Targeted microhabitat sampling and its role in conserving endemic freshwater taxa

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Abstract: North America harbors a rich freshwater fauna, but many of its taxa are narrowly-distributed and threatened with future range reductions from invasive species, habitat alterations, and water quality degradation. The importance of conserving narrowly-distributed freshwater species is well documented, but mechanisms responsible for their distributions are often poorly understood. Developing approaches to better address these species and their unique habitat requirements is critical for providing adequate conservation. We demonstrate the value of fine-scale sampling for narrowly-distributed taxa through a case study of the Coldwater Crayfish (\textit{Faxonius eupunctus}), a species endemic to the upper Eleven Point River drainage in Arkansas and Missouri, USA. We targeted the upper Eleven Point River drainage for sampling based on results from previously-constructed classification and regression tree species presence models. We estimated \textit{F. eupunctus} presence and density in shallow, wadeable habitats where lotic crayfishes are often surveyed (i.e., riffles), and we developed a novel approach to estimate \textit{F. eupunctus} presence and density in deeper, non-wadeable habitats (i.e., pools) where traditional crayfish sampling techniques can be difficult and have rarely been used. We quantified relationships between local \textit{F. eupunctus} density and surrounding physical microhabitat features in both pools and riffles to provide insights on the species’ habitat use and potential for future conservation. Our targeted sampling approach allowed us to detect a previously-undocumented spatial pattern in \textit{F. eupunctus} site density throughout the upper Eleven Point River drainage, and we identified important microhabitat variables for \textit{F. eupunctus} in both habitat types sampled. Our study highlights the benefits of fine-scale sampling in multiple habitat types for conserving narrowly-endemic species, and our methods provide a flexible framework for studying other benthic taxa at fine spatial scales.

Key words: density, Coldwater Crayfish, \textit{Faxonius eupunctus}, habitat use, pool sampling

North America harbors a rich freshwater fauna, containing more than 6\% of fishes (Page and Burr 2011, Brosse et al. 2013), 35\% of mussels (Graf and Cummings 2007, Williams et al. 2017), and 67\% of crayfishes (Richman et al. 2015, Crandall and De Grave 2017) currently described globally. Many of these organisms are rare and exhibit crypticism, narrow ranges, or low population densities (Taylor et al. 2007, Jelks et al. 2008, Johnson et al. 2013). Consequently, many of these species are understudied and lack the ecological information needed for adequate conservation (Lysne et al. 2008, Helms et al. 2013).

The importance of conserving narrowly-distributed species is well documented (Caldecott et al. 1996, Harvey et al. 2011), but mechanisms responsible for their distributions are often poorly understood (Kessler and Kluge 2008, Pearson and Raxworthy 2009, Gifford and Kozak 2012). Mechanisms influencing endemism patterns are inherently complex because they are hierarchical and can be influenced by ecological (e.g., competitive exclusion, habitat/resource specificity; see Wijesinghe and Brooke 2004), evolutionary (e.g., species divergence, dispersal from biogeographic centers, or a combination of both; see Crandall and Buhay 2008),
and statistical (e.g., variation in sampling effort across a region or among taxa; see Beck et al. 2007) elements. Research often addresses ecological mechanisms of endemism (Yu and Peters 2002, Purcell et al. 2014, Sheldon and Grubbs 2014) because some of these mechanisms, such as habitat or resource specificity, can be investigated through observational field studies. However, observational scale can affect study outcomes, potentially leading to inaccurate or general conclusions about habitat or resource specificity.

Narrowly-distributed species often exhibit greater habitat specificity than those more broadly distributed (Daniels 1993, Osier and Welsh 2007, Stites et al. 2017). Coarse-scale environmental features (e.g., catchment or reach scale), such as land cover, geology, or stream order, can be useful for explaining habitat specificity (Westhoff 2011, Dyer et al. 2013, Nolen et al. 2014), but investigating coarse features alone may fail to reveal key environmental features for species with small ranges. Coarse-scale environmental features can be relatively homogenous over small geographic areas (e.g., a single stream or catchment), so it can be difficult to identify relationships between coarse-scale variables and organism abundance or presence within these areas. In contrast, fine-scale environmental features (e.g., microhabitat features, such as current velocity or streambed substrate composition) can exhibit greater heterogeneity within small geographic areas (Kolasa 1989, Levin 1992, Pickett and Cadenasso 1995) and may have greater potential to explain fine-scale variations in organism abundance or presence. Fine-scale features can be of central importance to species whose narrow distributions are at least partially related to the presence or intensity of 1 or more microhabitat characteristics (Midway et al. 2010, Rhoden et al. 2017), so studies focusing on endemic organisms should routinely measure and include microhabitat variables in analyses.

