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Flood Pulse Characteristics and Physicochemical Influences on Harvested *Procambarus clarkii* and *Procambarus zonangulus* Populations in the Atchafalaya River Basin, Louisiana

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ABSTRACT

The majority of Louisiana's wild crayfish landings are harvested from the Atchafalaya River Basin (ARB) during floodplain inundation from the annual flood pulse. Spatial and temporal heterogeneity in ARB physicochemical characteristics are associated with flood pulse characteristics and floodplain inundation, and extensive areas of the ARB experience environmental hypoxia (dissolved oxygen [DO] $< 2.0\ \mathrm{mg \cdot L^{-1}}$) for several weeks to months during the annual flood pulse. The purpose of this research was to evaluate the effects of flood pulse characteristics and physicochemistry on harvested crayfish populations at 14 sites in the ARB that were sampled biweekly during the 2016 and 2017 crayfish seasons. Despite dissimilar 2016 and 2017 flood pulse characteristics, red swamp crawfish *Procambarus clarkii* and southern white river crawfish *P. zonangulus* carapace length and CPUE were similar between sample years. Comparisons of *P. clarkii* populations among physicochemical location groupings indicated that DO concentration, particularly chronically hypoxic water, is the principal abiotic variable influencing *P. clarkii* population characteristics. Although not significant, normoxic locations produced larger crayfish and yielded higher CPUE values for the majority of both crayfish seasons. Furthermore, hemolymph protein concentrations in *P. clarkii* from normoxic areas were significantly and consistently higher than individuals from chronically hypoxic locations.

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INTRODUCTION

Louisiana is the largest producer and consumer of crayfish in the United States (McClain et al. 2007), with a total industry value over $200 million (LSUAC 2016). Additionally, the crayfish industry supports thousands of commercial and recreational harvesters and processors throughout the state (Piazza 2014). The two commercially important crayfish species in Louisiana, the red swamp crawfish, *Procambarus clarkii* (Girard, 1852), and the southern white river crawfish, *Procambarus zonangulus* Hobbs and Hobbs, 1990, are harvested from managed ponds (farmed) and natural habitats (wild) throughout the state, with *P. clarkii* comprising the majority of landings.

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 1). The wild crayfish harvest season in the ARB coincides with the annual Atchafalaya River flood pulse, which varies from year to year (Bonvillain et al. 2008). Flood pulse timing, magnitude, and duration are all key abiotic factors that influence native crayfish population characteristics and the magnitude of landings (Bonvillain et al. 2013b). Absent or asynchronous floodplain inundation characteristics can have negative effects on crayfish populations and may cause crayfish starvation or desiccation inside the burrow, or premature emergence onto floodplain habitats that do not coincide with life history stages. Floodplain inundation provides optimal conditions for crayfish growth and mating and is an environmental cue for crayfish to emerge from burrows where females release attached juveniles. Brood survival affects population density and is dependent on water level fluctuations (Piazza 2014), with late and low magnitude flood pulses impacting population densities by increasing mortality of crayfish in burrows (McClain et al. 2007).

The flood pulse is the principle driving force in biotic productivity in the ARB, allowing floodplain nutrients to be recycled and river nutrients to be transported to floodplain habitats

