite assay kits. Additionally, laboratory analyses are time

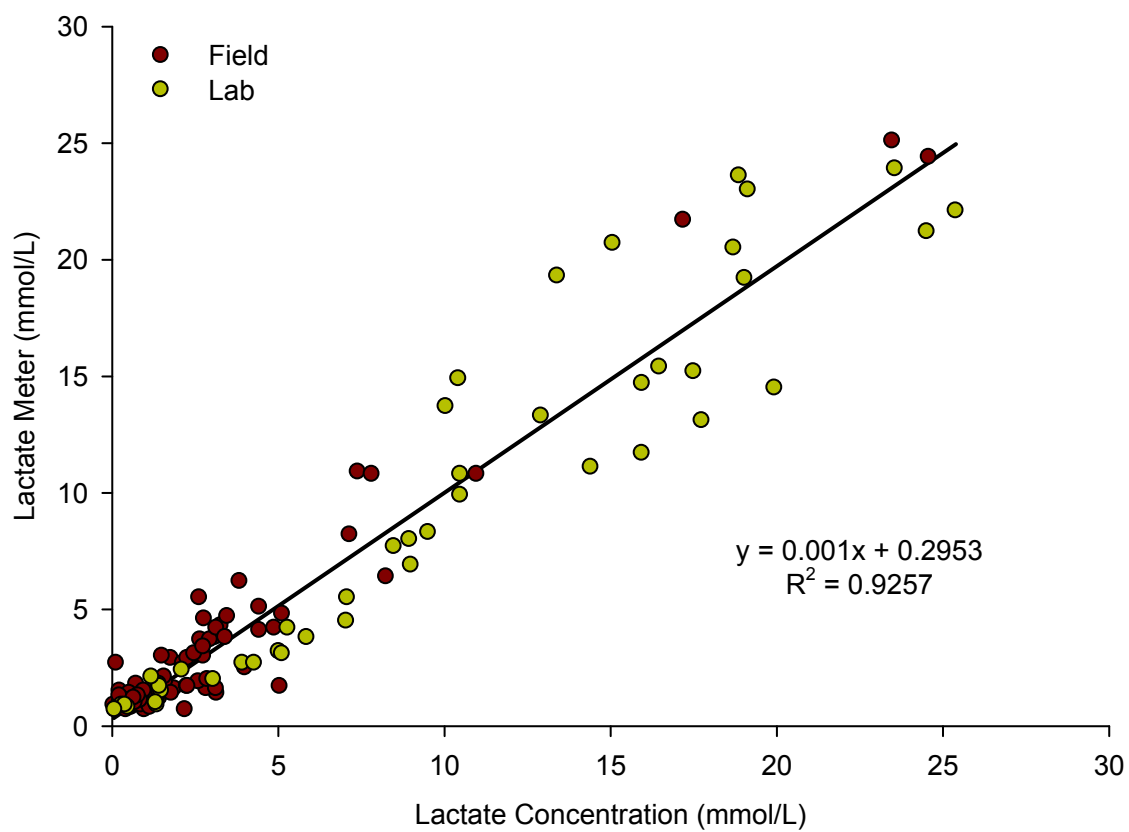


Figure 5.1. Relationship between hemolymph lactate concentrations determined by lactate meter and spectrophotometric measurements from field and laboratory sampled *Procambarus clarkii* ($n = 126$).