Freshwater crayfishes are known for their endemic nature (Taylor et al. 2007). More than 400 of the 669 crayfish species currently described globally are endemic to North America (Richman et al. 2015, Crandall and De Grave 2017). The United States of America (USA) east of the Rocky Mountains harbors more than 99% of North American crayfish species, and the southeast USA (including Arkansas and Missouri) is a known center of diversity and endemism for crayfishes (Taylor et al. 2007, Crandall and Buhay 2008). Accordingly, it has been estimated that approximately 48% of crayfishes in the USA and Canada warrant some form of conservation attention (Taylor et al. 2007), yet federal protection has been assigned to only 6 crayfish species in the USA. This discrepancy may be related to the large number of North American crayfishes currently lacking the ecological information required for reaching informed conservation and management decisions. For example, approximately 21% of the world’s crayfishes were assessed as data deficient (Richman et al. 2015), and it was estimated that more than 60% in the USA and Canada lack basic distributional or ecological data (Taylor et al. 2007, Moore et al. 2013).

The Coldwater Crayfish (Faxonius eupunctus, Williams 1952) is among the most narrowly-distributed crayfishes in the USA and is known only from the Eleven Point River (EPR) drainage in north-central Arkansas and south-central Missouri. (Fig. 1). Recent studies revealing cryptic endemism led to taxonomic revision that split F. eupunctus into 3 species, including F. eupunctus in the upper EPR drainage, the Eleven Point River Crayfish (F. wagneri, Fetznner and Taylor 2018) in the lower EPR drainage, and the Spring River Crayfish (F. roberti, Fetznner and Taylor 2018) in the nearby drainages of the Spring and Strawberry rivers. Previous studies of F. eupunctus used broad-scale sampling approaches across the range of the species complex and consequently may not have characterized the species’ habitat associations accurately (Pflieger 1996, Nolen et al. 2014, Magoulick et al. 2017). Other studies focused exclusively on lineages now known to constitute F. roberti (Flinders and Magoulick 2005, Larson and Magoulick 2008, Larson et al. 2009), so habitat requirements described for F. eupunctus reflect those of multiple species (Pflieger 1996, Nolen et al. 2014, Fetznner and Taylor 2018). Additionally, invasive Ringed Crayfish (F. neglectus, Faxon 1885), which have been implicated in the displacement and possible extirpation of F. roberti in Missouri (Flinders and Magoulick 2005, Magoulick and DiStefano 2007), have now been documented in 1 upper EPR tributary (Imhoff et al. 2012, DiStefano et al. 2015). In light of these findings, new research is needed to adequately conserve F. eupunctus and its limited habitats. A fine-scale evaluation of the newly-revised taxon’s range and habitat use could provide the spatial resolution needed to better identify abundance patterns and key environmental features relative to previous coarse-scale sampling approaches.

Our goal was to investigate the potential of fine-scale sampling across multiple habitat types for informing the conservation and management of narrowly-endemic crayfishes and other lotic organisms. We used a systematic approach to estimate F. eupunctus presence and density in shallow, wadeable habitats where lotic crayfishes are often surveyed (i.e., riffles), and we developed a novel sampling method to estimate F. eupunctus presence and density in deeper, non-wadeable habitats (i.e., pools) where traditional crayfish sampling techniques (e.g., seining) are often impractical and rarely used. We sought to quantify relationships between local F. eupunctus density and surrounding physical microhabitat features to provide insights relating to the species’ habitat use and potential for future conservation. Additionally, we hoped to provide a flexible and systematic framework for quantitatively sampling crayfishes and other narrowly-distributed lotic taxa at fine spatial scales and in non-wadeable pools of moderately-large streams.

**METHODS**

**Study area**

The EPR drainage covers ~3115 km² in the Ozark Highlands ecoregion (Omernik 1987) in Arkansas and
Missouri. The mainstem EPR is a relatively large 6th-order stream (Strahler 1957) at the confluence with its parent stream, the Spring River. Streams within the EPR drainage are generally characterized by well-developed riffle-pool complexes, cobble- and gravel-dominated streambeds, and abundant karst springs. Many of the EPR’s tributaries are prone to drying because of losing substantial surface flow to the groundwater system (Adamski et al. 1995). Four native lotic-dwelling crayfishes are known to occur in sympatry with *F. eupunctus*: Hubbs’ Crayfish (*Cambarus hubbsi*, Creaser 1931), the Ozark Crayfish (*F. ozarkae*, Williams 1952), the Spothanded Crayfish, (*F. punctimanus*, Creaser 1933), and the Eleven Point River Crayfish (*F. wagneri*). Introduced Ringed Crayfish (*F. neglectus*) have not yet been found in sympathy with *F. eupunctus*, and their distribution within the EPR drainage is currently limited to the upstream reaches of 1 small tributary (Imhoff et al. 2012).