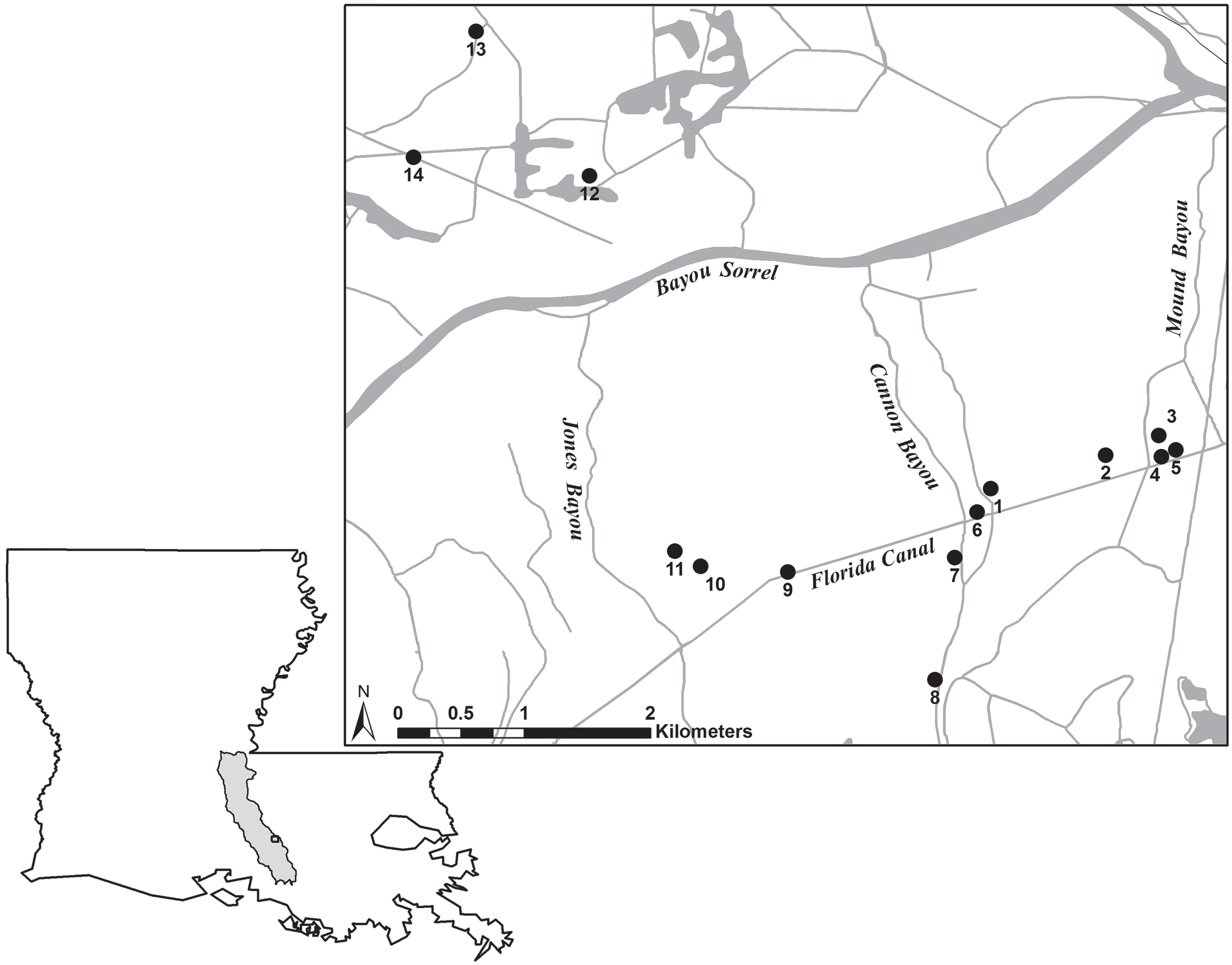

Figure 1. The Atchafalaya River Basin (shaded) in south-central Louisiana and crayfish sampling locations (n = 14) during the 2016 and 2017 crayfish seasons.

(Junk et al. 1989). However, spatial and temporal heterogeneity in ARB physicochemical characteristics are associated with floodplain inundation, rising water temperature, and microbial decomposition of floodplain organic matter (Junk et al. 1989; Bayley 1995; Sparks 1995; Bonvillain et al. 2015). Water quality characteristics can vary spatially depending on geomorphology, anthropogenic modifications, and rising or falling water levels, and extensive areas of the ARB experience environmental hypoxia (dissolved oxygen [DO] < 2.0 mg·L^{-1}) for several weeks to months during the annual flood pulse (Sabo et al. 1999b; Rutherford et al. 2001; Bonvillain et al. 2015). Additionally, the frequency, extent, and duration of hypoxia in the ARB continues to increase due to anthropogenic modifications. Levee and canal construction, distributary closures, dredging, and flood control structures have exacerbated poor water quality and hypoxic conditions by altering historic river-floodplain connectivity, water circulation, and natural flow patterns that isolate floodplain habitats from riverine inputs and result in persistent water stagnation (Sabo et al. 1999a, b; Piazza 2014).

Crayfish frequently encounter hypoxic and sub-optimal oxygen conditions in aquatic and burrow habitats in the ARB (Bonvillain et al. 2013b, 2015) and have evolved physiological and behavioral mechanisms to deal with sub-optimal oxygen environments (see McMahon 1986; Reiber 1995; McMahon 2002). Although *P. clarkii* can tolerate lower oxygen concentrations than other crayfish species (Nyström 2002) and can endure acute (days to several weeks) and moderate hypoxia (1.3 – 2.0 mg·L^{-1} DO), chronic (weeks to months), severe environmental hypoxia (DO < 1.2 mg·L^{-1}) can negatively affect *P. clarkii* population vigor (McClain 1999; Bonvillain et al. 2015). Additionally, hypoxic stress elicits multiple physiological responses in crayfish (Reiber 1995). Hemolymph protein concentration is a reliable biomarker of crayfish health and fluctuates in response to chronic hypoxia exposure in *P. clarkii* (Bonvillain et al. 2012, 2013a). *Procambarus clarkii* in chronically hypoxic environments in the ARB have lower hemolymph protein concentrations compared to individuals from normoxic areas (Bonvillain et al. 2012), likely due to reduced foraging activity as individuals move towards the air-water interface to respire (McMahon and Wilkes 1983; McMahon and Hankinson 1993; Morris and Callaghan 1998; McMahon and Stuart 1999).

Only recently have research efforts begun to investigate the ecology of *P. clarkii* populations in the ARB. Although these studies have shown chronic environmental hypoxia as a key abiotic factor influencing *P. clarkii* populations, little is known about the influences of other water quality variables on ARB crayfish population characteristics. Additionally, because of the lack of long-term crayfish population monitoring, it has been difficult to elucidate the effects of asynchronous flood pulse characteristics on crayfish populations. Understanding the effects of water quality characteristics on crayfish populations is critically important as federal, state, and private organizations implement water management projects to improve water quality and floodplain health in the ARB. Therefore, the objectives of this study were to quantify the effects of aquatic environmental characteristics on harvested crayfish populations in the ARB. Specifically, we compared crayfish carapace length (CL), catch per unit effort (CPUE), male size at maturity, and hemolymph protein concentration from ARB sample locations during the 2016 and 2017 crayfish seasons.