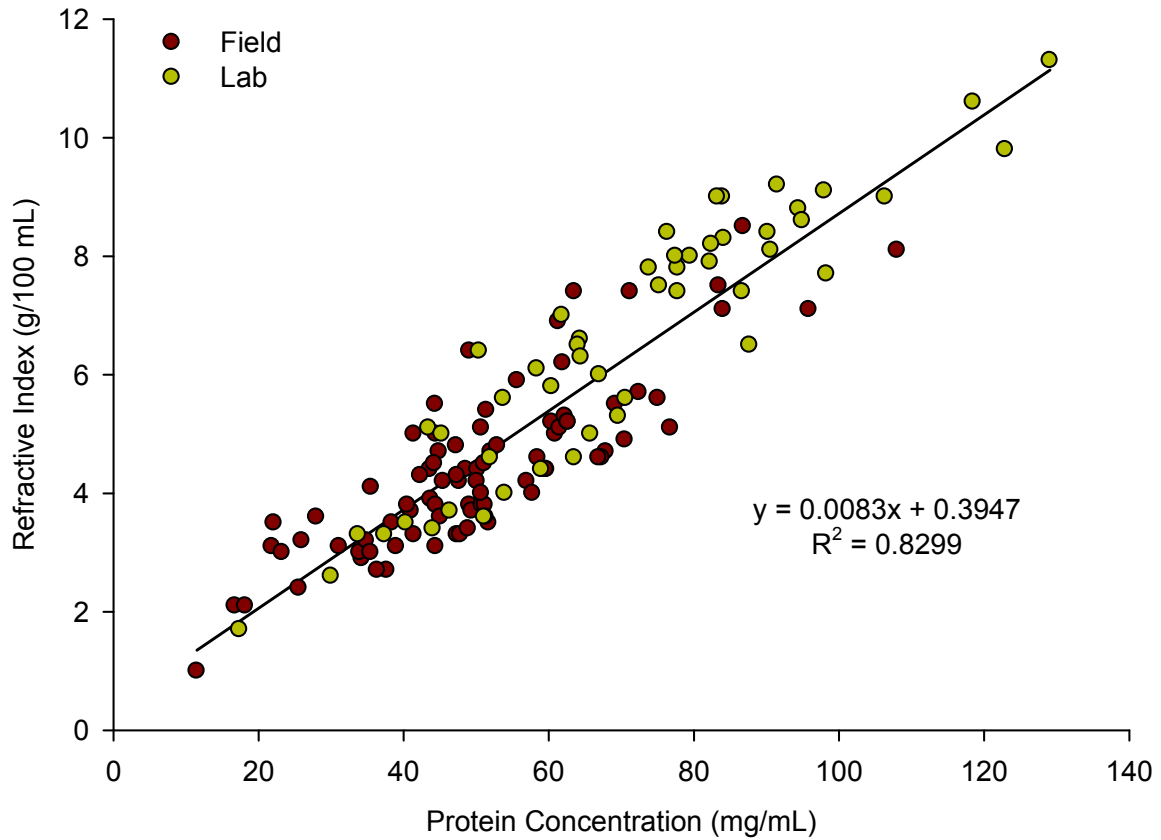


Figure 5.2. Relationship between hemolymph protein concentrations determined by refractometer and spectrophotometric measurements from field and laboratory sampled *Procambarus clarkii* (n = 136).

consuming and results are not immediately obtainable. Hand-held devices provide an affordable alternative to laboratory analyses. Non-digital refractometers are usually less than \$200 with no additional associated supply costs, whereas lactate meters are generally \$300-400 plus the purchase of individual test strips. Furthermore, these practical hand-held devices provide immediate, real-time information. This information can be used on site by commercial farmers and processors or resource agencies not only to monitor crayfish health and stress levels, but also to evaluate the success of water management activities designed to alleviate hypoxic conditions.

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CHAPTER 6

GENERAL SUMMARY AND CONCLUSIONS

Although the Atchafalaya River Basin (ARB) produces the largest commercial harvest of wild crayfish in Louisiana, ecological and physiological influences on crayfish populations are poorly understood. This dissertation investigated various biotic and abiotic effects on *Procambarus clarkii* population characteristics as well as the impacts of Hurricane Gustav on the ARB ecosystem. Additionally, because of the prevalence of hypoxia throughout the ARB, I assessed *P. clarkii* physiological responses during hypoxic exposure in both field and laboratory examinations.

Chapter 2 describes the effects of multiple biotic and abiotic variables on *P. clarkii* populations in the southeastern ARB throughout the 2008 and 2009 crayfish seasons. *P. clarkii* catch per unit effort (CPUE) at sampling locations increased nearly 600% between sample years despite similar hydrologic and physicochemical conditions. Increased allochthonous inputs and reductions in fish populations associated with the passage of Hurricane Gustav over the ARB between sample years may have contributed to the higher CPUE values observed during 2009. During 2008, CPUE was highest at sample locations characterized by higher dissolved oxygen (DO) concentrations and lowest at locations with relatively low DO levels. These results were mirrored when I compared chronically hypoxic and normoxic locations during 2008, with higher mean CPUE and carapace length values observed at normoxic locations. The increase in *P. clarkii* mean CPUE and decrease in mean carapace length observed during the 2009 crayfish season indicated density-dependent growth. While abiotic variables undoubtedly influenced crayfish population characteristics, relative density appeared to have a larger effect on *P. clarkii* carapace length and minimum size at maturity than the measured physicochemical characteristics.

Chapter 3 documented the physicochemical changes recorded in the southeastern ARB during and after the passage of Hurricane Gustav on 1 September 2008. Anticipating physicochemical shifts attributable to the combination of concentrated precipitation and wind stress generated by this strong category 2 storm, I deployed a continuous recording multiparameter water quality sonde in Little Bayou Jessie three days prior to landfall. Quarter-hourly physicochemical measurements taken over a two-week period indicated that DO, pH, and specific conductance all reached annual lows immediately following storm passage. The most pronounced post-storm physicochemical fluctuation involved DO. Daily mean DO concentration dropped to hypoxic level within three days of storm landfall, followed by near anoxic conditions within five days, which resulted in extensive system-wide fish kills. Within six weeks, DO returned to, and pH was near pre-storm levels. To evaluate the impact of Hurricane Gustav on ARB physicochemistry, I contrasted data on DO, pH, temperature, and specific conductance collected from the 16 lower ARB sampling sites over a 54-day interval prior to landfall with data collected during a 45-day period after the storm. A comparison of pre- and post-hurricane conditions revealed that water quality was highly dissimilar between the two periods. Although Hurricane Gustav initially produced rapid environmental oscillations, my observations suggested that both water quality and hydrology of the ARB resets relatively quickly after passage of a hurricane.

