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PRIMARY RESEARCH PAPER

A multigear protocol for sampling crayfish assemblages in Gulf of Mexico coastal streams

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Abstract Identifying an effective protocol for sampling crayfish in streams that vary in habitat and physical/chemical characteristics has proven problematic. We evaluated an active, combined-gear (backpack electrofishing and dipnetting) sampling protocol in 20 Coastal Plain streams in Louisiana. Using generalized linear models and rarefaction curves, we evaluated environmental and gear (separate and combined) effects on crayfish catch-per-unit-effort (CPUE), orbital carapace lengths, sex ratios, frequencies of rare species, and sample richness. Although pooled data from combined gears showed greater total numbers of crayfishes, CPUE, and richness compared to either gear individually, combined gear and backpack electrofisher results differed minimally. Overall, richness was negatively related to specific conductance, indicating potential agricultural influence. Neither crayfish sex ratios, lengths, nor frequencies of rare species differed by gear; however, combining data from both gears ensured crayfish were captured in all study streams, which was not found for electrofishing or dipnetting alone. Species accumulation and rarefaction curves indicated sampling was sufficient for recording crayfish diversity at the scale of the study and that adding streams (versus sites within streams) would be most effective for watershed-scale studies. Our results suggested the combined gear protocol was effective for assessing crayfish population and assemblage characteristics in these Coastal Plain streams.

Keywords Crayfish · Active sampling · Electrofishing · Undersampling · Gulf Coastal Plain

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Introduction

Warmwater streams in the southeastern United States are globally unique, containing the highest diversities of freshwater fishes, mussels, and crayfishes outside of the tropics (Isphording & Fitzpatrick, 1992; Neves et al., 1997; Warren et al., 2000; Thorp & Covich, 2009). More than 320 crayfish species (> 80% of the total crayfish diversity in the United States) occur in the Southeast, although many are vulnerable to ongoing alteration of riparian and aquatic habitats driven largely by agriculture and urbanization (Taylor et al., 2007). The conservation status of many rare or distributionally restricted crayfishes is often unknown, and evaluation and restoration efforts are hindered by deficient or antiquated survey data (Crandall & Buhay, 2008; Richman et al., 2015). Methods of sampling for crayfish have been well researched, but surprisingly, there are few standard recommendations for gear choice and sampling time (diurnal versus nocturnal) for particular research questions (Engelbert et al., 2016; Larson & Olden, 2016). Sampling methods have included manual excavation of burrows, baited traps, quadrat samplers, seines, dipnets, and electrofishing units (Rabeni et al., 1997; Harlioğlu, 1999; DiStefano et al., 2003), due in part to the diversity of habitat types occupied by crayfish, study goals (presence/absence versus population estimation), and type of data desired (quantitative or semiquantitative/qualitative, see Legendre and Legendre, 2012). Active sampling gears may be preferred for population and assemblage studies because of increased areal coverage and fewer site visits required compared to passive techniques (e.g., overnight trapping), but effort (i.e., crew size) and expense may be considerably greater (Dorn & Volin, 2009; Parkyn, 2015).

Depending on the research question, active sampling protocols that record relative abundance and catch-per-unit-effort (CPUE) are often reasonable alternatives to more time consuming and labor-intensive estimation of density (e.g., mark and recapture) or absolute abundance (e.g., depletion sampling). However, population and assemblage data derived from relative abundance and CPUE are biased by undersampling, as these data often do not represent a complete inventory of a stream community (Coddington et al., 2009; Beck & Schwanghart, 2010; Colwell et al., 2012). Integrating complementary sampling gears into a sampling protocol could reduce undersampling and improve statistical power (Sørensen et al., 2002), especially in situations where streams/reaches differ in habitat characteristics or crayfish species differ in habitat use and diel activity (Clifford & Casey, 1992; Knight & Bain, 1996; Paillisson et al., 2011). Despite additional onsite effort, integration of gears into a single unit of effort can also eliminate analytical problems associated with inter-gear comparisons and differences in biases among gear types (Weaver et al., 1993). Unfortunately, most studies that have compared sampling gear efficacy for crayfish used pairwise comparisons between gears, and no previously published studies have compared data pooled from combined gears with data obtained from gears singly.

