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PRIMARY RESEARCH PAPER

# Not all methods are created equal: assessment of sampling methods for crayfishes and fishes in southern Appalachian streams

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**Abstract** We compared the effectiveness of three sampling methods (kick seining, electrofishing, and nest trapping) to collect crayfishes and fishes, simultaneously, in southern Appalachian Mountain streams (Alabama, USA). For crayfishes, kick seining collected the highest species richness and most individuals. However, by combining kick seining and electrofishing collections, we decreased the number of sites needed to accurately assess crayfish richness relative to using one method. For 9 of the 13 species collected, no differences in crayfish sizes or sex ratios were detected between electrofishing and kick seining. In the remaining four species, electrofishing collected larger crayfishes and more females than kick seining. For fishes, electrofishing was most effective at assessing fish species richness. Sampled fish richness was higher when electrofishing in streams with higher water temperatures and width-to-depth ratios, as well as lower conductivities and smaller substrates. Electrofishing was the most effective sampling method for collecting Centrarchids, whereas kick seining was most effective at collecting Cyprinids. Nest traps were the least effective sampling method. We conclude that using a combination of kick seining and electrofishing is best for assessing stream fish and crayfish assemblages, simultaneously, which can improve management, biomonitoring, and understanding of the complex relationships between these faunal groups.

**Keywords** Electrofishing · Kick seine · Nest trap · Alabama · Species richness

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## Introduction

Fishes and crayfishes play critical roles in patterns of energy flow in stream ecosystems, including as potential prey, higher-order consumers, and by direct interactions with each through trophic encounters, and competitors for food and space (Stein, 1977; Rahel & Stein, 1988; Englund & Krupa, 2000; Reynolds, 2011). These interactions may influence crayfish and fish distributions, densities, behaviors, assemblage diversity, and size structure (Stein & Magnuson, 1976; Rahel & Stein, 1988; Garvey et al., 1994; Dorn & Mittelbach, 1999; Keller & Moore, 1999). For examination of their respective effects on a system, including on each other, reliable methods of sampling co-occurring fish and crayfish assemblages over a corresponding time frame are requisite. However, unlike for fishes (Kushlan, 1974; Jackson & Harvey, 1997; Bonar et al., 2009), standardized quantitative sampling methods are not well established for crayfishes (Barnett & Adams, 2018; Budnick et al., 2018). Numerous studies employ a single sampling method or set of sampling methods to assess crayfishes and fishes simultaneously (Hicks, 2003; Degerman et al., 2007; Dorn, 2008) without quantifying sampling method accuracy and biases, which could lead to erroneous conclusions. To better understand sampling biases and efficiently sample (i.e., reduce sampling time and cost for separate surveys) crayfish and fish assemblage structures simultaneously, we must establish quantitative sampling methods, evaluate methodological biases, and incorporate these findings into ecosystem management decisions (Black, 2011; Legendre & Legendre, 2012; Kusabs et al., 2018).

To accurately assess stream community structure (e.g., diversity, abundances, age classes), we must use sampling methods that gather precise data. Because most sampling methods have associated biases, combining data from multiple sampling methods can lead to more accurate estimates than using one sampling method (Onorato et al., 1998; Budnick et al., 2018). Sampling methods used in a study dictate sampling protocols, with decisions to intensely sample few sites or less intensely sample many sites often based on the sampling method used. Understanding how accuracy changes due to sampling intensity and the number of sites sampled can prevent under- and oversampling, allowing efficient use of time and money while collecting high quality data. Oversampling can also lead to greater environmental impacts, increasing disturbance and harm to stream habitats and organisms (Larson & Olden, 2016). Just as oversampling can negatively impact stream environments, sampling for multiple taxa within the same site during separate sampling events can also increase the chances of harming stream habitats and organisms. Thus, sampling for multiple taxa simultaneously can decrease sampling effort and site disturbance, while also reducing the probability of mistaken inferences regarding co-occurrence of different taxa.

Active (e.g., electroshocking, seining) and passive (e.g., trapping) sampling methods are used to assess stream crayfish and fish assemblages. Electrofishing is regarded as the most effective gear type for sampling stream fish assemblages, with both electrofishing and seining recommended when assessing fish size classes (Cowx et al., 2001; Poos et al., 2007; Bonar et al., 2009). However, few standard sampling method recommendations (i.e., best methods to sample particular habitat types or assess specific research questions) have been made for crayfishes (Engelbert et al., 2016; Larson & Olden, 2016; Budnick et al., 2018). Effectiveness of a sampling method is influenced by the characteristics and spatial extent of the habitat sampled, the abundance, diversity, and distribution of species, and their species-specific behavior (e.g., mobility, avoidance, hiding) or conspicuousness (size, coloration). Consequently, crayfish sampling method comparison studies generally focus on one target species (Olsen et al., 1991; Rabeni et al., 1997; Alonso, 2001; Gladman et al., 2010; Reid & Devlin, 2014; Williams et al., 2014). While streams in many geographic regions are occupied by only one crayfish species, streams of the southeastern U.S. tend to possess diverse crayfish assemblages (Richman et al., 2015). Thus, sampling methods are needed to effectively collect and accurately represent abundances and distributions of multiple, co-occurring crayfish species (DiStefano, 2000; Larson et al., 2008; Engelbert et al., 2016; Budnick et al., 2018). Few studies have compared the effectiveness of various crayfish sampling methods in species-rich streams, and results have been inconsistent across studies (Price & Welch, 2009; Engelbert et al., 2016; Budnick et al., 2018). Among these studies, sampling biases were noted in size, sex, and species sampled, with biases varying by sampling method, region, and habitat. No studies have assessed crayfish sampling methods in the southern Appalachian region of the southeastern U.S., the northern hemisphere center of crayfish diversity (Crandall & Buhay, 2008). Streams in this region have rocky substrates that are very different than the fine, silty/clay substrates in most southeastern, Coastal Plain streams (Williams & Amatya, 2016). Similarly, no studies have assessed the accuracy of different sampling methods for sampling stream crayfishes and fishes simultaneously, even though many studies, aimed at understanding crayfish abundances and distributions, targeted both faunal groups (Englund, 1999; Usio & Townsend, 2000; Garvey et al., 2003).To reduce biases, direct comparisons of sampling methods and integration of complementary sampling methods in heterogeneous, species-diverse stream habitats are needed (Barnett & Adams, 2018; Budnick et al., 2018).

In the present study, we examined the effectiveness of three sampling methods, electrofishing, kick seining, and nest trapping (Bechler et al., 2014), individually and combined, for sampling crayfishes in southern Appalachian streams. Because fish often play an integral role in stream crayfish distribution and abundance, we simultaneously examined how effectively our methods assessed fish populations. Our objectives were to (1) compare stream crayfish species richness, catch per unit effort (CPUE), sex ratios, and sizes between kick seining and electrofishing; (2) compare fish species richness and CPUE between kick seining and electrofishing; (3) identify fish families and species that were indicators of kick seining and electrofishing collections; (4) assess how environmental factors influenced the effectiveness of kick seining and electrofishing for crayfishes and fishes; and (5) determine the number of sites needed to accurately assess crayfish and fish species richness by nest trapping, electrofishing, kick seining, and the latter two methods combined. Our findings will benefit stream management and biomonitoring efforts in the southern Appalachian region by improving understanding of sampling method effectiveness in species-rich habitats, and the benefits and biases associated with simultaneously collecting crayfishes and fishes.

## Methods

### Study area

We sampled crayfishes and fishes in five streams in the Bear Creek (Tennessee River Basin) and Cahaba River (Mobile River Basin) drainages in the southern Appalachian region of Alabama, USA (Fig. 1). Both drainages are valuable ecological resources due to highly diverse aquatic faunal communities and numerous imperiled species (Allen, 2001; McGregor & Garner, 2003; Philip & Johnston, 2004). All streams sampled were wadeable, perennial streams with distinct pool-riffle complexes and channel widths ranging from 3 to 30 m. Streams were typical of the high to moderate gradient rocky, mountainous streams found throughout the southern Appalachian region (Wallace et al., 1992), marked by primarily large cobble and pebble substrate with varying amounts of sand and silt. Sites contained large woody debris and patches of aquatic vegetation with abundant detritus and small woody debris. Surrounding land uses were predominantly forest ($> 75\%$) intermixed with pasture, row crops and poultry production ($> 10\%$) in the Bear Creek drainage, and forest ($> 70\%$), low-density residential, medium intensity commercial ($> 10\%$), and pasture and row crops ($< 5\%$) in the Cahaba River drainage (Thom et al., 2013; Barnett unpublished data).

### Method comparison sampling

Among all five streams, 38 sites were sampled: 24 in the Bear Creek drainage and 14 in the Cahaba River drainage (Fig. 1). We selected sites at set intervals along streams, with six to ten sites per stream. If a predetermined location was inaccessible, we sampled the closest accessible site. Sites were dispersed over 26–55 km of streams lengths. We sampled in the spring/summer (“spring”; May–July) and fall/winter (“fall”; September–December) of 2015–2017 (hereafter, “sampling rounds”) (Table 1).

At each site, we sampled a linear reach 30 times the wetted width (established during each sampling round), unless wetted widths were less than 6 m or greater than 16 m, in which case minimum (200 m) or maximum (500 m) reach lengths were sampled (Simon, 2004). We sampled reaches by kick seining, electrofishing, and nest trapping. We kick seined or electrofished only in riffle and run habitats with maximum depths $\leq 1$ m ($\geq 85\%$ of each reach) because both sampling methods are more effective in shallow, flowing habitats (Larson & Olden, 2016). We restricted nest trapping to pools to prevent traps from filling with sand and dislodging in flowing waters (Bechler et al., 2014). We divided each reach equally into two subreaches. Each subreach consisted of multiple riffle-run complexes. We kick seined in downstream and electrofished in upstream subreaches to prevent kick seining from reducing electrofishing effectiveness by increasing turbidity. Because the effectiveness of kick seining does not depend on water clarity, increased turbidity due to simultaneously electrofishing upstream should not have decreased kick seining effectiveness. In both subreaches, we sampled pools with nest traps. We classified