Site selection: Riffles

We targeted the upper EPR drainage for sampling based on the results of Nolen et al. (2014), who used classification and regression tree models to show a high probability of *F. eupunctus* presence in that region. We identified a total of 13 focal streams within the upper EPR drainage based on locations in that region estimated by Nolen et al. (2014) to have >0% probability of harboring *F. eupunctus*. We used satellite imagery (ACME Mapper 2.1; https://mapper.acme.com/) to identify 114 candidate sites within these focal streams. Candidate sites were selected based on the presence of wadeable riffle habitats, which are likely conducive to *F. eupunctus* (Pflieger 1996, Nolen et al. 2014, Magoullick et al. 2017). We required a 5-river-km buffer between sites on tributary stream segments and a 3-river-km buffer between sites on mainstem EPR segments to bolster spatial independence between sampling locations. A smaller buffer was used for mainstem EPR segments because of limited riffle availability in mainstem segments relative to tributary segments. We used a random number generator to randomly select the maximum number of sites within each stream that would satisfy the spatial buffer criterion. These methods resulted in the selection of 54 sites within 2nd- to 6th-order stream segments (Fig. 1).

Site selection: Pools

Candidate pool sites were identified based on sites selected for riffle sampling. These sites were required to: 1) be located on the mainstem EPR, 2) be adjacent to a riffle that had at least 1 positive *F. eupunctus* detection during riffle
sampling, and 3) contain potentially non-wadeable pools visible from satellite imagery. We defined non-wadeable pools as those having reduced current velocities with little to no broken surface tension and depths too great (>1.5 m) to effectively collect crayfishes with a seine. These criteria were verified with on-site observations during riffle sampling and resulted in a total of 15 pools suitable for sampling. The portion of the mainstem EPR containing candidate pools was divided into 4 sampling reaches of similar length to prevent spatial clustering of sampling locations (Fig. 1). We randomly selected 2 reaches in which to sample 2 sites and sampled only 1 site in each of the remaining 2 reaches to accommodate time constraints. We randomly selected 1 site from each reach to serve as an alternate sampling location if a priority site was determined to be unsuitable for sampling upon arrival to that location. These methods resulted in the selection of 6 priority pools and 4 alternate pools (Fig. 1). We surveyed 2 additional non-randomly selected sites because of convenient access. Data collected from these sites were not included in statistical analyses.

**Sampling methods: Riffles**

Riffle sampling was conducted between 6 June and 2 August 2016. Site length was delineated by measuring wetted stream width at the most upstream run habitat and multiplying that value by 10 (adapted from Barbour et al. 1999). We chose to establish site length by measuring runs because riffles were often narrow and contained multiple channels, making a single wetted width measurement difficult to obtain. Runs were defined as areas having elevated water surface slope, visible surface flow, and reduced breaks in water surface tension relative to surrounding riffles (Jowett 1993). A maximum site length of 125 m was established to limit time spent at each survey site.

We used a quantitative kicknet method (Engelbert et al. 2016) to collect crayfish density (ind/m²) and habitat data from riffles at each site. Each subsample was taken within a 1-m² polyvinylchloride (PVC) quadrat frame constructed from 2.54-cm diameter PVC piping. Subsample locations were systematically placed on the streamed in 1-m increments moving from downstream to upstream on an alternating diagonal pattern (Fig. 2A, B) until all 12 subsamples were completed at each site. The number of subsamples taken in each riffle was allocated proportionally to riffle length when more than 1 riffle was present within a site. We used 0.5-m spacing between subsamples at sites with <10-m wetted stream width to collect all 12 subsamples within the length of the site.

We collected the following habitat data within each subsample location prior to collecting crayfishes: canopy closure (%), current velocity (m/s), depth (cm), and streambed substrate size. Canopy closure was measured using a Spherical Crown Densiometer (Convex Model A; Forestry Supplies, Jackson, Missouri). Current velocity was measured according to the United States Geological Survey’s six-tents-depth method (Turnipseed and Sauer 2010), where a flow meter (model FH950; Hach®, Loveland, Colorado) was placed at 60% of the total stream depth from the surface. Depth was measured to the nearest cm with a metal wading rod. Substrate size was evaluated with a 0.5 × 0.5-m metal substrate cross (Litvan et al. 2010), and a substrate classification was made at the center and at each tip of the cross (n = 5 locations) according to a modified Wentworth particle size scale (Bovee and Milhous 1978). Substrate scores were assigned as follows: 1 = sand/silt, 1.5 = bedrock, 2 = gravel, 3 = pebble, 4 = cobble, and 5 = boulder. Substrate classifications were averaged for each subsample location. Riffle width (m) was measured at the midpoint of each riffle using a meter tape. A site temperature reading (°C) was recorded at the most downstream boundary of the most downstream riffle at each site. We placed a YSI Pro 10 temperature probe (Yellow Spring Instruments, Yellow Springs, Ohio) at the water’s surface and allowed temperature readings to stabilize over a 1-min period before recording measurements. We chose not to collect temperature readings at the subsample level because in-stream temperature varied minimally within riffles at a single site. Crayfishes were collected using a 1.5 × 1.2-m mesh seine positioned immediately downstream from the quadrat frame. A technician disturbed substrate particles within the quadrat frame for 30 sec with their feet. The disturbance within the quadrat frame dislodged crayfishes from beneath substrate particles, and streamflow facilitated their movement into the seine.