Chapter 4 examined physiological biomarkers (hemolymph lactate, glucose, and protein concentrations) of hypoxic stress in *P. clarkii* from chronically hypoxic natural habitats and laboratory hypoxia experiments. *P. clarkii* from normoxic and hypoxic areas in the ARB were sampled monthly from April to July 2010. Laboratory experiments subjected *P. clarkii* to severe hypoxia (~1 mg/L DO), moderate hypoxia (~2 mg/L DO), or normoxic conditions (control: DO

> 7.5 mg/L) for 12, 24, and 48 hours. *P. clarkii* from normoxic and hypoxic natural habitats did not display significantly different hemolymph lactate or glucose concentrations, however, mean hemolymph protein concentration was significantly lower in crayfish from hypoxic areas. *P. clarkii* exposed to severe hypoxia in laboratory experiments had significantly higher hemolymph lactate and glucose concentrations for all three exposure times, whereas large differences in protein concentrations were not observed. These results suggested that elevated hemolymph lactate and glucose concentrations are responses to acute hypoxia in *P. clarkii*, while differences in protein concentrations were the result of chronic hypoxic exposure. Furthermore, *P. clarkii* from moderate hypoxia treatment tanks did not display significantly increased hemolymph lactate and glucose concentrations relative to control individuals, suggesting that the critical oxygen level (shift from oxygen regulator to oxygen conformer) for quiescent *P. clarkii* under laboratory conditions is between 1 and 2 mg/L.

In Chapter 5, I validated the accuracy of hand-held instruments for the determination of *P. clarkii* hemolymph lactate and protein concentrations. An Accutrend[®] lactate meter and a Reichert[®] Vet 360 temperature compensated refractometer were used to examine *P. clarkii* hemolymph lactate and protein concentrations, respectively, and the results were compared to measurements obtained from spectrophotometric analyses. Results from the lactate meter ($r^2 = 0.9257$) and the refractometer ($r^2 = 0.8299$) both demonstrated strong relationships with spectrophotometer measurements and are rapid, reliable methods for determining crayfish lactate and protein concentrations. These hand-held devices are an economical alternative to expensive laboratory analyses and information can be used on site by commercial farmers and processors or resource agencies not only to monitor crayfish health and stress levels, but also to evaluate the success of water management activities designed to alleviate hypoxic conditions.

Management and regulatory considerations for crayfish in the ARB include a commercial harvest season and various forms of trip ticket reporting (Issacs and Lavergne 2010). *P. clarkii* are a resilient species, one of the reasons they have established themselves as a highly invasive species around the world. The fact that crayfish populations continue to persist year after year despite the lack of harvest restrictions is evidence that a regulated season is unwarranted. However, while most crayfish harvesters are reluctant to allow trip ticket reporting, the information collected would provide more insight into how crayfish populations are structured in the ARB. Trip ticket reporting areas consisting of ARB water management units would allow federal and state resource agencies to assess the effects of ecosystem management projects on crayfish populations and harvests. Additionally, this information could help identify localized crayfish reductions or “crayfish droughts” and lead to a better understanding into the environmental and/or anthropogenic factors that structure these phenomena.

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APPENDIX A
CHAPTER 2 SUPPLEMENTAL TABLES AND FIGURES

Table A.1. Mean (\pm SE) metal concentrations (ppm) from water samples collected at intensive sampling locations ($n = 16$) in the lower Atchafalaya River Basin during 2008 and 2009.