Study Area

The 5,000 km^2 ARB in south-central Louisiana (Figure 1) is the largest bottomland hardwood river-floodplain system in North America (Lambou 1990) and is composed of diverse aquatic habitats including seasonally flooded swamps, shallow headwater and backwater lakes, and numerous natural bayous and excavated canals. The US Army Corps of Engineers operates two water control structures and a hydropower station that allow 30% of the combined volumes from the Mississippi and Red Rivers to flow into the Atchafalaya River. Although the timing, magnitude, and duration of the flood pulse varies annually, the ARB typically becomes inundated in the spring and dewaters from late summer to early fall (Bonvillain et al. 2008). The sample locations selected for this study are in a unique area of the ARB that, due to natural hydrology and anthropogenic modifications, experience relative variability in water quality characteristics in a moderately small area. Physicochemistry varied among sample locations due to site differences in timing and duration of hydrologic connectivity and water input sources.

MATERIALS AND METHODS

Field Collections

Fourteen locations in a 14 km^2 area of the ARB were selected for bi-monthly crayfish sampling (Figure 1). Crayfish were sampled every two weeks during the 2016 and 2017 crayfish seasons from 19 March to 9 June 2016 (eight sample dates) and from 7 May to 3 July 2017 (five sample dates). There is no official crayfish season set by resource managers in Louisiana, instead, the wild crayfish harvest is determined by Atchafalaya River water level. The crayfish season began when the Atchafalaya River level at Butte La Rose, Louisiana (U.S. Army Corps of Engineers gauge 03120) was greater than 3.5 m and floodplain habitats in the study area began to experience floodplain inundation. On every sample date at each sample location, Secchi disk depth (cm) was recorded and surface DO (mg·L^{-1}), temperature (°C), pH (2017 only), and specific conductance (mS·cm^{-1}) were measured with a handheld multiparameter water quality sonde (YSI model 556, Yellow Springs, Ohio). Surface DO concentrations represent total water column DO concentrations based upon previous sampling in the ARB, which demonstrated homogeneous DO levels throughout the inundated floodplain water column (Bonvillain et al. 2013b).

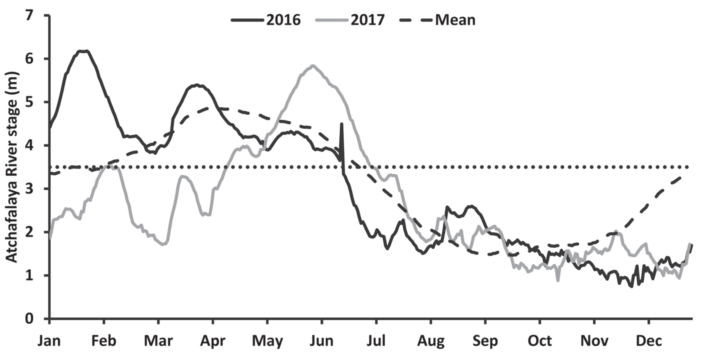

Figure 2. Daily Atchafalaya River stage at Butte La Rose, Louisiana (US Army Corps of Engineers gauge 03120) during the 2016 and 2017 sample years and the 59-year mean daily stage from 1959–2017. The horizontal dotted line represents floodplain inundation level (3.5 m) in the study area.

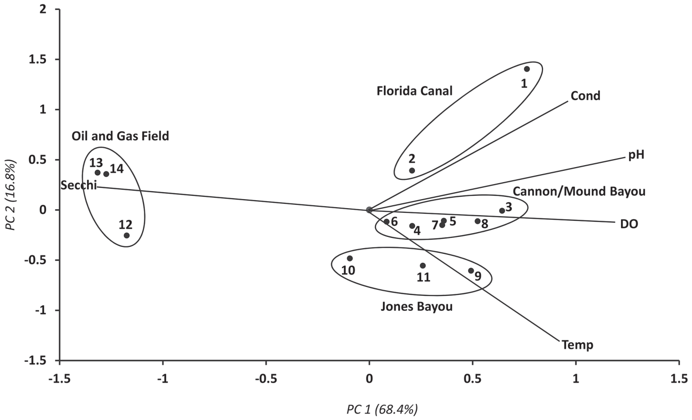

Figure 3. Biplot of principal components analysis of physicochemical parameters collected at crayfish sampling locations in the Atchafalaya River Basin during the 2016 and 2017 crayfish seasons.

Five pillow design traps per site were baited with 150 g (Beecher and Romaire 2010; Bonvillain et al. 2013b) of manufactured crayfish bait and allowed to fish overnight, with CPUE determined as the number of crayfish per trap set for 24 hours. Traps were the same style used by commercial crayfish harvesters and were constructed from 1.905 cm plastic-coated square mesh wire and were 122 cm in height with two 5.08 cm funnel openings on the bottom. Traps were deployed at least 5 m apart on the inundated floodplain and were secured to the surrounding trees or suitable vegetation. Species, reproductive form (males only), and standard CL (mm) were recorded for all captured crayfish.

Hemolymph Collection and Processing

To determine *P. clarkii* hemolymph protein concentration, hemolymph samples were collected from a minimum of 10

Table 1. Mean (± SE) carapace length (CL [mm]) and catch per unit effort (CPUE) of *Procambarus zonangulus* and *Procambarus clarkii* and Form I male CL (mm) and hemolymph protein concentration (g·100 mL^{-1}) of *P. clarkii* from sample locations during the 2016 and 2017 crayfish seasons. CPUE values were $\log_{(e)}$(CPUE + 1) transformed for analysis and are the geometric mean. F-value, degrees of freedom (df), and *P*-value are provided for each inter-annual population metric comparison.