At each site, we included an additional qualitative sampling component, which was conducted following the completion of quantitative kicknet sampling. Qualitative sampling was used to assess only _F. eupunctus_ presence in wadeable habitats not surveyed during quantitative sampling (i.e.,

![Figure 2. Patterns used for placing 1-m² quadrat subsamples within riffles (A) and non-wadeable pools (B) of the Eleven Point River drainage, Arkansas and Missouri, USA. One hollow square represents 1 subsample.](image-url)
wadeable forewaters, backwaters, and runs), and data collected with this sampling method were not included in statistical analyses. Qualitative sampling consisted of targeted, timed searches with a 3.0 × 1.2-m mesh seine (Engelbert et al. 2016) whereby crayfishes were collected as described for the quantitative sampling component. We targeted areas containing larger substrate particles more conducive to crayfishes and collected as many crayfishes as possible within a 15-min timed-search period. One timed-search period was used at sites <10-m wetted stream width, and 2 timed-search periods were used at sites ≥10-m wetted stream width.

All crayfishes were measured (carapace length, mm), sexed, and identified to species. Crayfishes were released after sampling, except for up to 2 F. eupunctus/site and up to 2 of each sympatric crayfish species/site. Retained specimens were collected in accordance with collection permits issued by the appropriate state government agencies and were accessioned into the Illinois Natural History Survey Crustacean Collection at the University of Illinois at Urbana–Champaign.

Sampling methods: Pools

Pool sampling was conducted between 11 July and 3 August 2017. Each site was delineated by measuring 125 m upstream from the riffle sampled during the previous field season. If pools identified for sampling were found to be unsuitable for sampling upon arrival at the site (e.g., shallower depth, elevated current velocity, and broken surface tension more reflective of runs), we moved the sampling location downstream to the next suitable non-wadeable pool. To maintain similarity to our riffle sampling approach, crayfishes and habitat data were collected from twelve 1-m² PVC quadrat frame subsamples at each site. Modifications to the sampling design were made to account for inherent difficulties associated with sampling deeper, non-wadeable habitats. Subsamples were partitioned evenly among 3 transects, each aligned approximately parallel to the wetted stream channel. One transect was located in the center of the channel, and the other 2 transects were located ~25% of the total wetted width from either margin (Fig. 2A, B). Crayfishes and habitat data were collected from 4 subsample sites along each transect, and subsamples within the same transect were spaced equidistantly in 10-m increments. For each subsample, we positioned an anchored aluminum jon boat containing a surface-air delivery system (model C270X; Brownie’s™ Third Lung, Pompano Beach, Florida) just upstream from the quadrat frame. Two divers descended to the streambed from the stern of the boat, collected habitat data and crayfishes, and returned to the surface. When a subsample was completed, the boat, divers, and all sampling equipment were relocated to the next subsample location by releasing the appropriate amount of anchor line from the boat. Subsamples were completed in the same order at each site, beginning with the most upstream subsample in the left-descending transect and moving downstream until all 4 subsamples within the transect were completed.

The following habitat data were collected within each subsample location: current velocity (m/s), depth (cm), in-stream temperature (°C), and substrate size. Divers measured temperature and substrate size from the center of each quadrat frame. Temperature was collected using a handheld mercury thermometer, and substrate size was evaluated using the same methodology described for riffle sampling. Current velocity and depth were recorded by a technician who remained in the boat. Current velocity was measured using the same methodology described for riffle sampling, except the flow probe was affixed to a 3-m PVC rod marked in 0.1-m increments. The PVC rod was used to estimate the appropriate depth at which to record velocity readings. Depth was measured using a meter tape with a lead weight affixed to the end. The 2 divers began searching for crayfishes after habitat data were collected. The divers slowly removed surface substrate particles with their hands, working from downstream to upstream within the quadrat frame. Divers used their hands to collect crayfishes found under substrate particles or displaced by removing substrate particles. Collected crayfishes were placed in a mesh bag with a drawstring to prevent escape and were brought to the surface for identification. All crayfishes were released after sampling, except for up to 4 F. eupunctus ind/site that were retained for analyses unrelated to the study. Retained specimens were collected and treated as described for riffle sampling.

