DISTRIBUTION, DENSITY, AND HABITAT ASSOCIATIONS OF THE NARROWLY ENDEMIC COLDWATER CRAYFISH (*FAXONIUS EUPUNCTUS*) IN THE ELEVEN POINT RIVER DRAINAGE, ARKANSAS AND MISSOURI, U.S.

BY

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THESIS

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ABSTRACT

North America harbors an exceptionally high number of rare or narrowly distributed crayfishes, and many of these species are threatened with displacement by a variety of biological, chemical, and physical factors. Detailed distributional and ecological data are lacking for many North American crayfishes, making the assignment of appropriate conservation protection designations difficult. A better understanding of the distribution, population densities, and habitat associations of rare and narrowly distributed crayfishes is therefore warranted to most effectively protect and manage these species. The purpose of this research was to assess the distribution and local population densities of the narrowly endemic Coldwater Crayfish (*Faxonius eupunctus*) in the Eleven Point River drainage, Arkansas and Missouri, U.S. My principle objectives were to: (1) assess the distribution of *F. eupunctus* within the Eleven Point River drainage to further evaluate its potential use of tributary streams; (2) estimate local *F. eupunctus* population densities to determine where the species is most abundant; and (3) investigate physical habitat variables relating to, and potentially influencing, local *F. eupunctus* densities. I used multiple sampling approaches, including environmental DNA sampling, to assess the distribution, density, and habitat of *F. eupunctus* throughout the Eleven Point River drainage. *Faxonius eupunctus* occurred primarily in the main stem Eleven Point River but was detected in a downstream reach of one of its largest tributary streams. *Faxonius eupunctus* site densities were highest in the middle reaches of the species' range in the main stem Eleven Point River, and my analyses showed that *F. eupunctus* density was strongly related to current velocity, in-stream temperature, and streambed substrate size. Results from this study corroborate evidence from previous research in supporting that *F. eupunctus* is restricted to large streams within its range. Further, I present new evidence that *F. eupunctus* is most abundant in the middle reaches of its range in the main stem Eleven Point

River, and I discuss a variety of local-scale physical habitat variables potentially influencing this noteworthy spatial abundance pattern. I also present support for the use of environmental DNA for the detection of rare lotic-dwelling crayfish species, although I highlight potential limitations of eDNA applications in lotic environments.

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CHAPTER 1: GENERAL INTRODUCTION

One of the goals of conservation biology is to identify geographic areas of high species endemism or richness in order to prioritize the conservation of rare or threatened taxa and their potentially sensitive habitats. Historically, the conservation of freshwater ecosystems and taxa has received proportionally less investigation than that of terrestrial ecosystems, although an increasing number of recent studies have shown that freshwater ecosystems and their taxa are disproportionately more threatened relative to their terrestrial counterparts (Richter *et al.*, 1997; Ricciardi & Rasmussen, 1999; Dudgeon *et al*., 2006; Strayer & Dudgeon, 2010). Freshwater ecosystems occupy approximately 0.01% of Earth's surface area (Dudgeon *et al*., 2006), and only about 0.26% of the Earth's freshwater is contained in lakes, reservoirs, and streams – the rest exists as groundwater, permafrost, or vapor (Carpenter *et al*., 2011). However, freshwater ecosystems harbor roughly 9.5% of the world's described animal species (Balian *et al*., 2008), many of which are threatened or are in need of conservation protection (IUCN, 2017). Significant threats to freshwater biodiversity include introduced and invasive species, nonpoint source pollution, and habitat alteration and fragmentation (Richter *et al*., 1997; Dudgeon *et al.*, 2006). These biological, chemical, and physical threats contributing to the loss or displacement of freshwater biodiversity are often exacerbated by narrow natural range sizes, thus elevating the risk for extinction or local extirpations in many species (Taylor *et al*., 1996; DiStefano *et al*., 2015).

Crayfishes are a diverse group of freshwater organisms known to exhibit high levels of endemism throughout much of the world (Richman *et al.*, 2015). To date, there are over 590 globally recognized crayfish species, approximately 70% of which occur in North America (Richman *et al*., 2015). In particular, the eastern United States (U.S.) harbors more than 60% of described crayfish species and has been identified as a region of high endemism and species

richness for crayfishes (Taylor *et al.*, 2007) and other freshwater taxa such as fishes (Abell *et al*., 2008), Unionid mussels (Williams *et al.*, 1993), and insects (Master *et al.*, 1998). The crayfish family Cambaridae comprises over 99% of described crayfish species in North America, and approximately two-thirds of crayfishes in the family Cambaridae are endemic to the eastern U.S. (Taylor *et al.*, 2007; Richman *et al.*, 2015). The restricted ranges and high species diversity associated with many of these crayfishes elevates their risk for extinction or extirpation. For example, it has been estimated that nearly 50% of known crayfish species in the U.S. and Canada warrant conservation protection or recognition (Taylor *et al*., 1996; 2007). Despite their threatened nature, only six crayfish species in the U.S. are currently assigned conservation protection under the U.S. Endangered Species Act.

Crayfishes in the U.S. are threatened by a variety of biological, chemical, and physical factors. Physical habitat alteration through channelization, dredging, and mining activities can incite population declines and range reductions by altering stream hydrologies and contributing to streambank erosion and sedimentation, greatly reducing the availability of the coarse substrates utilized by many crayfishes (Stein, 1977; Taylor *et al*., 2007). The widespread impoundment of lotic habitats in the U.S. throughout the twentieth century has fragmented the ranges of many narrowly endemic crayfishes (Westhoff *et al*., 2006; DiStefano *et al.*, 2008) and has resulted in increased abundances in some of their most prevalent predators (Riggs & Bonn, 1959; Martinez *et al.*, 1994). Chemical pollution, such as the deposition of heavy metals into streambed substrates, has exacerbated crayfish population declines, particularly in areas affected by mining activities (Allert *et al*., 2013). Non-indigenous crayfishes introduced through incidental stocking, bait releases, or natural migrations have contributed to range reductions, distributional shifts, and local extirpations of native crayfishes throughout the U.S. (Taylor & Redmer, 1996; Magoulick &

DiStefano, 2007; DiStefano *et al*., 2009). Documentation of non-indigenous crayfish introductions has become increasingly common in the 21st century (DiStefano *et al.*, 2015), and such introductions can have considerable impacts on ecosystem structure and function (Lodge *et al.*, 2012; Jackson *et al.*, 2014).

Crayfishes perform vital roles in freshwater ecosystems and have been said to function as ecosystem engineers, given their influence on both ecosystem structure and function (Momot *et al*., 1978; Creed, 1994; Momot, 1995; Rabeni *et al*., 1995; Creed & Reed, 2004). For example, crayfishes can influence detrital decomposition rates (Huryn & Wallace, 1987; Parkyn & Winterbourn, 1997; Usio, 2000; Schofield *et al.*, 2001), the bioturbation of fine organic and inorganic sediments (Statzner *et al*., 2000; Dorn & Wojdak, 2004), and the modification of interstitial matrices within streambed substrates (Johnson *et al*., 2010; 2011). Additionally, crayfishes often occupy an intermediate trophic position between other invertebrate consumers and fishes: they function as predators by consuming larval insects (Parkyn *et al.*, 2001), fishes (Rahel & Stein, 1988), and snails (Crowl & Covich, 1990), and they have been shown to alter primary production by consuming filamentous algae (Creed, 1994) and aquatic macrophytes (Nystrom & Strand, 1996). Further, crayfishes are vital prey items for popular Centrarchid game species such as rock bass (*Ambloplites rupestris*) and smallmouth bass (*Micropterus dolomieu*) (Momot *et al*., 1978; Rabeni, 1992), and some non-game fishes common throughout much of the eastern U.S. such as longear sunfish (*Lepomis megalotis*) and creek chubs (*Semotilus atromaculatus*) have been shown to consume smaller crayfishes (Newsome & Gee, 1978; Probst *et al.*, 1984).

The distributional and ecological data often required for making informed conservation decisions are lacking for many crayfishes in the U.S. (Taylor *et al.*, 2007; Moore *et al*., 2013), thus

making the assignment of appropriate conservation protection designations difficult in many cases. A better understanding of the distributions, densities, and habitat associations of rare and threatened crayfishes is therefore needed to provide policy makers and conservation managers with the means to make effective conservation decisions. The research presented in this thesis aims to determine the distribution and to estimate the population densities of the narrowly endemic Coldwater Crayfish (*Faxonius eupunctus*; Williams, 1952) in the Eleven Point River drainage of Arkansas and Missouri, U.S. *Faxonius eupunctus* is among the most narrowly distributed crayfishes in the world and is only known from the largest streams within the Eleven Point, Spring, and Strawberry River drainages of Arkansas and Missouri (Flinders & Magoulick, 2005; Nolen *et al.*, 2014). However, recent molecular data suggest *F. eupunctus* populations in the mid- and upper portions of the Eleven Point River drainage warrant classification as a distinct species (Fetzner *et al.*, 2013), and work is currently underway to reclassify *F. eupunctus* populations in the lower Eleven Point, Spring, and Strawberry River drainages as two new species. Accordingly, *F. eupunctus* is currently recognized as Threatened by the American Fisheries Society (Taylor *et al.*, 2007) and Vulnerable by the International Union for Conservation of Nature (Richman *et al.*, 2015), and the species is listed as Critically Imperiled (S1) and is a Species of Conservation Concern in Missouri. Further, *F. eupunctus* is currently being evaluated for federal conservation protection under the U.S. Endangered Species Act, partly due to its narrow range and apparent potential to experience displacement and future range reductions.

In this study, I conducted a comprehensive survey of the upper Eleven Point River drainage to assess the distribution, population densities, and habitat associations of populations constituting the nominal *F. eupunctus* species. I sampled sites on smaller, 2nd and 3rd order (Strahler, 1957) streams never before surveyed for *F. eupunctus* to evaluate the species' potential use of smaller

streams within the drainage. I used a conventional seine sampling approach to survey multiple habitat types and estimated site-scale *F. eupunctus* densities by collecting crayfish density data in riffle mesohabitats at each site. I also developed a novel approach to systematically estimate *F. eupunctus* presence and density in deeper, non-wadeable pool mesohabitats in the main stem Eleven Point River. Finally, I employed an emerging sampling technique for freshwater macrobiota, environmental DNA (eDNA), at most sites to determine the feasibility of the method in lotic ecosystems and to bolster confidence in my assessment of the distribution and abundance of *F. eupunctus*. I sought to determine if the ability of eDNA to estimate presence and abundance of *F. eupunctus* corresponded well with results obtained from conventional sampling, or instead, if downstream transport of detectable eDNA hindered the reliability of eDNA sampling. In addition to supplementing the conventional surveys for *F. eupunctus*, a better understanding of factors relating to eDNA detectability in lotic environments will help determine if eDNA sampling is a reliable method for sampling benthic, lotic-dwelling organisms such as crayfishes.

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CHAPTER 2: FINE-SCALE DISTRIBUTION, DENSITIES, AND HABITAT ASSOCIATIONS OF THE NARROWLY ENDEMIC COLDWATER CRAYFISH IN THE ELEVEN POINT RIVER DRAINAGE, ARKANSAS AND MISSOURI, U.S.

INTRODUCTION

Crayfishes assume vital roles in freshwater environments; they are capable of influencing ecosystem processes such as bioturbation (Dorn & Wojdak, 2004) and detrital processing (Schofield *et al.*, 2001), and they often serve as both predators (Parkyn *et al.*, 2001) and prey items (Rabeni, 1992). Despite their ecological importance, crayfishes are among the most at-risk groups of freshwater taxa globally (Richman *et al.*, 2015) and in North America (Taylor *et al.*, 1996, 2007). Major threats to native crayfishes in North America include destruction and degradation of physical habitats, non-point source chemical pollution, and potential for displacement by invasive crayfishes (Thorp & Covich, 2010; Richman *et al.*, 2015). These threats are often compounded by narrow natural range sizes (Taylor *et al*., 2007; Crandall & Buhay, 2008), and in some cases, low natural population densities (Stites *et al.*, 2017). Accordingly, it was estimated that approximately 48% of crayfishes in the United States (U.S.) and Canada warrant some form of conservation attention (Taylor *et al.*, 2007). However, federal conservation protection has only been assigned to six crayfishes in the U.S. and to none in Canada. This discrepancy is likely related to a disproportionate number of crayfishes currently lacking the ecological and distributional data often required to reach informed conservation and management decisions. For example, approximately 21% of the world's known crayfishes are currently assessed as "data deficient" (Richman *et al.*, 2015), and it is estimated that more than 60% of crayfishes in the U.S. and Canada lack basic ecological or distributional data (Taylor *et al.*, 2007; Moore *et al.*, 2013).

Species distribution and abundance data are critical components required in understanding the conservation status of aquatic taxa (Taylor *et al.*, 2007; Richman *et al*., 2015), and the need for

such data is particularly relevant to crayfishes given their often narrow range sizes and potential for displacement (Crandall & Templeton, 1999; Crandall & Buhay, 2008; Taylor *et al*., 2007). Recent crayfish studies have provided updated distribution, abundance, or habitat association assessments for rare crayfishes (e.g., DiStefano *et al.*, 2016; Stites *et al.*, 2017), and data from some such studies have been consulted in deliberations regarding conservation protection designations (e.g., Thoma *et al.*, 2014; Loughman *et al.*, 2015). Data collected from such studies may also be useful in explaining species distributions and can aid in the designation or mitigation of critical habitats. Additional research is therefore warranted to adequately assess the conservation status of crayfishes in the U.S., especially those with narrow natural range sizes.

This study sought to evaluate the distribution, population densities, and physical habitat associations of the narrowly endemic Coldwater Crayfish (*Faxonius eupunctus*; Williams, 1952) in the Eleven Point River drainage of Arkansas and Missouri, U.S. *Faxonius eupunctus* exhibits a narrow range in the Eleven Point, Spring, and Strawberry River drainages in Arkansas and Missouri (**Fig. 2.1**). However, recent molecular data suggest that *F. eupunctus* populations in the upper Eleven Point River drainage represent a distinct species (Fetzner *et al.*, 2013), and work is currently underway to reclassify populations inhabiting the lower Eleven Point, Spring, and Strawberry River drainages as two new species. Research to date has found that *F. eupunctus* primarily inhabits larger, perennially flowing streams within its range and is often associated with shallower, fast-flowing habitats (Hobbs, 1989; Pflieger, 1996). Further, *F. eupunctus* has been associated with local- and landscape-scale habitat variables such as current velocity, depth, stream order (Strahler, 1957), percent spring flow volume in local catchment, and rock fragment volume in local catchment (Flinders & Magoulick, 2005; Nolen *et al.*, 2014; Magoulick *et al.*, 2017).

However, physical habitat variables potentially influencing local *F. eupunctus* densities have not been evaluated at a fine scale within the Eleven Point River drainage.

Faxonius eupunctus is threatened by potentially rising water temperatures resulting from global climate change, and by chronic land use disturbances potentially increasing streambank erosion and sedimentation throughout the Eleven Point River drainage. Further, invasive Ringed Crayfish (*Faxonius neglectus chaenodactylus*), have been introduced into at least one stream within the Eleven Point River drainage (Imhoff *et al.*, 2012; DiStefano *et al.*, 2015)**.** Introduced *F. neglectus* populations in the nearby Spring River drainage have displaced native *F. eupunctus* from multiple streams (Flinders & Magoulick, 2005; Magoulick & DiStefano, 2007) and may pose a threat to the persistence of *F. eupunctus* populations in the Eleven Point River drainage**.** Accordingly, *F. eupunctus* is currently recognized as Threatened by the American Fisheries Society (Taylor *et al*., 2007) and Vulnerable by the International Union for Conservation of Nature (Richman *et al.*, 2015). *Faxonius eupunctus* is a Species of Greatest Conservation Need in Arkansas, and it is Critically Imperiled (S1) and is a Species of Conservation Concern in Missouri. *Faxonius eupunctus* is currently being evaluated for federal conservation protection under the U.S. Endangered Species Act, partly due to its narrow natural range and potential to experience future displacement and range reductions. Therefore, a comprehensive survey of the upper Eleven Point River drainage is warranted to evaluate whether *F. eupunctus* populations constituting the nominal *F. eupunctus* species are truly restricted to the drainage's larger, perennially flowing streams and to determine where the species occurs in high density. Further, an evaluation of local physical habitat associations of *F. eupunctus* may allow managers to identify areas in which to focus future conservation efforts.

This study was conducted to evaluate the presence, population densities, and habitat associations of *F. eupunctus* populations in the upper Eleven Point River drainage. The lower Eleven Point River drainage (downstream from Dalton, Arkansas) was excluded from this study given that *F. eupunctus* populations inhabiting this portion of the drainage will soon constitute a new species. My principle objectives were to: (1) assess the distribution of *F. eupunctus* within the Eleven Point River drainage to further evaluate its potential use of tributary streams; (2) estimate *F. eupunctus* population densities to determine where the species is most abundant; and (3) investigate local physical habitat variables relating to, and potentially influencing, local *F. eupunctus* densities.

METHODS

Study System

The Eleven Point River drainage covers approximately 3,115 km^2 in the Ozark Highlands ecoregion (Omernik, 1987) in northern Arkansas and southern Missouri (**Fig. 2.1**). The main stem Eleven Point River is a 6th order stream at its confluence with the Spring River. Streams within the Eleven Point River drainage are generally characterized by developed riffle-pool complexes and coarse streambed substrates, and portions of the drainage are heavily influenced by discharge from karst springs (Missouri Department of Conservation, 1994). Three lotic-dwelling crayfishes are known to occur in sympatry with *F. eupunctus*: Hubbs' Crayfish (*Cambarus hubbsi*), the Ozark Crayfish (*Faxonius ozarkae*), and the Spothanded Crayfish, (*Faxonius punctimanus*). Invasive *F. neglectus* introduced to the Eleven Point River drainage have not yet been reported to occur in sympatry with *F. eupunctus*, and the distribution of *F. neglectus* in the drainage is currently limited to the upstream reaches of one small tributary stream (Imhoff *et al.*, 2012).

Site Selection – Wadeable Riffles

I identified a total of 12 streams within the upper Eleven Point River drainage to sample based on locations in this region estimated by Nolen *et al.*, (2014) to have >0% probability of harboring *F. eupunctus* populations. I used satellite imagery (ACME Mapper 2.1, ACME Labs) to identify candidate sites within each stream based upon the presence of wadeable riffle mesohabitats, given the known habitat associations of *F. eupunctus* (Nolen *et al*., 2014; Magoulick *et al.*, 2017). Wadeable riffle habitats were identified based on the presence of broken surface tension in the stream channel visible from satellite imagery. To reduce spatial non-independence, I required a five-river km buffer between sites on tributary stream segments and a three-river km buffer between sites on main stem Eleven Point River stream segments. I randomly selected survey sites from the pool of 114 candidate sites using a random number generator and eliminated any remaining candidate sites within the spatial buffer (above). Accordingly, I selected 52 sites in stream reaches ranging from 2nd to 6th order (**Fig. 2.1**).

Site Selection – Non-wadeable Pools

Candidate pool sites were identified based on sites selected for riffle sampling (above). Candidate pool sites were required to: (1) be located on the main stem Eleven Point River; (2) have had at least one positive detection of *F. eupunctus* during riffle sampling; and (3) contain non-wadeable pool mesohabitats visible from satellite imagery. For the purposes of this study, I defined non-wadeable pools as habitats having reduced current velocities with little to no broken surface tension and depths too deep to effectively use a seine for collecting crayfish. Applying these criteria to all sites in which riffles were sampled resulted in a total of fifteen candidate sites suitable for non-wadeable pool sampling. I used a stratified approach to randomly select sites for sampling.

The length of the main stem Eleven Point River containing candidate sites was divided into four sampling reaches of similar length. This stratification was performed to ensure relatively uniform distribution of sampling sites throughout the known range of *F. eupunctus* in the Eleven Point River. Using a random number generator, I randomly selected two of these sampling reaches, within which, two sampling sites were randomly selected. I then randomly selected one site from each of the two remaining sampling reaches. I also randomly selected one site from each of the four total sampling reaches to serve as an alternate in the event that any previously selected site did not contain suitable habitat for sampling upon arrival to the site. These methods resulted in the selection of six-priority sampling sites and four alternate sampling sites.

Sampling Methods – Wadeable Riffles

Riffle sampling was conducted between 6 June and 2 August 2016. Site length was determined by measuring wetted stream width at the most upstream run mesohabitat and multiplying that number by 10 (adapted from Barbour *et al.*, 1999). I chose to establish site length using run mesohabitats, rather than riffle mesohabitats, given that riffle mesohabitats in the study system were, at times, narrow and contained multiple stream channels, making a single wetted width measurement difficult to obtain. A maximum site length of 125m was established to limit time spent at each individual survey site. I used a quantitative kicknet method (Engelbert *et al.*, 2016) to estimate site-scale *F. eupunctus* density for each site. I estimated site-scale *F. eupunctus* density for each site following recommendations from Engelbert *et al.* (2016) and averaged the number of *F. eupunctus* individuals collected from twelve $1m^2$ quadrat samples placed in riffle mesohabitats at each site. Engelbert *et al.* (2016) reported that twelve quadrat subsamples placed in riffle mesohabitats yielded crayfish density estimates within 50% of an estimated true density

at 70% confidence for crayfishes occurring in wadeable Missouri streams and was the most effective in balancing trade-offs between survey accuracy and on-site sampling effort.

To collect crayfish, an individual $1m^2$ polyvinyl chloride (PVC) quadrat frame was placed on the streambed, and a 1.5 x 1.2m 1/8" mesh seine was positioned immediately downstream from the quadrat frame. An investigator then disturbed streambed surface substrates within the quadrat with their feet while another investigator held the mesh seine in place. The disturbance within the quadrat dislodged crayfish from beneath streambed surface substrates, and streamflow facilitated their movement downstream into the mesh seine. Quadrats were systematically placed on the streambed moving from down- to upstream on a staggered diagonal pattern. That is, the first quadrat in each riffle was placed approximately 1m from the stream margin at the most downstream extent of the most downstream riffle, the second quadrat was placed 1m upstream in the approximate center of the stream channel, and the third quadrat was placed 1m upstream and approximately 1m from the opposite stream margin. This pattern was repeated until all 12 quadrats were completed at each site. The number of quadrats placed in each riffle was allocated proportionally to riffle length when more than one riffle was present within a site. Additionally, I used 0.5m upstream spacing between quadrats at sites with <10.0m wetted stream width in order to complete all 12 quadrats within the length of the site. All crayfish collected were measured, sexed, and identified to species. Only individuals ≥13.0mm carapace length were recorded to prevent misidentification of young-of-year crayfish.

I collected the following habitat data from the center of each quadrat: canopy closure (%), current velocity (m/s), depth (cm), and streambed surface substrate composition. Canopy closure was measured using a Convex Spherical Crown Densiometer (Forestry Suppliers, Model A). Current velocity was measured according to the U.S. Geological Survey (USGS) "six tens method"

where a flow probe (Hach FH950; Hach Company, Loveland, Colorado, U.S.A) was placed at a depth from the surface equal to 60% of the total stream depth. Depth was measured with a metal wading rod. Streambed surface substrate composition was evaluated using a 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) 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 quadrat. Additionally, riffle width (m) was measured at the midpoint of each riffle using a meter tape, and a site temperature reading was recorded at the most downstream point of the most downstream riffle at each site. Temperature readings were averaged over a period of one minute and were recorded using a YSI Pro 10 temperature probe (Yellow Spring Instruments Incorporated; Yellow Springs, Ohio, U.S.). I chose not to collect temperature readings at the quadrat level because in-stream temperatures would likely vary minimally within riffles at a single site.