Species	Population metric	2016	2017	F-value	df	*P*-value
P. zonangulus	CL	43.7 ± 0.3	44.1 ± 0.3	0.89	1,325.3	0.347
	CPUE	0.6 ± 0.1	0.5 ± 0.1	3.74	1,136.9	0.055
P. clarkii	CL	43.3 ± 0.1	43.4 ± 0.1	0.00	1,3387.8	0.947
	CPUE	1.4 ± 0.1	1.2 ± 0.1	2.03	1,106.3	0.158
	Form I Male CL	45.6 ± 0.2	43.4 ± 0.1	30.99	1,107.1	< 0.001
	Protein	5.2 ± 0.1	5.1 ± 0.1	1.12	1,696.9	0.291

intermolt individuals (or from all individuals if less than 10 were collected) at each sample location on every sample date. Crayfish hemolymph was collected by pericardial cavity puncture (20-gauge needle), transferred via capillary tube into a microcentrifuge tube, placed immediately on ice, and returned to the laboratory for storage at -4°C. In the laboratory, hemolymph clots were broken up, samples were centrifuged (2130 ×*g* for 15 minutes), and the supernatant serum was extracted. Hemolymph protein concentration was measured from a 100 µL hemolymph aliquot and analyzed with a Reichert® Vet360-Check temperature compensated digital refractometer (g·100 mL^{-1}; Reichert, Depew, New York). Refractometers provide a rapid and reliable measurement of *P. clarkii* hemolymph protein concentration (Bonvillain et al. 2013a).

Data Analysis

All statistical analyses were performed with SAS version 9.4 (SAS Institute Inc., Cary, North Carolina), and significance for all tests was determined with a Type I error rate of $\alpha = 0.05$. For all analyses, CPUE was $\log_{(e)}$(CPUE + 1) transformed, Secchi depth was $\log_{(e)}$(Secchi) transformed, and hemolymph protein concentration was square root transformed to stabilize error variances and meet distributional assumptions of parametric statistics. Homogeneity of variance was examined with Levene's test and Welch's analysis of variance (ANOVA) was used for unequal variance models.

To examine variability in water quality parameters among sampling locations, a principal component analysis (PCA) of DO, temperature, pH (2017 only), specific conductance, and Secchi disk depth was conducted. We retained principal components (PC) with eigenvalues greater than one and physicochemical variables with eigenvectors greater than 0.4. Site scores on PC1 and PC2 were plotted to illustrate clustering of sites with similar physicochemical characteristics. Because plotted location groupings for 2016 and 2017 were similar, both years were combined on the PCA. An inter-annual difference in physicochemical parameters (DO, temperature, specific conductance, and Secchi disk depth) between sample years was assessed with a one-way permutational multivariate ANOVA (PERMANOVA; Euclidean distance) using PRIMER software. A principal coordinates analysis (PCO) was conducted on water quality parameters to ordinate sample location Euclidean distances for visual comparisons of sample years and sites.

Separate ANOVAs with a Tukey-Kramer post hoc adjustment were used to test for inter-annual differences in *P. zonangulus* CL and CPUE and *P. clarkii* CL, CPUE, Form I male CL, and hemolymph protein concentration. Intra-annual differences in *P. clarkii* CL, CPUE, and Form I male CL at sampling locations were compared among four apparent physicochemical site groupings. Physicochemical groups for 2016 and 2017 were analyzed with separate nested general linear mixed models that included sample location as a random effect. A first order heterogeneous autoregressive covariance structure was used to account for temporal correlation between sample events for all CPUE and hemolymph protein analyses and a compound symmetry covariance structure was applied to CL and Form I male CL analyses. Comparisons among groups were examined with Tukey-Kramer *post hoc* adjustments.

Sample locations were classified as chronically hypoxic if mean DO concentration was less than 2.0 mg·L^{-1} during the sampling period (Bonvillain et al. 2015). Differences in CL, CPUE, and hemolymph protein concentration between hypoxic and normoxic locations were examined with the same methods as above.

RESULTS

Water Level and Physicochemistry

The 2016 Atchafalaya River flood pulse inundated floodplain habitats in the study area for more than six months, reaching a spring peak in March and declining to June (Figure 2). The 2017 flood pulse was a low magnitude and duration flood event compared to 2016, and restricted the sampling season from May to early July. Peak water levels did not occur until late May and rapidly declined through the beginning of July (Figure 2). Atchafalaya River stage at Butte La Rose remained at or above 3.5 m during the 2016 and 2017 crayfish seasons for 167 and 88 days, respectively. Comparison of physicochemistry at sample locations indicated a difference between 2016 and 2017 (pseudo-F = 91.7,

$P = 0.001$, 999 permutations). Temperature, specific conductance, and Secchi depth were higher, and DO was lower at all sample locations in 2017 compared to 2016 except at sites 1 (specific conductance), 12 (Secchi), and 2 (DO). In 2016, DO was higher at the start of crayfish season and slowly decreased throughout the sampling period. In contrast, DO during the 2017 crayfish season started out low, had a slight increase, and ended with a similar DO concentration as the first sample date.