Statistical analyses

We used generalized linear mixed-effects models to identify microhabitat features strongly associated with F. eupunctus density. Data collected from pools and riffles were independently analyzed. We centered and scaled all predictor variables of interest using a Z-score transformation. Predictor variables of interest for riffles included canopy closure, current velocity, depth, riffle width, substrate particle size, and in-stream temperature. These predictors were included because of their importance to other lotic crayfishes (Westhoff et al. 2006, Loughman et al. 2016, Noble and Fulton 2016) and their suspected importance to F. eupunctus (Pflieger 1996, Nolen et al. 2014, Magoullick et al. 2017). Predictor variables of interest for pools were the same as for riffles, except riffle width and canopy closure were not included. Canopy cover was not included in pool modeling because it was scarce in pools of the mainstem EPR. Only adult crayfishes (≥13-mm carapace length) were considered for calculating density estimates and conducting statistical analyses. This was done to avoid biasing results by young-of-year crayfishes, which can be abundant during late spring/early summer and often occupy different habitats than adults (Boyd and Page 1978, Riggert et al. 1999).
All statistical analyses were conducted in R (version 3.3.2; R Project for Statistical Computing, Vienna, Austria). Predictor variables were tested for multicollinearity using Spearman’s correlation coefficient ($p_s$). One variable from any pair of predictor variables having a $p_s \geq 0.60$ was excluded from the analysis. Candidate models were fit using the R package glmmADMB (Fournier et al. 2012). The response variable in each model was the number of $F. eupunctus$ collected within each 1-m$^2$ subsample. The response was modeled according to a zero-inflated Poisson distribution with a log link, and site was included as a random effect in each model to account for spatial autocorrelation. We fit a global model containing all fixed predictors remaining after multicollinearity analysis and included site as a random effect. We examined fit of the global model using a marginal $r^2$ and calculated the overdispersion parameter $c$-hat.

Model selection and averaging were conducted using the package MuMIn (Barton 2014). Candidate models were compared using Akaike’s Information Criterion (AIC) with a small sample size correction ($AIC_c$; Akaike 1974). Top models were defined as those having relative distance values ($\Delta AIC_c$) $\leq$2.0 (Burnham and Anderson 2004). We used the package performance (Lüdecke et al. 2019) to compute variance inflation factors (VIF) for assessing multicollinearity among fixed predictors in top models. All fixed predictors were required to have VIF $<5.0$ (James et al. 2013). Averaging of top models was used to derive model-averaged parameter estimates, and model parameters were examined at $\alpha = 0.05$. Full average parameter estimates were used to predict $F. eupunctus$ density for each covariate exhibiting a strong relationship with $F. eupunctus$ density, and partial dependence plots were generated to visualize the marginal relationships between these covariates and $F. eupunctus$ density. We calculated marginal $r^2$ values for top models to assess model fit. These values were not calculated for less-supported models because these models were not considered during model averaging or prediction. Model performance was further evaluated by regressing observed (y-axis) vs predicted (x-axis) values and comparing the resulting parameter estimates to a 1:1 line (Smith and Rose 1995, Piñeiro et al. 2008).

RESULTS

Riffles

We detected $F. eupunctus$ at 21 of 41 (~51%) surveyed sites, at 20 of 25 (80%) sites in the mainstem EPR, and at 1 of 16 (~6%) sites in tributary streams. The species was not detected at the 5 most upstream sites sampled in the mainstem EPR and was detected in just 1 tributary stream (Hurricane Creek) $<$2 stream km from its confluence with the mainstem EPR. We found no surface water at 13 sites and consequently did not sample those locations because of the aversion of $F. eupunctus$ to intermittent streams (Nolen et al. 2014). Additionally, we did not complete the quantitative portion of sampling at 3 sites. Of these sites, 1 had partially dried and did not contain flowing riffle habitats, and the other 2 were not completed because of time constraints. Data from these sites were not included in our analyses, but the sites are shown in Fig. 1. Site-averaged $F. eupunctus$ density ranged from 0.08 to 7.92 ind/m$^2$ at sites where it was detected. $Faxonius eupunctus$ density was highest in the middle reaches of its range within the mainstem EPR and generally decreased both downstream and upstream (Fig. 3A, B). We did not detect introduced $F. neglectus$ at any sites.