I included an additional, qualitative sampling component at each site, which was conducted following the completion of quantitative kicknet sampling. The qualitative sampling component was used to determine *F. eupunctus* presence in mesohabitats not surveyed during quantitative kicknet sampling (e.g., wadeable backwater pools, wadeable channel pools, and runs) and consisted of targeted, timed searches with a 3.0 x 1.2m 1/8" mesh seine (Engelbert *et al*., 2016). Deeper mesohabitats such as non-wadeable pools were not sampled using either quantitative or qualitative seine sampling methods because these approaches become difficult to implement in deeper mesohabitats. I targeted areas containing larger substrates or woody debris accumulations more conducive to *F. eupunctus* and collected as many crayfish as possible within a fifteen-minute timed-search period. Two timed-searches were conducted at sites with ≥10.0m wetted stream

width, and one timed-search was conducted at sites with <10.0m wetted stream width. All crayfish collected during both qualitative and quantitative sampling were measured, sexed, and identified to species. Only individuals ≥13.0mm carapace length were recorded to prevent misidentification of young-of-year crayfish. Crayfish collected during the qualitative sampling component were not used to estimate *F. eupunctus* site densities. All crayfish collected were released after sampling concluded, with the exception of up to two *F. eupunctus* per site and up to two of each sympatric crayfish species per site. Retained specimens were collected in accordance with scientific collection permits issued by the Arkansas Game and Fish Commission and the Missouri Department of Conservation and were deposited in the Illinois Natural History Survey Crustacean Collection at the University of Illinois at Urbana – Champaign.

Sampling Methods – Non-wadeable Pools

Pool sampling was conducted between 11 July and 3 August 2017. Each site was delineated by measuring 125m upstream from the approximate head of the riffle sampled during the previous field season. If pools identified for sampling via satellite imagery were unsuitable for sampling upon arrival to the site (e.g., shallower depth, increased current velocity, and broken surface tension more reflective of run mesohabitats), I moved the sampling location downstream to the next suitable non-wadeable pool mesohabitat.

To maintain similarity to the riffle sampling approach, crayfish and physical habitat data were collected from 12, $1m^2$ PVC quadrat frame samples at each site. Modifications to the sampling design were made to account for the inherent difficulties associated with sampling deeper, non-wadeable mesohabitats. Quadrats were partitioned evenly among three transects. Each transect was aligned approximately parallel with the stream channel, where one transect was

located in the approximate center of the stream channel and the other two were located approximately one quarter of the total wetted stream width from either stream margin. Data were collected from four quadrats along each transect line, and quadrats within the same transect line were spaced equidistantly, 10m apart. For each quadrat, an anchored aluminum jon boat containing a surface-air delivery system (Brownie's Third Lung model C270X) was positioned just upstream of each quadrat. Two divers descended to the streambed from the stern of the boat, collected habitat data and crayfish, and returned to the surface. Once a quadrat was completed, the boat, divers, and all sampling equipment were relocated to the next sampling location. Quadrats were completed in the same order at each site, beginning with the most upstream quadrat in the transect closest to the right upstream margin, moving downstream until all four quadrats were completed within the transect. This process was subsequently repeated for the mid-channel and left transects, respectively.

The following habitat data were collected at each quadrat location: current velocity (m/s), depth (cm), in-stream temperature (^{o}C), and streambed substrate size. Divers measured in-stream temperature (^{o}C) and streambed substrate size from the center of each quadrat. Temperature was collected using a handheld mercury thermometer. Streambed surface substrate composition was evaluated as previously described. Additionally, current velocity (m/s) and depth (cm) were recorded at each quadrat location by an investigator who remained in the boat. Current velocity was measured as previously described, although the flow probe was affixed to a 3m PVC rod which was marked in 0.1m increments. The PVC rod was used to estimate the appropriate depth at which to record current velocity readings. Depth was measured using a meter tape with a lead weight affixed to the end of the tape.

After habitat data for each quadrat had been collected, divers began searching for crayfish within the quadrat frame. Two divers slowly removed all streambed surface substrates within the quadrat with their hands working from downstream to upstream. Divers used their hands to collect crayfish found under removed substrates, or those displaced by removing substrates, and placed them in a mesh bag with a drawstring to prevent escape. Collected crayfish were brought to the surface and identified to species. All crayfish, with the exception of up to four *F. eupunctus* individuals per site were released unharmed at their point of capture. Retained *F. eupunctus* specimens were vouchered in accordance with scientific collection permits issued by the Arkansas Game and Fish Commission and the Missouri Department of Conservation and were deposited in the Illinois Natural History Survey Crustacean Collection at the University of Illinois at Urbana – Champaign.

I conducted additional sampling in riffle mesohabitats adjacent to non-wadeable pool sampling locations at nine sites to determine if *F. eupunctus* pool densities were significantly different than *F. eupunctus* riffle densities given that such information might reveal further evidence regarding the potential nature of *F. eupunctus* as a habitat specialist. Riffle mesohabitats were sampled concurrently with non-wadeable pool mesohabitats, although they were sampled by different investigators than those sampling non-wadeable pools. Crayfish were collected in accordance with the quantitative kicknet sampling method described in the preceding subsection, and all crayfish collected were released after being identified and recorded.

Statistical Analyses – Wadeable Riffles

I conducted all statistical analyses using version 3.3.2 of the statistical computing program R (R Core Development Team, 2016). I centered and scaled all predictor variables of interest prior

to conducting analyses. All predictor variables of interest were recorded in the field and included canopy closure (%), current velocity (m/s), depth (cm), riffle width (m), mean substrate size (above), and in-stream temperature (^{o}C). These habitat variables were included in the analysis given their relevance to the known physical habitat requirements of other lotic-dwelling crayfishes (Westhoff *et al.*, 2006; Loughman *et al.*, 2016; Noble & Fulton, 2016) and suspected habitat requirements of *F. eupunctus* reported by previous researchers (Pflieger, 1996; Flinders & Magoulick, 2005; Nolen *et al.*, 2014).

I tested predictor variables for multicollinearity using Spearman's correlation coefficient (ρ_s), and I excluded one variable from any pair of predictor variables having a Spearman correlation coefficient (ρ_s) $\geq$ 0.60. I used generalized linear mixed-effects models to examine relationships between *F. eupunctus* density and local physical habitat variables of interest. Candidate models were fit using R package *glmmADMB* (Fournier *et al.*, 2012). The response variable in each model was the number of *F. eupunctus* collected within each 1m^2 quadrat. 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 potential spatial autocorrelation from observations within the same site. Prior to fitting candidate models, I fit a global model containing all predictors remaining after multicollinearity analysis with site as a random effect. I examined fit of the global model using a marginal r^2 and calculated the overdisperion parameter, c-hat. Model selection and averaging was conducted using the R package *MuMIn* (Barton, 2014). Candidate models were compared using Akaike's Information Criterion with a small sample size correction (AICc; Akaike, 1974). Well-supported models were defined as those having ΔAICc values <2.0. I calculated marginal r^2 values for well-supported models to assess model fit. Marginal r^2 values were not calculated for less supported models since these models were not considered during

model averaging or prediction. Model averaging of well-supported models was used to derive model-averaged parameter estimates, and significance was examined at $\alpha = 0.05$. Full average parameter estimates were used to predict *F. eupunctus* density for each covariate in the well-supported models exhibiting a significant relationship with *F. eupunctus* density, and partial dependence plots were generated to visualize the marginal effects of these covariates on *F. eupunctus* density. Model validation was conducted by refitting each well-supported model using a training dataset containing 75% of the original observations and then using fitted models to predict *F. eupunctus* density for the remaining 25% of the original observations contained in a validation dataset.

Statistical Analyses – Non-wadeable Pools

Habitat modelling relating to *F. eupunctus* density in non-wadeable pools was conducted as described in the preceding subsection with the exception that riffle width (m) and canopy closure (%) were not included in the analyses.

Site-scale differences in *F. eupunctus* density between wadeable riffle and non-wadeable pool mesohabitats sampled concurrently were evaluated by comparing the Poisson rate parameter Lambda (λ; the rate of *F. eupunctus* occurrence) for each site. Significance tests were conducted using version 3.3.2 of the statistical computing program R (R Core Development Team, 2016) and significance was evaluated at $\alpha = 0.05$.

RESULTS

Survey Results – Wadeable Riffles

I found no surface water at 13 of 52 sites and consequently did not sample those sites with either quantitative or qualitative sampling approaches (**Fig. 2.1**) given the low desiccation tolerance of *F. eupunctus* and its related habitat aversion to intermittent streams (Flinders & Magoulick, 2005; Larson *et al.*, 2009). Additionally, I did not complete the quantitative portion of conventional sampling at two sites. Of these sites, one had partially dried and did not contain flowing riffle mesohabitats, whereas the other was not completed due to field time constraints. These sites were not included in statistical analyses but are included in **Fig. 2.1**. I detected *F. eupunctus* at 21 of the 39 (~54%) surveyed sites. *Faxonius eupunctus* was detected at 20 of 24 (~83%) sites located in the main stem Eleven Point River and at 1 of 15 (~7%) sites located in tributary streams. *Faxonius eupunctus* was not detected at the four most upstream sites sampled in the main stem Eleven Point River and was not detected anywhere in the drainage upstream from Thomasville, Missouri (**Fig. 2.1**). I detected *F. eupunctus* at one tributary site (Hurricane Creek), located within 2 stream kilometers of the stream's confluence with the Eleven Point River. Site-averaged *F. eupunctus* density ranged from 0.08-7.92 individuals/m^2 at sites where the species was detected (**Appendix A; Table A.1**). *Faxonius eupunctus* was most abundant in the middle reaches of its range in the main stem Eleven Point River, and site-averaged *F. eupunctus* density generally decreased moving both down- and upstream in the Eleven Point River (**Fig. 2.2**). Site averaged densities for the sympatric *C. hubbsi*, *F. ozarkae*, and *F. punctimanus* ranged from 0.17-1.42, 0.08-1.75, and 0.08-4.08 individuals/m^2, respectively, at sites where these species were detected. *Cambarus hubbsi* occurred in highest densities in the main stem Eleven Point River and rarely occurred in tributary streams (**Fig. 2.3**). *Faxonius ozarkae* and *F. punctimanus* occurred in higher

densities in smaller tributary streams (**Figs. 2.4; 2.5**). I did not detect introduced *F. neglectus* at any site.

Survey Results – Non-wadeable Pools

I successfully completed sampling at all ten randomly selected sites and sampled two additional, non-randomly selected sites to bolster confidence in my assessment of the distribution and density of *F. eupunctus* in non-wadeable pool mesohabitats. Data collected from non-randomly selected sites were excluded from statistical analyses. I detected *F. eupunctus* at 10 of 12 (~83%) sites. I did not detect *F. eupunctus* at the most down- and upstream sites in the Eleven Point River (**Fig. 2.6**). Site-averaged *F. eupunctus* density ranged from 0.08-5.17 individuals/m^2 at sites where the species was detected (**Appendix A; Table A.2**), and site averaged densities for sympatric crayfishes *C. hubbsi*, *F. ozarkae,* and *F. punctimanus* ranged from 0.08-1.17, 0.08-1.83, and from 0.08-0.17 individuals/m^2, respectively, at sites where these species were detected. I did not detect introduced *F. neglectus* at any site. *Faxonius eupunctus* was most abundant throughout the middle reaches of its range in the Eleven Point River, and site-averaged density generally decreased moving both down- and upstream in the Eleven Point River (**Fig. 2.6**).

Statistical Analyses – Wadeable Riffles

Multicollinearity analysis revealed that two pairs of predictor variables – (1) current velocity and depth and (2) riffle width and in-stream temperature – showed Spearman correlation coefficients (ρ_s) $\geq$ 0.60. I therefore chose to exclude depth (cm) and riffle width (m) from the analyses. The global model fit the data (marginal r^2 = 0.41) and showed slight underdispersion (c-hat = 0.75). Model Selection yielded two well-supported models (ΔAICc <2.0) for *F. eupunctus*

density, which included the global model (**Table 2.1**). Both well-supported models fit the data (marginal $r^2 > 0.40$). Well-supported models contained the predictor variables canopy closure, current velocity, in-stream temperature, and streambed substrate size. Current velocity, in-stream temperature, and streambed substrate size showed significant relationships with *F. eupunctus* density, while canopy closure did not (**Table 2.2**). Predicted *F. eupunctus* density was significantly positively related to streambed substrate size and was significantly negatively related to current velocity and in-stream temperature (**Fig. 2.7**). I did not detect *F. eupunctus* in riffle mesohabitats when the streambed substrate size mean score was below 2.0 (refer to substrate size classifications in methods; range = 1.4-4.8). Further, I did not detect *F. eupunctus* in riffles when current velocity was greater than 1.42m/s (range = 0.02-1.89 m/s) or when in-stream temperature was greater than 20.7ºC (range = 15.6-28.1ºC). Model validation revealed a strong relationship between predicted and observed *F. eupunctus* densities ($r^2 = 0.92$).

Statistical Analyses – Non-wadeable Pools

The global model fit the data well (marginal $r^2 = 0.56$) and showed slight underdispersion (c-hat = 0.89). Model Selection yielded two well-supported models (ΔAICc <2.0) for *F. eupunctus* density in non-wadeable pools and included the global model (**Table 2.3**). Both well-supported models fit the data (marginal $r^2 > 0.54$). Well-supported models contained the predictor variables current velocity, depth, in-stream temperature, and streambed substrate size. Current velocity, in-stream temperature, and streambed substrate size showed significant relationships with *F. eupunctus* density, while depth did not (**Table 2.4**). Predicted *F. eupunctus* density was significantly positively related to streambed substrate size and was significantly negatively related to current velocity and in-stream temperature (**Fig. 2.8**). I did not detect *F. eupunctus* in non-

wadeable pools when the streambed substrate size score was below 1.4 (refer to substrate size classifications in methods section; range = 1.0-4.8). Further, *F. eupunctus* was not detected in non-wadeable pools when current velocity was greater than 0.51m/s (range = 0.04-0.58m/s) or when in-stream temperature was greater than 24°C (range = 16.0-25.0^{o}C). Model validation yielded a strong relationship between predicted and observed *F. eupunctus* densities ($r^2 = 0.92$).

Additionally, the rate of *F. eupunctus* occurrence (λ) was significantly lower ($p \leq 0.05$) in non-wadeable pool mesohabitats than in adjacent riffle mesohabitats at five of nine sites in which both riffle and non-wadeable pools mesohabitats were sampled concurrently (**Table 2.5**).

DISCUSSION

Results from this study provide evidence that *F. eupunctus* is restricted to a limited portion of the main stem Eleven Point River and at least one of the drainage's largest tributary streams. These findings are consistent with previous research and collections of *F. eupunctus* within the Eleven Point River drainage (Pflieger, 1996; Imhoff *et al.*, 2012; Nolen *et al.*, 2014). My confidence in the narrow range size of *F. eupunctus* is further bolstered by the fact that I did not detect the species in any stream <4th order, and I only documented the presence of the species in one tributary stream, Hurricane Creek, despite visiting or sampling at 23 additional tributary stream locations. Moreover, Hurricane Creek was a relatively large stream (4th order) where *F. eupunctus* was detected, and the sampling site was located within two stream kilometers of Hurricane Creek's confluence with the Eleven Point River. All other sites where *F. eupunctus* was detected were located within stream segments $\geq 5^{th}$ order. This study's inability to detect *F. eupunctus* in tributaries of the Eleven Point River, and in the main stem Eleven Point River upstream from Thomasville, Missouri, suggests that *F. eupunctus* does not often utilize streams

<5th order. These findings corroborate evidence from previous studies in suggesting that *F. eupunctus* is a habitat specialist, primarily occupying large, perennially flowing streams and rivers (Flinders & Magoulick, 2005; Nolen *et al.*, 2014; Magoulick *et al.*, 2017). Further, this study found that *F. eupunctus* typically occurred in greater densities in riffle mesohabitats than in non-wadeable pool mesohabitats, although strong statistical differences in the rate of *F. eupunctus* occurrence (λ) were only observed at a five of nine sites in which both mesohabitat types were sampled concurrently. Therefore, I do not conclude that *F. eupunctus* is primarily a riffle-dwelling species.

Results from wadeable riffle sampling and non-wadeable pool sampling both revealed a noteworthy spatial pattern in *F. eupunctus* density. Site-averaged *F. eupunctus* densities in both mesohabitats largely agreed in showing that *F. eupunctus* occurred in higher densities in the reach of the Eleven Point River occurring approximately between the Missouri Route 19 and the U.S. Route 160 road-stream crossings. Further, the highest *F. eupunctus* densities recorded during this study for both sampling approaches occurred between the Turner Mill and Whitten stream access locations in the Mark Twain National Forest (**Figs. 2.2, 2.6**). This pattern in *F. eupunctus* density may be somewhat related to physical habitat conditions at the site level, given that my analyses showed strong relationships between *F. eupunctus* density and current velocity, in-stream temperature, and streambed substrate size in both wadeable riffle and non-wadeable pool mesohabitats.

My finding that *F. eupunctus* density was strongly related to current velocity is somewhat consistent with findings from Nolen *et al.* (2014) and Flinders & Magoulick (2005), although both of these studies reported a positive association between *F. eupunctus* presence and current velocity. This may be attributed to the respective designs of each of the aforementioned studies. For

example, Flinders & Magoulick (2005) collected crayfishes from wadeable riffle, run, and pool mesohabitats and noted a positive relationship with only small *F. eupunctus* (≤15.0mm carapace length). However, my study did not consider small crayfishes (<13.0mm carapace length), and previous studies have shown that smaller crayfishes can be associated with stronger current velocities (Clark *et al*., 2008). Further, Nolen *et al.*, (2014) collected crayfishes from riffle and run mesohabitats and presumably included crayfishes collected from both mesohabitat types in their analyses, whereas I conducted separate statistical analyses for both riffle and pool mesohabitats. Furthermore, *F. eupunctus* primarily inhabits large rivers and streams, so it is not surprising that *F. eupunctus* presence is often positively related to current velocity at segment-, reach-, or site-scales. However, at a more fine scale (i.e., a single riffle or pool in this study), *F. eupunctus* density is likely negatively often associated with current velocity given that increased flows in certain areas of the stream channel (e.g., thalweg) in a large river can potentially make the use of these habitats difficult for some crayfishes (Clark *et al.*, 2008; 2013).

Streambed substrate size is an important physical habitat requirement for many lotic-dwelling crayfishes (DiStefano *et al.*, 2016; Loughman *et al.*, 2016; Stites *et al.*, 2017), and previous researchers have reported that *F. eupunctus* is often associated with larger streambed substrates (e.g., Pflieger, 1996). To date, two studies have incorporated metrics of streambed substrate size into their statistical analyses (Nolen *et al.*, 2014; Magoulick *et al*., 2017). Interestingly, substrate size did not show a strong relationship with *F. eupunctus* detection probability in Magoulick *et al*., (2017), although Nolen *et al.*, (2014) reported a positive association between *F. eupunctus* presence and rock fragment volume in the local catchment. Anecdotal field observations suggest that streambed surface substrates were larger in the middle reaches of the Eleven Point River where *F. eupunctus* was most abundant, although I was unable

to document strong differences in mean substrate size at the site level due to high within-site variability in both sampled mesohabitats. Further, divers noted elevated volumes of fine particulate matter on the streambed in non-wadeable pools of the Eleven Point River near and downstream from the Arkansas/Missouri state border. Additionally, densities of sympatric crayfishes *C. hubbsi*, *F. ozarkae*, and *F. punctimanus* also declined, or these species were not present at all, in the downstream reaches of the Eleven Point River. These lower crayfish densities downstream coincide with a shift in surrounding land cover from heavily forested to field- and pasture-dominated. Elevated streambed sedimentation in the lower Eleven Point River resulting from chronic land use disturbances could therefore be reducing the availability of larger substrates often utilized by lotic-dwelling crayfishes (Burskey & Simon, 2010; Richman *et al.*, 2015). Accordingly, a more quantitative analysis of streambed substrate size in the main stem Eleven Point River is warranted to determine if sedimentation may be reducing the availability of larger substrates in the lower Eleven Point River.

Relationships between in-stream temperature and *F. eupunctus* presence have been investigated by both Nolen *et al.*, (2014) and Magoulick *et al*., (2017), although neither study reported significant relationships between the two variables. Differences observed in this study could be attributed to the much smaller spatial extent of interest here versus those in Nolen *et al.*, (2014) and Magoulick *et al.*, (2017). However, the distribution and densities of *F. eupunctus* observed in this study may be more closely related to the abundance of karst springs in the Eleven Point River drainage than in-stream temperature. Discharge from karst springs can influence seasonal flow stability (Adamski *et al.*, 1995), and *F. eupunctus* is intolerant of desiccation relative to sympatric congeners (Larson *et al.*, 2009) – both of which likely influence the species' distribution within the Eleven Point River drainage. For example, many sampling sites located in

smaller, 2nd and 3rd order streams in this study showed no signs of surface water, even during late spring (May-June). This is due to the unique fractured dolomite and limestone geology in the Eleven Point River drainage, which causes most of the drainage's streams to lose substantial amounts of surface flow to the groundwater system (Adamski *et al*., 1995). Furthermore, it has been reported that the lower two miles of Hurricane Creek and the main stem Eleven Point River downstream from Thomasville, Missouri are among the only stream reaches in the drainage that do not lose substantial surface flow to the groundwater system (Missouri Department of Conservation, 1994). Surface water lost to the groundwater system in the Eleven Point River drainage often emerges as discharge from karst springs, which are often abundant and in close proximity to the main stem Eleven Point River downstream from the Thomasville. The presence of these karst springs in the downstream reaches of Hurricane Creek and in the Eleven Point River downstream from Thomasville aligns closely with the distribution of *F. eupunctus*.

In this study, *F. eupunctus* was rarely encountered with other crayfishes in riffle mesohabitats, and I therefore chose to exclude sympatric crayfish densities as predictors of *F. eupunctus* density in my statistical analyses. It is possible that species-specific crayfish densities in the Eleven Point River drainage are affected by factors relating to stream size. For example, I observed that *F. punctimanus* was generally most abundant in the smaller, ephemeral and intermittent streams sampled, whereas *F. ozarkae* became dominant in slightly larger tributaries with more surface flow. *Faxonius eupunctus* and *C. hubbsi* showed similar distributions, only occurring in large, perennially flowing streams. *Faxonius ozarkae* and *F. punctimanus* were widespread in the Eleven Point River drainage, but they often occupied alternative habitats such as pools or backwaters when *F. eupunctus* was present. Similar phenomena have been observed in

crayfishes, with one or more species dominating headwater streams while others are restricted to larger streams and rivers (Flinders & Magoulick, 2005; Loughman *et al.*, 2016).