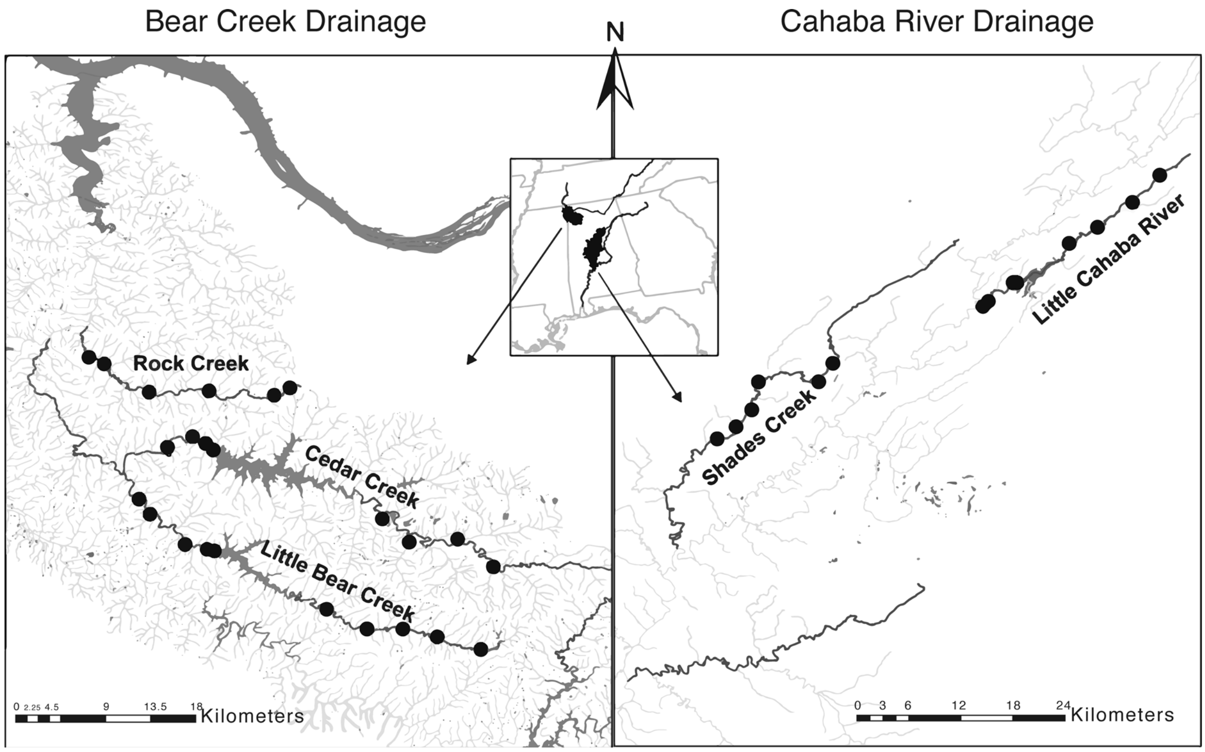



**Fig. 1** Map of the Bear Creek and Cahaba River drainages, Alabama, with collection sites represented by circles. Inset shows the Bear Creek and Cahaba River drainages with major rivers, Tennessee and Alabama rivers, respectively, within the southeastern United States

**Table 1** Number of sites sampled by each sampling method during each seasonal sampling round

| Drainage | Stream | Spring 2015 | Fall 2015 | Spring 2016 | Fall 2016 | Spring 2017 |
|---|---|---|---|---|---|---|
| Bear Creek | Cedar Creek | | | | | |
| | E-fishing and kick seining | 8 | 8 | – | – | – |
| | Nest trap | – | 8 | – | – | – |
| | Little Bear Creek | | | | | |
| | E-fishing and kick seining | 10 | 10 | – | 10 | 10 |
| | Nest trap | – | 10 | – | 10 | 10 |
| | Rock Creek | | | | | |
| | E-fishing and kick seining | 4 | 6 | – | 6 | 6 |
| | Nest trap | – | 6 | – | 6 | 6 |
| Cahaba River | Little Cahaba River | | | | | |
| | E-fishing and kick seining | – | – | 6 | 8 | 8 |
| | Nest trap | – | – | 6 | 8 | 8 |
| | Shades Creek | | | | | |
| | E-fishing and kick seining | – | – | 3 | 6 | 6 |
| | Nest trap | – | – | 3 | 6 | 6 |

A dashed line indicates that the method was not used in that sampling round

*E-fishing* electrofishing

macrohabitats based on channel characteristics and water velocity (Bisson et al., 1982).

Using two crews, we simultaneously made one pass of each subreach. Because we sampled multiple riffle-run complexes (replications within each subreach), we did not conduct multi-pass sampling to assess species community structures (Angermeier & Smogor, 1995; David et al., 2010). We conducted 20 kick seines per 100 m (Simon, 2004), sampling, on average, 15% of the subreach, and we electrofished 0.4 s/m$^2$ ($\bar{X}$ = 729 s; range = 216–1801s), sampling, on average, 70% of the subreach. We based electrofishing effort on the time necessary to sample riffle and run macrohabitats during preliminary sampling in several sites. To standardize efforts, we calculated total number of kick seines and time electrofishing, as well as the percentage of riffle and run macrohabitats in each subreach before sampling began. We apportioned sampling effort to macrohabitats by their proportion in the subreach. Once the target number of kick seines and time electrofishing was reached, sampling stopped. We sampled all habitat types within a subreach, including stream banks and mid-channels.

Kick seining was conducted by a 2–3 person crew, with each person alternating kicking within each subreach. One person kicked, disturbing the substrate and lifting any large rocks in a 2 m long × 1.5 m wide plot (measured with strings attached to seine brails) immediately upstream of the seine. Two people lifted the seine immediately after kicking was completed. The seine was 2.6 m wide x 1.6 m high with 3-mm mesh. After each kick, the seine was moved diagonally (from bank to bank) at least 2 m away from the previous plot.

We conducted single pass electrofishing using a Smith-Root backpack electrofisher (model 12A programmable output wave, battery-powered electrofisher set at 50–60 Hz, 4–5 ms pulse width, 300–400 V; Vancouver, Washington) with a circular anode covered with 3 mm mesh netting. Electrofisher settings were adjusted at each site based on stream conductivity and temperature. We used as low a voltage as possible to prevent loss of crayfish chela (Alonso, 2001). The sampling crew consisted of 3 people: the electrofisher operator who also collected crayfishes and two dip netters who collected crayfishes using 41 cm × 23 cm dip nets with 3 mm mesh. If stunned crayfish or fish were seen along the stream bottom but could not be easily collected with dip nets, the electrofisher operator would stop, lift the anode out of the water, and individuals would collect these crayfish by hand.

Nest traps were set in up to five pools per reach ($\bar{X}$ = 3 pools/reach). If a reach had five or fewer pools, all were sampled. If a reach had more than five pools, five pools were randomly sampled. Traps consisted of 30 cm lengths of 5 cm diameter polyvinyl chloride (PVC) irrigation pipes with three drain holes (1 cm diameter) 7 cm apart along the top and bottom and irrigation drain caps attached to each end. A semicircular opening (5.0 cm wide × 2.2 cm tall) in one cap allowed organisms to enter and leave the trap. This cap was attached with an eye bolt and nut for easy removal when checking the trap, and the other cap was glued on. Stream substrate placed in the lower halves of traps provided habitat. We placed nest traps in crevices and under large rocks, roots, and wood. Nylon parachute cords connected trap eye bolts to stable objects (e.g., root, metal stake in bank). The cords facilitated easy retrieval of traps in a vertical position, inhibiting organisms from escaping through the entrance hole. After retrieving a trap, all organisms were removed and the trap reassembled and placed back into the crevice. The number of traps per pool (range 2–6, $\bar{X}$ = 4) was determined by the pool size and number of available crevices. Traps (range 2–10 traps/subreach) were set for at least a week before first sampling and checked during every sampling round. Traps remained in sites until the study concluded (deployed for up to 24 months).

For all collections, we recorded crayfish and fish species and life stage (i.e., adult, juvenile). For crayfishes, we also recorded sex, adult reproductive form (form I male [reproductive], form II male [nonreproductive], female [without eggs], and ovigerous female [bearing eggs]), and postorbital carapace length (POCL). Most crayfishes and fishes were identified, measured, and released in the subreach of capture; all others were preserved in 5% formalin (fishes) or ≥ 70% ethanol (crayfishes) for further laboratory analyses.

## Environmental sampling

Environmental sampling quantified channel and substrate characteristics and water quality (Table 2).

**Table 2** Median (SD) values for environmental parameters from spring and fall sampling

| | | Little Bear | | Cedar | | Rock | | Little Cahaba | | Shades | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stream length (km) | 80 | | 82 | | 33 | | 45 | | 87 | |
| | Site length | 0.17 (0.5) | | 0.22 (0.1) | | 0.12 (0.5) | | 0.17 (0.1) | | 0.19 (0.5) | |
| Spring | *N* | 20 | | 8 | | 10 | | 14 | | 9 | |
| | Water temperature (°C) | 22.04 | (3.00) | 25.26 | (1.82) | 22.13 | (1.06) | 23.98 | (2.21) | 25.57 | (0.90) |
| | DO (mg/l) | 7.52 | (0.60) | 6.75 | (0.42) | 6.91 | (0.90) | 6.76 | (0.80) | 6.24 | (0.32) |
| | Conductivity (µS/cm) | 104.7 | (51.8) | 340.0 | (83.8) | 175.9 | (70.5) | 288.1 | (71.0) | 235.4 | (30.8) |
| | pH | 7.37 | (0.35) | 7.49 | (0.30) | 7.14 | (0.36) | 8.14 | (0.70) | 7.33 | (0.50) |
| | Wetted width (m) | 10.8 | (3.2) | 14.0 | (4.2) | 6.9 | (4.2) | 11.5 | (4.0) | 13.4 | (2.7) |
| | Depth (cm) | 20.6 | (9.1) | 25.7 | (15.5) | 16.9 | (16.5) | 21.0 | (7.8) | 33.4 | (18.0) |
| | Width to depth ratio | 0.48 | (0.24) | 0.59 | (0.61) | 0.33 | (0.55) | 0.58 | (0.36) | 0.40 | (0.14) |
| | D16 | 2.2 | (16.7) | 4.7 | (26.8) | 3.2 | (445.5) | 3.5 | (45.4) | 2.0 | (21.9) |
| | D84 | 98.0 | (656.6) | 64.3 | (854.0) | 63.7 | (900.3) | 300.5 | (895.4) | 995.8 | (965.0) |
| | Aquatic vegetation (%) | 9.8 | (9.1) | 15.7 | (9.3) | 19.8 | (13.3) | 11.1 | (20.1) | 11.0 | (8.1) |
| | Canopy cover (%) | 51.5 | (24.4) | 68.6 | (12.6) | 64.4 | (22.9) | 54.2 | (21.5) | 44.9 | (22.7) |
| | SWD (%) | 5.2 | (2.9) | 5.8 | (2.8) | 6.8 | (2.2) | 5.9 | (3.8) | 9.8 | (5.9) |
| | LWD (number) | 5.0 | (7.5) | 8.5 | (4.8) | 5.0 | (5.3) | 4.0 | (4.8) | 11.5 | (7.5) |
| Fall | *N* | 20 | | 8 | | 12 | | 8 | | 6 | |
| | Water temperature (°C) | 20.67 | (1.97) | 19.95 | (1.15) | 18.22 | (5.16) | 23.11 | (3.76) | 23.79 | (1.75) |
| | DO (mg/l) | 7.65 | (0.68) | 6.23 | (0.92) | 5.39 | (3.17) | 6.65 | (1.53) | 5.15 | (0.69) |
| | Conductivity (µS/cm) | 99.8 | (39.4) | 334.0 | (93.7) | 194.4 | (124.0) | 331.8 | (84.6) | 353.0 | (32.8) |
| | pH | 7.42 | (0.31) | 7.64 | (0.21) | 7.44 | (0.42) | 7.84 | (0.31) | 7.64 | (0.07) |
| | Wetted width (m) | 9.3 | (2.9) | 13.5 | (3.5) | 6.5 | (3.8) | 10.8 | (4.3) | 11.0 | (2.8) |
| | Depth (cm) | 15.7 | (7.5) | 16.6 | (18.1) | 15.5 | (18.2) | 18.0 | (9.4) | 20.7 | (9.4) |
| | Width to depth ratio | 0.62 | (0.52) | 0.65 | (0.54) | 0.34 | (0.48) | 0.73 | (0.44) | 0.50 | (0.15) |
| | D16 | 2.8 | (26.4) | 4.7 | (26.8) | 2.5 | (24.4) | 8.3 | (40.8) | 1.3 | (16.3) |
| | D84 | 121.8 | (750.3) | 81.9 | (842.8) | 45.4 | (788.1) | 309.1 | (894.4) | 917.4 | (1004.0) |
| | Aquatic vegetation (%) | 13.3 | (9.7) | 15.7 | (9.3) | 31.7 | (12.0) | 11.8 | (21.8) | 13.7 | (6.2) |
| | Canopy cover (%) | 46.4 | (19.3) | 35.6 | (16.1) | 49.3 | (14.8) | 57.3 | (24.9) | 71.8 | (22.3) |
| | SWD (%) | 7.6 | (2.6) | 5.8 | (3.0) | 9.2 | (3.6) | 8.8 | (4.1) | 13.5 | (6.0) |
| | LWD (number) | 7.0 | (6.7) | 8.5 | (4.8) | 5.0 | (5.6) | 6.5 | (4.5) | 18.0 | (8.1) |