The precision of data used to measure local/regional scale ecological characteristics (e.g., diversity, abundances, and traits) may or may not be improved by incorporating and pooling data from additional gears (see discussions by Jackson & Harvey, 1997; Ruetz et al., 2007). Thus, gear selection and number also play a role in the decision to apportion effort between intensive, multigear samplings of fewer locations versus sampling more locations with less effort. Adopting the wrong sampling gear and protocol can be costly in terms of effort, money, and data quality. Given finite resources and time, integrating multiple gears into a sampling protocol could potentially reduce the number of localities necessary for characterizing population and diversity patterns in a project area, although the sampling limitations of each complementary gear must also be considered. For example, surveying lotic crayfish with a single gear in Gulf of Mexico (hereafter Gulf) Coastal Plain streams can result in a mismatch between gear type and most effective habitat resulting in loss of efficacy, because stream habitat conditions can rapidly change from riffle and pool reaches with abundant woody debris and low specific conductance to deeper, channelized, higher specific conductance, and more turbid agricultural canals (Felley, 1992; Felley & Daniels, 1992; Isphording & Fitzpatrick, 1992; Brown et al., 2006; Kaller et al., 2013). Moreover, published literature pertaining to crayfish sampling protocols in this region of the United States is sparse, as is information on crayfish diversity, habitat associations, and population dynamics (Moore et al., 2013). Thus, it is unknown if published protocols for crayfish sampling in other ecoregions of the United States translate well to Gulf Coastal Plain streams. Active gears such as backpack electrofishers and dipnets have been successfully examined for semiquantitative and qualitative sampling of crayfish populations in a variety of studies (Rabeni et al., 1997; Alonso, 2001). However, deep water with high turbidity and low specific conductance can limit the effectiveness of backpack electrofishing (Reynolds & Koltz, 2013), and dipnets typically collect low numbers of smaller individuals (Price & Welch, 2009). Thus, integrating these gears as a single unit of effort could improve crayfish sampling effectiveness in terms of sample size, species richness, and species traits (e.g., size and sex) relative to a single-gear approach. (see reviews by Parkyn, 2015; Larson & Olden, 2016).

We examined the efficacy of an integrated backpack electrofisher and dipnet sampling protocol for lotic crayfishes in Gulf Coastal Plain streams of central Louisiana. In our preliminary sampling, other gears (e.g., seines and traps) showed low-to-mixed success in sampling any crayfish, and dipnets and backpack electrofishers were usually most effective. Thus, we selected these gears over others because we reasoned they were the most effective at sampling crayfish given the time constraints (i.e., investment of effort) and habitat characteristics (i.e., depth, substrate, and wood densities) that varied greatly among stream drainages. We also present a novel sampling protocol that allows for comparison of data collected by single gears with data pooled from multiple sampling gears as opposed to traditional pairwise comparison designs (e.g., Price & Welch, 2009). We expected that multiple gears employed simultaneously would generally outperform single gears when assessing sample richness, undersampling, and crayfish size and sex distributions. Preliminary field observations indicated that diel period might influence gear efficacy (Creed, 1994; Harlioğlu, 1999; DiStefano et al., 2003), so we also examined whether crayfish CPUE, richness, sex ratio, and size differed between diurnal and nocturnal samples. Our results inform protocols for lotic crayfish assemblage sampling designs in Gulf Coastal Plain streams.

Methods

Study area

We sampled crayfish assemblages from June through August 2014 in the central region of Louisiana's Gulf Coastal Plain. Sites were in the Calcasieu, Vermillion-Teche, Red, and Houston river drainages (Fig. 1), the latter being the only river known to harbor *Faxonius hathawayi blacki* (Walls 1972), a potential species of conservation concern (Holcomb et al., 2015). Study streams in the Calcasieu and Houston river drainages were predominantly forested with abundant woody debris and mostly sand substrates, whereas Vermillion-Teche drainage streams were agriculturally impacted with generally homogeneous clay substrates (Brown et al., 2006; Daigle et al., 2006; Felley, 1992), and Red River drainage streams included both agriculturally impacted and forested streams. From 40 sites that we initially assessed for habitat characteristics and access, we randomly selected five perennial streams in each of the four drainages (20 sites total). In Louisiana, similar to other regions of central and southern United States (Brown et al., 2006), ephemeral and intermittent streams tend to dry more frequently during summer than those during other times of the year (Moore, 1970; Felley, 1992), although the prevalence and distribution of such streams in our study area is poorly mapped. We only sampled perennial streams to ensure that streams (1) could be divided into sample reaches, and (2) were wide enough to reduce spatial influence from the gear operators as they sampled. Sampled streams were wadeable (1 m deep or shallower) with average wetted widths greater than 3 m.

Stream sampling design

We divided each stream site into two 120-m sample reaches, one for the diurnal samples and one for the nocturnal samples. We chose reach lengths primarily on sampling convenience and timing as well as to maintain consistency in the subreach lengths (explained below), although other project protocols may recommend lengths dependent on average wet width of the channel. Reaches were at least 30 m upstream of the bridge access point in all but two sites (which were placed downstream because of unsafe depths for effective electrofishing). We used identical protocols for diurnal and nocturnal crayfish sampling, but for safety reasons, the nocturnal reach was always nearest to the bridge. Before diurnal crayfish sampling, we recorded specific conductance (µS/cm), pH, temperature (°C), dissolved oxygen (mg/l), and turbidity (nephelometric turbidity units, NTUs) at a single point with a YSI® Sonde (Model No. 9130, YSI Inc.; calibrated bi-weekly) and analyzed 1-l water samples onsite for calcium and alkalinity concentrations (mg/l)