This study is the first to rigorously assess population densities of *F. eupunctus* in the Eleven Point River drainage at a fine scale. *Faxonius eupunctus* riffle densities in this study were similar those reported in previous studies. DiStefano *et al.* (2003a) reported mean crayfish densities ranging from 7.4-20.5 individuals/m^2 in the Jacks Fork River, Missouri, although these density estimates included all crayfish species and life stages. Further, Larson *et al.* (2008) reported an estimated overall mean crayfish density of 11.4 ± 0.9 individuals/m^2 in riffle mesohabitats across 12 sites in a small Ozark stream. Rabeni (1985) estimated an overall mean crayfish density of 9.1 individuals/m^2 and reported mean adult densities of 3.1 and 0.5 individuals/m^2 for *F. luteus* and *F. punctimanus*, respectively, from the Current River and Jacks Fork River in Missouri. I frequently observed similar site-scale crayfish densities for *F. eupunctus*, *F. ozarkae*, and *F. punctimanus*, but my overall density estimates for these species are likely lower than that of previous reports given that some species were not found at all sites. Furthermore, quantitative kicknet methods are commonly used to sample crayfishes from wadeable stream reaches in my study region (e.g., Nolen *et al.*, 2014; Engelbert *et al.*, 2016) and have shown detection probabilities approaching 1.0 in habitats <50cm depth for multiple *Faxonius* species, including *F. eupunctus* (Magoulick *et al*., 2017).

I designed a novel approach to estimate crayfish densities from non-wadeable pool mesohabitats in larger streams and rivers. Some studies have used SCUBA sampling approaches to estimate crayfish density or biomass in lentic waters (Capelli & Magnuson, 1983; Collins *et al.*, 1983; Somers & Stechey, 1986; France *et al.*, 1991; Olsen *et al.*, 1991), although these studies used SCUBA as a means with which to compare abundance estimates from baited trapping

sampling methods. A handful of studies have used various SCUBA sampling approaches to estimate crayfish density or biomass from streams and rivers (Light *et al.*, 1995; DiStefano *et al.*, 2003b; Brewer *et al.*, 2009; Stites *et al.*, 2017), although their results have varied. DiStefano *et al.*, (2003b) used SCUBA and enclosed 1-m^2 quadrat frames to estimate crayfish densities in deeper pools of the Big Piney and Jacks Fork rivers in Missouri. These authors reported mean crayfish densities of <1.0 individual/m^2 for *Faxonius luteus* and *F. punctimanus* adults collected from pools of the Big Piney River during summer. They further reported mean crayfish densities <1.0 individual/m^2 for *F. ozarkae* and *F. punctimanus* adults collected from pools of the Jacks Fork River during summer – similar to site density estimates for those species in my study. Further, site-averaged *F. eupunctus* densities showed similar spatial patterns in both wadeable riffle and non-wadeable pool mesohabitats, thus demonstrating that my non-wadeable pool sampling approach was able to detect areas of high *F. eupunctus* density similarly to that of my well-tested riffle sampling approach.

Findings from this study are relevant in that they may influence government agencies in deliberations regarding future conservation designations for *F. eupunctus*. The narrow range and tendency of *F. eupunctus* to occupy only large streams predisposes the species to imperilment and suggests that widespread changes in its chemical or physical habitat or colonization by invasive crayfishes could present considerable threats to its persistence. *Faxonius eupunctus* showed variable population densities across its range with the highest site densities occurring in a reach of the Eleven Point River heavily influenced by karst spring groundwater discharges. The abundance of karst springs in the Eleven Point River drainage near and upstream from this reach results in a main stem river that likely exhibits seasonal hydrologic and thermal stability relative to many of the drainage's tributary streams. These factors are likely closely related to the presence and

abundance of *F. eupunctus*. Accordingly, the monitoring and conservation of groundwater resources within the Eleven Point River drainage will likely benefit the species' long-term persistence and potential recovery. Large streambed substrates (e.g., cobble, boulder) are also important for *F. eupunctus*. Lower *F. eupunctus* densities observed in the downstream reaches of the mainstem Eleven Point River may be partially due to sedimentation from anthropogenic and natural streambank erosion. Thus, conservation practices aimed at reducing streambank erosion, such as the development of riparian buffer zones, may benefit *F. eupunctus* and allow its recovery in downstream reaches where it could have been previously abundant.

This study used multiple rigorous sampling approaches to estimate the distribution and densities of *F. eupunctus* throughout the Eleven Point River drainage. These results corroborate findings from previous research in suggesting that *F. eupunctus* is a large river habitat specialist and is largely confined to the main stem Eleven Point River and the downstream reaches of its largest tributaries. Sampling a variety of mesohabitat types revealed that *F. eupunctus* used multiple habitats within its range and highlights the importance of sampling multiple mesohabitat types during most freshwater surveys. Furthermore, my results revealed a noteworthy spatial pattern in *F. eupunctus* density in the main stem Eleven Point River and showed that local physical habitat variables current velocity, in-stream temperature, and streambed substrate size may influence *F. eupunctus* densities. Results from this study provide evidence for the continued use of traditional, field-based surveys in aquatic ecology and demonstrate that sampling methods well optimized for target organisms and study systems can reveal useful information pertaining to the conservation status of rare aquatic taxa.

TABLES AND FIGURES

Table 2.1: Candidate models for *Faxonius eupunctus* density (#/m^2) in wadeable riffles ranked by AIC$_c$. Well-performing models are defined as candidate models having ΔAICc <2.0. Models with Akaike weights (W$_i$) <0.01 are omitted. Marginal r^2 values were calculated for well-performing models to assess model fit.

Model	K	AICc	ΔAICc	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	N/A
SUBSTRATE+TEMP	3	775.7	6.14	0.027	N/A
CV+TEMP	3	777.1	7.54	0.013	N/A
CANOPY+CV+TEMP	4	777.7	8.14	0.01	N/A

Table 2.2: Model-averaged parameter estimates, standard errors (SE), and lower and upper 95% confidence interval (CI) bounds for *Faxonius eupunctus* wadeable riffle density. Parameter estimates with 95% confidence intervals not overlapping zero are considered significant.

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 (^{o}C)	-2.692	0.509	-3.689	-1.694

Table 2.3: Candidate models for *Faxonius eupunctus* density (#/m^2) in non-wadeable pools ranked by AIC$_c$. Well-performing models are defined as candidate models having ΔAICc <2.0. Models with Akaike weights (W$_i$) <0.01 are omitted. Marginal r^2 values were calculated for well-performing models to assess model fit.

Model	K	AICc	ΔAICc	W$_i$	Marginal r^2
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	N/A
SUBSTRATE+TEMP	3	206.7	5.63	0.034	N/A
CV+DEPTH+SUBSTRATE	4	207.0	5.94	0.029	N/A
DEPTH+SUBSTRATE+TEMP	4	208.6	7.57	0.013	N/A

Table 2.4: Model averaged parameter estimates, standard errors (SE), and lower and upper 95% confidence interval (CI) bounds for *Faxonius eupunctus* non-wadeable pool density. Parameter estimates with 95% confidence intervals not overlapping zero are considered significant.

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 (^{o}C)	-0.967	0.336	-1.626	-0.308

Table 2.5: Contrast of site-averaged *F. eupunctus* density in wadeable riffle mesohabitats and non-wadeable pool mesohabitats within the same site. Significance was examined at α = 0.05. P-values denoted by * represent a significant difference in the rate of *F. eupunctus* occurrence (λ) between riffle and pool mesohabitats at the site level.

Site ID	Lat.	Long.	Pool Density	Riffle Density	p-value
EPR_25	36.78469	-91.461744	0.00	0.00	1.00000
EPR_27	36.79929	-91.398548	0.25	0.25	1.00000
EPR_32	36.75360	-91.259526	1.08	2.67	0.00661*
EPR_33	36.74605	-91.237746	5.17	8.83	0.00044*
EPR_34	36.73224	-91.214036	3.75	2.92	0.31430
EPR_36	36.66197	-91.193601	0.33	4.75	4.86E-13*
EPR_38	36.61180	-91.173033	1.00	9.50	<2.20E-16*
EPR_40	36.56003	-91.179781	1.33	2.67	0.02930*
EPR_44	36.43284	-91.137583	0.00	0.25	0.25000

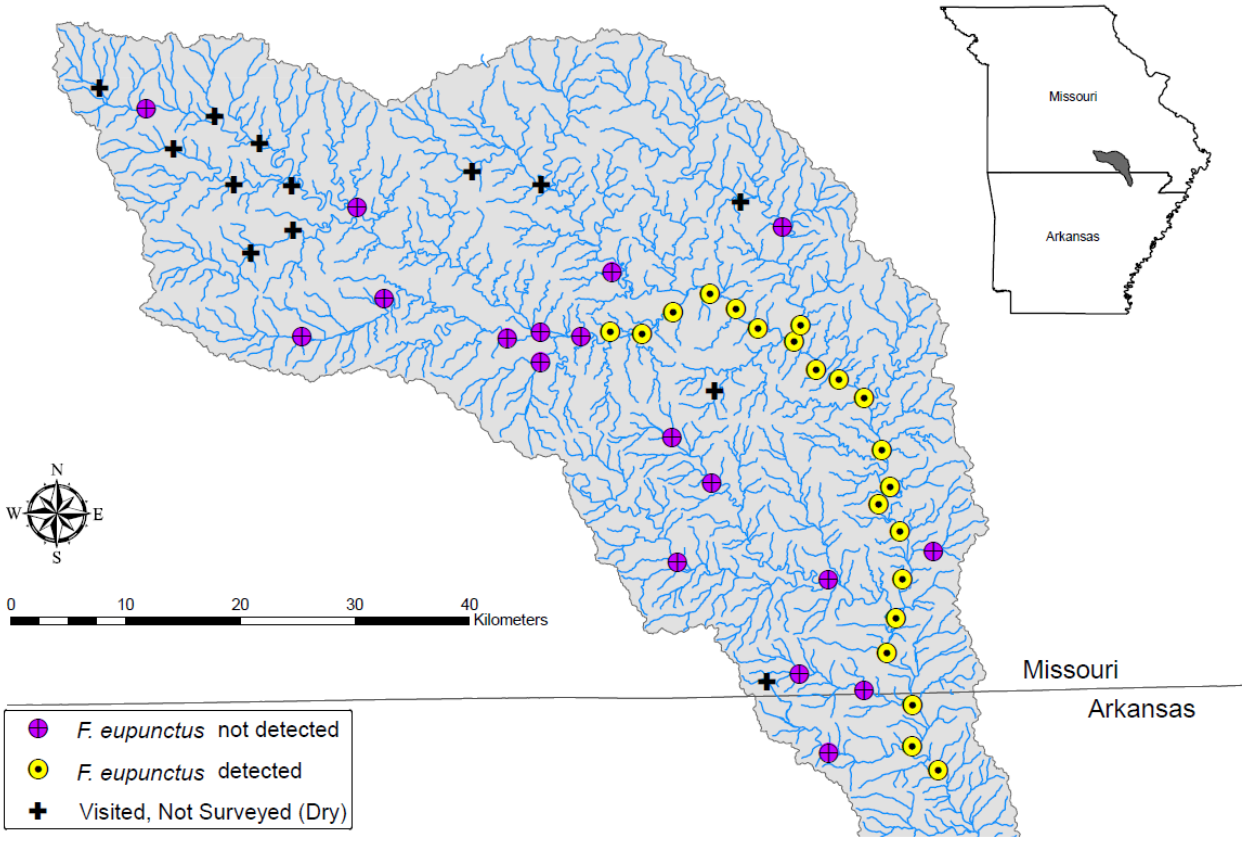

Fig. 2.1: Survey results for the presence of Coldwater Crayfish (*Faxonius eupunctus*) in wadeable habitats within the Eleven Point River drainage, Arkansas and Missouri, U.S. Crosses indicate sites visited but not surveyed due to a lack of surface water. Colored circles indicate results (detected or not detected).

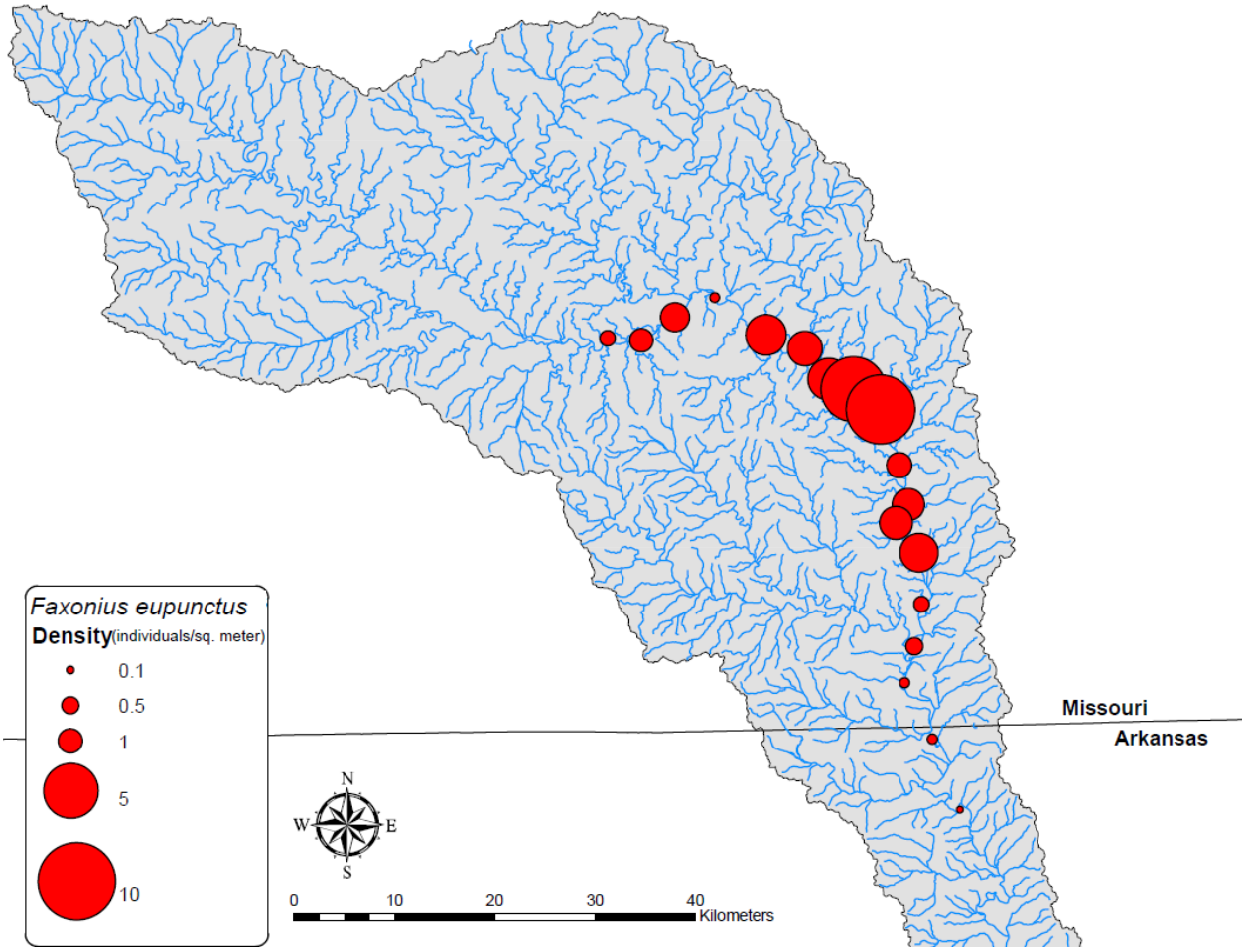

Fig. 2.2: Site-averaged *Faxonius eupunctus* densities as estimated from quantitative kicknet sampling in wadeable riffle mesohabitats within the Eleven Point River drainage, Arkansas and Missouri, U.S. Larger circles indicate greater site-averaged densities.

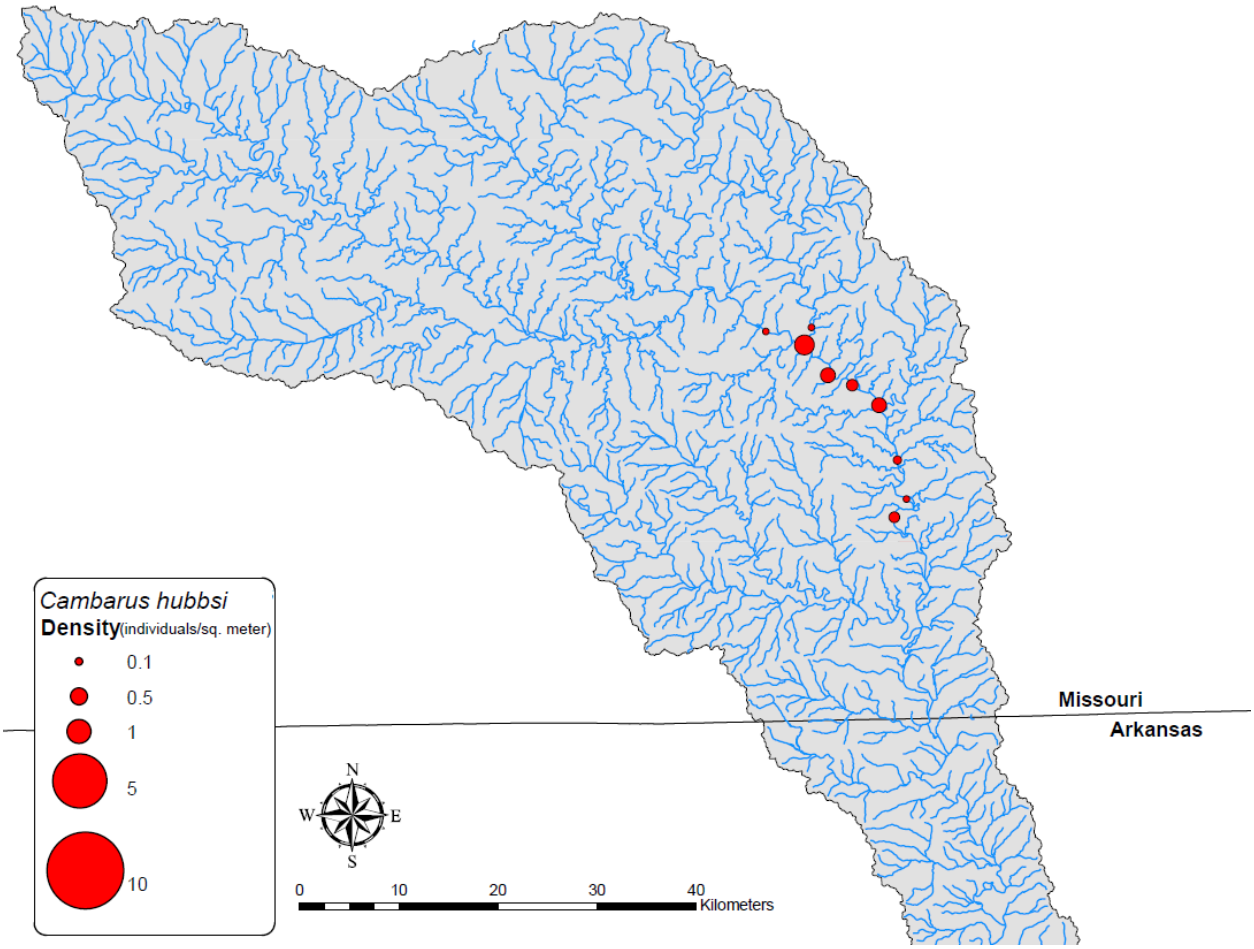

Fig. 2.3: Site-averaged *Cambarus hubbsi* densities as estimated from quantitative kicknet sampling in wadeable riffle mesohabitats within the Eleven Point River drainage, Arkansas and Missouri, U.S. Larger circles indicate greater site-averaged densities.

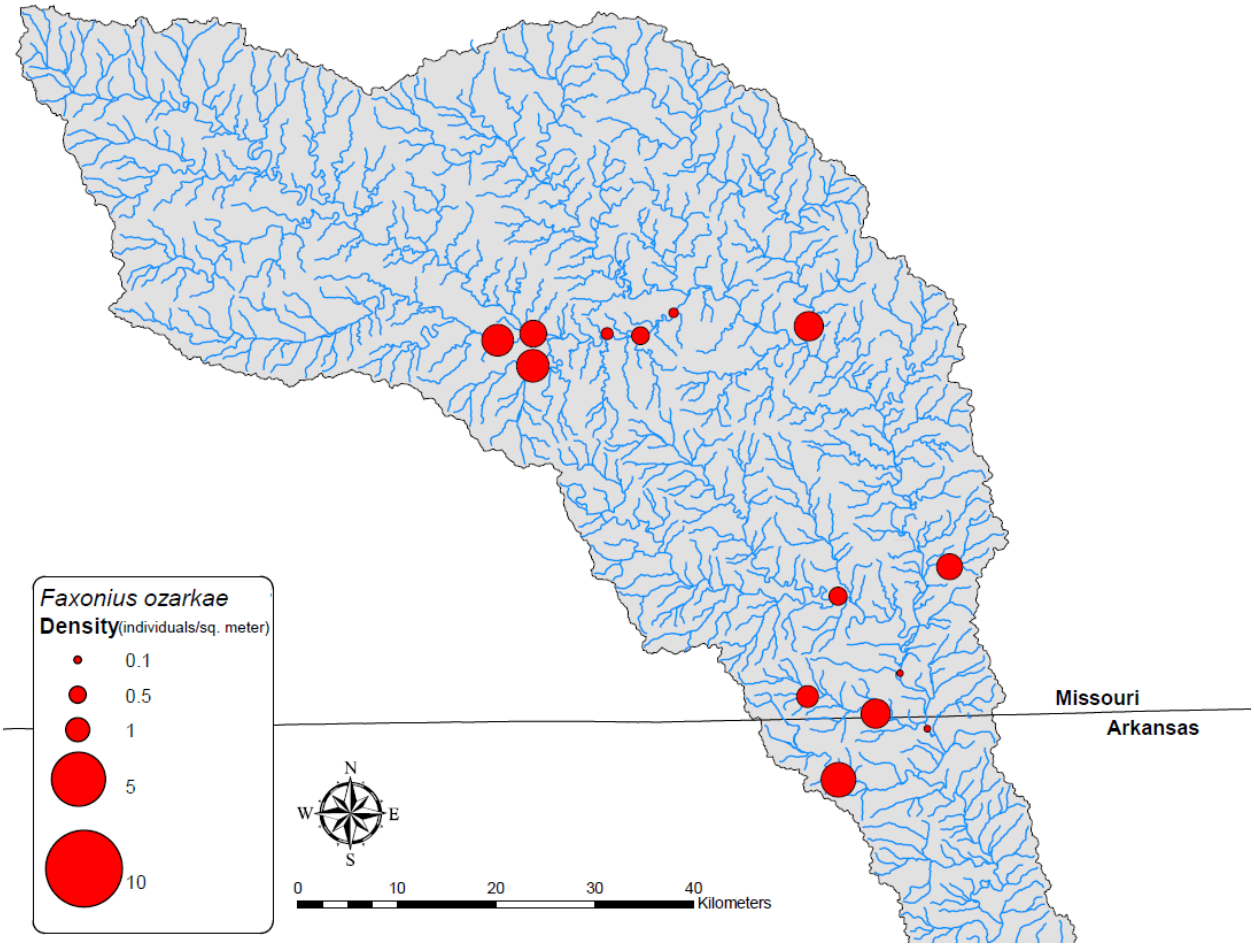

Fig. 2.4: Site-averaged *Faxonius ozarkae* densities as estimated from quantitative kicknet sampling in wadeable riffle mesohabitats within the Eleven Point River drainage, Arkansas and Missouri, U.S. Larger circles indicate greater site-averaged densities.