Population Characteristics

We collected 2048 *P. clarkii* and 315 *P. zonangulus* during the 2016 crayfish season and 1628 *P. clarkii* and 126 *P. zonangulus* during the 2017 season. No *P. zonangulus* were captured at sites 12, 13, and 14 in either 2016 or 2017. Inter-annual *P. zonangulus* CL and CPUE was similar between 2016 and 2017 (Table 1). Similar to *P. zonangulus*, there were no differences in *P. clarkii* CL or CPUE at sample locations between 2016 and 2017 (Table 1). However, Form I male *P. clarkii* were significantly larger in 2016 compared to 2017 (Table 1). Additionally, a difference in *P. clarkii* hemolymph protein concentration at sample locations between 2016 and 2017 was not detected (Table 1).

The PCA of water quality parameters at sample locations in 2016 and 2017 had two components with eigenvalues greater than 1.0 that explained 85% of the physicochemical variation among sample locations. Plots of site scores on PC1 and PC2 demonstrated that physicochemical characteristics closely followed spatial groupings (Figure 3). Florida Canal sites (1 and 2) were closest to Bayou Sorrel main channel inputs resulting in high specific conductance levels and lower temperatures. Cannon and Mound Bayou sites (3, 4, 5, 6, 7, and 8) received waters from Cannon and Mound Bayous and an artificial canal to the east that delivered water from the Intracoastal Waterway during rising water levels. These sites were characterized by high DO levels and low Secchi depths. Jones Bayou sites (9, 10, and 11) were the furthest inward floodplain sites in the study and had the highest temperatures and a mean DO concentration above 2 $mg \cdot L^{-1}$, but had several sample dates that experienced moderate hypoxia (three sample dates in 2016, two sample dates in 2017). Oil and Gas Field sites (12, 13, and 14) received low DO water from the surrounding floodplain that caused severe, chronic hypoxia ($[DO] < 1.0\ mg \cdot L^{-1}$) for most of the crayfish season. These sites were characterized by low flows and black water with high Secchi depths.

Procambarus clarkii CL was similar among the four physicochemical location groups in 2016 and 2017 (Table 2). However, Jones Bayou sites had the largest mean *P. clarkii* CPUE values during both sample years. In both 2016 and 2017, CPUE was significantly larger at Jones Bayou sites compared to Oil and Gas Field locations (Table 2). Oil and Gas Field sites had the lowest CPUE during both sample years and a significantly lower CPUE compared to all physicochemical location groups during 2017 (Table 2). Small sample sizes of Form I males in physicochemical groups during both sample years prohibited CL analyses among groups.

Eleven sample locations were classified as normoxic (1–11) and three locations were classified as chronically hypoxic (12–14; Figure 1). Mean DO concentrations at all chronically hypoxic locations were below 1.6 $mg \cdot L^{-1}$ during the 2016 crayfish season and below 0.7 $mg \cdot L^{-1}$ during the 2017 season. Chronically hypoxic locations only experienced normoxic conditions on the first sample date in 2016 (19 March) and site 14 was the only location with one normoxic sample date in 2017 (7 May). Beginning on 17 May and lasting until the end of the 2017 sample season, all chronically hypoxic locations had DO concentrations < 1.0 $mg \cdot L^{-1}$, and all but one location on a single sample date experienced DO concentrations < 0.5 $mg \cdot L^{-1}$ (site 13 on 7 June, DO = 0.9 $mg \cdot L^{-1}$). Although there was no difference ($F_{1,15.1} = 0.27$, $P = 0.609$) in *P. clarkii* least-squares mean CL between normoxic (42.6 ± 0.5 mm) and hypoxic (42.0 ± 1.0 mm) locations in 2016, normoxic sites produced larger individuals on all but one sample date (27 May). Normoxic sites had a higher mean CPUE (geometric = 1.5 ± 0.1; arithmetic = 4.8 ± 0.5) and produced higher CPUE values on every sample date in 2016 compared to hypoxic locations

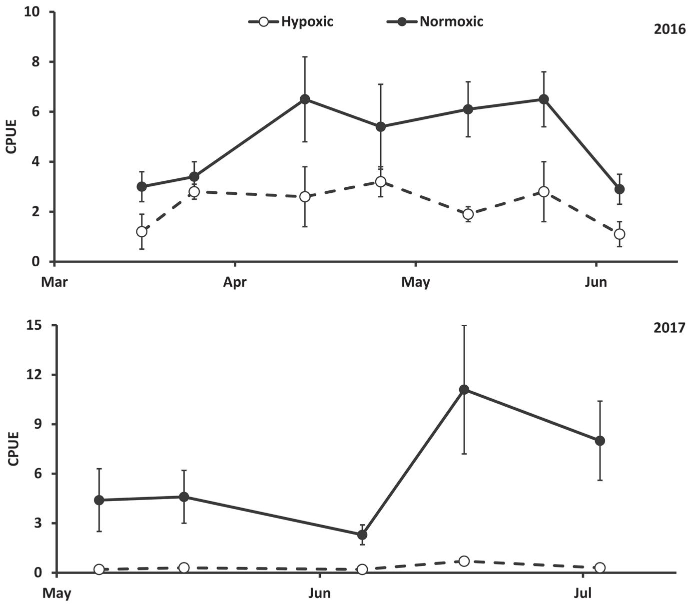

Figure 4. Mean (±SE) catch per unit effort (CPUE) of *Procambarus clarkii* from hypoxic and normoxic sample locations in the Atchafalaya River Basin during the 2016 and 2017 crayfish seasons.