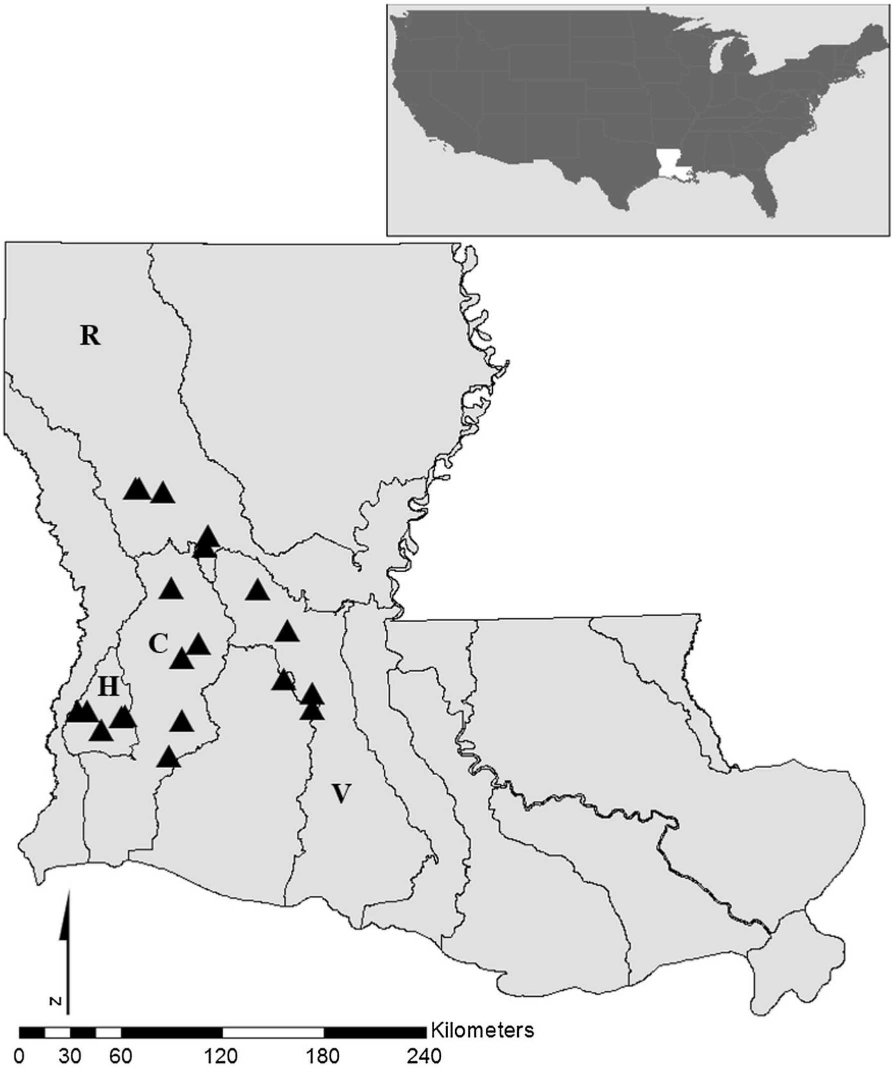

Fig. 1 Map of Louisiana delineating the major river drainages and sites sampled (black triangles) in summer 2014. Drainages labeled as follows: *R* Red River, *H* Houston/West Fork Calcasieu Rivers, *C* Calcasieu River, *M* Mermentau River, *V* Vermillion-Teche Rivers

with chemical titration (HACH® Calcium Hardness Category No. 1457-00; Alkalinity Category No. 20637-00). Time limitations resulted in our analyses using a single water quality measurement to characterize turbidity and specific conductance for both sample reaches. However, we found these parameters usually did not vary substantially between times of day, probably owing to the short time interval between taking diurnal and nocturnal samples (usually less than 5 h). Statistical analyses of other potentially interacting parameters could not be justified with a single diurnal measurement, therefore we reported the remaining water chemistry data as descriptive of stream conditions (Table S1). At the midpoints of six bank to bank transects spaced at 20-m intervals throughout each sample reach (12 measurements per site; Table S2), we recorded stream depth and velocity (75% stream depth; FlowTracker®, Sontek, Xylem Inc.), dominant substrate type (clay, silt, rock, sand, etc.), and woody debris density (number of wood pieces $\geq$ 10 cm in diameter within in a 0.5-m diameter circle).

Crayfish sampling

For each stream, we subdivided the diurnal and nocturnal sample reach into six 20-m subreaches and sampled crayfishes along opposite banks: one bank sampled by a person with a backpack electrofisher (Halltech HT2000B) and a dipnet (3040 cm net with

6.35 mm mesh), and the other bank by a person with only a dipnet to maintain independence of gears. Subreach lengths were standardized at 20 m to maintain sampling effort. For each subreach, operators simultaneously sampled stream margins and portions of the middle of the channel with two upstream zigzagging passes totaling approximately 10 min sampling time per subreach (approximately 2.5 min per person per pass). Dipnet sampling was performed by actively disturbing substrate and scooping through substrate and around tree roots. Electrofisher voltage and pulse frequency were adjusted to maintain an output of 1.2–1.6 amps to reduce crayfish limb loss (Rabeni et al., 1997). The subreach zone sampled by each operator was generally half the stream width, which we believed was sufficient to maintain independence of gear samples. During each pass, we deposited captured organisms into a bucket following each scoop with the dipnet and after approximately 5–10 s of continuous shocking. After the second pass, operators switched sides before sampling the next subreach. We sampled 4 reaches during each field day, with the second nocturnal (post-sunset) sample usually completed approximately 2 h after complete sunset. To minimize operator bias, the same person used each gear type across all samples. We used white LED headlamps during all nocturnal sampling, but the lights did not appear to affect crayfish behavior or movements.