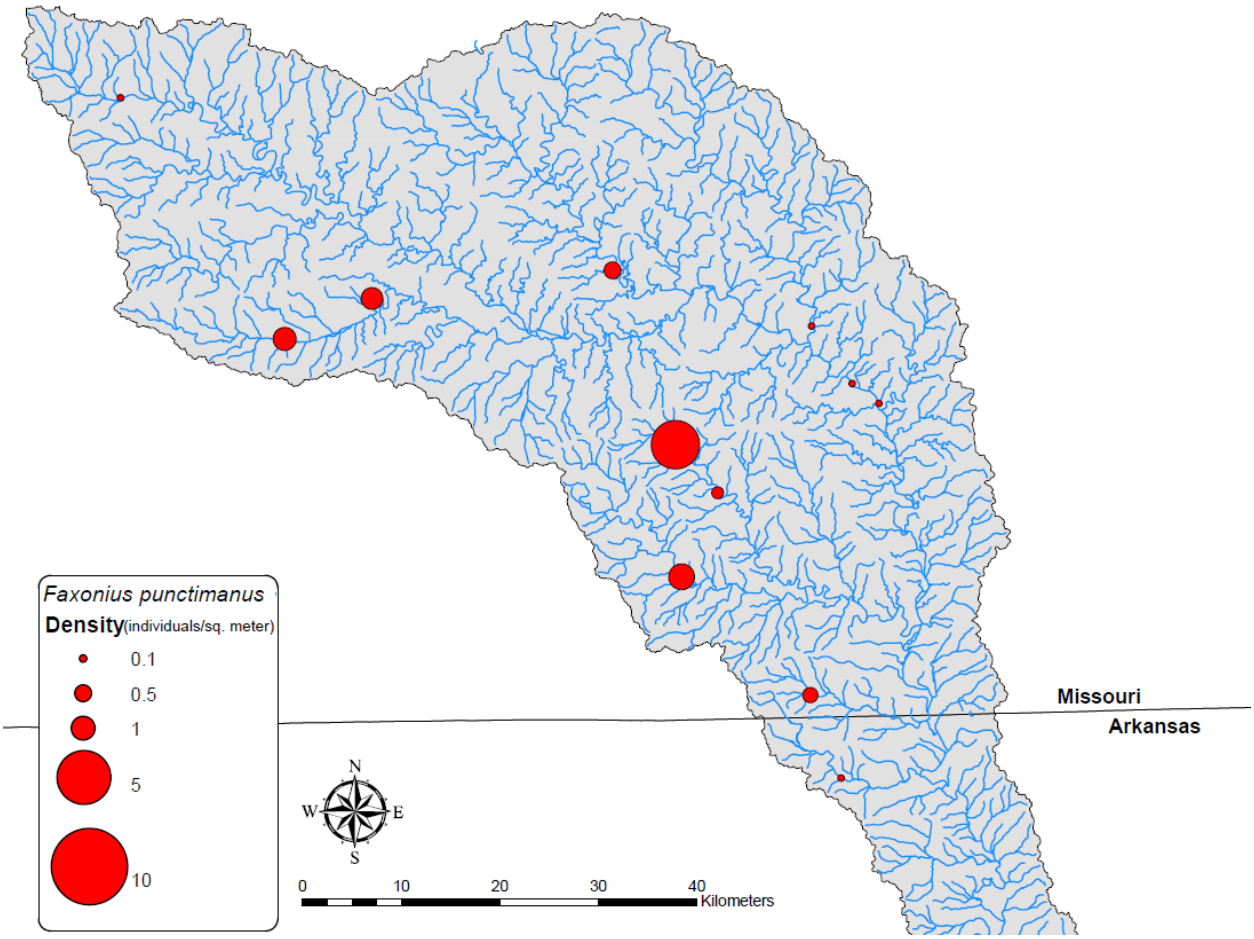

Fig. 2.5: Site-averaged *Faxonius punctimanus* densities as estimated from quantitative kicknet sampling in wadeable riffle mesohabitats within the Eleven Point River drainage, Arkansas and Missouri, U.S. Larger circles indicate greater site-averaged densities.

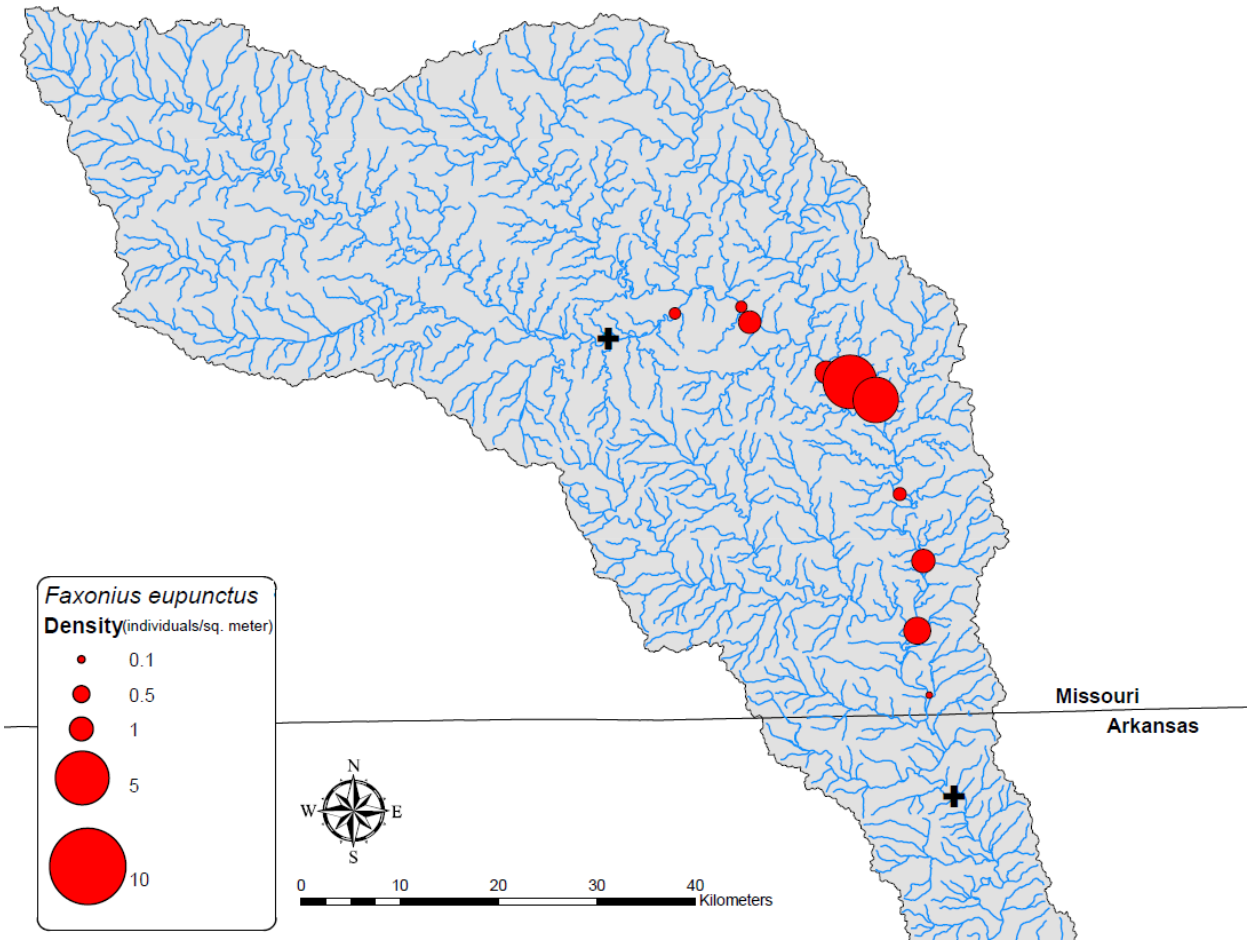

Fig. 2.6: Site-averaged *Faxonius eupunctus* densities as estimated from sampling in non-wadeable pool mesohabitats in the Eleven Point River, Arkansas and Missouri, U.S. Larger circles indicate greater site-averaged densities. Crosses indicate sites sampled but *F. eupunctus* was not detected.

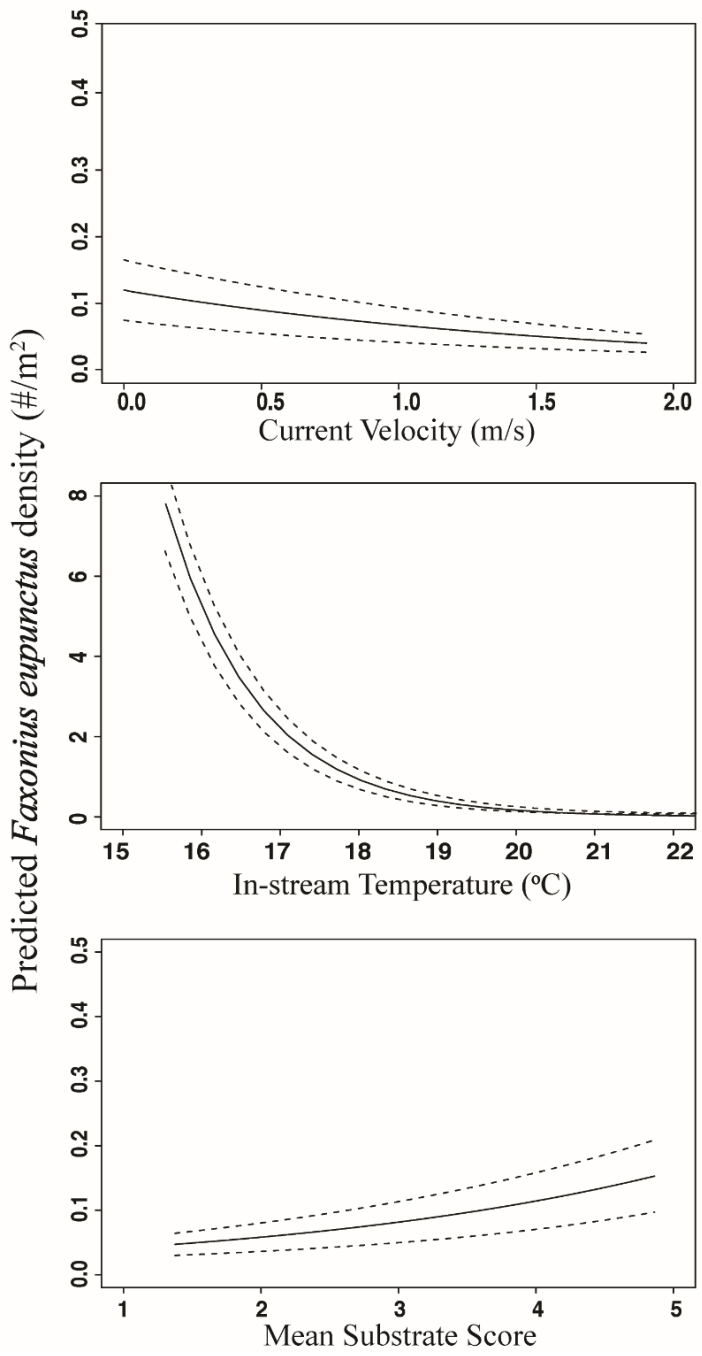

Fig. 2.7: Partial dependence plots showing relationships between predicted *Faxonius eupunctus* density (individuals/m^2) and physical habitat covariates in wadeable riffle mesohabitats within the Eleven Point River drainage, Arkansas and Missouri, U.S. Only significant relationships from well-performing models are shown (Table 2.1, Table 2.2). Solid lines are predicted *F. eupunctus* densities, and dashed lines are 95% confidence intervals.

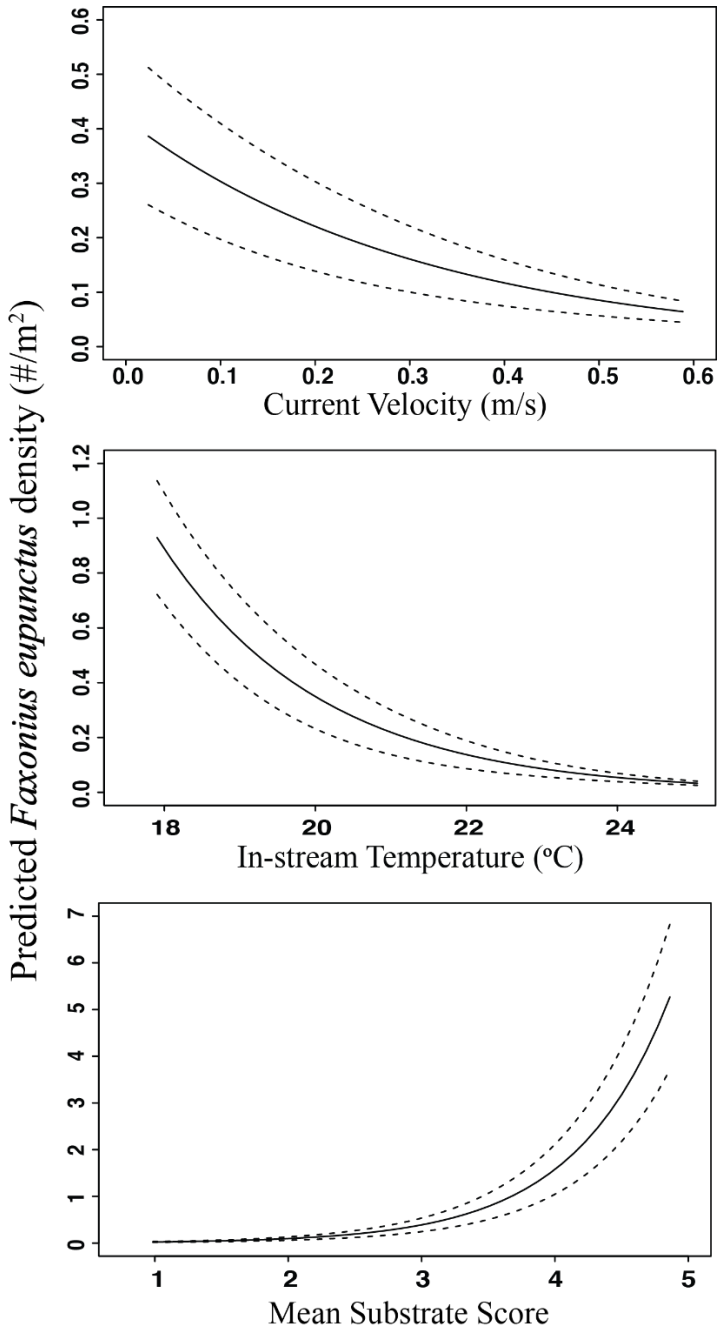

Fig. 2.8: Partial dependence plots showing relationships between predicted *Faxonius eupunctus* density (individuals/m^2) and physical habitat covariates in non-wadeable pool mesohabitats within the Eleven Point River drainage, Arkansas and Missouri, U.S. Only significant relationships from well-performing models are shown (Table 2.3, Table 2.4). Solid lines are predicted *F. eupunctus* densities, and dashed lines are 95% confidence intervals.

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CHAPTER 3: DOWNSTREAM TRANSPORT OVERWHELMS LOCAL ABUNDANCE IN EXPLAINING ENVIRONMENTAL DNA DETECTION PROBABILITY FOR A RARE CRAYFISH

INTRODUCTION

Environmental DNA (eDNA) is an emerging species surveillance method with the potential to revolutionize the management and conservation of freshwater species and ecosystems. This method is based on the principle that DNA extracted and identified from environmental samples can be used to infer the recent presence of species, sometimes with greater sensitivity than conventional sampling (Ficetola *et al*., 2008; Jerde *et al.*, 2011). Accordingly, eDNA may be a promising survey method for a variety of freshwater taxa, especially those that are rare or otherwise difficult to detect (Santas *et al.*, 2013; Fukumoto *et al.*, 2015). Despite its somewhat recent emergence for macrobiota in freshwater species surveys, eDNA has been successfully applied to a variety of freshwater taxa including amphibians (Goldberg *et al.*, 2011; Pilliod *et al.*, 2013), crayfishes (Tréguier *et al.*, 2014; Cai *et al.*, 2017), fishes (Minamoto *et al.*, 2012; Takahara *et al.*, 2013), gastropods (Goldberg *et al.*, 2013), insects (Thomsen *et al.*, 2012), mollusks (Egan *et al*, 2015), and reptiles (Piaggio *et al.*, 2014). Further, eDNA has shown promising results in both lentic and lotic environments (Thomsen *et al.*, 2012; Takahara *et al.*, 2013; Jane *et al.*, 2015), although its performance in lotic environments may be affected by eDNA transport through streamflow.

Rivers and streams may act as conveyor belts of molecular biodiversity information, transporting eDNA from both aquatic and terrestrial organisms downstream from its source (Deiner *et al.*, 2016). Detectable eDNA is thought to persist for just hours to days in freshwater (Dejean *et al.*, 2011; Barnes *et al.*, 2014), but it has the potential to move considerable downstream distances over that time, potentially resulting in the detection of taxa far from their actual locations

(Deiner & Altermatt, 2014). Few studies have evaluated the role of downstream transport on eDNA detection, and their results have varied (Deiner & Altermatt, 2014; Pilliod *et al*., 2014; Jane *et al*., 2015). As such, the potentially complex relationships between streamflow and eDNA detectability are not yet well understood (Wilcox *et al.*, 2016). A better understanding of the role that directional streamflow plays in downstream eDNA transport is necessary to begin effectively applying this tool to the conservation and management of rivers and streams and the taxa inhabiting them.

A high proportion of North American crayfishes are in need of conservation protection due to their high endemism and threats to their persistence, such as displacement by invasive species and habitat loss or degradation (Richman *et al.*, 2015). Further, many North American crayfishes in the family Cambaridae are difficult to detect or exhibit low population densities (Taylor *et al.*, 2016; Magoulick *et al.*, 2017; Stites *et al.*, 2017), thus making them excellent candidates for eDNA applications. To date, a handful of crayfish eDNA studies have shown promising results but have largely focused on lentic environments (e.g., Tréguier *et al.*, 2014; Dougherty *et al.*, 2016; Agersnap *et al.*, 2017; Cai *et al.*, 2017; Mauvisseau *et al.*, 2017), although one study (Ikeda *et al.* 2016) used eDNA to successfully detect endemic Japanese Crayfish (*Cambaroides japonicus*), in small headwater streams. Despite these encouraging results, it remains to be seen if eDNA can reflect presence and abundance of narrowly endemic crayfishes in larger, perennial streams where eDNA transport is more likely to be a problem (Deiner & Altermatt, 2014). Further, it is unknown how well eDNA applications in such systems will agree with data collected through conventional sampling methods well-suited for crayfishes inhabiting wadeable streams (Nolen *et al.*, 2014; Engelbert *et al.*, 2016; Magoulick *et al.*, 2017).

The Coldwater Crayfish (*Faxonius eupunctus*; Williams, 1952) is narrowly endemic to the Eleven Point, Spring, and Strawberry River drainages in northern Arkansas and southern Missouri, U.S. (Pflieger, 1996; Nolen *et al.*, 2014). However, recent molecular data suggest that *F. eupunctus* populations in the upper Eleven Point River drainage are genetically distinct (Fetzner *et al*., 2013), and work is currently underway to reclassify *F. eupunctus* populations in the lower Eleven Point, Spring, and Strawberry river drainages as new species. Accordingly, this study focuses on populations in the upper Eleven Point river drainage, which constitute the nominal *F. eupunctus* species. Within the Eleven Point River drainage, *F. eupunctus* is only known to inhabit a limited portion of the main stem Eleven Point River and the downstream reaches of a few of its direct tributaries (Pflieger, 1996; Fetzner *et al*., 2013. Research to date has found *F. eupunctus* primarily associated with higher order streams, greater groundwater spring flow volumes, and higher current velocity, thus making *F. eupunctus* a large river habitat specialist relative to sympatric congeneric crayfishes (Flinders & Magoulick, 2005; Nolen *et al.*, 2014; Magoulick *et al.*, 2017). *Faxonius eupunctus* is recognized as Threatened by the American Fisheries Society (Taylor *et al.*, 2007) and Vulnerable by the International Union for Conservation of Nature (Richman *et al.*, 2015), and it is currently being evaluated for potential listing under the United States Endangered Species Act, owed in part to its narrowly endemic range and risk for displacement by invasive crayfishes (Imhoff *et al.*, 2012; DiStefano *et al.*, 2015). Given the rarity and conservation need of this organism, eDNA may prove useful in better defining the distribution of *F. eupunctus* within the Eleven Point River drainage, including evaluating the species' potential use of tributary streams.

In this study, I developed a quantitative PCR (qPCR) assay for species-specific detection of *F. eupunctus* eDNA. I compared the capacity of eDNA to infer *F. eupunctus* presence and abundance to that of a conventional sampling method commonly used to survey crayfishes in

wadeable streams of Arkansas and Missouri (Engelbert *et al.*, 2016; Magoulick *et al.*, 2017). Further, I estimated *F. eupunctus* eDNA detection probabilities and examined relationships between eDNA detection probability and site-scale variables using a hierarchical occupancy and detection probability modeling framework (Schmidt *et al.*, 2013; Smart *et al.*, 2015). My study sought to evaluate whether results from eDNA corresponded well with the presence and abundance of *F. eupunctus* obtained via conventional sampling, or instead, if other factors such as downstream eDNA transport in a large river system might overwhelm the effect of local species abundance.

METHODS

Study System

The Eleven Point River drainage covers approximately 3,115 km^2 in the Ozark Highlands ecoregion (Omernik, 1987) in northern Arkansas and southern Missouri (**Fig. 3.1**). The main stem Eleven Point River is a 6th order stream (Strahler, 1957) at its confluence with the Spring River. Streams within the Eleven Point River drainage are generally characterized by developed riffle-pool complexes and coarse streambed substrates, and portions of the drainage are heavily influenced by discharge from karst springs. Three lotic-dwelling crayfishes are known to occur in sympatry with *F. eupunctus*: Hubbs' Crayfish (*Cambarus hubbsi*), the Ozark Crayfish, (*Faxonius ozarkae*), and the Spothanded Crayfish, (*Faxonius punctimanus*). Further, the invasive Ringed Crayfish (*Faxonius neglectus chaenodactylus*) has been introduced to a portion of the Eleven Point River drainage. However, *F. neglectus* is not known to occur in sympatry with *F. eupunctus* in the Eleven Point River drainage, and its distribution is currently limited to the upstream reaches of one small tributary stream (Imhoff *et al.*, 2012).

Site Selection

I identified a total of 12 streams within the Eleven Point River drainage to sample based on locations in this region estimated by Nolen *et al.* (2014) to have >0% probability of harboring *F. eupunctus* populations. I used satellite imagery (ACME Mapper 2.1, ACME Labs) to identify candidate sites within each stream based upon the presence of wadeable riffle mesohabitats visible in satellite images, given the known habitat associations of *F. eupunctus* (Nolen *et al.*, 2014; Magoulick *et al.*, 2017). To reduce spatial non-independence, I required a five-river km buffer between sites on tributary stream segments and a three-river km buffer between sites on Eleven Point River stream segments. I randomly selected sites from the pool of candidate sites using a random number generator and eliminated any remaining candidate sites within the spatial buffer (above). Accordingly, I selected 52 sites in stream reaches ranging from 2nd to 6th order (**Fig. 3.1**).