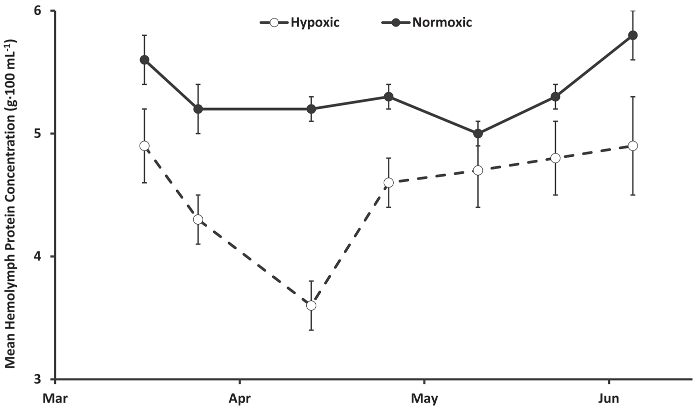

Figure 5. Mean (±SE) hemolymph protein concentration of *Procambarus clarkii* from hypoxic and normoxic sample locations in the Atchafalaya River Basin during the 2016 crayfish season.

Table 2. Least-squares means (± SE) carapace length (CL [mm]) and catch per unit effort (CPUE) of *Procambarus clarkii* from physicochemical classifications of sample locations during the 2016 and 2017 crayfish seasons. CPUE values were $\log_{(e)}$(CPUE+1) transformed for analysis and are the geometric mean. Physicochemical groups with different letters indicate a significant difference ($P < 0.05$) within a population metric (row) for each year. Table abbreviations include: FC (Florida Canal), CMB (Cannon/Mound Bayou), JB (Jones Bayou), and OGF (Oil and Gas Field).

		Physicochemical Group			
Year	**Population metric**	**FC**	**CMB**	**JB**	**OGF**
2016	CL	41.9 ± 1.1^{a}	42.0 ± 0.6^{a}	44.1 ± 0.8^{a}	42.0 ± 0.9^{a}
	CPUE	1.8 ± 0.2^{ab}	1.3 ± 0.1^{ab}	1.9 ± 0.2^{a}	1.1 ± 0.2^{b}
2017	CL	43.1 ± 0.8^{a}	43.1 ± 0.5^{a}	42.8 ± 0.7^{a}	43.0 ± 1.0^{a}
	CPUE	1.7 ± 0.2^{a}	1.0 ± 0.1^{b}	2.3 ± 0.2^{a}	0.3 ± 0.2^{c}

(geometric = 1.1 ± 0.3; arithmetic = 2.2 ± 0.3), but no difference was detected ($F_{1,\ 10.7}$= 2.27, P = 0.161; Figure 4).

In 2017, *P. clarkii* CL was similar between hypoxic (43.0 ± 0.9 mm) and normoxic (43.2 ± 0.3 mm) locations. However, except for one sample date, larger mean *P. clarkii* were collected from normoxic locations. Normoxic locations (geometric = 1.5 ± 0.2; arithmetic = 6.3 ± 1.2) had a significantly higher *P. clarkii* CPUE compared to hypoxic locations (geometric = 0.3 ± 0.4; arithmetic = 0.4 ± 0.1) during the 2017 crayfish season ($F_{1,\ 12.2}$= 8.43, P = 0.013) and higher CPUE values on every sample date (Figure 4). A comparison between *P. clarkii* Form I male CL at hypoxic and normoxic locations could not be completed for 2016 or 2017 because of the small sample size of individuals captured at hypoxic locations.

Mean *P. clarkii* hemolymph protein concentration was significantly lower ($F_{1,\ 14.4}$= 7.58, P = 0.015) in crayfish from hypoxic locations (4.5 ± 0.1 g·100 mL^{-1}) compared to individuals from normoxic sites (5.3 ± 0.1 g·100 mL^{-1}). Furthermore, *P. clarkii* from hypoxic locations had lower mean hemolymph protein concentrations on every sample date in 2016 (Figure 5). Statistical analysis of hemolymph protein concentration between hypoxic and normoxic sites in 2017 could not be performed because of few hypoxic samples.

DISCUSSION

The annual flood pulse is the key component that drives biotic productivity and nutrient cycling in river-floodplain systems and is reported to be the ultimate determinant of the magnitude of the annual ARB crayfish harvest (Junk et al. 1989; Bonvillain et al. 2013b). The timing, duration, and magnitude of the flood pulse are all important hydrologic regime constituents that influence ARB crayfish population and harvest characteristics (Alford and Walker 2013; Bonvillain et al. 2013b). Prolonged duration flood pulses with extended floodplain inundation periods provide a longer harvesting period and increased opportunities for crayfish growth and access to floodplain resources (Bonvillain et al. 2013b). Conversely, years with low magnitude or duration flood events experience decreased spatial and temporal floodplain inundation, below average crayfish harvests, and potential negative crayfish population responses (Bonvillain 2012). Although floodplain inundation began in January, 2016 still experienced a typical flood pulse in the ARB, with floodplain inundation for 167 days and water levels declining in early to mid-June. However, 2017 was an atypical flood pulse, with a late timing and short duration floodplain inundation event during the late spring and summer that resulted in floodplain inundation for only 88 days. Atypical flood pulses are not uncommon in the ARB and can result in below average crayfish harvests (Bonvillain et al. 2008; Bonvillain 2012) and can cause water quality deviations from the norm (Pasco et al. 2016).