Crayfish from both passes within a subreach were combined so that each sample was specific to the subreach, gear type, and time of day. Following sampling, we recorded species, sex, and adult male form (reproductive [Form I] versus nonreproductive [Form II]) and measured orbital carapace length (mm; posterior edge of thorax to eye orbital) and wet weight (g; on an electronic scale; Sartorius Type 1B16000S). Specimens were then preserved in 95% ethanol and deposited in a voucher collection at the Louisiana State University School of Renewable Natural Resources.

Data analysis

For each gear and time of day at each site, we combined the total number of individuals sampled in all subreaches to calculate catch per unit effort (CPUE; crayfish/reach standardized to an hour) and species richness. Samples with rare species (1 or 2 individuals) for each gear and time of day were noted for further analysis of undersampling effects. We used generalized linear and generalized linear mixed models (GLM and GLMM, respectively) and contrasts between gears (separate and combined) to explore relationships of CPUE, species richness, size, and sex between gear types and times of day. For analyses of richness and CPUE, we contrasted data from each gear singly against data pooled from both gears because we were interested in whether a single gear would perform as well as combined gears. Time of day, substrate, turbidity, depth, specific conductance, and median wood density (the latter four standardized with mean = 0 and variance = 1) were included as model covariates, and we used AICc and Pearson X^2/df criteria to determine the best fitting distributions and link functions. There were no significant covariable interactions with gear or time of day, so we fit separate slopes models (i.e., no interaction terms). We also used likelihood ratio tests (LRTs) of model deviances to determine whether random effects (stream, drainage, or both as random variables) improved model fit over fixed-effects-only GLMs. If LRTs supported inclusion of random effects in a GLMM, we further compared the marginal (variance explained by fixed effects only) and conditional (variance explained by both fixed and random effects) R^2 values to quantify how much variance was captured by the fixed and random components of the model (Nakagawa & Schielzeth, 2013).

After examining results of the LRTs, we fit GLMMs to the CPUE (marginal $R^2 = 0.544$, conditional $R^2 = 0.898$) and size data (marginal $R^2 = 0.0138$, conditional $R^2 = 0.430$), and GLMs to the richness (marginal $R^2 = 0.295$) and sex data (marginal $R^2 = 0.103$). Both GLMMs incorporated stream as a random variable. The CPUE data were fit with a negative binomial error distribution and a log canonical link function; richness data were fit with a Poisson error distribution and log link function; size data were fit with a Gaussian error distribution and identity link function; and sex data were fit with a binomial error distribution and logit link function. Assumptions of the linear model were evaluated before model selection. All models and figures were analyzed and produced using R statistical software (Venables & Ripley, 2002; Kuznetsova et al., 2016; Lefcheck, 2016; R Core Team, 2016).

We examined undersampling with sample- and individual-based rarefaction curves and loglinear analysis. The GLM indicated no significant diel effects on richness, so we pooled richness data from both times of day and calculated species accumulation and rarefaction curves (Oksanen et al., 2017) that examined the rate of species accumulation as a function of the number of streams, and the number of individuals, sampled for each gear type. These analyses examined asymptotic behavior of the richness data that would indicate sufficient sampling given the effort employed (Gotelli & Colwell, 2011). Comparisons among sample- and individual-based curves also indicated how effective the gears were in capturing diversity in the study area when standardized by level of effort and number of individuals. Finally, we used a GLM (Poisson error and log link function) to perform a loglinear analysis to determine if the frequency of rare species (number of singleton or doubleton species recorded in a sample) was associated with a particular gear or time of day (Table 1).

Results

Crayfish samples

We collected 676 crayfishes (dipnet = 154, electrofisher = 522) representing 11 stream species expected to occur in the region (Walls, 2009). Site richness was low, with samples typically yielding one (3 sites), two (12 sites), three (1 sites), or four (4 sites) species. Four crayfishes (*F. h. blacki* (Walls 1972), *Procambarus pentastylus* Walls and Black 2008, *Faxonius maletae* (Walls 1972), and *Procambarus natchitochae* Penn 1953) that were distributionally restricted to a single drainage accounted for 60% of the total catch, with the majority being either *P. pentastylus* or *P. natchitochae* (Table 2). All but three species (*F. maletae*, *Procambarus zonangulus* Hobbs and Hobbs 1999, and *Cambarellus puer* Hobbs 1945) were found in two or more streams. Sexes were evenly represented among samples (339 males, 342 females), with 292 form II and 47 form I males.