Date	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	P	Zn
6-Mar-08	29.849	0.0005	0.006	0.05	3.5	9.444	0.03	20.8397	0.043	0.0103
	± 0.21	± 0.00	± 0.00	± 0.01	± 0.0	± 0.10	± 0.00	± 0.35	± 0.00	± 0.00
28-Mar-08	29.464	0.0015	0.006	0.26	2.3	8.913	0.04	21.4230	0.037	0.1719
	± 0.14	± 0.00	± 0.00	± 0.01	± 0.1	± 0.04	± 0.00	± 0.25	± 0.00	± 0.00
9-Apr-08	32.068	0.0025	0.010	0.23	2.6	8.742	0.05	18.1861	0.057	0.1931
	± 0.19	± 0.00	± 0.00	± 0.02	± 0.1	± 0.09	± 0.00	± 0.66	± 0.00	± 0.04
2-May-08	31.538	0.0019	0.007	0.23	1.9	8.418	0.02	15.4998	0.043	0.2019
	± 0.29	± 0.00	± 0.00	± 0.01	± 0.0	± 0.08	± 0.00	± 0.24	± 0.00	± 0.01
22-May-08	33.599	0.0014	0.004	0.13	2.4	9.633	0.05	17.5907	0.088	0.2084
	± 0.13	± 0.00	± 0.00	± 0.01	± 0.0	± 0.05	± 0.00	± 0.14	± 0.00	± 0.02
10-Jun-08	37.250	0.0012	0.002	0.10	2.7	10.934	0.14	19.0581	0.130	0.3000
	± 0.28	± 0.00	± 0.00	± 0.02	± 0.0	± 0.09	± 0.02	± 0.26	± 0.01	± 0.03
25-Jun-08	40.683	0.0012	0.003	0.09	2.9	11.574	0.11	19.3599	0.101	0.3332
	± 0.45	± 0.00	± 0.00	± 0.01	± 0.1	± 0.12	± 0.01	± 0.53	± 0.01	± 0.03
9-Jul-08	43.059	0.0009	0.003	0.09	2.7	11.957	0.06	18.5096	0.061	0.3341
	± 0.15	± 0.00	± 0.00	± 0.01	± 0.1	± 0.06	± 0.01	± 0.24	± 0.00	± 0.02
28-Jul-08	45.908	0.0011	0.007	0.54	2.9	12.958	0.11	16.9572	0.071	0.3049
	± 0.25	± 0.00	± 0.00	± 0.21	± 0.1	± 0.05	± 0.02	± 0.05	± 0.00	± 0.02
12-Aug-08	39.484	0.0009	0.004	0.23	3.1	11.950	0.12	16.8090	0.112	0.2210
	± 0.75	± 0.00	± 0.00	± 0.13	± 0.1	± 0.24	± 0.02	± 0.42	± 0.01	± 0.04
10-Sep-08	31.632	0.0008	0.003	1.05	7.6	9.508	1.46	13.5122	0.575	0.0345
	± 0.75	± 0.00	± 0.00	± 0.14	± 0.3	± 0.22	± 0.12	± 0.36	± 0.05	± 0.01
24-Sep-08	27.047	0.0006	0.001	0.11	6.1	8.054	0.25	11.5622	0.209	0.0104
	± 0.60	± 0.00	± 0.00	± 0.02	± 0.2	± 0.20	± 0.02	± 0.15	± 0.01	± 0.00
16-Oct-08	29.134	0.0013	0.006	0.06	4.6	8.356	0.10	14.3838	0.097	0.0133
	± 0.22	± 0.00	± 0.00	± 0.01	± 0.0	± 0.08	± 0.01	± 0.21	± 0.00	± 0.00