Multicollinearity analysis revealed that 2 pairs of predictor variables, current velocity and depth and riffle width and temperature, exhibited $p_s \geq 0.60$. We excluded depth and riffle width from the analysis based on their hypothesized reduced importance to $F. eupunctus$ and because exploratory analyses revealed redundancy in models fitting these variables. The global model fit the data (marginal $r^2 = 0.41$) and showed slight underdispersion ($c$-hat = 0.75). Model selection yielded 2 top models ($\Delta AIC_c < 2.0$) for $F. eupunctus$ density, which included the global model (Table 1). Both top models fit the data (marginal $r^2 > 0.40$), and all VIF scores were $<2.0$. Top models contained the predictor variables canopy closure, current velocity, temperature, and substrate size. Current velocity, temperature, and substrate size showed strong relationships with $F. eupunctus$ density, while canopy closure did not (Table 2). Predicted $F. eupunctus$ density was positively related to substrate size and negatively related to current velocity and temperature (Fig. 4A, B). We did not detect $F. eupunctus$ in riffle habitats when the substrate size mean score was below 2.0 (refer to substrate size classifications in Methods, range = 1.4–4.8). Further, we did not detect $F. eupunctus$ in riffles when current velocity was $>1.42$ m/s (range = 0.02–1.89) or when temperature was $>20.7^\circ$C (range = 15.6–28.1). Model validation revealed a strong relationship between predicted and observed $F. eupunctus$ densities ($r^2 = 0.92$).

Pools

We detected $F. eupunctus$ at 10 of 12 (~83%) sites. We did not detect $F. eupunctus$ at the most downstream and upstream sites (Fig. 3A, B). Site-averaged $F. eupunctus$ density ranged from 0.08 to 5.17 ind/m$^2$ at sites where the species was detected. Density was greatest within the middle reaches of the mainstem EPR and generally decreased both downstream and upstream from this area (Fig. 3A, B). We did not detect introduced $F. neglectus$ at any sites.

The global model fit the data well (marginal $r^2 = 0.56$) and showed slight underdispersion ($c$-hat = 0.89). Model selection yielded 2 top models ($\Delta AIC_c < 2.0$) for $F. eupunctus$ density in non-wadeable pools and included the global model (Table 3). Both top models fit the data (marginal $r^2 > 0.54$), and all VIF scores were $<2.0$. Top models contained the predictor variables current velocity, depth, temperature, and
substrate size. Current velocity, temperature, and substrate size showed strong relationships with *F. eupunctus* density, whereas depth did not (Table 4). Predicted *F. eupunctus* density was positively related to substrate size and negatively related to current velocity and temperature (Fig. 4A, B). We did not detect *F. eupunctus* in non-wadeable pools when the substrate score was $<1.4$ (range $5-1.0$–$4.8$). Further, *F. eupunctus* was not detected in non-wadeable pools when current velocity was $>0.51$ m/s (range $=0.04–0.58$) or when temperature was $>24^\circ$C (range $=16.0–25.0$). Model validation yielded a strong relationship between predicted and observed *F. eupunctus* densities ($r^2 = 0.92$).

**DISCUSSION**

Our targeted sampling approach allowed us to document distributional and ecological information not captured by previous research and sampling of *F. eupunctus*. We identified an undocumented spatial pattern in *F. eupunctus* density throughout the upper EPR drainage in both habitat types (rifles and pools) where crayfish density was quantified.

### Table 1. Candidate models for Coldwater Crayfish (*Faxonius eupunctus*) density (ind/m²) in rifles ranked by Akaike’s Information Criterion with a small sample size correction (AICc). Top models were defined as models having relative distance values ($\Delta$AICc) $<2.0$. Marginal $r^2$ values were calculated for top models to assess model fit. Models with Akaike weights ($W_i$) $<0.01$ were omitted. A total of 16 candidate models were constructed. $K$ represents the number of parameters in each model, including the intercept term.

| Model                     | $K$ | AICc  | $\Delta$AICc | $W_i$ | Marginal $r^2$ |
|---------------------------|-----|-------|--------------|-------|----------------|
| CANOPY + CV + SUBSTRATE + TEMP | 5   | 769.5 | 0.00         | 0.581 | 0.42           |
| CV + SUBSTRATE + TEMP     | 4   | 770.7 | 1.15         | 0.328 | 0.41           |
| CANOPY + SUBSTRATE + TEMP | 4   | 775.0 | 5.43         | 0.038 | –              |
| SUBSTRATE + TEMP          | 3   | 775.7 | 6.14         | 0.027 | –              |
| CV + TEMP                 | 3   | 777.1 | 7.54         | 0.013 | –              |
| CANOPY + CV + TEMP        | 4   | 777.7 | 8.14         | 0.01  | –              |

Figure 3. Site-averaged Coldwater Crayfish (*Faxonius eupunctus*) densities for rifles (A) and non-wadeable pools (B) of the Eleven Point River drainage, Arkansas and Missouri, USA.
Table 2. Model-averaged parameter estimates, standard errors (SE), and lower and upper 95% confidence interval (CI) bounds for Coldwater Crayfish (Faxonius eupunctus) density (ind/m²) in riffles.