Conventional Sampling Methods

Conventional sampling was conducted between 6 June 2016 and 2 August 2016. Site length was determined by measuring wetted stream width at the most upstream run mesohabitat and multiplying that number by 10 (adopted from Barbour *et al.*, 1999). I chose to establish site length using run mesohabitats, rather than riffle mesohabitats, given that riffle mesohabitats in my study system were, at times, narrow and contained multiple stream channels, making a single wetted width measurement difficult to obtain. A maximum site length of 125m was established to limit time spent at each individual survey site. I used a quantitative kicknet method (Engelbert *et al.*, 2016) to estimate a site-scale *F. eupunctus* density for each site. Quantitative kicknet methods are effective for collecting crayfishes from wadeable stream reaches and have shown detection probabilities approaching 1.0 in habitats <50cm depth for multiple *Faxonius* species, including *F.*

eupunctus (Magoulick *et al.*, 2017). I estimated a site-scale *F. eupunctus* density for each survey site following recommendations from Engelbert *et al.*, (2016) and averaged the number of *F. eupunctus* individuals collected from twelve $1m^2$ quadrat subsamples placed in riffle mesohabitats at each site. The aforementioned authors reported that twelve quadrat subsamples placed in riffle mesohabitats yielded crayfish density estimates within 50% of an estimated true density at 70% confidence for crayfishes in Missouri streams. These authors further recommended the use of twelve quadrat subsamples per site to most effectively minimize trade-offs between survey accuracy and effort.

Prior to collecting crayfish from each quadrat subsample, percent canopy closure within each quadrat frame was estimated using a Convex Spherical Crown Densiometer (Forestry Suppliers, Model A). To collect crayfish from individual quadrat subsamples, a $1m^2$ polyvinyl chloride (PVC) quadrat frame was placed on the streambed, and a 1.5 x 1.2m 1/8" mesh seine was placed immediately downstream from the quadrat frame. An investigator then disturbed streambed surface substrates within the quadrat with their feet, while another investigator held the mesh seine in place. The disturbance within the quadrat dislodged crayfish from beneath streambed surface substrates, and streamflow facilitated their movement downstream into the mesh seine. Quadrats were systematically placed on the streambed moving from downstream to upstream on a staggered diagonal pattern. That is, the first quadrat in each riffle was placed approximately 1m from the stream margin at the most downstream extent of the most downstream riffle, the second quadrat was placed 1m upstream in the approximate center of the stream channel, and the third quadrat was placed 1m upstream and approximately 1m from the opposite stream margin. This pattern was repeated until all 12 quadrats were completed at each site. The number of quadrats placed in each riffle was allocated proportionally to riffle length when more than one riffle was present within a

site. Additionally, we used 0.5m upstream spacing between quadrats at sites with <10.0m wetted stream width in order to complete all 12 quadrats within the length of the site.

I included an additional, qualitative sampling component, which was conducted at each survey site following the completion of quantitative kicknet sampling. The qualitative sampling component was used to infer *F. eupunctus* presence in mesohabitats not surveyed during quantitative kicknet sampling (e.g., wadeable backwater pools, wadeable channel pools, and runs) and consisted of targeted, timed searches with a 1.2. x 3.0m mesh seine. Deeper mesohabitats such as non-wadeable pools were not sampled using either quantitative or qualitative conventional sampling methods because *F. eupunctus* was previously thought to occur less frequently in deeper, less swift mesohabitats (Pflieger, 1996) and because the approach becomes difficult to implement in deeper mesohabitats. Investigators targeted areas containing larger substrates more conducive to *F. eupunctus* and collected as many crayfish as possible within a 15-minute timed-search period. Two timed-searches were conducted at sites with ≥10.0m wetted stream width, and one timed-search was conducted at sites with <10.0m wetted stream width. All crayfish collected during both qualitative and quantitative sampling were measured, sexed, and identified to species. Only individuals ≥13.0mm carapace length were recorded to prevent misidentification of young-of-year crayfish. Crayfish collected during the qualitative sampling component were not considered when estimating site-scale *F. eupunctus* densities.

Environmental DNA Sampling

I did not conduct eDNA and conventional sampling concurrently to reduce the risk of eDNA sample contamination. Instead, eDNA samples were collected by returning to sites later in the summer, between 27 July 2016 and 13 September 2016. Although conventional sampling

continued until 2 August 2016 (above), eDNA sampling occurring during this time was conducted by a separate group of field workers not involved in conventional sampling during those dates. I did not anticipate substantial changes in site-scale *F. eupunctus* abundance over the relatively short period between conventional and eDNA sampling given the known biology and life history of lotic-dwelling *Faxonius* species (Hazlett, 1979; Riggert *et al.*, 1999; Larson & Magoulick, 2008). Environmental DNA water samples were collected at each site where conventional sampling took place. All eDNA sample collection gear was decontaminated with 10% bleach solution and rinsed with deionized water prior to use. Prior to collecting water samples from each site, surface water temperature (^{o}C) was measured in the most downstream riffle and readings were averaged over one minute. Temperature was measured mid-channel using a YSI Pro 10 temperature probe (Yellow Spring Instruments Incorporated; Yellow Springs, Ohio, U.S.). Investigators collected four 250mL surface water samples from the most downstream riffle at each site. One sample was collected ~1m from the right stream margin, two samples were collected mid-channel, and one sample was collected ~1m from the left stream margin. Investigators filled one additional 250mL collection bottle with store-bought water at each survey site to serve as a negative field control. Further, investigators wore nitrile gloves while collecting water samples, and changed gloves between each study site. Water samples were stored on-ice in a cooler and were filtered later the same day through 1.0µm pore diameter cellulose nitrate filter membranes using a peristaltic pump. All filtering and filter membrane transfers were conducted with site-specific nitrile gloves and forceps. Once filtering was completed, filter membranes were preserved in 95% EtOH, stored in 1.5mL microcentrifuge tubes, and were transported back to the laboratory at room temperature. Filtered samples were stored at 4°C until DNA extraction.

DNA Isolation

I extracted eDNA from filtered water samples within two months of completed sample collection. All laboratory equipment and surfaces used were sanitized with 10% bleach solution prior to DNA extraction. Each cellulose nitrate filter was halved; one-half remained in 95% EtOH and the other was used during eDNA extraction. Extractions were conducted using a DNeasy Blood and Tissue Kit (QIAGEN GmbH, Hilden, Germany) following the standard EtOH extraction protocol outlined by the manufacturer. To minimize contamination risk, samples were extracted by site and in order from lowest to highest *F. eupunctus* density as determined via conventional sampling. Additionally, I added one extraction control for each day eDNA extractions were performed in order to identify potential laboratory contamination. Each extraction control consisted of a clean, unused cellulose nitrate filter membrane, which underwent each step of the eDNA extraction process. Extracted eDNA from each sample was stored in an individual 1.5mL microcentrifuge tube and was sealed with Parafilm all-purpose laboratory film. Sealed microcentrifuge tubes were then stored at -20°C until qPCR amplification.

Primer Design and Testing

I obtained cytochrome c oxidase subunit I (COI) sequences for *F. eupunctus* and known sympatric crayfishes *C. hubbsi*, *F. ozarkae*, and *F. punctimanus* from museum holdings. Individuals used to derive the aforementioned COI sequences were collected from within the Eleven Point River drainage. I downloaded additional COI sequences for *C. hubbsi*, *F. ozarkae*, and *F. punctimanus* from GenBank (National Center for Biotechnology Information; **Table 3.1**). I aligned all COI sequences using the alignment software Sequencher 5.4 (Gene Codes Corporation; Ann Arbor, Michigan) and designed multiple candidate primer pairs for *F. eupunctus*

using PrimerHunter (Duitama *et al.*, 2009). Candidate primer pairs were prepared by IDT (Integrated DNA Technologies; Coralville, Iowa, U.S.A) and were tested in the laboratory for successful amplification with tissue-derived *F. eupunctus* DNA and for null or reduced amplification with tissue-derived DNA from non-target crayfishes *C. hubbsi*, *F. ozarkae*, and *F. punctimanus*. All tissue-derived DNA was diluted by a factor of 10 before primer testing. A 496 base pair, synthetic *F. eupunctus* COI fragment (gBlock; gBlocks® Gene Fragments) was synthesized by IDT, and a 1:10 serial dilution ranging from 1×10^{-4} to 1×10^{-10} was used on each qPCR plate to generate a standard curve. I required both a qPCR amplification curve and a quantification cycle (Cq) value <40 (limit of detection) to diagnose a positive amplification. Further, I required a qPCR melt curve consistent with *F. eupunctus* DNA (T_m=79.3-79.7°C) and Sanger sequencing of purified qPCR products to confirm the amplification of *F. eupunctus* DNA. My preferred primer pair, EUCOI_5_F (5'**GGA GTT GGG ACA GGC TGA ACA G** 3') and EUCOI_5_R (5'**ACT GAA CCA AGA ATA GAA GAA ACC** 3'), consistently amplified tissue-derived *F. eupunctus* DNA and showed reduced amplification with tissue-derived DNA from sympatric non-target species, as well as distinct melt curves for *C. hubbsi*, *F. ozarkae*, and *F. punctimanus*. This primer pair amplified a 124 base pair amplicon and showed 100% identity to all *F. eupunctus* COI sequences used during primer design (**Table 3.1**).

DNA Amplification

I amplified field samples within three months of eDNA extraction using a QuantStudio 3 Real-Time PCR System (Applied Biosystems; Foster City, California, U.S.A). Three replicate 20uL qPCR reactions were run for each field replicate, field control, and extraction control. A 1:10 serial dilution of the gBlock ranging from 1×10^{-4} to 1×10^{-10} served as a positive control on each

qPCR plate and was used to generate a standard curve for quantification of copy number. Three additional no-template controls (NTCs) were run on each qPCR plate to identify contamination. Each 20uL qPCR reaction consisted of the following reagents: 10uL iTaq Universal SYBR Green Mix, 5uL H_20, 4uL template, and 1uL of primer mastermix. Primer mastermix consisted of 270uL H_20, 15uL forward primer, and 15uL reverse primer. NTCs did not receive template but instead received 4uL of laboratory grade H_20. All qPCR runs were completed with a cover temperature of 105^oC, and cycling parameters were kept constant across all qPCR runs as follows: an initial denaturing stage at 95^oC for 3 minutes, 45 cycles of denaturation at 95^oC for 15 seconds, followed by annealing at 60^oC for 1 minute, and a melt curve analysis that increased consistently from 60^oC to 95^oC over a period of 3 minutes. I required an r^2 >0.99 and a qPCR efficiency 95.0-110.0% for each qPCR run. Amplification of any of the three-qPCR replicates – accompanied by a melting curve and a melting temperature (T_m) in the range appropriate to *F. eupunctus* – was used to identify a positive detection for the species. Product from positive qPCR replicates was purified using ExoSAP-IT (Affymetrix, Inc. Cleveland, OH, U.S.A) and was sequenced using Sanger sequencing to verify the amplification of *F. eupunctus* eDNA.

Detection Probability Modeling

I employed the hierarchical occupancy rate estimation models described by MacKenzie *et al.* (2002) to investigate relationships between *F. eupunctus* eDNA detection probability and site-scale variables anticipated to affect the production, persistence, and transport of eDNA (Schmidt *et al.*, 2013; Barnes & Turner, 2015). I included local *F. eupunctus* density as the sole predictor of *F. eupunctus* eDNA occupancy (psi) based on the prediction that both presence and abundance of the *F. eupunctus* would correspond with the presence of the species' eDNA. Further, I included

canopy closure (%), in-stream temperature (°C), site-averaged *F. eupunctus* density (number of individuals/m^2), and upstream river distance (km) as predictors of *F. eupunctus* eDNA detection probability (p).

I anticipated that more open canopies would increase UV exposure to the stream channel and reduce eDNA persistence times and transport distances, and I expected cooler temperatures, a particularly relevant predictor in my study system, to allow for increased eDNA persistence times and transported distances (Strickler *et al.*, 2015). I predicted that if eDNA transport distances were relatively short, local *F. eupunctus* densities would relate strongly to eDNA detection probability and that the rigor of my sampling protocol would potentially lead to stronger relationships between eDNA and conventional sampling than has been observed in past crayfish eDNA studies (Tréguier *et al.*, 2014; Dougherty *et al.*, 2016; Agersnap *et al.*, 2017; Larson *et al.*, 2017). Upstream river distance was derived using ArcMap version 10.4 (Environmental Systems Research Institute; Redlands, California, U.S.A) and indicated the total amount of stream kilometers upstream from each survey site, tributary streams included. Upstream river distance was included as a proxy for the risk of eDNA transport from upstream *F. eupunctus* populations, given that *F. eupunctus* is a known large river specialist (Nolen *et al.*, 2014; Magoulick *et al.*, 2017). I anticipated that *F. eupunctus* would be more likely to be present upstream from any given location as upstream river distance increased, and accordingly, transport risk of *F. eupunctus* eDNA would similarly increase with increasing upstream river distance.

Candidate models were fit using the package *unmarked* (Fiske & Chandler, 2011) in version 3.3.2 of the statistical computing program R (R Core Development Team, 2016). I fit a global model containing site-averaged *F. eupunctus* density as a predictor of occupancy (psi) and included canopy closure, in-stream temperature, site-averaged *F. eupunctus* density, and upstream

river distance as predictors of detection probability (p). All covariates were standardized and analyzed for multicollinearity prior to analysis. I used Pearson's Chi-square statistic to examine model fit and calculated the overdispersion statistic, c-hat, using the R package *AICCModavg* (Mazerolle, 2015). Model selection and model averaging were conducted using the R package *MuMIn* (Barton, 2014). QuasiAICc (*Q*AICc) scores adjusted to account for overdispersion were calculated for each candidate model, and their parameter estimates and standard errors were adjusted accordingly. Well-supported models were defined as those having Δ*Q*AICc values <2.0, and I calculated a likelihood-ratio based pseudo-r-squared to assess model fit for each well-supported model. Likelihood-ratio based pseudo-r-squared values were not calculated for less supported models given that these models were not considered during model averaging and prediction. Model averaging of well-supported models was used to derive model-averaged parameter estimates, and significance was examined at $\alpha = 0.05$. Model-averaged parameter estimates were used to predict detection probabilities for covariates exhibiting significant relationships with *F. eupunctus* eDNA detection probability, and partial dependence plots were then generated to visualize the marginal effects of these relationships.

RESULTS

Conventional Sampling

I found no surface water at 13 of 52 sites and consequently did not sample those sites with either conventional or eDNA methods (**Fig. 3.1**). It is unlikely that *F. eupunctus* was present at these sites given the species' low desiccation tolerance and related habitat aversion to intermittent streams (Flinders & Magoulick, 2005; Larson *et al.*, 2009). Additionally, I did not complete the quantitative portion of conventional sampling at two sites. Of these sites, one had partially dried

and did not contain flowing riffle mesohabitats, while the other was not completed due to field time constraints. These sites were excluded from statistical analyses but were included in **Fig. 3.1** to enhance my confidence in inferring the range of *F. eupunctus* within the Eleven Point River drainage. I detected *F. eupunctus* at 21 out of 39 remaining sites by the conventional sampling method (**Fig. 3.1**). Twenty of 21 sites with *F. eupunctus* detections were located on the main stem Eleven Point River, whereas one survey site was located on the lower reaches of a tributary stream. I detected non-target crayfishes *C. hubbsi*, *F. ozarkae*, and *F. punctimanus* at 10, 27, and 35 sites, respectively, and found no other crayfish species during my surveys. Site-averaged *F. eupunctus* densities ranged from 0.08-7.92 individuals/m^2 at sites where detected, and the species' site-averaged densities were lowest at the most down- and upstream portions of its range in the main stem Eleven Point River (**Appendix B; Table B.1**).

Environmental DNA Sampling

I detected *F. eupunctus* eDNA at 19 of 39 sites, all of which were located on the main stem Eleven Point River (**Fig. 3.1**). I did not observe DNA amplification in any of my field or laboratory controls. Amplified DNA identified as *F. eupunctus* by melt curve temperatures always Sanger sequenced to the species. I attempted to quantify eDNA concentration (copies/uL) for each positive qPCR replicate based on my gBlock serial dilution sequence, but late DNA amplification following my last dilution sequence for all positive qPCR replicates at 30 of 39 (77%) sites prohibited accurate quantification. The conventional sampling method detected *F. eupunctus* at two sites where eDNA did not. One survey site resulting in a false negative eDNA detection was the most upstream observation of *F. eupunctus* individuals in the main stem Eleven Point River, while the other was in Hurricane Creek, the only tributary where *F. eupunctus* was observed during

my study (**Fig. 3.1**). Alternatively, all of the remaining 19 detections of *F. eupunctus* eDNA were at locations where the species was detected by conventional sampling.

Detection Probability Modeling

The global model fit the data (Pearson's Chi-square P = 0.21), although it exhibited some overdispersion (c-hat = 1.27). Therefore, AICc scores were converted to *Q*AICc scores using the overdispersion parameter, c-hat. *Q*AICc scores were then used to determine well-supported candidate models. Model selection yielded two well-supported models (Δ*Q*AICc <2.0) for *F. eupunctus* eDNA detection probability (**Table 3.2**). Both well-supported models fit the data and exhibited likelihood-ratio based pseudo-r^2 values >0.70 (**Table 3.2**). Well-supported models contained the predictor variables canopy closure, in-stream temperature, and upstream river distance. In-stream temperature and upstream river distance showed significant relationships with eDNA detection probability, although canopy closure did not (**Table 3.3**). Local *F. eupunctus* density showed little relation to both eDNA detection probability and eDNA occupancy and was not included in either of the well-supported models. Predicted *F. eupunctus* eDNA detection probabilities were significantly positively related to upstream river distance and significantly negatively related to in-stream temperature (**Fig.3.3**).

DISCUSSION

My results demonstrate eDNA is a largely reliable tool for estimating the presence of benthic organisms in large (i.e., 6th order) freshwater rivers, although the ability of this approach to accurately reflect species presence or abundance in flowing waters may be hindered by the downstream transport of detectable eDNA in some cases. I found a poor relationship between *F.*

eupunctus eDNA detection probability and local *F. eupunctus* density, despite using a rigorous and well-studied conventional field method to estimate *F. eupunctus* densities (Nolen *et al.*, 2014; Engelbert *et al.*, 2016; Magoulick *et al.*, 2017). Alternatively, I found a strong relationship between eDNA detection probability and upstream river distance, which I used as a proxy for the risk of downstream transport of eDNA from upstream *F. eupunctus* populations. My findings supplement an ever-growing body of recent studies that have shown promise in documenting and better understanding the complex relationships between downstream eDNA transport and eDNA performance as a species detection method (Jerde *et al.*, 2016; Wilcox *et al.*, 2016; Shogren *et al.*, 2017).

Many studies to date have sought to compare performance of eDNA to conventional sampling methods in estimating both occupancy and abundance of target organisms. In general, eDNA has performed well for representing occupancy of species (Pilliod *et al.*, 2013; Smart *et al.*, 2015; Dougherty *et al.*, 2016), and some studies have found strong agreement between abundance or biomass of organisms and abundance of eDNA in the environment (Doi *et al.*, 2016; Klobucar *et al.*, 2017). Specific to crayfishes, studies have generally found eDNA to accurately represent occupancy of crayfishes, potentially with more sensitivity than some conventional methods, but eDNA has performed poorly in reflecting crayfish abundance (Dougherty *et al.*, 2016; Agersnap *et al.*, 2017; Cai *et al.*, 2017; Larson *et al.*, 2017). In most of these cases, the poor performance of eDNA relative to conventional sampling methods could be related to weaknesses of the conventional methods; for example, baited trapping of crayfishes has known biases and limitations that could fail to accurately reflect crayfish biomass (Stuecheli, 1991; Larson & Olden, 2016). I hoped that by using a rigorous, well-tested conventional method for sampling lotic-dwelling crayfishes in my study region (Nolen *et al.*, 2014; Engelbert, *et al.*, 2016; Magoulick *et al.*, 2017)

I would find stronger relationships between local *F. eupunctus* density and both eDNA detection probability and abundance of eDNA itself. Unfortunately, I were not able to reliably quantify eDNA copy number at all sites, and crayfish densities estimated from the quantitative kick seining approach were not a good predictor of eDNA detection probability relative to other attributes of the stream environment. Accordingly, I conclude that downstream transport is overwhelming local abundance in explaining eDNA detection probability for this narrowly endemic, large river crayfish.

Environmental DNA applications to lotic environments differ from applications to lentic environments due in part to the role directional streamflow may play in transporting eDNA downstream from the true locations of target organisms. I did not directly quantify transport distances for *F. eupunctus* eDNA, although several recent studies have attempted to quantify eDNA transport distances for other freshwater taxa in streams and rivers. Results from these studies have varied with observed transport distances ranging from <50m to over 12km. For example, Pilliod *et al.* (2014) reported positive detections of Idaho Giant Salamander (*Dicamptodon aterrimus*) eDNA 5m downstream from caged individuals but were unable to detect the species' eDNA at distances greater than 50m downstream. These authors also reported *D. aterrimus* eDNA was no longer detectable within one hour following the removal of caged individuals from the stream, suggesting that eDNA transport from the study reach was rapid. Alternatively, Deiner & Altermatt (2014) observed eDNA transport distances up to 9.1 km for eDNA from Swollen River Mussels (*Unio tumidus*) and 12.3 km for eDNA from a planktonic crustacean, *Daphnia longispina.* Further, both Jane *et al.* (2015) and Wilcox *et al.* (2016) reported detection of Brook Trout (*Salvelinus fontinalis*) eDNA at distances greater than 200m. In particular, Wilcox *et al.* (2016) showed evidence of downstream eDNA detections approaching

1km. These results suggest that eDNA can be transported downstream considerable distances in lotic environments, although variability in observed transport distance estimates seemingly indicates that relationships between eDNA detectability and streamflow may be system- and taxon- specific.

Given that *F. eupunctus* is primarily a large river inhabitant, I anticipated that greater upstream river distances might increase the downstream transport of eDNA from upstream populations. I found upstream river distances to be a strong predictor of eDNA detection probability for *F. eupunctus*, although cooler in-stream temperatures were also associated with higher eDNA detection probabilities. Cooler in-stream temperatures may facilitate increased downstream eDNA transport by slowing eDNA degradation rates (Moyer *et al.*, 2014; Strickler *et al.*, 2015). Relatively cool summer in-stream temperatures (16-21°C) are characteristic of much of the main stem Eleven Point River; due in part to the abundance of natural karst springs found in portions of the drainage. Therefore, it may be possible for detectable *F. eupunctus* eDNA to travel considerable downstream distances from its point of origin as a result of slowed degradation. If so, *F. eupunctus* eDNA detected at any stream location in my study system is likely to be at least partially representative of *F. eupunctus* populations in upstream locations. For such an organism, downstream transport of eDNA to larger receiving rivers could lead to substantial overestimation of its range size, although in my case I did not sample beyond the known extant range of *F. eupunctus* (Nolen *et al.*, 2014). Regardless, potential range overestimation from eDNA transport is a risk for many narrowly endemic species found in similar freshwater ecosystems (Page *et al.*, 1992; Boschung & Mayden, 2004).