Table continued

Date	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	P	Zn
11-Nov-08	39.315	0.0024	0.006	1.26	7.4	12.311	0.15	23.1445	0.122	0.0465
	± 0.48	± 0.00	± 0.00	± 0.39	± 0.3	± 0.19	± 0.03	± 0.73	± 0.01	± 0.00
7-Jan-09	29.959	0.0011	0.004	1.02	5.5	9.258	0.14	19.9293	0.140	0.0525
	± 0.76	± 0.00	± 0.00	± 0.12	± 0.2	± 0.21	± 0.02	± 0.53	± 0.01	± 0.00
2-Mar-09	36.021	0.0012	0.006	1.10	4.0	11.001	0.10	21.8452	0.109	0.0566
	± 0.37	± 0.00	± 0.00	± 0.18	± 0.1	± 0.11	± 0.01	± 0.22	± 0.01	± 0.00
18-Mar-09	35.050	0.0011	0.005	0.86	3.7	10.675	0.19	20.9266	0.147	0.0619
	± 0.25	± 0.00	± 0.00	± 0.09	± 0.1	± 0.07	± 0.03	± 0.27	± 0.01	± 0.01
1-Apr-09	31.065	0.0011	0.006	0.71	3.9	9.428	0.10	18.3167	0.119	0.0745
	± 0.53	± 0.00	± 0.00	± 0.13	± 0.1	± 0.16	± 0.01	± 0.52	± 0.00	± 0.02
15-Apr-09	30.821	0.0008	0.007	0.69	3.5	9.303	0.10	17.3332	0.133	0.0621
	± 0.29	± 0.00	± 0.00	± 0.11	± 0.0	± 0.07	± 0.01	± 0.17	± 0.00	± 0.00
28-Apr-09	32.815	0.0010	0.008	0.85	3.4	9.805	0.23	16.7695	0.163	0.0656
	± 0.32	± 0.00	± 0.00	± 0.22	± 0.0	± 0.10	± 0.04	± 0.10	± 0.01	± 0.01
14-May-09	35.102	0.0014	0.007	0.48	4.1	10.417	0.12	18.1987	0.144	0.0578
	± 0.35	± 0.00	± 0.00	± 0.08	± 0.1	± 0.12	± 0.01	± 0.32	± 0.01	± 0.00
26-May-09	33.151	0.0015	0.006	0.37	4.3	9.161	0.05	16.7095	0.091	0.0158
	± 0.23	± 0.00	± 0.00	± 0.20	± 0.1	± 0.13	± 0.00	± 0.59	± 0.01	± 0.00
11-Jun-09	34.540	0.0009	0.001	0.15	3.7	9.434	0.07	18.2517	0.086	0.0121
	± 0.08	± 0.00	± 0.00	± 0.04	± 0.0	± 0.03	± 0.00	± 0.28	± 0.00	± 0.00
25-Jun-09	34.340	0.0012	0.004	0.11	3.3	9.828	0.17	18.8233	0.123	0.0131
	± 0.17	± 0.00	± 0.00	± 0.01	± 0.1	± 0.04	± 0.03	± 0.11	± 0.00	± 0.00
13-Jul-09	32.865	0.0011	0.003	0.21	3.0	9.702	0.19	17.0388	0.110	0.0158
	± 0.37	± 0.00	± 0.00	± 0.02	± 0.1	± 0.09	± 0.04	± 0.28	± 0.00	± 0.00
30-Jul-09	33.229	0.0004	0.002	0.14	3.1	10.362	0.09	17.5795	0.056	0.0141
	± 3.26	± 0.00	± 0.00	± 0.02	± 0.3	± 1.04	± 0.02	± 2.93	± 0.01	± 0.00
10-Aug-09	37.289	0.0002	0.001	0.12	3.4	11.805	0.08	16.5011	0.043	0.0155
	± 0.85	± 0.00	± 0.00	± 0.02	± 0.1	± 0.33	± 0.01	± 0.60	± 0.00	± 0.00