| Parameter          | Estimate | SE  | Lower 95% CI | Upper 95% CI |
|--------------------|----------|-----|--------------|--------------|
| Intercept          | -2.523   | 0.484 | -3.472       | -1.574       |
| Canopy closure (%) | 0.094    | 0.096 | -0.282       | 0.094        |
| Current velocity (m/s) | -0.192 | 0.071 | -0.331       | -0.053       |
| Substrate          | 0.222    | 0.074 | 0.077        | 0.367        |
| In-stream temperature (°C) | -2.692 | 0.509 | -3.689       | -1.694       |

Faxonius eupunctus site densities were greatest throughout the middle reaches of the EPR and generally tapered both downstream and upstream from those locations. Previous research may not have detected this pattern because of discrepancies in design relative to the present study. For example, Nolen et al. (2014) and Magoulick et al. (2017) conducted multi-species surveys of the Black River drainage in Arkansas and Missouri, but by design, each study sampled just 1/3 of the EPR drainage locations visited or sampled during this study. Consequently, these previous studies did not likely attain the spatial resolution needed to uncover fine-scale density patterns or microhabitat variation. Other studies of F. eupunctus (Pflieger 1996) differ from ours in that they emphasized species presence and did not report empirical data relating to crayfish abundance or density. Despite these disparities in study design, our findings corroborate evidence from previous research suggesting that F. eupunctus is a narrowly-endemic habitat specialist, relative to sympatric congeners, that primarily occupies large perennial streams within the upper EPR drainage (Pflieger 1996, Nolen et al. 2014, Magoulick et al. 2017).

Current velocity, in-stream temperature, and substrate size were strongly associated with F. eupunctus density in both riffles and pools. While differences in methods between riffle and pool sampling prevented rigorous statistical comparison of these data, visual examination of the resulting species–habitat associations showed clear disparities between habitat types (Fig. 4A, B). For example, F. eupunctus showed a stronger association with large substrate particles in pools relative to riffles, perhaps reflecting the greater availability of these particles in deep, bluff-adjacent pools of the mainstem EPR. Similarly, F. eupunctus showed a stronger negative association with current velocity in pools, although the range of velocities the species used in pools was smaller than in riffles. These findings provide evidence that F. eupunctus may not be as strongly associated with riffles as previously hypothesized (Pflieger 1996) and affirm the need to sample alternative habitat types (i.e., not just riffles/runs) in surveys for crayfishes. Further, our findings supplement a growing body of literature highlighting the importance of physical microhabitat features for endemic crayfishes (Usio 2007, Welsh and Loughman 2015, Loughman et al. 2017, Stites et al. 2017). However, we acknowledge that other environmental features not investigated here, such as water chemistry (Allert et al. 2008) or density-dependent factors (Stein 1977), can similarly affect crayfish densities.

Our study provides a framework for quantifying crayfish density and microhabitat features in lotic habitats often considered too deep for conventional crayfish sampling methods. We hoped to provide a method that would perform similarly in pools to the quantitative kicknet approaches commonly used for sampling crayfishes in wadeable riffle and run habitats ( Larson et al. 2008). While a handful of studies have used diving methods similar to ours (e.g., SCUBA, surface-supplied air, etc.) to collect crayfishes from lentic waters (Capelli and Magnuson 1983, Collins et al. 1983, Somers and Stechey 1986, France et al. 1991, Olsen et al. 1991), relatively few have applied these methods in lotic ecosystems (Rabeni 1985, Roell and Orth 1992, Light et al. 1995, DiStefano et al. 2003, Brewer et al. 2009, Stites et al. 2017). Of these studies, even fewer have quantified crayfish densities in non-wadeable pools (DiStefano et al. 2003, Brewer et al. 2009). DiStefano et al. (2003) quantified crayfish density in pools from 2 Ozark streams but reported lower site-scale crayfish density estimates for Faxonius crayfishes than our...
study. These differences could be attributed to a variety of factors, including variability in sampling effort, disparities in target species ecology, or inherently-heterogeneous crayfish densities (Raben 1985). Nonetheless, we believe our approach for sampling non-wadeable pools was effective because of its consistency with our riffle sampling in revealing longitudinal *F. eupunctus* density trends throughout the upper EPR.