*N* total sites sampled, *DO* dissolved oxygen, *D16* size (mm) that 16% of particles were smaller than, *D84* size (mm) that 84% of particles were smaller than, *SWD* small woody debris, *LWD* large woody debris (number of pieces)

During each sampling round, we measured channel characteristics (wetted width, depth, and percent canopy cover) at four evenly spaced transects, ranging from 50 to 125 m apart, within each reach (2 transects per subreach). Depth was measured mid-channel and 10 cm from right and left edges. Percent canopy cover was measured mid-channel with a convex spherical densiometer. Streambed composition across the bank-full channel width was assessed using pebble counts (Wolman, 1954; Harrelson et al., 1994) once per year. Pebble count data were collected from at least ten diagonal transects (five per subreach) with ten sample points equally spaced along each transect. The first transect began along a stream bank at one end of a reach. At each sample point, we blindly chose and measured one pebble and a woody debris sample, if present, at the boot tip. We visually estimated percentages of the streambed covered by vegetation and small woody debris (SWD, < 10 cm diameter) (Bain & Stevenson, 1999) between points and counted large woody debris (LWD, ≥ 10 cm diameter). Before sampling, we measured water quality parameters (water temperature, conductivity, dissolved oxygen [DO], and pH) at one location within each site with a Hydrolab Quanta (OTT Hydrolab, Loveland, Colorado). We calibrated the Hydorolab before each sampling round for all parameters and daily for DO.

## Data analyses

Data analyses consisted of three main components. First, we compared methods by identifying differences between kick seining and electrofishing in collections of crayfish (species richness, CPUE, sex ratios, sizes) and fish (species richness, CPUE, indicator species, percent relative abundance) assemblages, separately. Next, for crayfish and fish variables that differed between sampling methods, we used statistical models to relate differences to stream environmental characteristics. Finally, we compared the sampling effort needed by each sampling method singly and combined to assess crayfish and fish species richness within streams based on Chao-1 (Chao, 1984) estimates. For all models, histograms of model residuals did not depart from normality.

### *Method comparison analyses*

We compared crayfish and fish species richness and CPUE ($N$/100 $m^2$ [total area within subreach]) between electrofishing and kick seining in each macrohabitat, stream, and season. Because nest traps sampled a different macrohabitat type (pools), nest trap captures were not statistically compared to results from other sampling methods. We excluded from analyses age-0 individuals (individuals during their first year of life) that were not identifiable to species. We calculated $\log_e$ CPUEs (+0.001) of the most widespread crayfish species (present in ≥ 35% of sites) and of total fish. We compared CPUEs (response variable) between sampling methods, streams, seasons, and macrohabitats (fixed effects) using linear mixed-effect (LME) models, with site as a random effect. Sampling methods interaction with stream, season, and macrohabitat were included in models. Only differences in sampling methods or its interactions were interpreted to understand if sampling method CPUE differences varied between streams, seasons, and macrohabitats. The same approach was repeated using species richness as the response variable. If significant interactions were detected between sampling methods and streams, indicating sampling method effectiveness differed among streams, we then investigated correlations between stream habitat characteristics and sampling method effectiveness; those analyses are described below in the Methods, Analyses of environmental effects section. Analyses were performed with the *lmerTest* package (Kuznetsova et al., 2015) in R software version 3.4.2 (R Core Team, 2013), using Tukey's HSD post hoc tests for comparing means.

We compared lengths and age class structures (i.e., age-0, age-1) of the most abundant crayfish species ($N > 25$ individuals/season) between electrofishing and kick seining. We compared crayfish $\log_e$ POCLs (response variable), separately for each abundant species, between sampling methods using LME models as in the above CPUE comparisons.

We also used POCLs to estimate age compositions of crayfish species. We estimated the number of age classes separately for spring and fall collections (all sites within a drainage combined), using mixed distribution analysis (*flexmix* R package) of length-frequency (POCL) data (France et al., 1991; Leisch, 2014; Barnett et al., 2017). We did not analyze *Cambarus striatus* Hay, 1902 age classes within the Cahaba River drainage due to low numbers there. We used a maximum of four age classes for each species (Weagle & Ozburn, 1972; Page, 1985) except *C. striatus,* for which seven classes were used (Camp et al., 2011). We ran models with 1,000 iterations, used integrated completed likelihoods to select the best models (Biernacki et al., 2000), and qualitatively compared the number of age classes estimated from kick seining and electrofishing model results.

For the most abundant crayfish species within each drainage, we compared differences in ratios of adult reproductive forms (i.e., form I males, form II males, and females) between kick seining and electrofishing collections, macrohabitats, and seasons. We analyzed data separately for each drainage and species using a log-linear model computed with the 'glm' function in the *MASS* package (Ripley, 2014) in R. We did not include *C. striatus* form I males collected in the Bear Creek drainage in analyses due to low numbers collected ($N = 2$). Because adult reproductive form ratios varied little across streams and years, we pooled data across both. To balance sampling effort between seasons, sites sampled in spring 2016 (6 in Little Cahaba River and 3 in Shades Creek; Table 1) were excluded. Because the focus of this model was to assess differences in ratios of crayfish adult reproductive forms between sampling methods across macrohabitat and season, only sampling method or its interactions (e.g., sampling method interaction with macrohabitat) were interpreted. Data were not overdispersed (dispersion tests: $P$ values = 0.07–0.90).

To determine whether one sampling method consistently collected higher percent relative abundances (PRA) of a certain group, we compared differences in six different fish groupings by families (Cyprinidae, Catostomidae, Percidae, Centrarchidae, and Ictaluridae, and "Other", including all fishes belonging to all other families) between kick seining and electrofishing collections. Fish PRAs were calculated separately for each drainage, using data pooled across streams and years. We compared PRAs between electrofishing and kick seining using a log-linear model just as in crayfish sex ratio comparisons. Because the focus of this model was to assess differences in fish PRAs between sampling methods across season, only sampling method or its interactions with season was interpreted. Data were not overdispersed (dispersion tests: $P$ values = 0.7–0.9).

We identified if any fish species was associated more with kick seining than electrofishing by applying an indicator value analyses (IndVal, Dufrêne & Legendre, 1997). The IndVal analysis (R package *labdsv*; Roberts, 2016) measured the association between a species and sampling method by calculating the product of specificity (mean CPUE of a species within one sampling method compared to the other sampling method) and fidelity (the number of sites a species is collected from using one sampling method). The maximum IndVal of 100% is reached when a given species is collected at all sites by only one method. Statistical significance levels of indicator species were tested by 9999 random permutations of the samples.

*Analyses of environmental effects*

To assess the relationship between stream environmental characteristics and sampling effectiveness for collecting crayfishes and fishes, we created LME models. This relationship was investigated only for fish and crayfish parameters (CPUE and richness) in models with significant interactions (sampling methods interacted with streams) in the "method comparison" section. Sampling methods interactions with streams indicated that the effectiveness of each sampling method may be driven by stream characteristics. We constructed separate LME models for kick seining and electrofishing to understand what environmental variables were correlated with a difference in the effectiveness of sampling methods among streams. We assumed that sampling methods were more effective in environmental characteristics that were different between kick seining and electrofishing models.

In the kick seining and electrofishing LME models, CPUE or richness was the response variable, and site was the random effect. Independent variables included season, percent macrohabitat, $\log_e$ transformed channel characteristics, $\log_e$ transformed streambed coverage (vegetation, SWD, LWD), $\log_e$ transformed water quality parameters (water temperature, conductivity, and DO), pH, and substrates sizes. Because only two macrohabitat types were kick seined and electrofished, only one macrohabitat percentage (riffles) was used in models. Channel characteristics and streambed coverage were averaged for each subreach. Three substrate metrics were derived from pebble counts from each subreach: the median particle size (D50), and the particle sizes that 16% (D16) and 84% (D84) of particles were smaller than (Olsen et al., 2005). Models were fit with maximum likelihood estimations. We used the *MuMIn* R package (Barton & Anderson, 2002) to analyze all possible models. Model selection was based on corrected Akaike information criterion ($AIC_c$) because sample sizes were small relative to the number of estimated parameters (Burnham & Anderson, 2004). We compared alternative models by weighting their level of data support (Hurvich & Tsai, 1989), with delta $AIC_c$ values $\leq 2$ representing the best-supported models. We calculated relative variable importance (RVI) scores for each predictor variable, based on variables appearance in the $AIC_c$-best models. Predictors with RVI $> 0.5$ were considered most important. To assess the fit of each model, we calculated marginal $R^2$s, the proportion of variance explained by the fixed effects, and conditional $R^2$s, the proportion of variance explained by the fixed and random effects (Nakagawa & Schielzeth, 2013; Johnson, 2014).