Sampling effects of gear type and diel period

Gear type significantly affected CPUE, but time of day and other covariates did not (Table 2; Fig. 2). There

Table 1 Crayfish species sampled from 40 Louisiana sample reaches (20 streams with 1 diurnal sample reach and 1 nocturnal sample reach) during summer of 2014 with a backpack electrofisher and dipnet

	Number captured (#)	Prevalence (# of streams)	Proportion of total sample (%)
Cambarellus puer Hobbs 1945	14	1	2.1
Faxonius hathawayi blacki[a] (Walls 1972)	16	2	2.3
Faxonius lancifer (Hagen 1870)	153	11	22.5
Faxonius palmeri longimanus (Faxon 1898)	6	3	0.9
Faxonius palmeri palmeri (Faxon 1884)	70	4	10.3
Faxonius maletae[a] (Walls 1972)	16	1	2.3
Procambarus acutus (Girard 1852)	26	3	3.8
Procambarus clarkii (Girard 1852)	22	7	3.2
Procambarus natchitochae[a] Penn 1953	176	4	25.8
Procambarus pentastylus[a] Walls & Black 2008	208	6	30.5
Procambarus zonangulus Hobbs and Hobbs 1990	1	1	0.1
Grand Total	681		

Data represent pooled totals across both gears and times of day for the entire effort

[a] Species that are reported to be distributionally restricted to a single drainage (Walls, 2009)

Table 2 Tests of fixed effects (Wald Chi-square) of generalized linear and linear mixed effects models quantifying effects of gear type (backpack electrofisher, dipnet, and both) and time of day (day and night) on richness and crayfish catch-per-unit-effort (CPUE), respectively

Response	Variable	Wald Chi-square	df	*P*
CPUE	Gear type	78.966	2	< 0.001*
	Time of day	1.082	1	0.298
	Gear type × time of day	1.056	2	0.590
	Substrate	3.119	1	0.077
	Wood density	0.655	1	0.418
	Specific conductance	0.819	1	0.819
	Depth	0.117	1	0.117
	Turbidity	0.835	1	0.835
Richness	Gear type	5.552	2	0.062
	Time of day	0.741	1	0.389
	Gear type × time of day	1.040	2	0.594
	Substrate	1.923	1	0.165
	Wood density	0.753	1	0.385
	Specific conductance	6.380	1	0.012*
	Depth	0.326	1	0.568
	Turbidity	0.617	1	0.432

Substrate (sand and clay), median wood density (#/m^2), specific conductance (mS/cm), depth (m), and turbidity (NTU) were treated as covariates in the models. The latter four variables were standardized with mean = 0 and standard deviation = 1

*Indicate significant *P*-values ($\alpha = 0.05$)

were no differences in CPUE between the electrofisher (13.49 ± 10.76 SD) and the combined gears (17.16 ± 12.73; $Z = -1.616$, $P = 0.238$), but average CPUE of the electrofisher and the combined gears were higher than dipnetting alone (3.65 ± 3.61; $Z = -6.913$ and $Z = -8.553$ respectively; both $P < 0.001$). We found no significant pairwise differences in mean richness between gears (electrofisher 1.68 ± 0.90; dipnet 1.18 ± 0.73; combined gear 1.81 ± 0.89) or time of day (diurnal samples 1.42 ± 0.93; nocturnal 1.72 ± 0.81; all $P > 0.05$). Of the covariates, only specific conductance was negatively related to site richness (slope = − 0.228 ± 0.090 SE, $Z = -2.526$, $P = 0.012$).

Mean orbital carapace length was larger in electrofisher (20.59 ± 8.72 SD) than in dipnet (17.77 mm ± 8.17; $P = 0.004$) samples. Nocturnal samples produced larger crayfish (20.31 mm ± 8.81) than did diurnal samples (19.43 mm ± 7.72; $P = 0.004$; Table 3), although the length difference was small and size variability high for gears and sampling periods. Sex ratios did not differ among gears or times of day (both $P > 0.05$).

Undersampling

Both individual- and sample-based rarefaction curves approached asymptotes, suggesting the sampling effort we used was sufficient for characterizing richness in terms of individuals and sites sampled (Fig. 3). We saw no significant gear differences in either set of curves (overlapping confidence intervals), further indicating that richness estimates were similar among gears given a level of effort or crayfish abundance. Both sets of curves differed in the point where the curves decelerated to an asymptote. Fewer individuals were recorded with the dipnet than the electrofisher and combined gear, yet the dipnet reached an asymptote at 10 species with far fewer individuals than the electrofisher and combined gears. In addition, sample-based rarefaction curves rose slowly (∼ 1 additional species captured per 2 sites sampled) and approached asymptotes at relatively