I observed eDNA false negatives at two sites relative to my conventional sampling method. *Faxonius eupunctus* eDNA concentrations at these sites were likely below my detection threshold

due to the proximity of each to the upstream extent the species' range. Accordingly, these sites likely experienced reduced eDNA contributions from upstream habitats less optimal for *F. eupunctus* (e.g., tributaries). However, false negatives can occur in eDNA studies for many reasons (Wilcox *et al.*, 2013; Moyer *et al.*, 2014) and have often been attributed to sampling design choices like water sample volume or replication level in the field or laboratory (Ficetola *et al.*, 2015). Design improvements such as increased sample volume or replication may have allowed for improved detection in my study (Moyer *et al.*, 2014), although such adjustments also elevate the risk for false positives (Ficetola *et al.*, 2015). Similarly, the collection location of water samples within the water column may affect eDNA detectability, given that some aquatic sediments are known to harbor higher eDNA concentrations than those found in surrounding waters (Jerde *et al.*, 2016; Shogren *et al.*, 2017). However, surface water samples have been shown to work well for crayfish eDNA in a number of systems (Dougherty *et al.*, 2016; Ikeda *et al*., 2016; Cai *et al*., 2017). Further, I collected water samples from riffle mesohabitats because *F. eupunctus* is a known riffle associate (Pflieger, 1996; DiStefano *et al.*, 2010) and because hydraulic mixing in riffle mesohabitats is thought to suspended eDNA throughout the water column (Pilliod *et al.*, 2013). Additionally, I collected surface water samples given that humic compounds found in some aquatic sediments can produce false negatives though PCR inhibition (Matheson *et al.*, 2010).

My results highlight some limitations of eDNA applications in lotic environments and potential implications for other lotic-dwelling taxa, particularly those that are habitat specialists or have narrow range sizes. Accordingly, more work is urgently needed to characterize how system and study organism attributes relate to eDNA transport in lotic environments. Future efforts relating to eDNA detectability in lotic environments should examine physical stream characteristics at and upstream of sampling locations in order to better understand the hydrologic

processes governing downstream movement and retention of eDNA molecules. A handful of recent studies have begun investigating some of these relationships, such as the role of streambed substrates on the retention and resuspension of eDNA (Jerde *et al.*, 2016; Shogren *et al.*, 2016, 2017) or the importance of flow direction in determining eDNA detection probability (Song *et al.*, 2017). However, physical stream characteristics potentially influencing downstream transport rates of particulate matter, such as current velocity or depth (Reynolds *et al.*, 1990; Miller & Georgian, 1992), remain understudied for eDNA. As these studies accumulate over time, I recommend researchers be mindful of the potential risk of eDNA transport in lotic ecosystems, and apply statistical tools like hierarchical occupancy estimation with detection probability modeling to quantify both false negatives and false positives (Schmidt *et al.*, 2013; Ficetola *et al.*, 2015; Dougherty *et al.*, 2016).

TABLES AND FIGURES

Table 3.1: Similarity of preferred primer pair to *Faxonius eupunctus* and sympatric crayfishes *Cambarus hubbsi*, *F. ozarkae*, and *F. punctimanus*. Cytochrome c oxidase subunit I (COI) sequences denoted by † were obtained from museum holdings. All other COI sequences used during primer design were obtained from GenBank (National Center for Biotechnology Information) and associated accession numbers are included.

Species	Forward Primer	Reverse Primer	Identity	Accession #(s)
EUCOI_5 (Primer)	5'GGAGTTGGGACAGGCTGAACAG 3'	5'ACTGAACCAAGAATAGAAGAAACC 3'		
F. eupunctus†	GGAGTTGGGACAGGCTGAACAG	ACTGAACCAAGAATAGAAGAAACC	100	N/A
F. ozarkae	GGAGTTGGTACAGGATGAACAG	ACTGACCCTAAAATAGAAGAAACC	89	AY701242.1
F. ozarkae†	GGAGTTGGTACAGGATGGACGG	ACTGACCCTAAAATAGAAGAAACC	85	N/A
F. punctimanus	GGAGTTGGTACAGGATGGACGG	ACTGACCCTAAAATAGAAGAAACC	85	KT759604.1; KT759524.1
F. punctimanus†	GGAGTAGGTACAGGATGGACAG	ACTGATCCTAAAATAGAAGAAACC	85	N/A
C. hubbsi	GGAGTAGGTACTGGGTGAACTG	ACTGACCCTAAAATAGAAGAAACC	83	JX514446.1
C. hubbsi†	GGAGTAGGTACTGGGTGAACTG	ACTGACCCTAAAATAGAAGAAACC	83	N/A

Table 3.2: Candidate models for *Faxonius eupunctus* eDNA occupancy (psi) and detection probability (p) ranked by quasi AIC_c (*Q*AICc). Well-performing models are defined as candidate models having Δ*Q*AICc <2.0. Models with Akaike weights (W_i) <0.01 are omitted. Likelihood ratio-based pseudo r-squared (r^2) values were calculated for well-performing models to assess model fit.

Model	K	(*Q*)AIC	Δ(*Q*)AIC	W_i	r^2
psi(.), p(DISTANCE + TEMP)	5	89.2	0.00	0.396	0.704
psi(.), p(CANOPY + DISTANCE + TEMP)	6	90.5	1.21	0.216	0.735
psi(.), p(DISTANCE + EU.DENSITY + TEMP)	6	92.1	2.81	0.097	N/A
psi(EU.DENSITY), p(DISTANCE + TEMP)	6	92.1	2.86	0.095	N/A
psi(.), p(CANOPY + DISTANCE + EU.DENSITY + TEMP)	7	93.5	4.24	0.048	N/A
psi(EU.DENSITY), p(CANOPY + DISTANCE + TEMP)	7	93.5	4.27	0.047	N/A
psi(EU.DENSITY), p(CANOPY + TEMP)	6	94.6	5.35	0.027	N/A
psi(EU.DENSITY), p(DISTANCE + EU.DENSITY + TEMP)	7	95.1	5.87	0.021	N/A

Table 3.3: Model averaged parameter estimates, standard errors (SE), and lower and upper 95% confidence interval (CI) bounds for *Faxonius eupunctus* eDNA detection probability. Parameter estimates with 95% confidence intervals not overlapping zero are considered significant.

Parameter	Estimate	SE	Lower 95%	Upper 95% CI
Intercept	-2.029	0.447	-2.905	-1.152
Canopy Closure (%)	-0.211	0.376	-1.404	0.211
Upstream River Distance (km)	1.448	0.389	0.685	2.210
In-stream Temperature (^oC)	-1.505	0.428	-2.343	-0.666

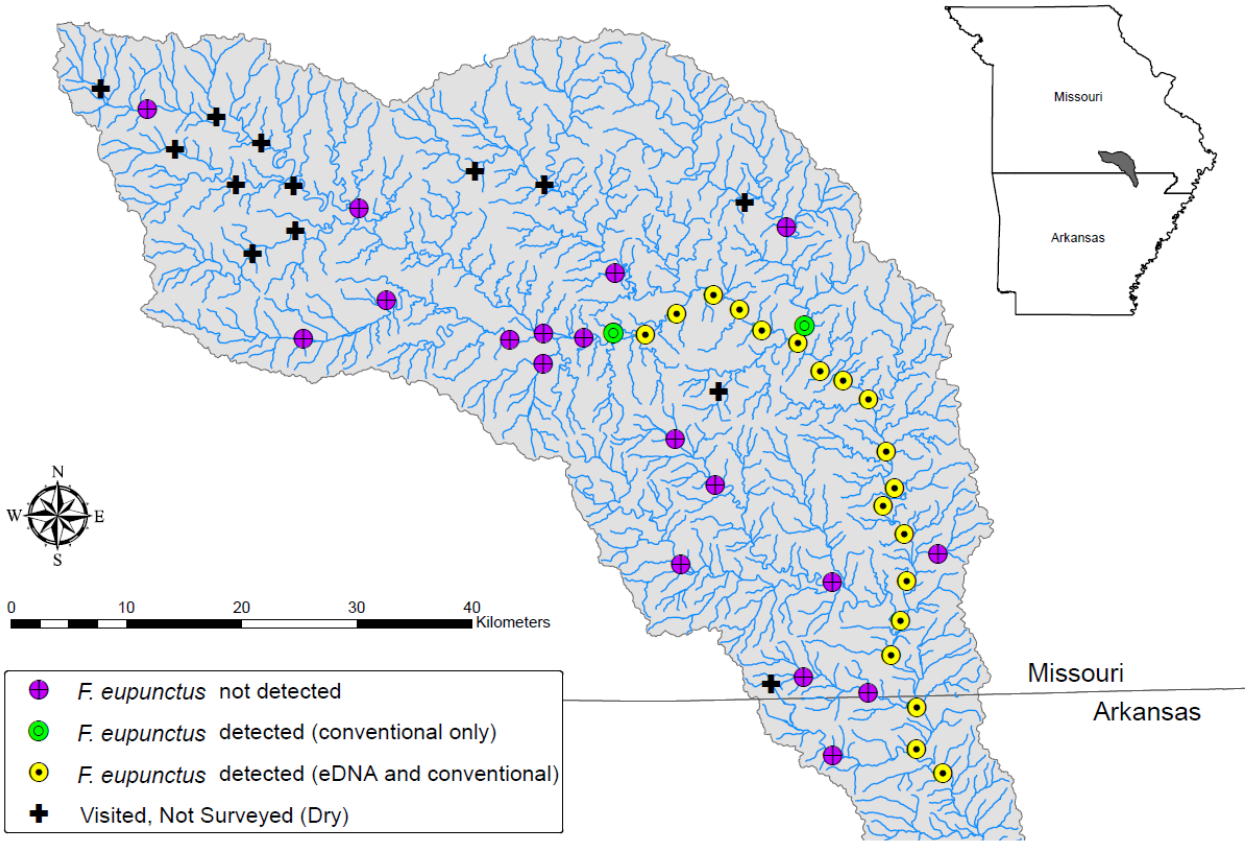

Fig. 3.1: Survey results using conventional and eDNA sampling for Coldwater Crayfish (*Faxonius eupunctus*) in the Eleven Point River drainage, Arkansas and Missouri, U.S. Crosses indicate sites visited but not surveyed using either method due to a lack of surface water. Colored circles indicate results (detected or not detected) at sites surveyed for *F. eupunctus* using both eDNA and conventional sampling methods.

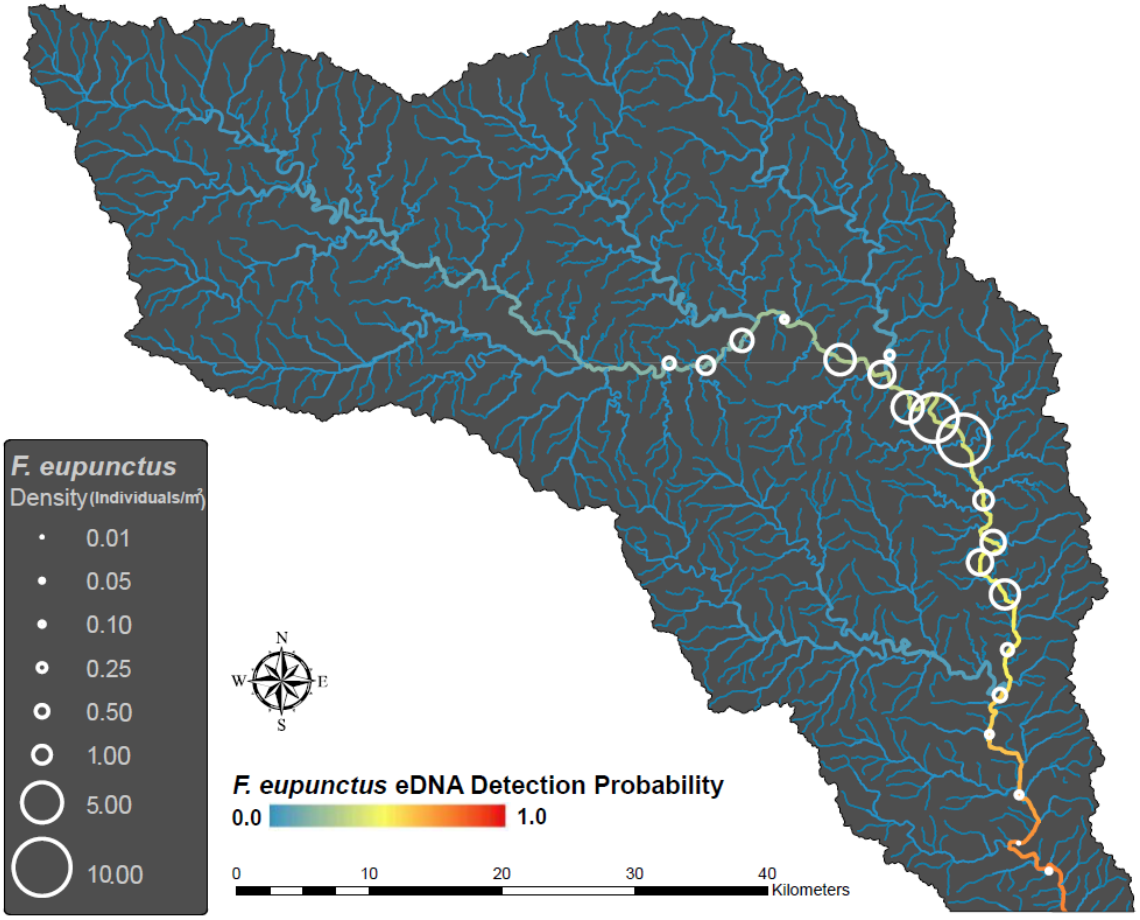

Fig. 3.2: Predicted *Faxonius eupunctus* eDNA detection probabilities in the Eleven Point River drainage, Arkansas and Missouri, U.S.A based on upstream river distance (km; Table 3.2, Table 3.3). Site-scale *F. eupunctus* density estimates (hollow circles; number of individuals/m^2) from conventional sampling are overlaid to illustrate the poor relationship between local *F. eupunctus* density and *F. eupunctus* eDNA detection probability.

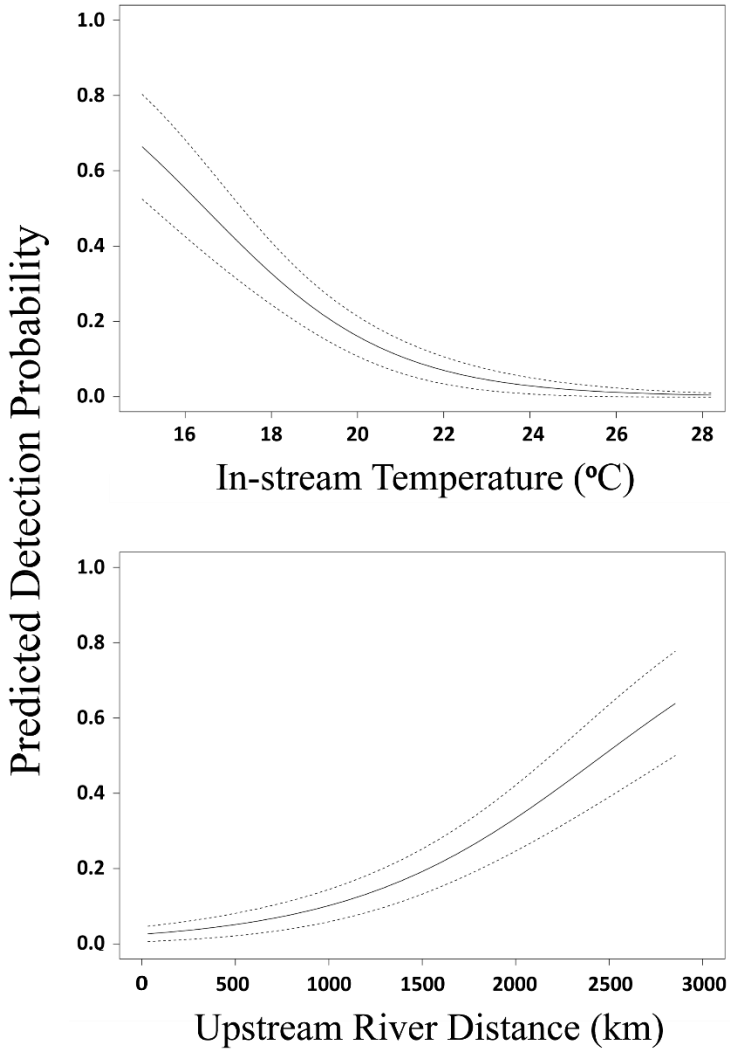

Fig. 3.3: Partial dependence plots showing relationships between predicted *Faxonius eupunctus* eDNA detection probability and site-scale covariates in the Eleven Point River drainage of Arkansas and Missouri, U.S. Only significant relationships from well-performing models are shown (Table 3.2, Table 3.3). Solid lines are predicted *F. eupunctus* eDNA detection probabilities, and dashed lines are 95% confidence intervals.

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SUMMARY

This study is the first to rigorously assess the fine-scale distribution, population densities, and physical habitat associations of the narrowly endemic Coldwater Crayfish (*Faxonius eupunctus*) in the Eleven Point River drainage. I found that *F. eupunctus* primarily occurred in a limited portion of the main stem Eleven Point River and only occurred in the downstream reaches of one of its direct tributary streams. *Faxonius eupunctus* occurred in higher densities within a limited reach of the main stem Eleven Point River, and the species' densities generally decreased moving both down- and upstream from this range in the Eleven Point River. Current velocity, in-stream temperature, and streambed substrate size were strong predictors of local *F. eupunctus* density in both wadeable riffle mesohabitats and non-wadeable pool mesohabitats and may influence where *F. eupunctus* occurs in high densities. These findings have the potential to influence the conservation and management of *F. eupunctus* by aiding government agencies in their deliberations regarding conservation protection for the species. This study therefore provides compelling evidence for the continued use of traditional, field-based surveys in aquatic ecology and demonstrates that sampling methods well optimized for target organisms and study systems can reveal useful information pertaining to the conservation status of rare aquatic taxa.

Results from eDNA sampling revealed that the ability of eDNA to estimate *F. eupunctus* presence rivaled that of my conventional seining approach, but the ability of eDNA to reflect patterns in local *F. eupunctus* abundances could not be evaluated due to low *F. eupunctus* eDNA concentrations at most sites. I found that *F. eupunctus* eDNA detection probability related poorly to local *F. eupunctus* density and instead related strongly to upstream river distance, which served as a proxy for the risk of downstream transport of eDNA. These results bolstered confidence in my assessment of the range and distribution of *F. eupunctus* using conventional sampling methods. Furthermore, I was able to highlight some of the technical limitations of

eDNA applications in lotic environments through my analysis of eDNA detection probability. These findings support much of the current literature in demonstrating that eDNA is a reliable technique for sampling a variety of aquatic macrobiota, although the risk for downstream eDNA transport in some lotic environments suggests that investigators should exercise caution when applying eDNA in rivers and streams.

APPENDIX A: CRAYFISH SAMPLING SUPPLEMENTARY MATERIAL

Table A.1: Number of *Faxonius eupunctus* (EU) collected and physical habitat variables recorded for each quadrat completed in wadeable riffle mesohabitats. Geographic coordinates are unique to the upstream extent of each site rather than individual quadrats. "Canopy" represents the proportion of each quadrat sampler covered by overhead foliage. "Substrate" is the average of five streambed substrate size estimates recorded at each point of a substrate cross (n = 5) according to the following scale: 1 = sand; 1.5 = bedrock; 2 = gravel; 3 = pebble; 4 = cobble; 5 = boulder.

Site_ID	Lat./Long.	EU	Canopy	CV (m/s)	Depth (cm)	Width (m)	Temp (ºC)	Substrate
BFK_1	36.7623, -91.5286	0	0.0016	1.139	24	7.6	26.45	2.8
BFK_1	"	0	0.0848	0.519	13	7.6	26.45	2
BFK_1	"	0	0.064	1.012	22	7.6	26.45	2.8
BFK_1	"	0	0.376	0.35	16	7.6	26.45	3
BFK_1	"	0	0.0328	0.527	21	7.6	26.45	2.2
BFK_1	"	0	0.0016	0.493	26	7.3	26.45	3
BFK_1	"	0	0.0016	0.598	26	7.3	26.45	2.2
BFK_1	"	0	0.012	0.988	26	7.3	26.45	2.6
BFK_1	"	0	0.0224	0.455	15	7.3	26.45	2.6
BFK_1	"	0	0.0952	0.511	20	9.4	26.45	2
BFK_1	"	0	0.1056	0.952	21	9.4	26.45	2.6
BFK_1	"	0	0.0016	0.886	28	9.4	26.45	4.8
DC_2	36.4519, -91.2540	0	0.4592	0.349	13	1.6	28.1	3.4
DC_2	"	0	0.4592	0.49	24	1.6	28.1	3.1
DC_2	"	0	0.4176	0.573	12	1.6	28.1	3.2
DC_2	"	0	0.7712	0.598	12	1.6	28.1	3
DC_2	"	0	0.844	0.251	6	1.6	28.1	3
DC_2	"	0	0.9584	0.705	17	1.3	28.1	4.2
DC_2	"	0	0.8752	0.469	10	1.3	28.1	4
DC_2	"	0	0.5216	0.174	4	5.5	28.1	3.6
DC_2	"	0	0.6672	0.332	4	5.5	28.1	3.6
DC_2	"	0	0.5424	0.435	6	5.5	28.1	3
DC_2	"	0	0.8752	0.144	2	5.5	28.1	2.6
DC_2	"	0	0.9584	0.342	5	5.5	28.1	3.2
EPR_8	36.9653, -91.9108	0	0.0952	0.883	10	5	24.72	2.8
EPR_8	"	0	0.3864	0.724	6	5	24.72	3.8
EPR_8	"	0	0.584	0.685	13	5	24.72	2.2
EPR_8	"	0	0.449	0.339	8	5	24.72	3.4
EPR_8	"	0	0.1888	1.239	22	3.5	24.72	3.6
EPR_8	"	0	0.48	0.892	16	3.5	24.72	3
EPR_8	"	0	0.948	0.851	3.5	2	24.72	3
EPR_8	"	0	0.7504	0.882	11	8.6	24.72	4.2
EPR_8	"	0	0.8752	0.787	9	8.6	24.72	3.6
EPR_8	"	0	0.5112	0.805	9	8.6	24.72	4.8
EPR_8	"	0	0.22	0.616	14	8.6	24.72	3.2
EPR_8	"	0	0.4904	0.367	14	8.6	24.72	4.2