*Assessment of sampling effort adequacy*

To evaluate the differences in the effort needed by each sampling method to accurately assess crayfish and fish species richness, we compared Chao-1 estimates of effort required by each sampling method singularly versus combined to estimate crayfish richness within streams. Nest traps caught 0–2 fish species in each stream, so we did not assess the effort required to estimate fish species richness for this sampling method. We estimated the rate of species accumulation as a function of the number of sites sampled for each sampling method, separately, and for kick seining and electrofishing combined. Combined kick seining and electrofishing sampled twice the area at each site relative to each sampling method alone. To account for the differences in areas sampled, we also compared stream length (rkm) needed per site to accurately estimate species richness among sampling methods. We extrapolated stream species composition (counts of individuals and species in each collection) to estimate species richness for 100 sites with the Chao-1 method using EstimateS 9.1.0 (Colwell, 2013). The Chao-1 method estimates species richness by extrapolating the probability of undetected species within each site from the number of rare species captured (i.e., singletons). The program randomly reordered and resampled collections 100 times (Colwell, 2013; Engelbert et al., 2016) and gave $S_{est}$-values and 95% confidence intervals. The $S_{est}$-value was the average number of species estimated in a stream during the 100 resampling events. We used $S_{est}$-values to determine species accumulation by method in each stream (Engelbert et al., 2016).

## Results

We collected 13 crayfish species (Table 3) and 87 fish species (Online Resource 1), including 88% of the crayfish species known from both drainages, and 64% and 36% of the fish species known from the Bear and Cahaba River drainages, respectively. We collected crayfishes and fishes from all sites, with a maximum of 5 crayfish and 29 fish species in a single collection. The five most abundant crayfish species, constituting 97% of total collections, were *C. striatus*, *Faxonius erichsonianus* (Faxon, 1898), *F. validus* (Faxon, 1914), *F. virilis* (Hagen, 1870), and *F. compressus* (Faxon, 1884), with the first four also being the most widespread in sampled streams. The most abundant fish species, constituting 87% of total collections, were in Cyprinidae (38%), Percidae (32%), and Centrarchidae (17%) families (Fig. 2).

### Method comparisons

*Species richness and CPUE comparisons*

Kick seining, electrofishing, and nest trapping collected crayfishes in 97% ($N$ = 1,799 individuals), 89% ($N$ = 1,007 individuals), and 39% ($N$ = 33 individuals) of collections, respectively. Of the 13 species collected, 92%, 85%, and 54% were collected by kick seining, electroshocking, and nest trapping, respectively. We recovered 60% of nest traps across all sampling dates, resulting in 418 trap examinations, of which 11% ($N$ = 47) were occupied by crayfishes. No trap was occupied by more than one crayfish.

Crayfish species richness was 30% higher in kick seining than electrofishing collections (LME $F_{1,392} = 22.26$, $P < 0.001$). We identified no significant differences in crayfish CPUE between kick seining and electrofishing for any species ($P$ values range: 0.23–0.82) except *F. erichsonianus.* The differences in *F. erichsonianus* CPUE between kick seining and electrofishing collections were inconsistent across macrohabitat (Fig. 3a; LME $F_{1,290} = 7.36$, $P < 0.01$) and stream (Fig. 3b; $F_{4,290} = 4.38$, $P < 0.01$), but overall, for all streams and macrohabitats, *F. erichsonianus* CPUEs were two times higher from kick seining than electrofishing (LME $F_{1,291} = 13.33$, $P < 0.001$).

Kick seining and electrofishing both collected fishes in 99% of collections, and nest trapping collected fishes in 9% of collections. Five percent of nest traps were occupied by fishes. Of the species collected, 87% were captured by kick seining, 100% by electrofishing, and 9% by nest trapping. Both juveniles and adults of larger-bodied Centrarchid and Cyprinid species were more vulnerable to electrofishing than kick seining (61% of individuals collected by electrofishing), whereas small-bodied benthic fish species (e.g., Darters, Madtoms, Sculpins) were more vulnerable to kick seining than electrofishing (54% of

**Table 3** Number of crayfishes captured by each sampling method in the Bear Creek and Cahaba River drainages during seasonal sampling, with total number of sites (*N*) containing each species indicated

| Drainage | Crayfish (*N*) | Electrofishing | Kick seining | Nest trapping | Total |
|---|---|---|---|---|---|
| Bear Creek (24 sites) | *Lacunacambarus aff. diogenes* (Girard, 1852) (6) | 0/2 | 6/11 | 0/1 | 20 |
| | *C. striatus* (12) | 14/25 | 61/28 | 2/2 | 132 |
| | *Faxonius compressus* (6) | 18/11 | 35/55 | 2/0 | 121 |
| | *F. erichsonianus* (22) | 98/78 | 221/274 | 1/11 | 683 |
| | *F. etnieri* species complex (Taylor et al., 2014) (1) | 4/2 | 8/0 | 0/0 | 14 |
| | *F. validus* (24) | 489/128 | 422/185 | 1/9 | 1,234 |
| | *Procambarus hayi* (Faxon, 1884) (1) | 2/0 | 0/0 | 0/0 | 2 |
| Cahaba River (14 sites) | *L. acanthura* Hobbs, 1981 (1) | 0/0 | 1/0 | 0/0 | 1 |
| | *C. coosae* Hobbs, 1981 (4) | 4/2 | 14/2 | 0/0 | 22 |
| | *C. striatus* (4) | 1/0 | 7/1 | 1/0 | 10 |
| | *F. erichsonianus* (9) | 23/20 | 153/63 | 1/0 | 260 |
| | *F. spinosus* (Bundy, 1877) (1) | 0/0 | 1/1 | 0/0 | 1 |
| | *F. virilis* (13) | 70/8 | 198/46 | 5/9 | 336 |
| | *P. acutus* (Girard, 1852) (3) | 0/8 | 3/0 | 1/0 | 4 |
| | *P. clarkii* (Girard, 1852) (6) | 3/5 | 2/2 | 0/0 | 12 |
| | Total | 726/667 | 1,132/667 | 14/32 | 2,852 |

Numbers indicate individuals captured in spring and fall (spring/fall) sampling rounds

individuals collected by kick seining). Seventy-one percent of fish species not collected by kick seining were larger-bodied pelagic fishes (Fig. 2). Additionally, cavity-spawners (e.g., Madtoms, Catfish) dominated the fishes caught in nest traps (67% of individuals collected). Fish CPUE was three times higher in electrofishing than kick seining collections (LME $F_{1,392} = 8.31$, $P < 0.001$). The differences in fish species richness between sampling methods were inconsistent across streams (Fig. 3c; $F_{4,392} = 3.56$, $P < 0.01$); however, overall 40% more species were collected by electrofishing than kick seining (LME $F_{1,393} = 82.80$, $P < 0.001$).

### *Crayfish size, age class, and reproductive form comparisons*

Crayfish sizes ranged from 3.2 to 37.0 mm POCL ($\bar{X} = 11.8$ mm) in kick seining, 3.8–52.9 mm ($\bar{X} = 13.0$ mm) in electrofishing, and 5.5–33.9 mm ($\bar{X} = 17.6$ mm) in nest trapping collections. Although there were significant differences in POCL among methods ($P$ value $< 0.05$), no method consistently collected larger or smaller crayfish among all species (Fig. 4). Nonetheless, electrofishing collected crayfishes of similar sizes or larger than kick seining in each stream and season.

We estimated two age classes for all species except *C. striatus* (Online Resource 2). Age class estimates from kick seining versus electrofishing were similar for all species except *F. erichsonianus* and *F. virilis* in Cahaba River drainage fall collections, where low numbers of crayfishes were collected by electrofishing ($N < 25$). In both kick seining and electrofishing collections, growth of age-0 crayfishes was documented from spring to fall for all species except *C. striatus*, with larger age-0 crayfishes collected later in the year.

Ratios of adult reproductive forms did not differ between sampling methods for most crayfishes. Females were the most abundant adult reproductive form collected ($N = 676$), consisting of 57% of total adult collections. No ovigerous females or females with young were collected. Form I male collections