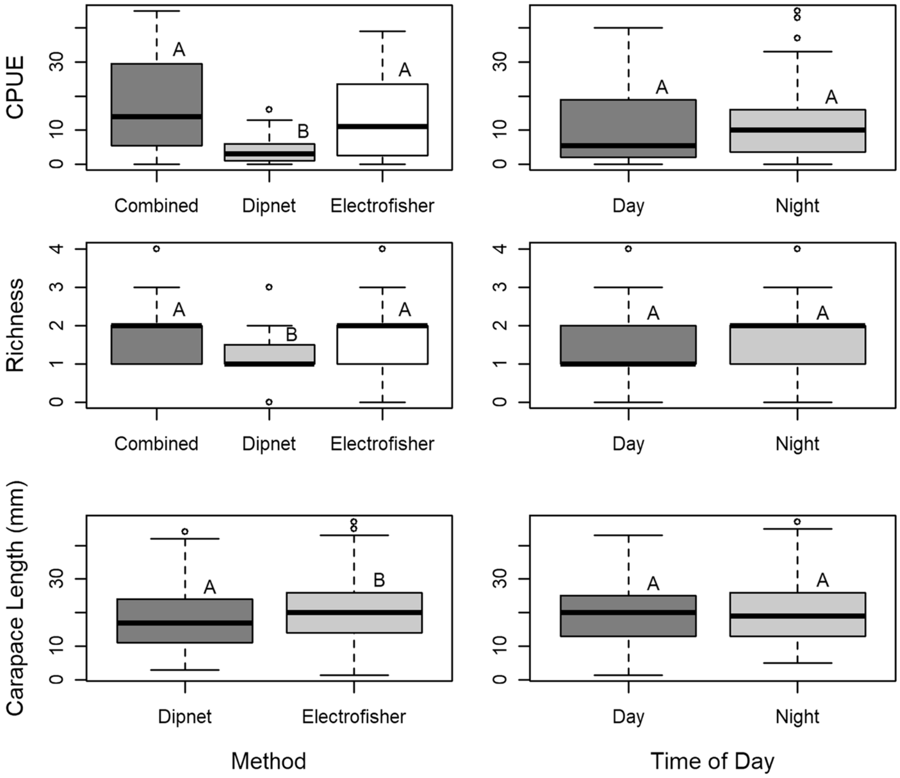

Fig. 2 Boxplots of crayfish catch per unit effort (CPUE, # individuals per sample), species richness, and carapace length (mm) by gear type and time of day. Thick horizontal bars within boxes represent medians. Different letters indicate significant pairwise differences determined with a priori contrasts of group means analyzed with generalized linear (richness data) and linear mixed models (CPUE and carapace length). Small points plotted above or below the whiskers indicate observations beyond the 1.5 interquartile ranges

Table 3 Tests of fixed effects (Wald Chi-square) of generalized linear and linear mixed effects models to assess potential gear (backpack electrofisher or dipnet) and time of day (day or night) biases on orbital crayfish carapace length or sex

Response	Variable	Wald Chi-square	df	*P*
Carapace length	Gear type	12.082	1	< 0.001*
	Time of day	6.239	1	0.013*
	Gear type × time of day	1.7441	1	0.187
Sex	Gear type	0.202	1	0.653
	Time of day	0.803	1	0.370
	Gear type × time of day	0.866	1	0.352

*Indicate significant *P*-values (α = 0.05)

high effort (~ 18 sites). Loglinear analysis did not indicate any relationship between gear type and the presence of rare species (i.e., no interactions among fixed effects). However, dipnetting failed to sample any individuals in four sites, compared to one site for electrofishing. Combining data from both gears ensured crayfish were collected in all site samples.

Discussion

The goal of this study was to investigate the effectiveness of a backpack electrofishing and dipnetting sampling protocol for estimating crayfish CPUE, carapace length, sex ratios, and species richness in 20 Gulf Coastal Plain stream sites in Louisiana. Although the CPUE and richness data recorded with the backpack electrofisher did not statistically differ from pooled data, adding a dipnet ensured the protocol

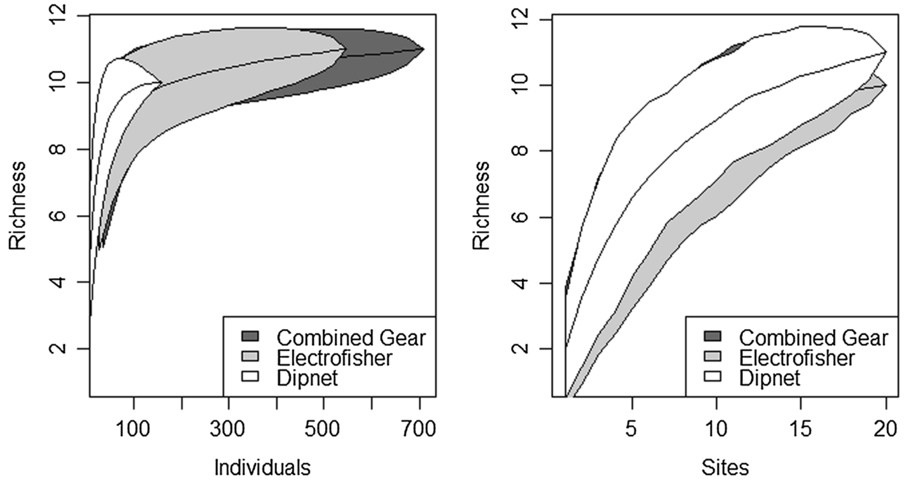

Fig. 3 Individual-based (left panel) and sample-based (right panel) rarefaction curves calculated to assess potential undersampling of crayfish richness by gear type (beige = dipnet, dark gray = backpack electrofisher, light gray = both gears). 95% confidence intervals are shown with colored polygons