This study builds upon the pool sampling framework described in DiStefano et al. (2003) by providing a method for systematically subsampling non-wadeable pools of moderately-large streams. Equidistant subsample placement along longitudinal transects allowed us to easily locate and move between subsample locations, reducing sampling time and habitat disturbance relative to field-randomized subsamples. By using a surface-supplied air approach rather than traditional SCUBA oxygen tanks, we eliminated the need for oxygen tank refills that would have been impractical in the remote study area. Our method instead required infrequent fueling of the generator supplying power to the oxygen compressor. In addition to quantifying crustaceans, our pool sampling framework is likely well suited for other less mobile benthic organisms, including gastropods, mollusks, and larger aquatic insect larvae. Simple modifications, such as exchanging the 2-dimensional quadrat sampler for a 3-dimensional enclosure with mesh netting, could allow for quantitative assessment of benthic fishes, such as those in the genera *Etheostoma* or Noturus.

Despite its benefits, pool sampling presented several limitations relative to riffle sampling. Most importantly, open-water diving requires technical expertise and specialized equipment that may not be readily accessible to some collectors. The gear- and labor-intensive nature of under-water sampling also made this method more time consuming than riffle sampling. For example, we were able to comfortably sample 1 pool/d vs 2 or sometimes 3 riffles/d. Both sampling methods were limited by travel time, but pool sampling was considerably more limited by the time needed for gear setup and breakdown. Additionally, turbidity is an important factor in determining whether diving should be used in pools, given that collectors rely heavily on eyesight for locating and capturing target organisms. However, the 1-to-2-m underwater visibility range in our study system was not problematic for collecting crayfishes or habitat data. Overall, pool sampling provided new and beneficial ecological information for our target species, but the limitations of this method mean that sampling non-wadeable pools may be best suited for declining or under-sampled endemics or for those whose habitat requirements have not been well studied.

Our findings demonstrate that such targeted, fine-scale sampling for narrowly-distributed species can be useful for uncovering ecological information not captured by coarse-scale sampling efforts. For example, we identified a noteworthy spatial pattern in *F. eupunctus* density relative to previous work (Nolen et al. 2014, Magoulick et al. 2017), and we documented potentially-important microhabitat features for the species in multiple habitat types. Fine-scale density information uncovered through such sampling efforts will allow managers to better define critical habitats for conservation protection or identify stream locations for mitigation

| Table 3. Candidate models for Coldwater Crayfish (*Faxonius eupunctus*) density (ind/m²) in pools ranked by Akaike’s Information Criterion with a small sample size correction (AICc). Top models were defined as models having relative distance values (∆AICc) < 2.0. Marginal r² values were calculated for top models to assess model fit. Models with Akaike weights (Wi) < 0.01 were omitted. A total of 16 candidate models were constructed. K represents the number of parameters in each model, including the intercept term. CV = current velocity (m/s), DEPTH = depth (cm), SUBSTRATE = streamed particle size score, TEMP = in-stream temperature (°C). |
| Model                          | K | AICc | ∆AICc | Wi  | Marginal r² |
| CV + SUBSTRATE + TEMP          | 4 | 201.0 | 0.00  | 0.565 | 0.55 |
| CV + DEPTH + SUBSTRATE + TEMP  | 5 | 202.5 | 1.46  | 0.273 | 0.56 |
| CV + SUBSTRATE                | 3 | 204.8 | 3.81  | 0.084 | –   |
| SUBSTRATE + TEMP              | 3 | 206.7 | 5.63  | 0.034 | –   |
| CV + DEPTH + SUBSTRATE        | 4 | 207.0 | 5.94  | 0.029 | –   |
| DEPTH + SUBSTRATE + TEMP      | 4 | 208.6 | 7.57  | 0.013 | –   |

| Table 4. Model-averaged parameter estimates, standard errors (SE), and lower and upper 95% confidence interval (CI) bounds for Coldwater Crayfish (*Faxonius eupunctus*) density (ind/m²) in pools. |
| Parameter          | Estimate | SE   | Lower 95% CI | Upper 95% CI |
|--------------------|----------|------|--------------|--------------|
| Intercept          | -1.743   | 0.497| -2.717       | -0.769       |
| Current velocity (m/s) | -0.417    | 0.149| -0.709       | -0.125       |
| Depth (cm)         | -0.036   | 0.086| -0.205       | 0.133        |
| Substrate          | 1.261    | 0.140| 0.987        | 1.535        |
| In-stream temperature (°C) | -0.967    | 0.336| -1.626       | -0.308       |
or restoration (Rosenfeld and Hatfield 2006). Similarly, species–habitat associations resulting from fine-scale surveys can be used to inform spatial habitat availability models at multiple scales (Hatten and Parsley 2009, Jacobson et al. 2009, Erwin et al. 2017). Additionally, this study presents an adaptable framework for sampling crayfishes and other narrowly-distributed lotic taxa in multiple habitat types and at fine spatial scales. The methods presented herein can be readily applied to numerous benthic taxa and, with careful consideration, can be applied to a variety of species beyond the benthos.

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