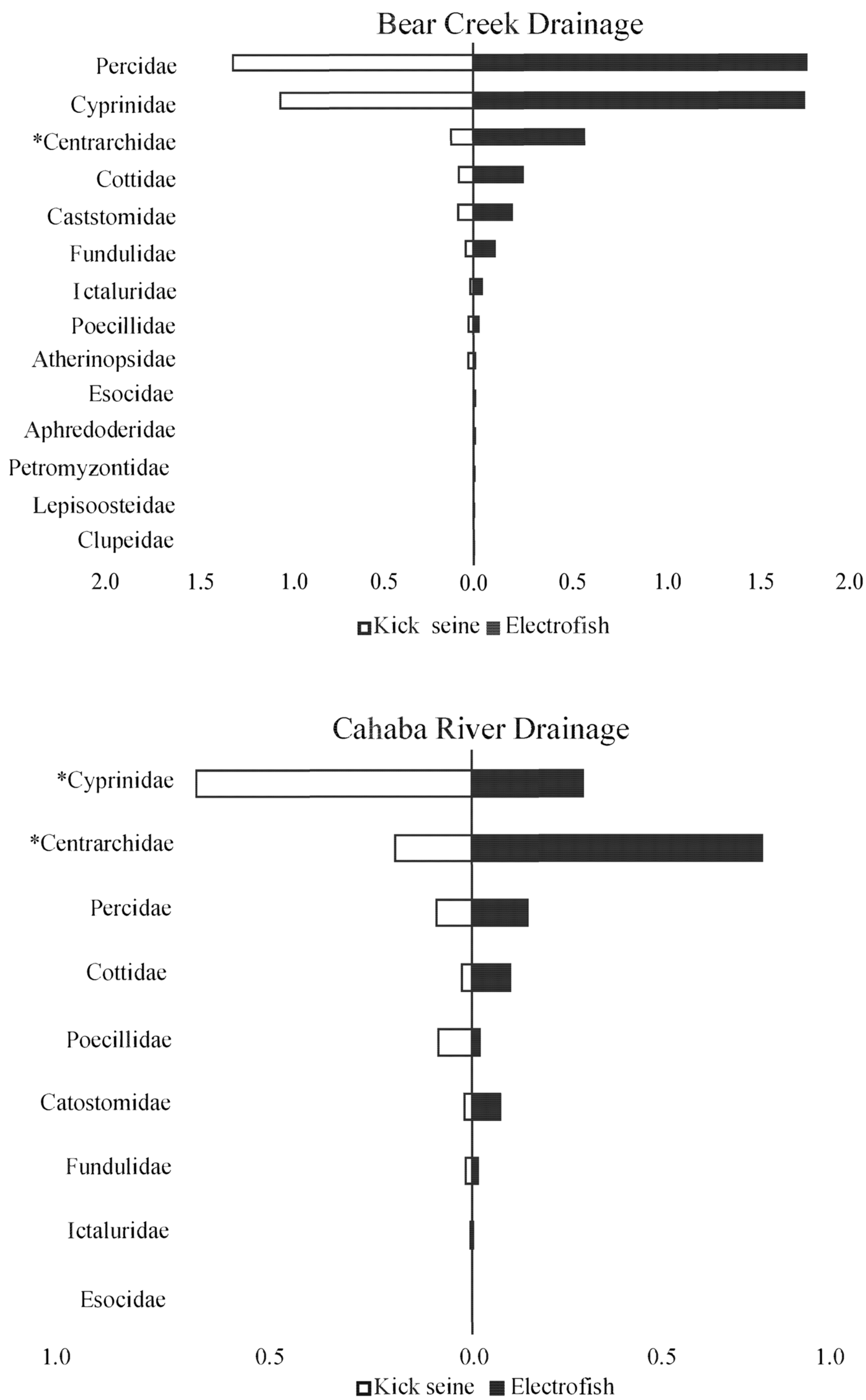
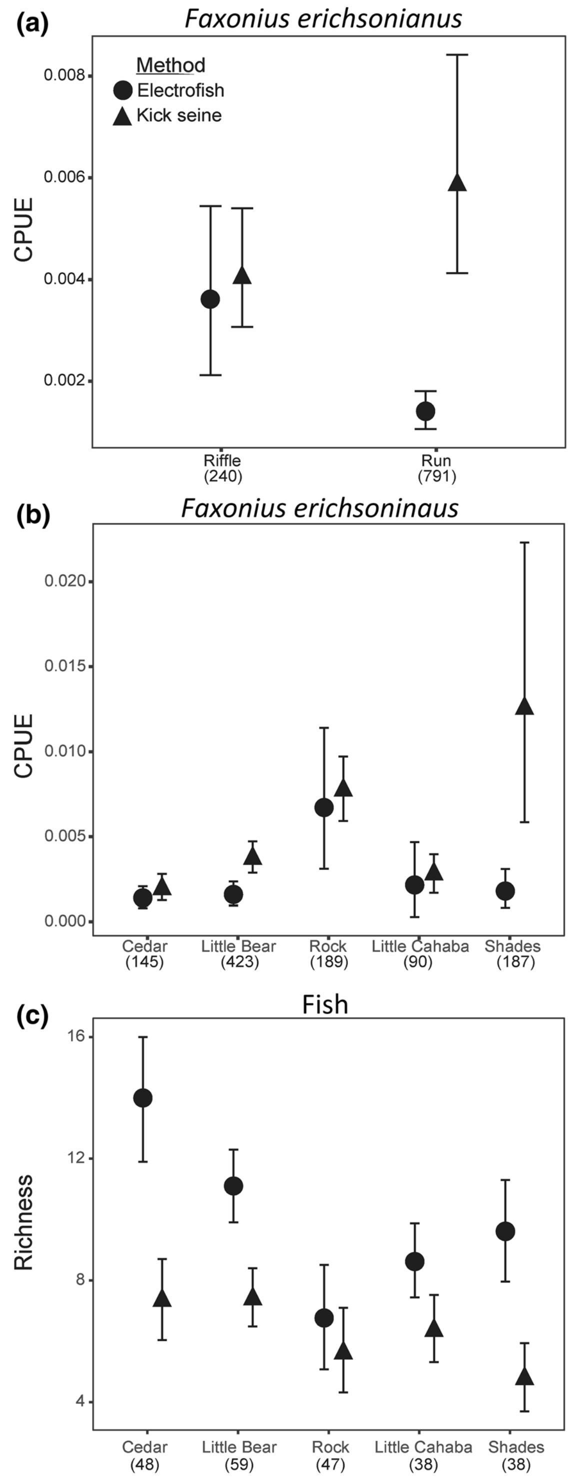



**Fig. 2** Horizontal mirrored histogram with total CPUE ($N$/100 $m^2$) for each fish family collected in the Bear Creek and Cahaba River drainages by kick seining and electrofishing. Asterisks represent families with significantly different ($P > 0.05$) electrofishing and kick seining collections in percent relative abundance analyses. Note x-axis differ for each drainage




◀**Fig. 3** Sampling method comparisons of mean (± 95% CI) catch per unit effort (CPUE; *N*/100 $m^2$) (**a**, **b**) and richness (**c**) of *Faxonius erichsonianus* and fishes, respectively, among macrohabitats (**a**) and streams (**b**, **c**), with number of individuals [CPUE] or species [richness] collected in parenthesis. $Log_e$ transformed data were used in analyses. Only relationships with significant interactions in linear mixed-effect models are displayed

increased during fall sampling for each species and sampling method. No differences were detected in ratios of reproductive forms between kick seining and electrofishing collections for any species ($X^2$, *P* values range: 0.22–0.41) except *F. virilis*. Female *F. virilis* were 2.7 times more likely to be collected than form I or II males when electrofishing ($X^2_2 = 4.08$, $P = 0.05$), but no differences were detected among reproductive forms in kick seining collections ($X^2_2 = 0.01$, $P = 0.91$).

*Fish family and species comparisons*

Percent relative abundance did not vary between kick seining and electrofishing for most fish groupings in the Bear Creek or Cahaba River drainage. In both drainages, Centrachid PRAs were higher when electrofishing than kick seining (Bear: $Z = 2.4$, $P = 0.01$; Cahaba: $Z = 3.3$, $P < 0.01$; Fig. 2). In the Cahaba River Drainage, Cyprinid PRAs were higher when kick seining than electrofishing ($Z = -2.9$, $P < 0.01$; Fig. 2).

Among the 88 fish species collected, 10 were identified as significant indicators (Table 4). Nine species (7 pelagic; 2 benthic) were indicative of electrofishing collections. One benthic species was indicative of kick seining collections (Table 4). Relative abundance was more important than relative frequency (fidelity > 50%; Table 4) in driving the indicator values of all but three species.

Environmental effects

Kick seining and electrofishing effectiveness varied by stream for *F. erichsonianus* CPUE and fish richness, indicating that stream environmental factors impacted the effectiveness of sampling methods. The CPUE of *F. erichsonianus* was higher when kick seining in sites with greater percentages of aquatic vegetation and smaller particle sizes, as well as when electrofishing in cooler streams (Table 5). Fixed effects explained

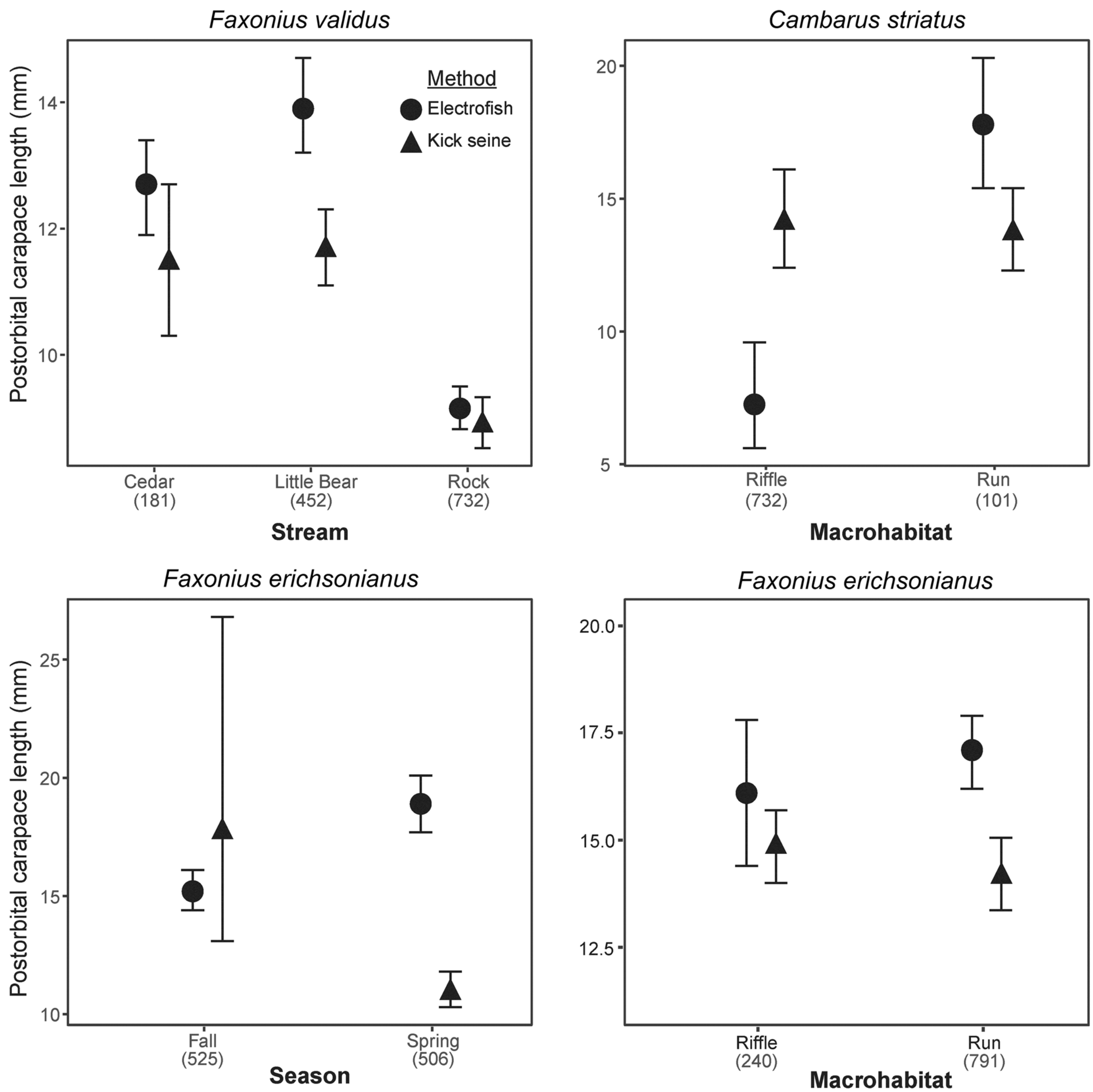



**Fig. 4** Sampling method comparisons of crayfish postorbital carapace lengths (± 95% CI) among streams (*N*; number of individuals), macrohabitats (*N*), and seasons (*N*). $Log_e$ transformed data were used in analyses. Only relationships with significant interactions in linear mixes-effect models are displayed

4–11% of the variation in the dependent variable, indicating that other unmeasured variables may be important in the effectiveness of each sampling method. Fish species richness was positively correlated with water temperature and negatively correlated with particle size for both sampling methods (Table 5). When electrofishing, fish species richness was higher in streams with higher width-to-depth ratios and lower conductivities, but when kick seining, richness was higher in sites with lower DO and SWD (Table 5).