collected crayfishes in every sample reach. The dipnet tended to sample smaller individuals, but sizes varied greatly within gears. Night sampling produced slightly larger individuals. We found our sampling protocol with these gears could be especially useful for studying richness patterns and range delineation of crayfish because both gears were effective in evaluating species richness. However, further studies should compare combined gears with single gears in sampling species abundances, an especially important parameter for biodiversity conservation goals. Overall, combining the two gears provided an effective and adaptable protocol for assessing crayfish assemblage characteristics in the Gulf Coastal Plain streams.

Selection of sampling gear(s) for crayfish studies should be based on study objectives and comparisons of gear efficacy and bias under conditions that may be encountered during the study (Parkyn, 2015). Here, our research focused on comparing the performance of two gears, which we chose to compare because we believed these would be best suited for sampling diversity among our project streams given the project context. Our study protocol can be easily extended to make these comparisons across more than two gears; however, project constraints and habitat conditions may preclude the extra effort required for trapping (e.g., overnight deployment and retrieval of traps), or additional active sampling (e.g., extra personnel for seining). We note that although investigators may require different sampling gears than what we compared in this study (e.g., quadrat samplers and traps, Dorn et al., 2005), our protocol can be easily adapted to examine performance of those gears in collecting relevant crayfish assemblage data and assist with gear selection.

We also anticipated that nocturnal sampling in these streams would yield more crayfish than diurnal sampling, but the data indicated no effect of diel period on sampling efficiency. Importantly, our nocturnal samples were restricted to post-sunset hours (generally ending sampling before midnight), and sampling later at night may have yielded different results. However, active gears that can adequately sample crayfish taking shelter in benthic debris can compensate for potential differences in diel encounter rates (see Distefano et al., 2003), and the similarity in richness among diel periods suggests that in these streams, diurnal sampling is adequate to describe crayfish assemblage composition.

Gear bias in this study was similar to that reported by Price & Welch (2009) for South Carolina streams in that the electrofisher produced greater CPUE. Additionally, the dipnet produced smaller individuals, although this relationship was very noisy because the random effect for carapace length accounted for nearly half of the total model variance. This suggested that other unmeasured temporal, spatial, or environmental factors at the stream level probably influence the effect of gear type and time of day on carapace length. The lack of sex bias we found was similar to results of other studies that employed active gears (e.g., throw traps, Dorn et al., 2005) and contrasted with widely reported male sex bias in passive gears, which could be influenced by temperature (Mason, 1975; Somers & Stechey, 1986) and seasonality. We also observed no sex biases by gear or time of day when we applied the model to each species (13 GLMs, all $P \gg 0.05$), indicating active gears ensured representative samples of both sexes regardless of species during the course of a survey. The rarity of Form I males among our samples suggested that additional approaches (e.g., incorporating passive gears or accounting for seasonality) may be needed if obtaining Form I males is a research goal.

Interestingly, covariate effects were only significant in the model for richness. This was surprising because woody debris, turbidity, and stream depth were also expected to be correlated with crayfish CPUE (Kershner & Lodge, 1995; Flinders & Magoulick, 2007). We may have underestimated woody debris density (see Wallace & Benke, 1984), because we quantified wood in the middle of the stream channel instead of at the stream margins (Flinders & Magoulick, 2007) or littoral zones (Kershner & Lodge, 1995). However, examination of large woody debris in nearby streams indicated little difference between margin and channel habitats when debris was abundant (analysis of data in Kaller & Kelso, 2007). Therefore, given differences in methodology and geography, it is not clear whether the lack of relationship with woody debris in this study was due to a methodological difference or was an inherent feature of this woody debris-rich region. Depths did not appear to affect either gear's sampling performance, but covariance of depth with stream velocity may need to be considered and assessed with future studies. Most of the study streams we sampled were generally shallow and slow flowing; however, deeper or faster flowing streams could impact crayfish catchability and gear efficacy.