Differences in inter-annual flood pulse timing, magnitude, and duration influenced mean annual physicochemistry at sample locations between sample years. The delayed 2017 flood pulse caused peak water levels and the floodplain inundation period to occur during the warmer, late spring and summer, which influenced physicochemistry, notably DO. Reduced hydrologic connectivity and warmer water temperatures with the concomitant increased microbial activity contributed to lower mean DO concentrations in 2017 (Minami et al. 1999; Sierra et al. 2017). Except for site 2, all sample locations experienced lower mean DO concentrations in 2017 compared to 2016. Furthermore, hypoxia generally develops in the ARB when water temperatures exceed 20ºC (Bonvillain et al. 2015). Mean water temperature was above this threshold for all samples dates in 2017, whereas mean water temperatures in 2016 did not reach this threshold until the fourth sample date on 29 April. Additionally, DO at sample locations was typically higher when hydrologically connected to the nearest main channel as oxygen-rich riverine water mixed with backwater areas (Bonvillain et al. 2013b). Flood pulse magnitude affected sample locations that were hydrologically connected to the nearest main channel during the 2016 and 2017 sampling seasons. Sample locations had higher DO concentrations when they were hydrologically connected as they received oxygenated waters from main channels. Most sites became hydrologically disconnected from the main water channel by mid-April in 2016, but were disconnected in the beginning of May and June in 2017. Sites 12, 13, and 14 were not hydrologically connected to a water body that brought in oxygenated water and DO was not influenced by main channel connectivity at these sites. Although the 2017 flood pulse was a delayed, reduced duration event compared to 2016, floodplain inundation was sufficient to support a condensed crayfish season that lasted until late July.

Despite dissimilar crayfish seasons in terms of flood pulse timing and duration, *P. zonangulus* and *P. clarkii* harvest characteristics at our sample locations were similar between sample years. However, *P. zonangulus* CPUE between sample years approached significance (Table 1) with 13% of the total crayfish catch in 2016 comprised of *P. zonangulus* while the number dropped to 7% in 2017. Although there is limited research on wild *P. zonangulus* populations, the different flood pulse characteristics in 2016 and 2017 may have influenced the CPUE differences between sample years. *Procambarus zonangulus* in aquaculture systems thrive during later pond flooding with cooler temperatures (Huner 2002; McClain et al. 2007), acquire food more effectively (Croll and Watts 2004), and exhibit higher growth rates at lower water temperatures (Romaire and Lutz 1989). *Procambarus zonangulus* likely benefited from cooler water temperatures experienced during the early 2016 flood pulse and may have been inhibited by higher water temperatures during the late 2017 flood pulse.

Compared to 2016, the delayed and short duration 2017 flood pulse did not appear to affect *P. clarkii* CPUE or harvest size despite having a warmer mean water temperature (2016 = 22.6°C, 2017 = 24.4°C) and a longer period with favorable water temperatures above 24°C for *P. clarkii* growth (2016 = 13 days, 2017 = 31 days; McClain 2010). *Procambarus clarkii* Form I male size was the only harvest metric that showed a difference between sample years, with a larger mean size in 2016. The longer crayfish season in 2016 may have provided crayfish with increased time and resources to attain the larger size observed, although this is not reflected in the total harvest population CL. Although we only measured mature male *P. clarkii*, it is reasonable to expect a similar response in the female population. Additionally, because fecundity is a function of size (Nobblitt et al. 1995), the 2017 crayfish cohort likely benefited from larger 2016 maturation sizes.

Despite the variation present among physicochemical groups, the absence in *P. clarkii* CL differences among physicochemical groups during both sample years was not surprising given how water quality parameters structured sites in the PCA. Florida Canal, Cannon and Mound Bayou, Jones Bayou, and Oil and Gas Field sites grouped spatially, and location proximity to main channel and canal inputs likely influenced site physicochemical characteristics and the observed PCA location groupings. The Oil and Gas Field locations group were characterized by chronically low DO concentrations and large Secchi depths characteristic of black water sites throughout the flood pulse regardless of water level, connectivity, or temperature. The three other physicochemical groups, Jones Bayou, Cannon and Mound Bayou, and Florida Canal, were separated by specific conductance and slight temperature and DO differences. These results corroborate other studies, both within the ARB and other systems, and suggest that the range of specific conductance, water clarity, and minimal temperature differences (< 3°C) experienced by crayfish in the ARB do not influence *P. clarkii* size or CPUE (Nyström 2002; Trouilhe et al. 2007; Bonvillain et al. 2013b). Additionally, although there were large variations in mean DO concentrations at Jones Bayou, Cannon and Mound Bayou, and Florida Canal locations (1.7 – 5.9 mg·L^{-1}), DO concentrations did not decline to *P. clarkii* stress threshold. Physiological stress and lethal DO concentrations for adult *P. clarkii* is near 1.0 mg·L^{-1} (Avault et al. 1975; McClain et al. 2007; Bonvillain et al. 2012) and dead *P. clarkii* in this study were only observed in completely submerged traps at chronically hypoxic locations with DO levels less than 1.0 mg·L^{-1}. However, chronic, severe hypoxia (DO < 1.0 mg·L^{-1}) is reported to reduce crayfish abundance (Bonvillain et al. 2013b, 2015) and this was observed at Oil and Gas Field locations, which had the lowest CPUE values among physicochemical groups during both sample years, particularly in 2017.