## Sampling effort adequacy

We collected 86–100% of crayfish species estimated by the Chao-1 method in each stream (Fig. 5). For most streams, confidence intervals on species richness

**Table 4** Specificity (%), fidelity (%), and indicator values (IndVal; %) for fish species identified as indicators of electrofishing and kick seining collections

| | Specificity | Fidelity | IndVal |
|---|---|---|---|
| Electrofishing | | | |
| *Lepomis megalotis* (Rafinesque, 1820) | 78 | 77 | 78** |
| *Lepomis macrochirus* Rafinesque, 1810 | 87 | 66 | 76** |
| *Lepomis cyanellus* Rafinesque, 1819 | 83 | 58 | 69** |
| *Ambloplites rupestris* (Rafinesque, 1817) | 98 | 38 | 61** |
| *Percina caprodes* (Rafinesque, 1818)† | 78 | 29 | 48** |
| *Micropterus punctulatus* (Rafinesque, 1819) | 81 | 24 | 45** |
| *Lepomis miniatus* (Jordan, 1877) | 86 | 22 | 43** |
| *Ameiurus natalis* (Lesueur, 1819)† | 85 | 14 | 34** |
| *Lepomis microlophus* (Günther, 1859) | 100 | 6 | 25* |
| Kick seining | | | |
| *Etheostoma stigmaeum* (Jordan, 1877)† | 90 | 15 | 36** |

Higher IndVal indicates a stronger indicator species. † indicates benthic species; * = $P$ values < 0.01; ** = $P$ values < 0.001

accumulation curves overlapped for electrofishing, kick seining, and the two sampling methods combined, indicating similarity between sampling methods. Nest traps collected significantly fewer crayfishes per stream (20–67% of species) than other sampling methods (Fig. 5). For Rock and Cedar creeks, estimated species richness was higher when using combined sampling methods. Additionally, species richness accumulation curves for kick seining alone and combined sampling methods rose more steeply or started with a higher richness than those for electrofishing and nest trapping in all streams (Fig. 5). To collect the maximum species estimated, combined sampling methods required sampling 1.5–5.1 rkm (3–6% of total stream length) and 31–68% fewer sites (6–15 sites) than electrofishing or kick seining alone.

We collected 68–92% of fish species estimated by the Chao-1 method (Fig. 6). Species richness accumulation curves were not distinguishable between electrofishing and a combination of kick seining and electrofishing for all streams (Fig. 6). Kick seining collected significantly fewer fish species than electrofishing in all streams except Rock Creek and Little Cahaba River. Electrofishing was the most effective single sampling method at capturing all species in all streams except Rock Creek, requiring 14–61 sites (2.6–10 rkm [sampling 4–13% of the stream]) to capture 100% of species. Kick seining was the most effective single sampling method in Rock Creek, requiring 38 sites (4.4 rkm [sampling 14% of the stream]) to capture 100% of species.

## Discussion

Sampling methods should be selected based on study objectives, targeted faunal groups, and effectiveness of sampling methods in habitat types sampled (Bonar et al., 2009; Parkyn, 2015). Characterization of crayfish and fish communities differed among kick seining, electrofishing, and nest trapping collections, in rocky, southern Appalachian Mountain streams. As found in previous studies, combining sampling methods provided a better overall representation of the crayfish and fish populations in the Bear Creek and Cahaba River drainages relative to using a method individually (Onorato et al., 1998; Barnett & Adams, 2018; Budnick et al., 2018). The current and previous studies also show that combining sampling methods offset sampling biases (e.g., sex, habitat, size), providing more accurate, robust data (Onorato et al., 1998; Barnett & Adams, 2018; Budnick et al., 2018). Additionally, conducting one survey for both crayfish and fish reduced the effort, time, cost, and stream disturbance associated with conducting multiple surveys.

Kick seining is commonly used for characterizing the relative abundance of crayfishes occupying shallow streams (Williams et al., 2014) as well as small benthic (Weddle & Kessler, 1993) and open water column fishes (Tiemann & Tiemann, 2004), such as Percids and schooling Cyprinids, respectively. In our study, kick seining consistently collected the most crayfishes and was the most effective single sampling method for documenting crayfish species richness. In contrast, in South Carolina, electrofishing was more

**Table 5** Results from linear mixed-effect repeated-measure models within 2 $AIC_C$ units of the best model

| Model | M-$R^2$ | C-$R^2$ | *N* | Estimate | SE | RVI | Model | M-$R^2$ | C-$R^2$ | *N* | Estimate | SE | RVI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *F. erichsonianus* | | | | | | | *F. erichsonianus* | | | | | | |
| Kick seining CPUE | 0.11 | 0.53 | 40 | | | | Electrofishing CPUE | 0.04 | 0.28 | 15 | | | |
| D50 | | | | − 0.193 | 0.106 | 0.70 | Water temp (°C) | | | | − 0.391 | 0.188 | 0.89 |
| Aquatic vegetation (%) | | | | 0.256 | 0.135 | 0.70 | D84 | | | | | | 0.41 |
| Water temp (°C) | | | | | | 0.49 | LWD | | | | | | 0.20 |
| SWD | | | | | | 0.36 | pH | | | | | | 0.19 |
| D84 | | | | | | 0.18 | Width to depth ratio | | | | | | 0.12 |
| Conductivity (μS/cm | | | | | | 0.10 | Canopy cover (%) | | | | | | 0.10 |
| D16 | | | | | | 0.08 | D50 | | | | | | 0.05 |
| pH | | | | | | 0.06 | SWD | | | | | | |
| Canopy cover (%) | | | | | | 0.04 | | | | | | | |
| Width to depth ratio | | | | | | 0.02 | | | | | | | |
| LWD | | | | | | 0.02 | | | | | | | |
| Fish | | | | | | | Fish | | | | | | |
| Kick seining richness | 0.30 | 0.57 | 21 | | | | Electrofishing richness | 0.21 | 0.62 | 11 | | | |
| DO | | | | − 0.705 | 0.270 | 1.00 | D50 | | | | − 0.096 | 0.034 | 1.00 |
| SWD | | | | − 0.521 | 0.145 | 1.00 | Water temp (°C) | | | | 0.557 | 0.223 | 1.00 |
| Water temp (°C) | | | | 0.843 | 0.289 | 1.00 | Width to depth ratio | | | | 0.472 | 0.204 | 1.00 |
| D50 | | | | − 0.136 | 0.062 | 0.97 | Conductivity (μS/cm) | | | | − 1.137 | 0.662 | 0.58 |
| D84 | | | | − 0.109 | 0.054 | 0.80 | SWD | | | | | | 0.49 |
| pH | | | | | | 0.46 | pH | | | | | | 0.22 |
| LWD | | | | | | 0.28 | LWD | | | | | | 0.14 |
| Conductivity (μS/cm) | | | | | | 0.23 | Canopy cover (%) | | | | | | 0.08 |
| Canopy cover (%) | | | | | | 0.20 | Aquatic vegetation (%) | | | | | | 0.07 |
| Riffles (%) | | | | | | 0.16 | | | | | | | |
| D16 | | | | | | 0.03 | | | | | | | |

Models show estimates for stream environmental variables that best explain sampling method effectiveness for collecting *F. erichsonianus* CPUE and total fish richness across all sites and drainages (negative estimates indicate a negative correlation with method effectiveness). Only estimates for important variables (relative variable importance [RVI] > 0.50), averaged across models, are displayed

*M-$R^2$* marginal $R^2$ of important variables, *C-$R^2$* conditional $R^2$ of important variables, *N* number of models within 2 $AIC_C$ units of the best model, *SE* standard error, *RVI* relative variable importance (parameters with RVI of 1.00 were included in all of the best models), *DO* dissolved oxygen (mg/l), *D16* size (mm) that 16% of particles were smaller than, *D50* median particle size (mm), *D84* size (mm) that 84% of particles were smaller than, *SWD* small woody debris (%), *LWD* large woody debris (number of pieces)