Table A.1 cont'd

Site_ID	Lat./Long.	EU	Canopy	CV (m/s)	Depth (cm)	Width (m)	Temp (ºC)	Substrate
EPR_16	36.8856, -91.7057	0	0.584	0.941	18	3.6	17.61	3.4
EPR_16	"	0	0.6568	0.782	16	3.6	17.61	3.4
EPR_16	"	0	0.7608	0.459	13	3.6	17.61	2.6
EPR_16	"	0	0.9376	0.357	15	3.6	17.61	2.8
EPR_16	"	0	0.948	0.88	22	3.6	17.61	2.4
EPR_16	"	0	0.9168	0.754	28	3.6	17.61	2.8
EPR_16	"	0	0.9584	0.822	28	3.6	17.61	2.6
EPR_16	"	0	0.8128	0.845	35	3.6	17.61	2.4
EPR_16	"	0	0.5216	0.762	20	3.6	17.61	2.6
EPR_16	"	0	0.3032	0.394	12	3.6	17.61	3.2
EPR_16	"	0	0.584	0.375	21	4.3	17.61	2.6
EPR_16	"	0	0.5112	0.408	22	4.3	17.61	3.2
EPR_23	36.7859, -91.5279	0	0.3136	0.289	6	11.9	20.71	2.2
EPR_23	"	0	0.8024	0.851	60	11.9	20.71	2.4
EPR_23	"	0	0.6464	0.254	7	11.9	20.71	2.4
EPR_23	"	0	0.8648	1.313	92	11.9	20.71	2.8
EPR_23	"	0	0.4904	0.395	13	11.9	20.71	3
EPR_23	"	0	0.9584	0.331	86	11.9	20.71	2.4
EPR_23	"	0	0.3552	0.458	14	11.9	20.71	1.8
EPR_23	"	0	0.8896	1.318	73	11.9	20.71	2.2
EPR_23	"	0	0.6568	0.427	15	11.9	20.71	2.8
EPR_23	"	0	0.7816	1.123	68	11.9	20.71	2.6
EPR_23	"	0	0.0432	0.526	22	11.9	20.71	2.8
EPR_23	"	0	0.1472	0.605	15	11.9	20.71	2.6
EPR_24	36.7818, -91.4886	0	1	0.424	27	9.6	20.67	2.4
EPR_24	"	0	1	0.717	24	9.6	20.67	2.6
EPR_24	"	0	0.3448	0.713	39	9.6	20.67	2.2
EPR_24	"	0	0.4904	0.433	20	9.6	20.67	2.4
EPR_24	"	0	0.8544	0.636	13	9.6	20.67	3.4
EPR_24	"	0	0.792	0.636	13	9.6	20.67	3.2
EPR_24	"	0	0.4592	0.996	16	9.6	20.67	2.8
EPR_24	"	0	0.324	0.927	19	9.6	20.67	3.2
EPR_24	"	0	0.792	1.034	22	9.6	20.67	3.8
EPR_24	"	0	0.8648	0.781	20	9.6	20.67	2.2
EPR_24	"	0	0.22	0.646	30	9.6	20.67	2.8
EPR_24	"	0	0.1784	0.751	45	9.6	20.67	3.4
EPR_25	36.7853, -91.4598	1	0.0016	0.699	27	24	20.63	3.8
EPR_25	"	0	0.0744	0.908	31	24	20.63	3.6
EPR_25	"	0	0.1368	0.794	28	24	20.63	3.2
EPR_25	"	0	0.0744	0.814	32	24	20.63	3.2
EPR_25	"	1	0.012	0.855	34	24	20.63	4
EPR_25	"	0	0.0016	0.804	24	24	20.63	3.4

Table A.1 cont'd

Site_ID	Lat./Long.	EU	Canopy	CV (m/s)	Depth (cm)	Width (m)	Temp (ºC)	Substrate
EPR_25	"	0	0.064	0.873	20	24	20.63	3
EPR_25	"	0	0.0016	1.041	22	24	20.63	3.6
EPR_25	"	1	0.0016	1.041	28	24	20.63	3.2
EPR_25	"	0	0.012	0.821	30	24	20.63	3.2
EPR_25	"	1	0.0224	0.727	24	24	20.63	3.4
EPR_25	"	1	0.3136	0.683	21	24	20.63	2.2
EPR_26	36.7833, -91.4290	1	0.25	0.56	20	18.5	20.62	3
EPR_26	"	0	0.116	0.557	35	18.5	20.62	3.2
EPR_26	"	1	0.116	0.88	42	18.5	20.62	4.2
EPR_26	"	2	0.261	0.328	27	18.5	20.62	4.4
EPR_26	"	0	0.4384	0.209	26	18.5	20.62	4.2
EPR_26	"	1	0.6152	0.445	27	18.5	20.62	3
EPR_26	"	1	0.3136	0.867	61	18.5	20.62	3
EPR_26	"	2	0.7504	0.615	32	18.5	20.62	4.4
EPR_26	"	1	0.5632	0.332	23	18.5	20.62	4
EPR_26	"	2	0.9792	0.609	37	18.5	20.62	4.8
EPR_26	"	0	1	0.84	66	18.5	20.62	3.8
EPR_26	"	0	0.9272	0.56	26	18.5	20.62	4.4
EPR_27	36.7998, -91.3981	0	0.0328	0.83	28	9.9	20.6	2.8
EPR_27	"	1	0.0224	1.04	38	9.9	20.6	3
EPR_27	"	0	0.0536	0.896	40	9.9	20.6	2.4
EPR_27	"	0	0.1888	0.594	44	9.9	20.6	2.8
EPR_27	"	2	0.012	0.542	35	9.9	20.6	2.4
EPR_27	"	0	0.168	0.582	38	9.9	20.6	2.6
EPR_27	"	0	0.1784	0.269	36	9.9	20.6	2.8
EPR_27	"	2	0.3136	0.906	23	9.9	20.6	2.8
EPR_27	"	1	0.324	0.847	24	9.2	20.6	2.8
EPR_27	"	4	0.0328	0.96	25	9.2	20.6	2.6
EPR_27	"	8	0.012	0.958	28	9.2	20.6	3.4
EPR_27	"	0	0.0744	0.907	31	9.2	20.6	2.8
EPR_28	36.8137, -91.3619	0	0.0016	1.89	20	14.1	20.57	3.6
EPR_28	"	0	0.0016	1.03	13	14.1	20.57	3.2
EPR_28	"	0	0.0016	1.055	16	14.1	20.57	3.2
EPR_28	"	0	0.0016	1.416	31	14.1	20.57	4
EPR_28	"	0	0.012	1.02	20	14.1	20.57	3.4
EPR_28	"	1	0.012	1.041	16	14.1	20.57	2.8
EPR_28	"	0	0.0016	0.407	11	11.6	20.57	2.8
EPR_28	"	0	0.012	0.884	28	11.6	20.57	2.2
EPR_28	"	0	0.0536	1.103	30	11.6	20.57	2
EPR_28	"	1	0.116	1.111	34	11.6	20.57	2
EPR_28	"	0	0.012	0.898	22	11.6	20.57	2.6
EPR_28	"	0	0.064	0.998	34	11.6	20.57	2.4

Table A.1 cont'd

Site_ID	Lat./Long.	EU	Canopy	CV (m/s)	Depth (cm)	Width (m)	Temp (°C)	Substrate
EPR_30	36.7857, -91.3157	4	0.0848	0.444	16	34.3	15.6	3
EPR_30	"	5	0.064	0.657	46	34.3	15.6	3
EPR_30	"	4	0.0016	0.752	75	34.3	15.6	3.2
EPR_30	"	4	0.116	0.689	50	34.3	15.6	3.4
EPR_30	"	2	0.952	0.73	43	34.3	15.6	2.6
EPR_30	"	1	0.0536	0.793	74	34.3	15.6	3.2
EPR_30	"	2	0.0328	0.842	54	34.3	15.6	3.2
EPR_30	"	1	0.0016	0.72	60	34.3	15.6	3.2
EPR_30	"	1	0.0952	0.666	48	34.3	15.6	3.8
EPR_30	"	5	0.0744	0.563	64	34.3	15.6	3.2
EPR_30	"	2	0.0224	0.72	82	34.3	15.6	3.6
EPR_30	"	2	0.012	0.522	57	34.3	15.6	3.4
EPR_31	36.7752, -91.2805	10	0.0016	0.994	32	39	15.7	3.8
EPR_31	"	1	0.0016	0.815	36	39	15.7	4
EPR_31	"	1	0.0016	1.261	28	39	15.7	3.4
EPR_31	"	1	0.0016	0.968	21	39	15.7	2.6
EPR_31	"	5	0.0016	0.699	16	39	15.7	2.8
EPR_31	"	3	0.0016	0.726	19	39	15.7	3.4
EPR_31	"	0	0.0016	1.321	36	39	15.7	3.6
EPR_31	"	0	0.0016	0.824	21	39	15.7	3.4
EPR_31	"	1	0.0016	0.665	14	39	15.7	4
EPR_31	"	1	0.0016	1.195	22	39	15.7	3.2
EPR_31	"	0	0.0016	0.801	24	39	15.7	3.6
EPR_31	"	1	0.0016	1.397	36	39	15.7	4
EPR_32	36.7525, -91.2594	0	0.0016	0.483	9	36.2	15.9	2.4
EPR_32	"	2	0.0016	0.273	8	36.2	15.9	3
EPR_32	"	1	0.0016	1.415	36	36.2	15.9	3.8
EPR_32	"	3	0.0016	0.911	26	36.2	15.9	3.2
EPR_32	"	1	0.0328	0.345	17	36.2	15.9	2.4
EPR_32	"	4	0.0016	0.664	23	36.2	15.9	3
EPR_32	"	3	0.8232	0.43	15	20	15.9	3
EPR_32	"	8	0.9584	0.867	27	20	15.9	3.4
EPR_32	"	6	0.3136	0.884	46	20	15.9	3.4
EPR_32	"	6	0.8752	1.085	35	20	15.9	2.8
EPR_32	"	1	0.636	1.315	37	20	15.9	3.2
EPR_32	"	2	0.1885	0.871	18	20	15.9	3.4
EPR_33	36.7449, 91-2371	0	0.1472	0.249	14	10.1	16.2	2.6
EPR_33	"	4	0.8788	0.696	25	10.1	16.2	2.6
EPR_33	"	6	0.22	0.674	22	10.1	16.2	2.8
EPR_33	"	7	0.0016	0.893	20	10.1	16.2	3.4
EPR_33	"	16	0.0016	0.67	27	10.1	16.2	3.6
EPR_33	"	12	0.0016	0.704	27	10.1	16.2	3.2

Table A.1 cont'd

Site_ID	Lat./Long.	EU	Canopy	CV (m/s)	Depth (cm)	Width (m)	Temp (ºC)	Substrate
EPR_33	"	10	0.0016	0.53	23	10.1	16.2	3
EPR_33	"	7	0.0016	0.678	44	10.1	16.2	2.8
EPR_33	"	4	0.0016	0.513	50	10.1	16.2	2.4
EPR_33	"	3	0.0016	0.735	43	10.1	16.2	4
EPR_33	"	5	0.0016	0.672	14	10.1	16.2	3.6
EPR_33	"	10	0.0016	0.683	26	10.1	16.2	3
EPR_34	36.7297, -91.2129	0	0.324	0.947	33	25.9	16.6	2.8
EPR_34	"	0	0.324	1.151	27	25.9	16.6	2.8
EPR_34	"	1	0.324	1.14	36	25.9	16.6	2.6
EPR_34	"	0	0.2824	1.147	30	25.9	16.6	3.4
EPR_34	"	0	0.3552	1.253	35	25.9	16.6	2.8
EPR_34	"	4	0.1888	1.162	26	25.9	16.6	2.6
EPR_34	"	15	0.0016	0.315	13	30	16.6	3.2
EPR_34	"	14	0.0016	0.711	28	30	16.6	2
EPR_34	"	16	0.0016	1.106	37	30	16.6	4
EPR_34	"	14	0.0016	0.704	33	30	16.6	3.6
EPR_34	"	15	0.0016	0.235	16	30	16.6	3.8
EPR_34	"	16	0.0016	0.85	28	30	16.6	3.4
EPR_35	36.6888, -91.1969	5	0.0016	0.651	45	14.7	16.8	2.6
EPR_35	"	2	0.0016	0.931	44	7.38	16.8	3
EPR_35	"	2	0.0016	0.805	53	14.7	16.8	2
EPR_35	"	2	0.0016	0.759	36	7.38	16.8	3
EPR_35	"	1	0.0224	0.855	65	14.7	16.8	3
EPR_35	"	1	0.0224	0.988	28	7.38	16.8	2.6
EPR_35	"	0	0.0016	0.782	44	19.17	16.8	2.8
EPR_35	"	0	0.0016	0.543	47	19.17	16.8	1.8
EPR_35	"	0	0.0224	0.547	46	19.17	16.8	1.4
EPR_35	"	0	0.0224	0.617	33	19.17	16.8	1.4
EPR_35	"	0	0.0016	0.729	34	19.17	16.8	2.2
EPR_35	"	0	0.0536	0.681	27	19.17	16.8	2.6
EPR_36	36.6599, -91.1893	1	0.0016	0.739	42	44.6	18.2	2.4
EPR_36	"	3	0.0016	0.989	85	44.6	18.2	3
EPR_36	"	2	0.0016	1.224	60	44.6	18.2	2.8
EPR_36	"	0	0.0016	1.168	57	44.6	18.2	2.8
EPR_36	"	4	0.0016	0.717	38	44.6	18.2	3.2
EPR_36	"	2	0.0016	1.152	56	44.6	18.2	3.6
EPR_36	"	2	0.0016	0.986	48	44.6	18.2	3.2
EPR_36	"	2	0.0016	1.086	51	44.6	18.2	2.4
EPR_36	"	0	0.0016	0.848	38	44.6	18.2	2.4
EPR_36	"	5	0.0016	1.168	46	44.6	18.2	2.4
EPR_36	"	0	0.0016	1.444	54	44.6	18.2	2.8
EPR_36	"	0	0.0016	0.889	47	44.6	18.2	2.8

Table A.1 cont'd

Site_ID	Lat./Long.	EU	Canopy	CV (m/s)	Depth (cm)	Width (m)	Temp (ºC)	Substrate
EPR_37	36.6467, -91.2008	4	0.0016	0.596	34	28.9	19.21	3.6
EPR_37	"	0	0.0016	0.553	28	28.9	19.21	3.2
EPR_37	"	2	0.0016	0.704	18	28.9	19.21	3
EPR_37	"	0	0.0016	0.865	19	28.9	19.21	3.8
EPR_37	"	2	0.0016	1.278	34	28.9	19.21	3.2
EPR_37	"	2	0.0016	0.85	18	28.9	19.21	3.4
EPR_37	"	2	0.0016	0.834	16	28.9	19.21	2.8
EPR_37	"	1	0.0016	0.935	24	28.9	19.21	3.6
EPR_37	"	1	0.0016	1.194	44	28.9	19.21	2.6
EPR_37	"	1	0.0016	0.751	30	28.9	19.21	3.8
EPR_37	"	4	0.0016	0.571	20	28.9	19.21	3
EPR_37	"	3	0.0016	0.895	38	28.9	19.21	3.6
EPR_38	36.6246, -91.1804	6	1	0.471	29	5.1	19.58	2.6
EPR_38	"	6	1	0.812	24	5.1	19.58	2.8
EPR_38	"	11	1	0.909	27	5.1	19.58	2.8
EPR_38	"	4	1	0.618	37	5.1	19.58	2.6
EPR_38	"	1	0.12	0.97	40	30.7	19.58	2.6
EPR_38	"	0	0.0016	1.222	48	30.7	19.58	3.2
EPR_38	"	1	0.0016	1.131	46	30.7	19.58	3
EPR_38	"	0	0.0016	1.28	58	30.7	19.58	2.8
EPR_38	"	0	0.012	0.903	36	13.1	19.58	3
EPR_38	"	0	0.1056	0.047	40	13.1	19.58	2.8
EPR_38	"	1	0.1264	0.837	36	13.1	19.58	2.4
EPR_38	"	0	0.0744	0.857	47	13.1	19.58	2.8
EPR_39	36.5871, -91.1791	0	1	0.694	41	11.9	19.98	1.6
EPR_39	"	0	0.4696	0.607	39	11.9	19.98	2.8
EPR_39	"	0	0.0952	0.677	46	11.9	19.98	2.4
EPR_39	"	2	0.5008	0.495	36	11.9	19.98	2.2
EPR_39	"	0	0.688	0.878	46	11.9	19.98	2.2
EPR_39	"	1	0.3344	0.508	37	11.9	19.98	2.4
EPR_39	"	0	0.0224	0.807	38	11.9	19.98	3
EPR_39	"	0	0.9792	0.618	26	11.9	19.98	2.4
EPR_39	"	1	0.3136	0.751	58	11.9	19.98	2.8
EPR_39	"	0	0.3552	0.831	27	11.9	19.98	3.8
EPR_39	"	0	0.064	1.067	50	11.9	19.98	3
EPR_39	"	1	0.1472	0.911	46	11.9	19.98	2.6
EPR_40	36.5564, -91.1861	0	0.0016	0.573	28	7.94	20.39	2.2
EPR_40	"	0	0.0016	0.883	40	7.94	20.39	2.8
EPR_40	"	1	0.0016	1.139	64	7.94	20.39	3.2
EPR_40	"	0	0.0016	0.533	38	12.95	20.39	2
EPR_40	"	0	0.0016	0.823	22	12.95	20.39	2
EPR_40	"	1	0.0016	0.622	20	12.95	20.39	2.6

Table A.1 cont'd

Site_ID	Lat./Long.	EU	Canopy	CV (m/s)	Depth (cm)	Width (m)	Temp (°C)	Substrate
EPR_40	"	1	0.0016	0.274	10	11.5	20.39	2.8
EPR_40	"	1	0.0016	0.893	27	11.5	20.39	2.6
EPR_40	"	0	0.0016	0.408	12	11.5	20.39	2.4
EPR_40	"	1	0.0016	0.659	30	11.5	20.39	2.6
EPR_40	"	0	0.0016	0.811	28	11.5	20.39	2.8
EPR_40	"	1	0.0016	0.615	28	11.5	20.39	2.2
EPR_41	36.5299, -91.1957	0	0.0432	1.023	53	12.7	19.98	3
EPR_41	"	0	0.0848	1.124	51	12.7	19.98	3
EPR_41	"	0	0.7088	0.813	66	12.7	19.98	2.4
EPR_41	"	0	0.0016	1.106	50	12.7	19.98	3
EPR_41	"	0	0.0016	0.854	42	12.7	19.98	2.6
EPR_41	"	1	0.0016	0.63	35	23.2	19.98	2.6
EPR_41	"	0	0.0016	0.933	40	23.2	19.98	3.6
EPR_41	"	0	0.0016	1.143	48	23.2	19.98	3
EPR_41	"	1	0.0016	0.945	42	23.2	19.98	3.6
EPR_41	"	0	0.0016	0.983	36	23.2	19.98	3.4
EPR_41	"	0	0.0016	1.038	44	23.2	19.98	3.2
EPR_41	"	0	0.0016	1.182	46	23.2	19.98	3.8
EPR_42	36.4885, -91.1716	0	0.0848	0.297	32	35.5	19.7	2.6
EPR_42	"	0	0.0016	0.357	14	35.5	19.7	2.6
EPR_42	"	0	0.1576	0.5	49	35.5	19.7	2.4
EPR_42	"	0	0.0016	0.587	16	35.5	19.7	1.8
EPR_42	"	0	0.0016	1.021	24	35.5	19.7	2.8
EPR_42	"	1	0.7088	0.464	24	35.5	19.7	2.6
EPR_42	"	0	0.0016	0.53	28	35.5	19.7	3.2
EPR_42	"	0	0.0016	0.636	18	35.5	19.7	3.8
EPR_42	"	1	0.0016	0.837	30	35.5	19.7	2.6
EPR_42	"	0	0.0016	0.896	36	35.5	19.7	2.8
EPR_42	"	0	0.064	0.982	18	35.5	19.7	3.2
EPR_42	"	0	0.0016	0.759	39	35.5	19.7	2.8
EPR_43	36.4559, -91.1728	0	0.0016	0.343	10	29.3	20	2.6
EPR_43	"	0	0.0016	0.478	11	29.3	20	2
EPR_43	"	0	0.0016	0.595	14	29.3	20	2.2
EPR_43	"	0	0.0016	0.737	38	29.3	20	2.6
EPR_43	"	0	0.0016	1.117	48	29.3	20	2.2
EPR_43	"	0	0.0016	1.025	40	29.3	20	3.4
EPR_43	"	0	0.0016	0.748	29	29.3	20	2.4
EPR_43	"	0	0.0016	0.697	22	29.3	20	2.8
EPR_43	"	0	0.0016	0.849	28	29.3	20	2
EPR_43	"	0	0.0016	1.149	40	29.3	20	2.4
EPR_43	"	0	0.0016	1.005	46	29.3	20	3
EPR_43	"	0	0.0016	0.534	51	29.3	20	2

Table A.1 cont'd

Site_ID	Lat./Long.	EU	Canopy	CV (m/s)	Depth (cm)	Width (m)	Temp (ºC)	Substrate
EPR_44	36.4366, -91.1477	0	0.0016	0.266	16	13.6	20.7	2.6
EPR_44	"	0	0.0016	0.697	40	13.6	20.7	2.4
EPR_44	"	0	0.0016	1.115	48	13.6	20.7	2.4
EPR_44	"	0	0.0016	0.575	24	13.6	20.7	2.8
EPR_44	"	1	0.0016	0.306	22	10.3	20.7	2.4
EPR_44	"	0	0.0016	1	38	10.3	20.7	2
EPR_44	"	0	0.0016	1.456	76	10.3	20.7	2.6
EPR_44	"	0	0.0016	1.109	47	10.3	20.7	2.6
EPR_44	"	0	0.0016	0.666	22	10.3	20.7	2.8
EPR_44	"	0	0.0016	1.14	40	10.3	20.7	3.2
EPR_44	"	0	0.0016	1.378	42	10.3	20.7	2.6
EPR_44	"	0	0.0016	1.456	49	10.3	20.7	3
FRC_2	36.5879, -91.2513	0	0.948	0.936	30	2.6	24.9	2.4
FRC_2	"	0	0.5424	1.64	48	2.6	24.9	3.8
FRC_2	"	0	0.4072	1.266	42	2.6	24.9	3
FRC_2	"	0	0.272	1.853	52	2.6	24.9	3.4
FRC_2	"	0	0.8232	0.903	24	2.6	24.9	2.2
FRC_2	"	0	0.0224	1.287	32	2.6	24.9	3.8
FRC_2	"	0	0.9792	0.953	21	2.6	24.9	1.8
FRC_2	"	0	0.0016	1.27	40	2.6	24.9	4.8
FRC_2	"	0	0.8752	0.921	18	2.6	24.9	2.6
FRC_2	"	0	0.012	1.082	24	2.6	24.9	2.8
FRC_2	"	0	0.9798	0.939	27	2.6	24.9	3
FRC_2	"	0	0.0016	0.937	43	2.6	24.9	3.2
FRC_12	36.6036, -91.3979	0	0.0952	0.27	44	9.2	21.9	2.8
FRC_12	"	0	0.4504	0.74	47	9.2	21.9	2.2
FRC_12	"	0	0.584	0.706	41	9.2	21.9	2.4
FRC_12	"	0	0.4488	0.61	35	9.2	21.9	3.8
FRC_12	"	0	0.1888	0.452	15	9.2	21.9	2
FRC_12	"	0	0.48	0.744	24	9.2	21.9	2.8
FRC_12	"	0	0.948	0.791	33	9.2	21.9	3.6
FRC_12	"	0	0.7504	0.519	23	9.2	21.9	3
FRC_12	"	0	0.8752	0.934	23	11.7	21.9	3.2
FRC_12	"	0	0.5112	0.509	26	11.7	21.9	3.8
FRC_12	"	0	0.22	0.705	17	11.7	21.9	3.4
FRC_12	"	0	0.4904	0.703	22	11.7	21.9	2.4
HC_1	36.6082, -91.1483	0	0.6256	0.213	4	4.4	22	3.6
HC_1	"	0	0.8856	0.302	4	4.4	22	3.6
HC_1	"	0	0.5944	0.116	5	4.4	22	3.4
HC_1	"	0	0.6568	0.17	6	4.4	22	3
HC_1	"	0	0.8856	0.088	12	4.4	22	2.8
HC_1	"	0	0.636	0.617	10	5.65	22	3.6