Dissolved oxygen is a key abiotic factor that influences crayfish population characteristics and extended periods of environmental hypoxia can adversely affect crayfish survival, growth, physiology, and population health (McClain 1999; Reynolds 2002; King et al. 2012; Bonvillain et al. 2013b, 2015). Although *P. clarkii* commonly inhabit hypoxic environments in the ARB and have evolved behavioral and physiological adaptations to cope with hypoxic stress (see Reiber 1995; McMahon 2002), prolonged exposure to severe hypoxia can reduce CL, size at maturity, and CPUE (Bonvillain et al. 2015). Although there was no statistical difference in *P. clarkii* CL between normoxic and hypoxic sample locations in 2016 and 2017, normoxic locations produced larger crayfish on most sample dates. Additionally, normoxic sites consistently yielded higher CPUE values compared to hypoxic sites on every sample date during both sample years (Figure 4). Crayfish exposed to higher DO concentrations typically grow faster and have shorter intermolt periods (Jussila and Evans 1997), whereas chronic hypoxic exposure, especially in juveniles, can suppress growth (McClain 1999). Lower *P. clarkii* CPUE in chronically hypoxic areas may be attributed to increased mortality from physiological stress (McClain 1999; Bonvillain et al. 2012), increased susceptibility to pathogens (Shields 2012), or limited movement into traps (Hessen et al. 2004).

Although some normoxic locations experienced ephemeral periods of hypoxia, particularly in 2017, hypoxic periods were relatively brief (three or fewer sample dates) with DO concentrations remaining above 1.5 mg·L^{-1} during sampling times. *Procambarus clarkii* at chronically hypoxic locations were subjected to severe environmental hypoxia (< 1.0 mg·L^{-1}) for the entire crayfish season in both sample years, and most sample dates had DO levels below 0.5 mg·L^{-1}. While the lower DO concentrations experienced in 2017 associated with the late flood pulse did not lower DO concentrations at normoxic sites to levels that would impact *P. clarkii* population attributes, DO reduction at already chronically hypoxic habitats can have negative population responses. Mean CPUE at hypoxic locations was already low in 2016 with a mean DO concentration for the crayfish season at 1.6 ± 0.2 mg·L^{-1}. However, the late 2017 flood pulse resulted in an observed mean DO concentration of 0.6 ± 0.2 mg·L^{-1} and the lowest mean CPUE observed during this study. The effects of delayed flood pulse characteristics on crayfish populations are likely amplified in chronically hypoxic habitats where individuals are already living at physical and physiological limits.

The effects of chronic hypoxic exposure on *P. clarkii* is evident when examining hemolymph protein concentrations. Hemolymph protein is an indicator of chronic hypoxic stress and population health in crayfish (Silva-Castiglioni et al. 2007; Bonvillain et al. 2012), and hemolymph protein concentrations in *P. clarkii* from normoxic areas were consistently higher than individuals from hypoxic locations throughout the 2016 crayfish season. Active foraging and food consumption may be reduced in hypoxic waters (Bernatis et al. 2007; Paschke et al. 2010) as crayfish spend more time at the air-surface interface to access atmospheric oxygen (McMahon and Stuart 1999), which would require individuals to use hemolymph protein concentrations as organic reserves (Oliver and MacDiarmid 2001) and reduce concentrations in starved individuals (Wen et al. 2007). Lower hemolymph protein concentrations in crayfish from chronically hypoxic habitats may also be a contributing factor to reduced crayfish populations documented in these areas. Reduced food consumption, especially protein, adversely affects crayfish survival and growth. Dietary protein levels in breeding females enhance growth and survival of juveniles under adverse environmental conditions, such as hypoxia (Rodríguez-González et al. 2014). Additionally, although not documented in this study, crayfish have delayed maturity (McClain 1999) and mature at smaller sizes in hypoxic environments (Romaire and Lutz 1989; Bonvillain et al. 2013b, 2015), and the number of eggs produced by *P. clarkii* females are directly proportional to the size of the individual (Penn 1943; Reynolds 2002). Thus, smaller females in hypoxic environments may produce fewer offspring compared to crayfish in normoxic environments.

Characteristics of the annual flood pulse in the ARB are the ultimate drivers of crayfish populations and harvests in this system. The timing, magnitude, and duration of the flood pulse are all key hydrologic components that influence harvested crayfish populations. Other than the obvious influence of water level fluctuations on crayfish populations, flood pulse timing can structure water quality characteristics in the ARB (Pasco et al. 2016) and can ultimately influence harvested crayfish populations. Our results support other research efforts on the effects of water quality parameters on *P. clarkii* populations in the ARB and provide novel information that strengthen the theory that DO concentration, particularly chronically hypoxic water, is the principal abiotic variable influencing harvested crayfish population characteristics in this dynamic system (Bonvillain et al. 2013b, 2015). This information is critical to stakeholders and resource managers as efforts to improve water quality and reduce the severity and duration of hypoxia in the ARB move forward (Piazza 2014).

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