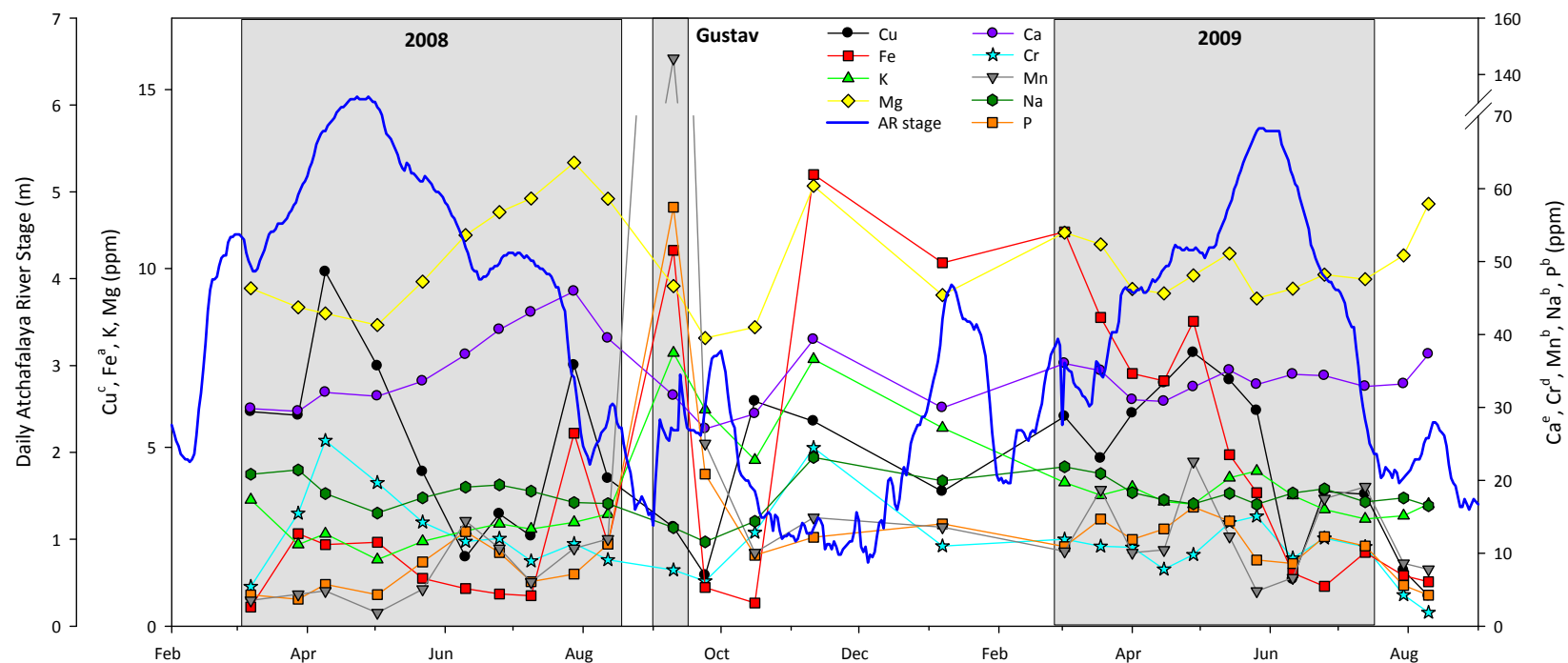



Figure A.1. Mean metal concentrations from water samples collected at intensive sampling locations ($n = 16$) in the lower Atchafalaya River Basin from 6 March 2008 to 11 August 2009 and daily stage of the Atchafalaya River at Butte La Rose, Louisiana (U.S. Army Corps of Engineers recording gauge 03120). The shaded box to the far left represents the 2008 crayfish season from 6 March to 12 August 2008 while the far right shaded area represent the 2009 crayfish season from 2 March to 13 July. The small shaded box in the center depicts 10 September 2008 samples collected after the passage of Hurricane Gustav across the Atchafalaya River Basin on 1 September 2008. Note: ^appm $\times 10^1$, ^bppm $\times 10^2$, ^cppm $\times 10^3$, ^dppm $\times 10^4$, ^eppm $\times 10^{-1}$.

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APPENDIX C MEAN MONTHLY AND ANNUAL ATCHAFALAYA RIVER STAGE

Appendix C. Mean monthly and annual Atchafalaya River stage (m) at Butte La Rose, Louisiana (USACE gauge 03120) from 1959-2011. Values in bold represent the maximum recorded means while shaded values represent the lowest recorded means.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
1959	3.20	6.40	6.66	6.11	5.30	4.91	3.27	3.37	2.49	4.22	3.54	4.42	4.48
1960	6.21	6.26	6.21	6.87	6.44	5.61	5.10	3.10	2.73	2.26	2.53	3.19	4.70
1961	3.92	3.59	7.29	7.92	7.74	7.16	4.71	4.34	3.38	3.76	4.04	6.20	5.35
1962	6.53	7.30	7.73	7.99	6.62	4.97	3.67	2.82	2.56	2.85	2.68	2.64	4.85
1963	2.95	2.64	5.04	6.46	3.69	3.18	1.84	1.53	1.04	0.73	0.69	0.88	2.55
1964	1.26	1.98	4.95	6.62	3.22	0.87	0.90	1.00	1.08	1.64	1.17	3.73	2.37
1965	4.24	5.16	6.14	6.96	5.98	3.89	2.78	1.79	2.35	3.43	1.70	1.51	3.82
1966	2.87	2.78	3.00	3.33	6.63	4.32	1.78	1.34	1.13	1.01	1.15	2.20	2.63
1967	2.41	2.26	3.64	4.63	5.46	4.83	4.12	2.15	1.30	1.19	2.02	3.52	3.13
1968	5.27	5.12	3.64	6.48	5.49	6.09	3.51	2.47	1.49	1.37	1.58	3.81	3.85
1969	3.88	6.24	5.19	5.76	6.12	3.89	4.16	2.84	1.46	1.76	1.73	2.00	3.74
1970	3.58	3.33	4.58	5.08	6.36	4.71	2.33	1.63	1.40	2.87	3.12	2.43	3.45
1971	3.79	3.55	5.76	3.77	3.70	2.95	1.83	2.13	1.54	1.39	1.46	3.68	2.96
1972	4.84	3.85	4.49	4.08	5.47	2.55	2.55	2.13	1.66	2.12	4.33	5.70	3.65
1973	6.26	6.38	6.23	7.75	8.19	7.29	4.92	3.01	2.49	3.23	3.36	5.50	5.38
1974	6.03	6.81	5.84	5.96	5.50	6.06	4.98	2.11	3.45	2.73	3.86	4.77	4.82
1975	5.39	5.82	6.55	7.41	6.66	5.61	3.83	2.22	1.62	1.82	1.89	2.32	4.25
1976	3.26	3.09	5.07	4.44	3.07	2.19	2.39	0.94	0.68	0.71	0.96	1.07	2.32
1977	1.25	1.17	3.61	4.45	2.61	1.12	1.38	1.11	1.64	2.16	2.60	3.85	2.25
1978	3.17	3.28	2.98	5.23	4.84	3.61	2.11	1.69	1.29	1.05	0.94	3.29	2.79
1979	4.31	4.19	5.67	6.64	6.44	4.74	2.86	2.72	2.67	2.60	2.17	3.57	4.04
1980	3.26	2.68	3.22	5.77	4.15	3.01	1.78	1.23	1.33	1.00	0.91	1.15	2.45
1981	0.41	1.19	2.28	1.65	2.50	4.32	3.02	2.02	1.28	1.10	1.74	1.22	1.90
1982	2.18	4.24	4.35	4.60	3.17	4.12	3.05	1.84	1.69	1.25	1.43	5.14	3.08
1983	5.75	4.45	4.18	5.19	6.32	6.08	2.96	1.42	0.95	0.89	1.79	4.45	3.70
1984	2.94	2.96	4.67	5.54	5.89	4.83	3.04	1.65	1.01	1.90	3.80	3.93	3.51