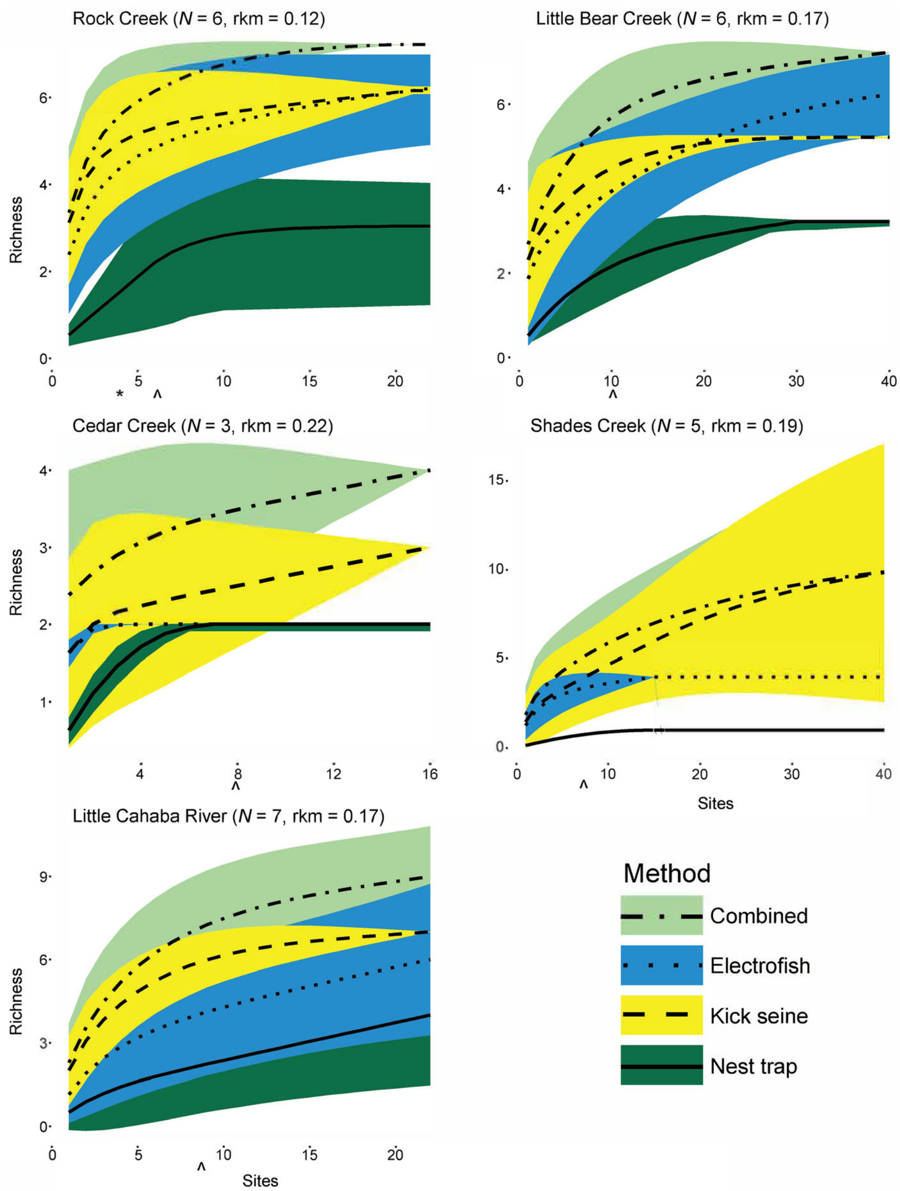



**Fig. 5** Species accumulation curves (Chao, 1984) calculated to assess sampling effort (number of sites) needed to accurately estimate crayfish richness within streams. Colored polygons represent 95% confidence intervals. With combined gears (kick seining and electrofishing together), we sampled double the area and expended twice the effort relative to a single gear used at each site. *N* = number of species collected in each stream; rkm = average site length (km) for single sampling method; carets indicate number of sites sampled during the study; asterisks indicate number of sites sampled during first sampling round (if different from other rounds)

effective than drag seining at collecting the most crayfishes and documenting crayfish species richness (5 species; Price & Welch, 2009). The target crayfish species in South Carolina (*Procambarus* spp. and *Fallicambarus* sp.; Price & Welch, 2009) differed from the species in Alabama streams (*Faxonius* spp. and *Cambarus* spp.). Species differences may lead to behavioral and habitat use differences and increased susceptibility to electrofishing or seining. Different seining techniques also impact crayfish collections, with small, bottom-dwelling species more susceptible to kick seining than drag seining (Pflieger et al., 1982).

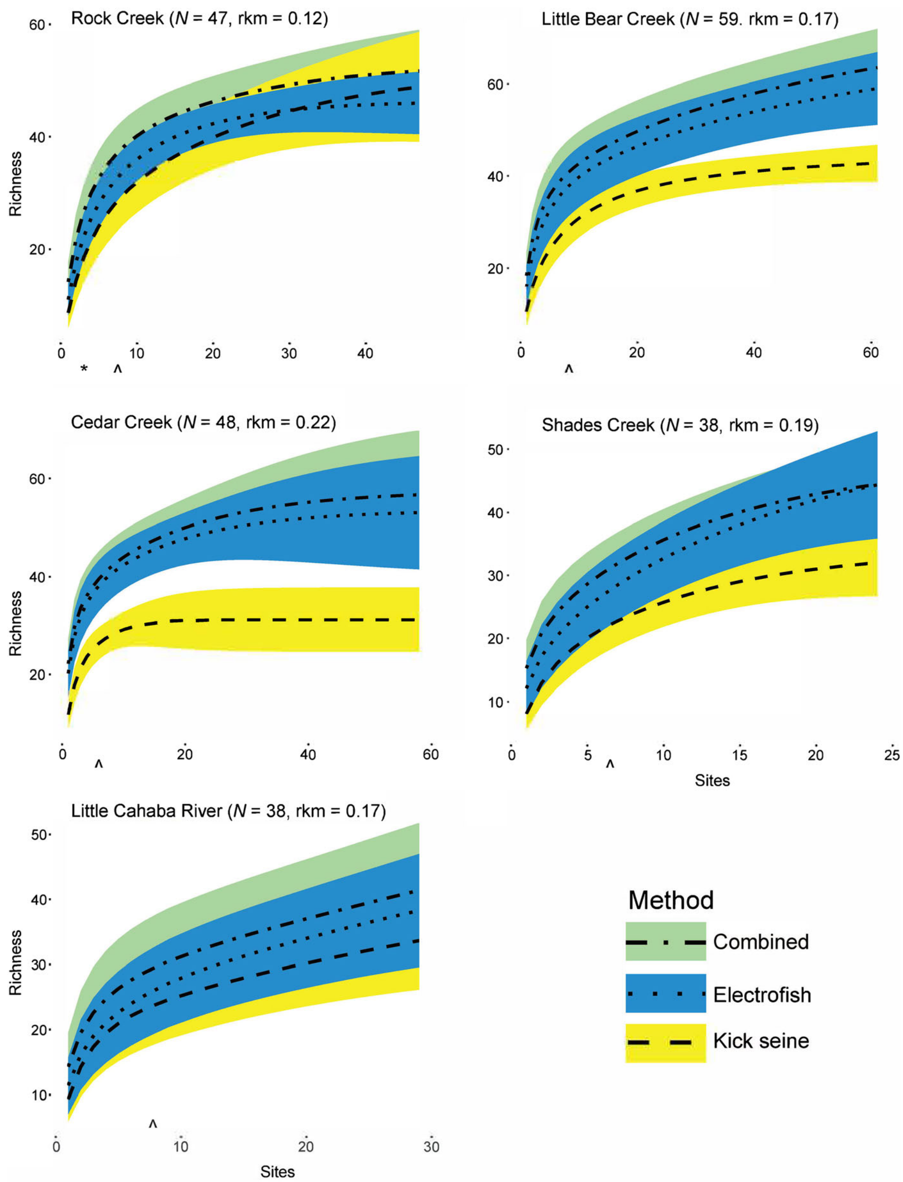



**Fig. 6** Species accumulation curves (Chao, 1984), calculated to assess sampling effort needed to accurately estimate fish richness within streams. Colored polygons represent 95% confidence intervals. With combined gear use (kick seining and electrofishing together), we sampled double the area and expended twice the effort relative to single gear use at each site. Abbreviations as in Fig. 5

Similarly, in the current study, small and bottom-dwelling fishes were more susceptible to kick seining than electrofishing. For instance, a Percidae species, *Etheostoma stigmaeum* (Jordan, 1877), was the indicator species for kick seining collections and Cyprinid PRAs were greater in kick seining than electrofishing collections in the Cahaba River Drainage. Conversely, in Washington (Dauble & Gray, 1980), more individuals and species of Cyprinids were collected by electrofishing than drag seining. The small sizes of the Cyprinids in Alabama streams and their preference for open water habitat makes them less susceptible to electrofishing than the larger individuals collected in Washington (Onorato et al., 1998; Reynolds and Koltz, 2013). Smaller fishes are harder to see and more likely to be overlooked when electrofishing than larger fishes (Reynolds & Koltz, 2013). These differences demonstrate how sampling method efficiency can vary when sampling different species in different physiographic regions.

While electrofishing is effective at collecting crayfishes in some physiographic regions (Price & Welch, 2009; Budnick et al., 2018), most crayfish species in southern Appalachian Mountain streams were less susceptible to electrofishing than kick seining. Electrofishing collected only the two most dominant species in 72% of collections where crayfishes were present. The relative ineffectiveness of electrofishing in the current study may be largely associated with the larger, cobble substrate in southern Appalachian mountain streams compared to smaller, silty/clay substrate in Coastal Plain streams previously studied (Zhao et al., 2006; Wohl et al., 2011; Price & Welch, 2009; Budnick et al., 2018). Cobble substrate makes netting stunned crayfishes harder because crevices create more opportunities for individuals to lodge under substrate and escape capture. Complex habitats (e.g., woody debris, aquatic vegetation), as seen in our study sites, also created conditions where some crayfish avoided capture due to complex cover. Increasing electrofishing passes may increases crayfish chances of being dislodged from cover and subsequently collected (Alonso, 2001; Barnett et al., 2020). Barnett et al. (2020) found that in southern Appalachian mountain streams, crayfish species richness increased during a second electrofishing pass, indicating that more than one electrofishing pass may be necessary to accurately assess richness. Thus, increasing the number of electrofishing passes conducted in the current study may have led to similar crayfish richness results between electrofishing and kick seining collections.

High electrical conductivities ($\bar{X}$ = 246 µS/cm) in our study may have negatively impacted the effectiveness of electrofishing. Electrofishing was more effective in Coastal Plain streams than in our study streams (Price & Welch, 2009; Budnick et al., 2018). Conductivities are commonly higher in southern Appalachian mountain versus Coastal Plain streams (Griffith, 2014). Because very low and very high conductivities can decrease electrofishing efficiency, future studies assessing the impacts of conductivity on electrofishing efficiency when collecting crayfishes are needed.

For fishes, electrofishing was the most effective method at assessing species richness and CPUEs. Fish species composition plays an important role in the effectiveness of sampling methods, with larger-bodied pelagic fishes more susceptible to electrofishing than kick seining (Matthews & Marsh-Matthews, 2017). A higher PRA of centrarchids was collected by electrofishing, and most electrofishing indicator species were centrarchids. Centrarchids were less susceptible to kick seining because they occupied areas with larger rocks that make kicking more difficult. Centrarchids are crayfish predators that influence stream crayfish behavior and habitat choices (Collar & Wainwright, 2009), and crayfishes, in turn, prey on Centrarchids eggs (Reynolds, 2011). Therefore, underrepresentation of Centrarchids by kick seining could lead to incorrect estimations of fish community structure and inhibit understanding of ways that predator–prey interactions structure crayfish communities. Conversely, electrofishing was not as effective at collecting Cyprinids in the Cahaba River drainage. Although this was the only family more susceptible to kick seining, Cyprinids are numerically dominant in the Cahaba River drainage (Shepard et al., 1994; Onorato et al., 1998). Several Cyprinids were endemic to the Cahaba River Drainage (e.g., *Cyprinella callistia* (Jordan, 1877), *Phenoacobius catostomus* Jordan, 1877*)* and served as indicator species of healthy streams (e.g., *C. trichroistia* (Jordan and Gilbert, 1878), *Notropis volucellus* (Cope, 1865)) because of their sensitivity to degradation of water and habitat quality (Pierson et al., 1989). Additionally, some Cyprinids and crayfishes compete for aquatic invertebrates and small fish prey (Wood et al., 2017). To maximize the probability of collecting Cyprinids and other benthic fishes that are less susceptible to electrofishing and give a better understanding of stream community structure, kick seining and electrofishing should be used when sampling fishes.