Specific conductance can affect electrofishing performance (Reynolds & Koltz, 2013) as well as the abundance and diversity of many stream organisms, including crayfish (Burskey & Simon, 2010), fish (Gorman & Karr, 1978; Kimmel & Argent, 2010) and freshwater mussels (Allen & Vaughn, 2010). Elevated specific conductance may be also indicative of anthropogenically impaired habitat due to runoff and increased sedimentation from adjacent lands (Walser & Bart, 1999). During our initial qualitative assessments of physical habitat prior to sampling (8/9 sites, see also Felley, 1992), we observed that many study sites surrounded by agriculture had more clay in the substrate and higher specific conductance relative to nonagricultural sites. High specific conductance was not related to crayfish CPUE but was negatively related to richness. In the same agricultural watersheds, Fitzgerald (2012) did not find a meaningful difference between dominant substrate types assessed visually versus quantitatively. Therefore, we interpreted elevated specific conductance as evidence of agricultural impact disproportionately affecting crayfish species in these sites, although biological responses to land-use disturbances can vary based on position within the watershed (Gomi et al., 2002; Allan, 2004). We welcome further studies that employ our methodology to study gear efficacy and gear complementarity in the face of land-use influences (e.g., eutrophication vs environmental heterogeneity) in documenting crayfish diversity and abundance patterns.

Importantly, the lack of a significant gear effect in richness analyses supported the patterns observed in the species accumulation curves and rarefaction curves. In short, the data recorded with our protocol suggest that the dipnet, electrofisher, or both gears can yield similar estimates of richness with similar effort (i.e., number of sites or individuals sampled). Thus, for questions of richness, using a dipnet can be as effective as the electrofisher. In 24 out of the 120 total subreaches of our study, one of the gears (usually the dipnet) failed to detect any crayfish within the subreach. Thus, we potentially observed species differences in vulnerability to these gears, which could significantly bias survey data. We did not explore if increasing the number of subreaches in each sample reach could overcome differences in species vulnerabilities to being detected by our gears and yield greater sample richness. This is an important consideration because patchy crayfish distributions within structurally complex stream habitats can substantially increase the risk of undersampling due to gear sampling characteristics. For example, the electrofisher failed to detect any *C. puer* (a small-bodied species) across all six subreaches in a diurnal sample of Windham Creek, even though the species was collected in dipnet samples. Consequently, based on our sample-based rarefaction curves, a protocol with an electrofisher alone may need to sample more subreaches (i.e., longer sample reach lengths) in order to detect this species. Conversely, although dipnet sampling was usually less effective at sampling crayfish, it did allow easy sampling of habitats such as leaf packs that could be swept and sorted, and thus resulted in increased sample richness at some sites. Although the relative ease of the dipnet to access specific habitats increased richness in some cases, additional subreaches would be necessary for the dipnet alone to sample richness as effectively as combined gears, again based on our sample-based rarefaction curves. Therefore, employing complementary gears simultaneously may reduce the reach length (i.e., reduce time investment needed to detect crayfish in these Gulf coastal plain streams.

Crayfish did not appear to be abundant in our study streams, and low sample abundances, although consistent among reaches and streams, characterized all of our sample sites, a pattern supported by observations from Walls (2009). The asymptotes of species accumulation and rarefaction curves suggest that crayfish studies in our study region should attempt to maximize the number of streams sampled to ensure sufficient sampling of richness (Gotelli & Colwell, 2011). Unfortunately, richness, relative abundance, and density estimates for most Louisiana crayfish communities have not been published, and comparison of our data with previously documented estimates of crayfish assemblage composition (Dorn et al., 2005; Engelbert et al., 2016) is not possible. Data combined from both gears had high precision among subreaches, and although the accuracy of the abundance data remains unknown, our samples were consistent with distributional data for most of the species in this region (Walls, 2009). We did not collect four other species (*Faxonius hathawayi hathawayi* Penn 1952; *Procambarus kensleyi* Hobbs 1990; *Procambarus acutus* (Girard 1852); and *Cambarellus shufeldtii* (Faxon 1884)) that inhabit the study area (Walls, 2009). True range distributions are unknown for many crayfish, including Louisiana species. Thus, we cannot determine whether our protocols simply missed the species or if they were absent from the study streams.

The literature comparing the efficacy of different crayfish sampling gears continues to grow (see review by Larson & Olden, 2016), but standardization of methods is still problematic. Crayfishes in Gulf Coastal Plains streams remain poorly studied (Taylor et al., 2007; Moore et al., 2013), and matching appropriate gear(s) with research questions and stream habitat conditions, particularly regarding comparison of results among studies, has not been adequately addressed. An advantage of combining gears in our study design was that it allowed for the assessment of relative gear efficiency while still permitting the pooling of gears into a single unit of timed effort. Similar stream conditions, including fine substrates lacking rock and cobble and low slopes, which occur along the Gulf and Atlantic Coasts (Felly, 1992; Isphording & Fitzpatrick, 1992) and in the central United States (Brown et al., 2006), eastern Asia (Dudgeon, 1995), eastern Australia (Lake, 1995), and southeastern Europe along the Black Sea (Birk & Hering, 2009) offer opportunities for adopting this or similar protocols. Integrating additional active gears into a similar sampling protocol would likely increase personnel requirements and project costs, but might be justified if sample accuracy could be increased. Therefore, we urge that future studies exploring gear efficacy adapt and improve upon our protocol so that more accurate recommendations can be made given project goals and constraints.

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