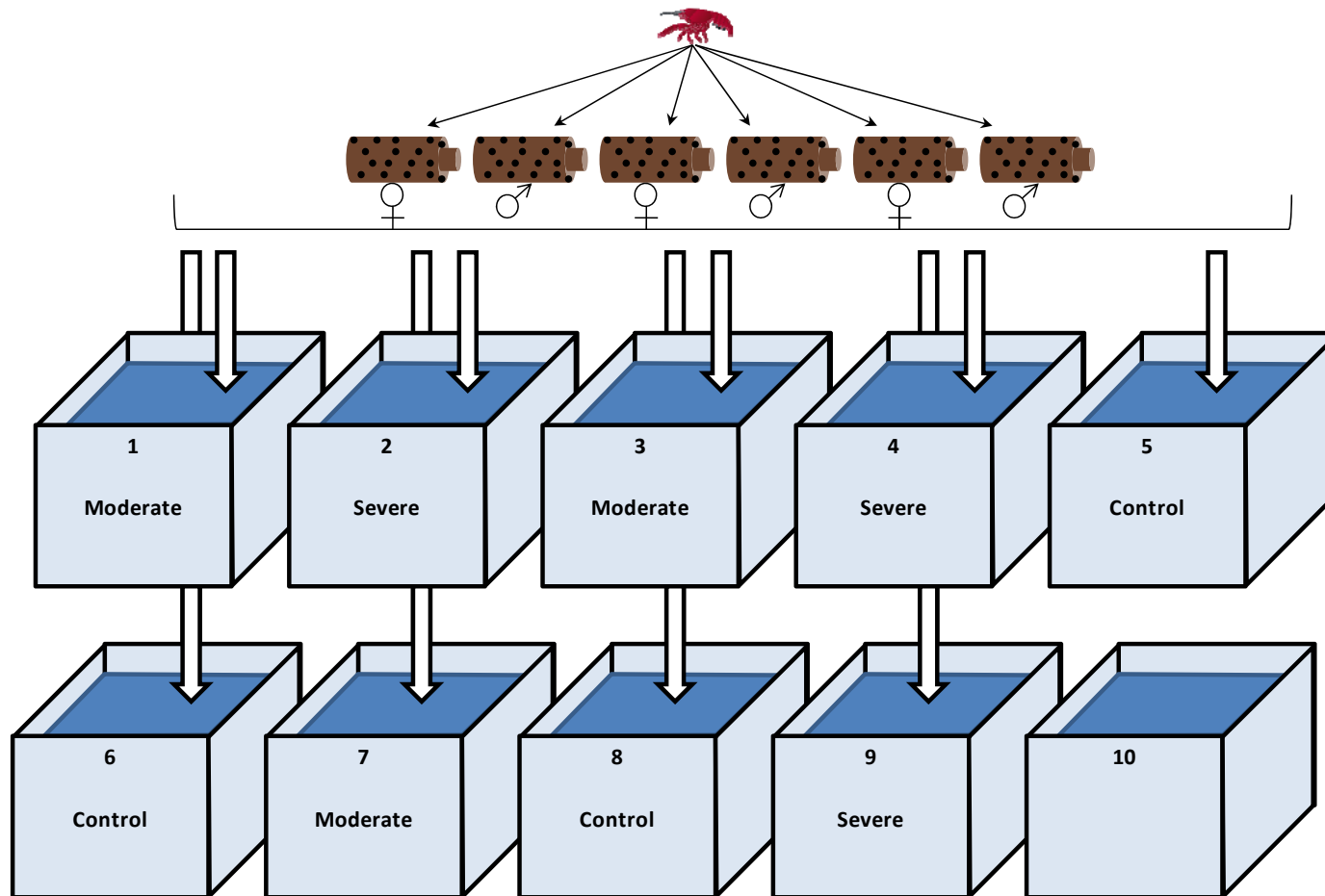
Table continued

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
1985	4.52	3.35	5.61	5.33	3.97	2.57	1.66	1.25	1.47	1.80	3.31	5.22	3.34
1986	2.34	3.20	3.31	3.17	2.85	3.65	2.29	1.23	1.14	3.47	3.91	4.45	2.92
1987	2.81	2.78	4.56	3.77	2.31	1.90	1.42	0.83	0.84	0.66	1.15	1.89	2.07
1988	4.24	4.08	3.46	3.70	1.66	0.66	0.47	0.50	0.63	0.63	0.94	1.95	1.90
1989	3.69	3.97	5.41	4.87	3.64	4.06	4.26	1.68	1.62	1.56	1.36	1.12	3.10
1990	2.25	4.66	5.26	4.51	4.68	5.52	3.28	1.86	1.46	1.32	1.38	2.09	3.18
1991	5.53	4.63	4.73	4.87	5.46	4.33	2.15	0.97	0.90	0.83	1.93	3.92	3.35
1992	3.65	2.61	3.73	3.23	2.55	2.15	2.10	2.75	1.41	1.36	1.75	3.83	2.60
1993	4.68	3.96	4.62	5.45	5.61	4.26	3.76	4.47	3.10	3.24	2.64	4.23	4.18
1994	2.96	4.58	5.39	5.23	5.50	2.70	2.15	1.56	1.05	1.10	1.56	2.64	3.03
1995	2.44	3.06	3.61	2.91	4.25	5.57	3.56	1.96	1.08	1.02	1.35	1.27	2.67
1996	1.75	3.25	2.71	3.35	4.27	4.92	2.61	1.95	1.18	1.62	2.67	4.27	2.88
1997	3.58	4.33	5.65	5.58	4.33	4.01	2.90	1.28	1.03	0.97	1.06	1.40	3.00
1998	3.79	4.03	4.59	4.80	5.07	3.45	3.54	1.92	1.18	1.49	1.81	1.99	3.13
1999	2.88	5.12	4.25	3.78	4.31	3.33	2.74	1.27	0.76	0.70	0.60	0.96	2.54
2000	0.83	0.75	2.50	3.13	1.88	1.87	2.44	1.01	0.75	0.75	1.06	1.35	1.53
2001	1.94	2.94	4.90	3.68	2.71	3.56	1.93	1.20	1.04	1.28	1.20	3.66	2.50
2002	2.97	3.83	2.87	4.97	4.70	4.35	1.57	1.03	1.02	1.58	1.78	2.02	2.71
2003	2.90	1.98	4.57	2.68	3.62	4.16	2.38	1.61	1.45	1.17	1.39	2.72	2.56
2004	3.10	3.72	3.79	3.04	3.52	4.06	3.57	1.44	1.46	1.62	2.65	4.46	3.03
2005	4.72	4.95	3.57	3.42	2.17	1.68	1.27	0.75	1.21	0.88	0.74	0.90	2.17
2006	1.15	2.53	2.02	2.05	2.32	1.21	0.82	0.66	0.78	1.37	2.01	1.90	1.56