Neither crayfish nor fish populations were accurately represented by nest trap collections. Nest traps in this study were not as efficient as nest traps in Coastal Plain Georgia streams (Bechler et al., 2014). Occupancy rates in the traps were four times lower in our study than in Georgia streams. The difference between nest trap efficiencies in the current and previous study may be due to differences in habitat complexity and macrohabitat types. Whereas we deployed traps in pools with high habitat complexity, Bechler et al. (2014) installed them in runs with low habitat complexity. Thus, crayfishes in the Georgia sites had less available shelter from predators, potentially making nest traps more desirable as refugia. Furthermore, sampling in streams with high habitat heterogeneity likely decreased our nest trap sampling efficiency. In the current study, nest traps sampled pools, and electrofishing and kick seining sampled riffles and runs, yet species collected in nest traps were also collected by kick seining and electrofishing. As in other nest trap studies (Bechler et al., 1990, 2014), small cavity-spawners dominated the fishes caught in nest traps, and the most abundant crayfish species in nest traps represented the most abundant crayfishes within the stream system. Unlike our results, nest traps in Georgia streams collected multiple crayfish per trap, as well as fishes and crayfishes simultaneously in traps (Bechler et al., 2014). Because crayfishes and cavity spawning fishes (e.g., Ictalurids) prey on other crayfishes and fishes, these predator–prey relationships may have decreased the number of individuals in traps when collected. Macrohabitats are often partitioned among crayfish and fish species and size classes (DiStefano et al., 2003), thus collection differences may represent differences in macrohabitats use by crayfishes and fishes, with runs sampled in Georgia streams (Bechler et al., 2014) and pools sampled in the current study. Future studies using sampling methods adequate for all macrohabitat types are needed to assess differences in crayfish macrohabitat use and to compare nest trap efficiency in all macrohabitat types.

Combining kick seining and electrofishing decreased the number of sites needed to assess crayfish species richness. While neither method alone collected all species present within all sampled streams, using both methods together increased the number of species collected and decreased the number of sites needed to accurately assess stream's crayfish richness. Unlike other studies (Pflieger, 1996; Engelbert et al., 2016), actual crayfish richness in each stream was unknown in our study. However, Chao-1 estimates were used to estimate crayfish richness. Sampling effort needed to assess crayfish species richness has been evaluated for kick seining (Engelbert et al., 2016) and electrofishing (Budnick et al., 2018), but not for the two methods combined. Among the individual sampling methods, kick seining required the fewest sites to assess crayfish species richness in our study. In Missouri streams, the precision of kick seining was comparable to that of quadrat sampling (Williams et al., 2014) and provided a repeatable and statistically supported tool for assessing stream crayfish species richness (Engelbert et al., 2016). Species accumulation analyses from kick seining in Missouri streams suggests that sampling fewer sites with greater site lengths (rkm) detects a lower percentage of a drainage's species richness (Engelbert et al., 2016). Conversely, we found that when combining electrofishing and kick seining to assess species richness, sampling fewer sites with greater site lengths increased our ability to accurately assess species richness. Similarly, in Louisiana streams, Budnick et al. (2018) inferred that combining electrofishing and dip netting would reduce site lengths needed to assess species richness. Using more than one sampling method offsets biases associated with each method and increases the ability to detect crayfishes in structurally complex stream habitats.

For numerous crayfish assemblage characteristics (e.g., CPUE, size, and age classes), kick seining and electrofishing collections were comparable. Similarly, Price & Welch (2009) collected similarly sized crayfishes by electrofishing and kick seining in South Carolina. Although collections of small crayfishes often vary by sampling methods (Parkyn et al., 2011; Barnett & Adams, 2018), in the current study both sampling methods collected and documented growth of age-0 crayfishes. The present study is the first comparison of kick seining versus electrofishing assessment of seasonal changes in age-0 crayfishes. Because temporal changes of age-0 crayfishes' abundance and growth should be expected (Brewer et al., 2009), understanding the effectiveness of sampling methods across seasons is essential.

Biased crayfish sex ratios have been reported for all three sampling methods (Alonso, 2001; Price & Welch, 2009; Hightower & Bechler, 2013; Bechler et al., 2014; Reid & Devlin, 2014). Nonetheless, biased sex ratios do not necessarily indicate biased sampling methods (Barnett & Adams, 2018). In the current study, females dominated collections of all sampling methods, with reproductive form ratio differences between sampling methods detected for only one species. Thus, sex ratios may not have been 1:1 for most species. This also indicates that sampling methods were likely not biased towards any reproductive form.

In the Cahaba River drainage, *F. virilis*, an introduced species, was the most abundant and widespread crayfish. Its native range is largely confined to the upper Mississippi River and Great Lakes drainages (Hobbs, 1959; Schwartz et al., 1963; Hamr, 2002). *Faxonius virilis* was the only species we collected for which sex ratios and age class estimates differed between electrofishing and kick seining collections. Age-class and sex ratio estimates from kick seining collections were similar to other reports for this species, with *F. virilis* living 2–3 years in equal or male-dominated populations (Momot, 1967; Momot & Gowing, 1972). Furthermore, because kick seining results were most similar to previous *F. virilis* population studies, kick seining was the less biased sampling method for monitoring *F. virilis*. If managers are only interested in the presence or absence of this species in a stream system, nest traps would provide a less labor-intensive sampling method, as 80% of individuals collected from nest traps in the Cahaba River drainage were *F. virilis*, and nest traps collected *F. virilis* in 71% of sites where they occurred.

The heterogeneity of macrohabitats found in wadeable streams throughout a drainage can be highly variable and create challenges when employing one sampling method to assess a stream system. For instance, because kick seining and backpack electrofishing are more effective in shallow-water habitats, we limited our collections to shallow-water species. Additionally, habitat complexity decreased the effectiveness of our sampling methods, with *Faxonius erichsonianus* CPUE higher when kick seining at sites with smaller substrate sizes, and fish richness higher when kick seining and electrofishing at sites with smaller substrate sizes. Cobbles provide more interstitial space for crayfishes and fishes than does very small substrate. However, when kick seining, large cobbles can be difficult to move and kick through, and when electrofishing, stunned animals can get stuck under large cobble and go undetected. The median substrate size in this study was large cobble (115 mm), and sampling methods were more efficient in habitats with smaller substrates, indicating a possible nonlinear relationship, with very small and very large substrate yielding a lower abundance of crayfishes and fewer fish species. Sampled fish richness was also higher when electrofishing at sites with lower conductivities (study conductivity range 46–538 μS/cm; Table 2). Although electrical currents are more readily transmitted in waters with medium to high conductivities (> 150 μS/cm), higher watts (i.e. > 500 w; more than the maximum output of our backpack electrofisher) are needed to stun fishes, indicating a nonlinear relationship, with electrofishing ineffective in streams with very high and very low conductivities (< 20 μS/cm) (SFCC, 2007; Allard et al., 2014).

Species distributions are shaped by numerous biotic and abiotic factors including stream size, substrate composition, and habitat along a stream's length. To help ensure accurate assessments of species richness and prevent oversampling, estimations of stream sampling length (reach length) and number of sampling sites from this study can be used on similar stream types within the region. Because sampling methods most efficient at collecting crayfish and fish species richness differed and certain species were more susceptible to one method than another, a combination of kick seining and electrofishing sampling methods are recommended for accurate sampling of both taxa. Sampling both taxa simultaneously decreases the time needed to conduct separate surveys. However, simultaneously sampling for both taxa when electrofishing can be more difficult than sampling for one taxon, due to differences in taxa responses. Unlike most fishes that float to the water's surface when exposed to the electrofisher's electrical field, crayfishes often move erratically through the water column to escape the electrical field or remain stunned at the bottom of the stream (Westman et al., 1978; Burba, 1993). Thus, collectors need to focus on numerous parts of the water column to ensure collection of both taxa, which could possibly reduce collection efficiency for both taxa. Electrofishing efficiency may increase with multiple electrofishing passes (Rabeni et al., 1997; Shank et al., 2016; Barnett et al., 2020); thus, future studies should assess the effectiveness of multi-pass electrofishing collections of crayfishes and fishes simultaneously. Additionally, because both taxa use similar habitats and both sampling methods disturb these habitats, conducting separate surveys within the same sites could create biased samples and lead to inaccurate conclusions. Likewise, using only one sampling method to sample both taxa will likely also lead to biased samples for at least one of the taxa.

While kick seining and electrofishing were more effective than nest trapping at collecting crayfishes and fishes, they both come with drawbacks. Kick seining in rocky, highly vegetated streams is time- and energy-intensive, and fewer sites can be sampled per time when kick seining (average = 2 sites/day; ≤ 100 kick seines/day) than electrofishing (average = 3.5 sites/day; shocking ≤ 3300 s/day). Electrofishing also sampled more of each subreach ($\bar{X}$ = 70%) than kick seining ($\bar{X}$ = 15%) in the same or less time. Kick seining and electrofishing cause more harm to organisms than nest trapping due to greatly disturbing habitats and possibly crushing organisms when kick seining (Larson & Olden, 2016), as well as loss of crayfish chelae, and broken spines and bruising of fishes when electrofishing (Westman et al., 1978; Alonso, 2001; Snyder, 2003; Miranda & Kidwell, 2010). We did not record such injuries but sometimes observed them during sampling.

To increase the efficiency of nest trapping, traps should be deployed for less than 12 months. We documented a 50% decrease in trap recovery when traps were deployed for more than 12 months. Lower recovery rates may be due to displacement of traps during heavy winter and early spring rains. If so, removal and resetting of traps between fall and spring sampling may increase the number of traps recovered.

In conclusion, a priori consideration of the relative effectiveness, costs, and benefits of different sampling methods is key to the success of a stream faunal survey. Commonly used methods for sampling crayfish and fish vary in their collection efficiency depending on habitat type and target species. For example, when sampling for crayfishes, kick seining may be more effective in streams with aquatic vegetation, while electrofishing may be preferable in streams with small substrates (e.g., sand, pebbles). Additionally, when sampling for fishes, kick seining may be more effective when targeting small-bodied, benthic fishes, while electrofishing may be preferable when targeting large bodied, pelagic fishes. In our study area, while electrofishing alone was well-suited to assessing fish species richness, kick seining and electrofishing, used in tandem, was superior for simultaneous determination of stream crayfish species richness and fish community structure. Using methods in tandem to assess fish and crayfish populations offsets biases associated with individual methods, reduces sampling effort, and contributes to a better understanding of stream organism composition.

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**Data availability** The data generated and analyzed during the current study are available from the corresponding author upon request.

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