2007	3.93	3.22	2.87	3.33	3.27	1.90	2.31	1.33	1.30	0.95	1.08	1.71	2.26
2008	2.48	3.11	4.55	5.84	5.35	4.31	3.80	1.94	2.39	1.69	1.03	1.55	3.17
2009	3.23	2.36	3.07	4.12	5.01	4.79	2.45	1.84	1.49	3.26	4.64	4.03	3.36
2010	3.97	4.91	3.80	4.08	3.83	3.70	3.15	2.25	1.54	1.36	1.10	1.54	2.92
2011	1.29	1.32	3.99	4.40	6.05	5.42	3.43	1.83	1.45	1.09	1.34	3.91	2.97

APPENDIX D

EXPERIMENTAL DESIGN FOR CRAYFISH HYPOXIA TRIALS

Appendix D. Experimental design utilized during laboratory crayfish hypoxia experiments. An individual crayfish was placed into a 1-L polyethylene amber bottle with 0.635-cm holes over the entire bottle. Six bottles with crayfish (3 males and 3 females) were then placed into each of the 9 aquaria with randomly assigned dissolved oxygen (DO) treatments. Control: DO > 7.5 mg/L; Moderate: DO ~ 2.0 mg/L; Severe: DO ~ 1.0 mg/L.



VITA

Christopher P. Bonvillain was born in October 1979, in Thibodaux, Louisiana. After graduating from Thibodaux High School in 1997, Chris attended Nicholls State University and Louisiana State University. Chris graduated from Nicholls State University in May of 2004 with a Bachelor of Science degree in biology with an environmental biology concentration and a minor in chemistry. In May of 2006, Chris received his Master of Science degree in marine and environmental biology from Nicholls State University. Chris then continued his education by enrolling in the wildlife and fisheries doctorate program in the School of Renewable Natural Resources at Louisiana State University. Chris will receive his Doctor of Philosophy degree in August 2012.