Table A.1 cont'd

Site_ID	Lat./Long.	EU	Canopy	CV (m/s)	Depth (cm)	Width (m)	Temp (°C)	Substrate
HC_1	"	0	0.0792	0.45	6	5.65	22	3.4
HC_1	"	0	0.5424	0.277	8	5.65	22	2.8
HC_1	"	0	0.2616	0.12	3	5.65	22	3.8
HC_1	"	0	0.5111	0.083	7	5.65	22	2.6
HC_1	"	0	0.8648	0.174	12	5.65	22	2.8
HC_1	"	0	0.4696	0.069	16	5.65	22	4.4
HC_10	36.7881, -91.2737	0	0.844	0.424	28	6.2	20.6	3
HC_10	"	0	0.896	0.576	17	6.2	20.6	3.2
HC_10	"	1	0.9792	0.464	17	6.2	20.6	2.6
HC_10	"	0	0.9064	0.51	21	6.2	20.6	2.8
HC_10	"	0	0.9498	0.467	9	5.3	20.6	2.8
HC_10	"	0	0.9498	0.632	30	5.3	20.6	3.8
HC_10	"	0	0.4488	0.961	16	4.9	20.6	3.4
HC_10	"	2	0.5424	0.753	16	4.9	20.6	3.4
HC_10	"	0	0.4196	0.7	14	4.9	20.6	3
HC_10	"	0	0.6776	0.665	11	6.3	20.6	1.8
HC_10	"	0	0.0952	0.697	12	6.3	20.6	2.8
HC_10	"	0	0.636	0.234	11	6.3	20.6	2
MC_1	36.5140, -91.2810	0	1	0.168	8	4.5	19.11	2.6
MC_1	"	0	0.0016	0.308	6	4.5	19.11	2.4
MC_1	"	0	0.0536	0.295	6	4.5	19.11	2
MC_1	"	0	0.2512	0.117	6	4.5	19.11	2.6
MC_1	"	0	0.0016	0.054	4	4.5	19.11	2
MC_1	"	0	0.3448	0.337	16	1.7	19.11	3.8
MC_1	"	0	0.1472	0.357	11	1.7	19.11	4.2
MC_1	"	0	0.6618	0.439	9	1.7	19.11	3.4
MC_1	"	0	0.5632	0.227	8	1.7	19.11	4
MC_1	"	0	0.9376	0.348	14	2.8	19.11	3.6
MC_1	"	0	0.0016	0.315	6	2.8	19.11	2.4
MC_1	"	0	0.0328	0.093	6	2.8	19.11	2.4
MC_3	36.5004, -91.2187	0	0.4904	0.333	7	7.4	27.13	2
MC_3	"	0	0.394	0.234	5	7.4	27.13	1.6
MC_3	"	0	0.3032	0.313	5	7.4	27.13	1.6
MC_3	"	0	0.4904	0.317	8	7.4	27.13	1.6
MC_3	"	0	0.4176	0.018	18	7.15	27.13	3.4
MC_3	"	0	0.0016	0.326	5	7.15	27.13	2
MC_3	"	0	0.0952	0.167	5	7.15	27.13	2
MC_3	"	0	0.1264	0.394	12	7.15	27.13	2.8
MC_3	"	0	0.0848	0.67	12	7.15	27.13	2.8
MC_3	"	0	0.0016	0.394	10	8.87	27.13	2.8
MC_3	"	0	0.0016	0.058	8	8.87	27.13	3
MC_3	"	0	0.0016	0.204	10	8.87	27.13	2.6

Table A.1 cont'd

Site_ID	Lat./Long.	EU	Canopy	CV (m/s)	Depth (cm)	Width (m)	Temp (ºC)	Substrate
MF_3	36.7846, -91.7614	0	0.77712	0.479	22	6.7	24.71	2.8
MF_3	"	0	0.6152	0.206	27	6.7	24.71	2.6
MF_3	"	0	0.5362	0.296	19	6.7	24.71	3.4
MF_3	"	0	0.5736	0.041	16	6.7	24.71	3.4
MF_3	"	0	0.6152	1.12	15	6.9	24.71	3.6
MF_3	"	0	0.5008	0.88	13	6.9	24.71	4.2
MF_3	"	0	0.5008	0.15	10	6.9	24.71	4.2
MF_3	"	0	0.896	0.212	23	4.1	24.71	1.5
MF_3	"	0	0.8024	0.693	19	4.1	24.71	1.5
MF_3	"	0	0.7504	0.624	24	4.1	24.71	1.5
MF_3	"	0	0.896	0.651	20	4.1	24.71	1.5
MF_3	"	0	0.7608	0.64	32	4.1	24.71	1.5
MF_6	36.8138, -91.6802	0	0.7608	1.038	34	6.6	22.24	4.4
MF_6	"	0	0.948	0.845	26	6.6	22.24	4.4
MF_6	"	0	0.8024	0.561	22	6.6	22.24	4.2
MF_6	"	0	0.6672	1.174	28	6.6	22.24	3.8
MF_6	"	0	0.4384	0.351	32	6.6	22.24	3.8
MF_6	"	0	0.3448	0.67	32	6.6	22.24	3.4
MF_6	"	0	0.8752	0.998	17	10.5	22.24	3.2
MF_6	"	0	0.7269	0.292	21	10.5	22.24	4.8
MF_6	"	0	0.636	0.932	17	10.5	22.24	3.6
MF_6	"	0	0.864	1.11	26	8.8	22.24	4.4
MF_6	"	0	0.8866	0.319	37	8.8	22.24	4.8
MF_6	"	0	0.7816	0.535	32	8.8	22.24	3.8
MF_7	36.7815, -91.5608	0	0.0016	1.14	42	2.3	23.5	3.6
MF_7	"	0	0.0016	1.098	38	5.6	23.5	3.4
MF_7	"	0	0.0224	1.244	46	2.3	23.5	2.8
MF_7	"	0	0.0016	1.21	36	2.3	23.5	3.4
MF_7	"	0	0.0744	1.143	32	5.6	23.5	3
MF_7	"	0	0.1056	1.052	52	2.3	23.5	2.6
MF_7	"	0	0.012	0.993	36	5.6	23.5	3.2
MF_7	"	0	0.0016	1.03	29	5.6	23.5	2.6
MF_7	"	0	0.0016	1.16	42	5.6	23.5	2.4
MF_7	"	0	0.064	1.189	48	2.3	23.5	3.4
MF_7	"	0	0.012	1.469	48	2.3	23.5	3.2
MF_7	"	0	0.064	1.13	38	5.6	23.5	4.2
PC_3	36.6654, -91.3633	0	0.376	0.726	18	4.3	23.9	3.8
PC_3	"	0	0.3968	0.161	21	4.3	23.9	4
PC_3	"	0	0.428	0.579	26	4.3	23.9	2.4
PC_3	"	0	0.5632	0.684	22	4.3	23.9	2.8
PC_3	"	0	0.9168	0.523	22	4.3	23.9	2.4
PC_3	"	0	0.584	0.712	20	4.3	23.9	4

Table A.1 cont'd

Site_ID	Lat./Long.	EU	Canopy	CV (m/s)	Depth (cm)	Width (m)	Temp (ºC)	Substrate
PC_3	"	0	0.3448	0.648	16	4.3	23.9	3
PC_3	"	0	0.8336	0.657	21	4.3	23.9	2.8
PC_3	"	0	0.9688	0.308	19	4.3	23.9	2.4
PC_3	"	0	0.9772	0.404	19	4.3	23.9	2.8
PC_3	"	0	0.5424	0.331	19	4.3	23.9	3
PC_3	"	0	0.8752	0.289	12	4.3	23.9	2.6
PC_6	36.7017, -91.4015	0	0.0016	0.589	21	8.57	24.4	2.8
PC_6	"	0	0.0016	0.6	7	8.57	24.4	1.6
PC_6	"	0	0.0016	0.133	7	8.57	24.4	1.8
PC_6	"	0	0.0016	0.112	12	8.57	24.4	2.2
PC_6	"	0	0.0016	0.352	8	8.57	24.4	3
PC_6	"	0	0.0016	0.256	12	8.57	24.4	2.6
PC_6	"	0	0.0016	0.276	4	8.57	24.4	2.8
PC_6	"	0	0.0016	0.126	3	8.57	24.4	2.4
PC_6	"	0	0.0016	0.224	7	8.57	24.4	2.4
PC_6	"	0	0.0016	0.132	4	8.57	24.4	3
PC_6	"	0	0.116	0.116	6	8.57	24.4	3
PC_6	"	0	0.0016	0.078	6	8.57	24.4	2.6
SPC_6	36.8321, -91.4571	0	0.5632	0.385	14	2.3	21.14	2.8
SPC_6	"	0	0.6364	0.263	16	2.3	21.14	2.8
SPC_6	"	0	0.6152	0.383	10	2.3	21.14	3
SPC_6	"	0	0.5008	0.261	12	2.3	21.14	2.6
SPC_6	"	0	0.1784	0.122	8	2.3	21.14	3.4
SPC_6	"	0	0.8238	0.52	13	3.8	21.14	2.4
SPC_6	"	0	0.8232	0.369	11	3.8	21.14	2.2
SPC_6	"	0	0.9896	0.27	8	3.8	21.14	2.2
SPC_6	"	0	0.8336	0.499	10	6.36	21.14	3.6
SPC_6	"	0	0.7192	0.318	8	6.36	21.14	4.2
SPC_6	"	0	0.8024	0.356	10	6.36	21.14	3.4
SPC_6	"	0	0.74	0.216	8	6.36	21.14	4.6

Table A.2: Number of *Faxonius eupunctus* (EU) collected and physical habitat variables recorded for each quadrat completed in non-wadeable pool mesohabitats. Geographic coordinates are unique to each site rather than individual quadrats. "Substrate" is the average of five streambed substrate size estimates recorded at each point of a substrate cross (n = 5) according to the following scale: 1 = sand; 1.5 = bedrock; 2 = gravel; 3 = pebble; 4 = cobble; 5 = boulder.

Site_ID	Lat./Long.	EU	CV (m/s)	Depth (cm)	Temp (ºC)	Substrate
EPR_25	36.7847, -91.4617	0	0.059	154	24	3.2
EPR_25	"	0	0.037	142	23	3.6
EPR_25	"	0	0.04	147	23	2.6
EPR_25	"	0	0.068	140	23	2.6
EPR_25	"	0	0.086	173	24	2.6
EPR_25	"	0	0.068	157	23	2
EPR_25	"	0	0.083	146	23	1.2
EPR_25	"	0	0.094	136	24	1.6
EPR_25	"	0	0.085	160	24	1.6
EPR_25	"	0	0.079	153	24	1.6
EPR_25	"	0	0.091	141	23	1.8
EPR_25	"	0	0.09	133	24	1.6
EPR_27	36.7992, -91.3985	2	0.279	122	24	4
EPR_27	"	0	0.237	131	24	4.4
EPR_27	"	1	0.243	134	24	4.4
EPR_27	"	0	0.232	284	24	4.2
EPR_27	"	0	0.374	140	24	4
EPR_27	"	0	0.299	118	24	3.8
EPR_27	"	0	0.315	173	24	2.6
EPR_27	"	0	0.357	80	24	1.2
EPR_27	"	0	0.298	68	25	1.2
EPR_27	"	0	0.301	81	25	1.6
EPR_27	"	0	0.263	78	25	2.2
EPR_27	"	0	0.099	123	25	2
EPR_29	36.8033, -91.3370	0	0.097	118	22	2.4
EPR_29	"	0	0.06	128	22	3
EPR_29	"	1	0.14	155	22	3.4
EPR_29	"	0	0.19	180	22	3.8
EPR_29	"	0	0.31	109	23	2.4
EPR_29	"	1	0.32	121	23	2.4
EPR_29	"	1	0.26	130	23	3
EPR_29	"	0	0.254	127	23	2.2
EPR_29	"	0	0.43	84	23	2
EPR_29	"	0	0.4	101	23	2
EPR_29	"	0	0.35	111	23	2.2
EPR_29	"	0	0.38	103	23	2.2
EPR_32	36.7536, -91.2595	0	0.354	97	18	2.8
EPR_32	"	0	0.318	100	18	2

Table A.2 cont'd

Site_ID	Lat./Long.	EU	CV (m/s)	Depth (cm)	Temp (ºC)	Substrate
EPR_32	"	1	0.298	106	18	2
EPR_32	"	0	0.229	178	18	2.2
EPR_32	"	0	0.438	154	18	2.2
EPR_32	"	0	0.38	217	18	2.2
EPR_32	"	0	0.398	218	18	2.2
EPR_32	"	1	0.398	218	18	2.6
EPR_32	"	0	0.163	249	19	1
EPR_32	"	0	0.286	290	19	1
EPR_32	"	4	0.185	332	19	3.2
EPR_32	"	7	0.216	351	19	4.4
EPR_33	36.7461, -91.2377	4	0.218	282	18	2.4
EPR_33	"	5	0.27	190	18	2.4
EPR_33	"	7	0.239	150	19	3.2
EPR_33	"	19	0.218	111	19	3
EPR_33	"	0	0.4	255	19	2
EPR_33	"	1	0.406	221	19	2
EPR_33	"	10	0.453	184	19	3.8
EPR_33	"	13	0.392	155	19	3.6
EPR_33	"	0	0.267	188	19	1
EPR_33	"	0	0.301	175	19	1
EPR_33	"	0	0.285	148	20	1.4
EPR_33	"	1	0.466	110	20	2.4
EPR_34	36.7322, -91.2140	7	0.168	290	19	3
EPR_34	"	14	0.156	359	19	3
EPR_34	"	7	0.153	345	18	3.6
EPR_34	"	16	0.106	327	18	3.4
EPR_34	"	0	0.262	312	18	2.4
EPR_34	"	0	0.238	310	18	2.6
EPR_34	"	0	0.201	312	17	2
EPR_34	"	0	0.188	318	17	1.6
EPR_34	"	1	0.189	312	18	1.4
EPR_34	"	0	0.156	346	18	1.4
EPR_34	"	0	0.131	329	18	1
EPR_34	"	0	0.117	338	18	1
EPR_36	36.6619, -91.1936	0	0.394	325	20	2.8
EPR_36	"	1	0.324	258	20	2.2
EPR_36	"	1	0.333	234	20	2.4
EPR_36	"	1	0.234	230	20	2.8
EPR_36	"	0	0.334	245	20	2.8
EPR_36	"	0	0.29	234	21	2.4
EPR_36	"	0	0.327	215	21	2.4
EPR_36	"	0	0.434	210	21	2.4

Table A.2 cont'd

Site_ID	Lat./Long.	EU	CV (m/s)	Depth (cm)	Temp (ºC)	Substrate
EPR_36	"	0	0.036	130	21	1.6
EPR_36	"	0	0.065	127	21	1.8
EPR_36	"	0	0.082	122	22	2
EPR_36	"	1	0.09	94	22	2
EPR_38	36.6118, -91.1730	6	0.508	184	22	3.4
EPR_38	"	2	0.478	219	22	3.4
EPR_38	"	3	0.241	231	22	3
EPR_38	"	1	0.431	179	22	3.2
EPR_38	"	0	0.387	152	23	1.6
EPR_38	"	0	0.476	159	22	1.4
EPR_38	"	0	0.5	146	22	1.6
EPR_38	"	0	0.465	152	23	2
EPR_38	"	0	0.2	130	23	1.6
EPR_38	"	0	0.234	125	23	1.6
EPR_38	"	0	0.25	123	23	2
EPR_38	"	0	0.24	108	23	2.2
EPR_40	36.5600, -91.1798	3	0.242	186	19	4.4
EPR_40	"	2	0.217	197	20	4.8
EPR_40	"	4	0.23	235	20	4.8
EPR_40	"	3	0.107	268	20	3.6
EPR_40	"	3	0.284	255	20	3.6
EPR_40	"	0	0.298	268	21	2.6
EPR_40	"	0	0.257	268	21	2.4
EPR_40	"	1	0.294	284	20	2.6
EPR_40	"	0	0.257	253	20	1.6
EPR_40	"	0	0.261	262	21	1.2
EPR_40	"	0	0.258	282	21	1
EPR_40	"	0	0.229	319	21	1.6
EPR_44	36.4329, -91.1376	0	0.293	210	22	1.4
EPR_44	"	0	0.103	292	22	2.6
EPR_44	"	0	0.133	227	22	2.6
EPR_44	"	0	0.119	234	22	2.2
EPR_44	"	0	0.402	215	22	2.8
EPR_44	"	0	0.401	236	22	2.2
EPR_44	"	0	0.423	230	22	2
EPR_44	"	0	0.333	223	22	2
EPR_44	"	0	0.508	153	21	1.8
EPR_44	"	0	0.508	157	22	1.8
EPR_44	"	0	0.58	163	22	1.8
EPR_44	"	0	0.483	176	22	1.6
Greer Access	36.7918, -91.3294	0	0.317	162	17	1
Greer Access	"	0	0.284	227	17	2.6

Table A.2 cont'd

Site_ID	Lat./Long.	EU	CV (m/s)	Depth (cm)	Temp (ºC)	Substrate
Greer Access	"	0	0.272	226	17	2.4
Greer Access	"	0	0.3	175	17	1.8
Greer Access	"	0	0.526	155	18	1.4
Greer Access	"	0	0.424	143	17	1.4
Greer Access	"	0	0.449	140	18	1.8
Greer Access	"	0	0.424	130	17	1.2
Greer Access	"	0	0.245	150	16	2.6
Greer Access	"	2	0.225	135	16	2.4
Greer Access	"	4	0.28	124	16	3.4
Greer Access	"	4	0.368	124	16	3.2
Myrtle Access	36.5120, -91.1698	0	0.231	268	22	4.2
Myrtle Access	"	0	0.149	249	21	1.5
Myrtle Access	"	0	0.131	248	21	1.5
Myrtle Access	"	0	0.16	256	21	1.5
Myrtle Access	"	0	0.268	220	21	1.6
Myrtle Access	"	0	0.231	244	21	1.2
Myrtle Access	"	0	0.301	256	21	1
Myrtle Access	"	0	0.326	237	21	1.2
Myrtle Access	"	0	0.312	186	21	1.6
Myrtle Access	"	0	0.279	165	21	1.6
Myrtle Access	"	0	0.269	168	21	1.8
Myrtle Access	"	1	0.259	162	21	1.6

Table A.3: Number of *Faxonius eupunctus* (EU) collected from quadrats in riffle mesohabitats located within 125m of non-wadeable pool sampling sites. Geographic coordinates are unique to each site rather than individual quadrats.

Site_ID	Lat./Long.	Quadrat	EU
EPR_25	36.7847, -91.4617	1	0
EPR_25	"	2	0
EPR_25	"	3	0
EPR_25	"	4	0
EPR_25	"	5	0
EPR_25	"	6	0
EPR_25	"	7	0
EPR_25	"	8	0
EPR_25	"	9	0
EPR_25	"	10	0
EPR_25	"	11	0
EPR_25	"	12	0
EPR_27	36.7992, -91.3985	1	0
EPR_27	"	2	1
EPR_27	"	3	0
EPR_27	"	4	0
EPR_27	"	5	1
EPR_27	"	6	0
EPR_27	"	7	0
EPR_27	"	8	0
EPR_27	"	9	0
EPR_27	"	10	0
EPR_27	"	11	1
EPR_27	"	12	0
EPR_32	36.7536, -91.2595	1	0
EPR_32	"	2	1
EPR_32	"	3	0
EPR_32	"	4	1
EPR_32	"	5	1
EPR_32	"	6	1
EPR_32	"	7	2
EPR_32	"	8	7
EPR_32	"	9	4
EPR_32	"	10	10
EPR_32	"	11	5
EPR_32	"	12	0
EPR_33	36.7461, -91.2377	1	9
EPR_33	"	2	10
EPR_33	"	3	5
EPR_33	"	4	4

Table A.3 cont'd

Site_ID	Lat./Long.	Quadrat	EU
EPR_33	"	5	4
EPR_33	"	6	4
EPR_33	"	7	7
EPR_33	"	8	10
EPR_33	"	9	19
EPR_33	"	10	5
EPR_33	"	11	9
EPR_33	"	12	4
EPR_34	36.7322, -91.2140	1	1
EPR_34	"	2	4
EPR_34	"	3	5
EPR_34	"	4	8
EPR_34	"	5	6
EPR_34	"	6	1
EPR_34	"	7	2
EPR_34	"	8	4
EPR_34	"	9	0
EPR_34	"	10	1
EPR_34	"	11	1
EPR_34	"	12	2
EPR_36	36.6619, -91.1936	1	1
EPR_36	"	2	1
EPR_36	"	3	13
EPR_36	"	4	6
EPR_36	"	5	1
EPR_36	"	6	12
EPR_36	"	7	5
EPR_36	"	8	1
EPR_36	"	9	2
EPR_36	"	10	0
EPR_36	"	11	12
EPR_36	"	12	1
EPR_38	36.6118, -91.1730	1	6
EPR_38	"	2	21
EPR_38	"	3	13
EPR_38	"	4	11
EPR_38	"	5	12
EPR_38	"	6	31
EPR_38	"	7	3
EPR_38	"	8	4
EPR_38	"	9	9
EPR_38	"	10	0

Table A.3 cont'd

Site_ID	Lat./Long.	Quadrat	EU
EPR_38	"	11	1
EPR_38	"	12	4
EPR_40	36.5600, -91.1798	1	11
EPR_40	"	2	8
EPR_40	"	3	1
EPR_40	"	4	0
EPR_40	"	5	1
EPR_40	"	6	0
EPR_40	"	7	0
EPR_40	"	8	1
EPR_40	"	9	1
EPR_40	"	10	4
EPR_40	"	11	1
EPR_40	"	12	5
EPR_44	36.4329, -91.1376	1	0
EPR_44	"	2	0
EPR_44	"	3	0
EPR_44	"	4	0
EPR_44	"	5	2
EPR_44	"	6	0
EPR_44	"	7	0
EPR_44	"	8	0
EPR_44	"	9	0
EPR_44	"	10	0
EPR_44	"	11	1
EPR_44	"	12	0

APPENDIX B: ENVIRONMENTAL DNA SAMPLING SUPPLEMENTARY MATERIAL

Table B.1: Number of eDNA water samples per site with positive *Faxonius eupunctus* eDNA detections along with crayfish and habitat data collected at each site. "eDNA" refers to the number of positive eDNA water samples per site. "EU Detected" refers to whether *F. eupunctus* was detected with conventional sampling methods. "Density" is a site-averaged density estimate for *F. eupunctus* in wadeable riffles. "Canopy" represents an estimate of the proportion of each site covered by overhead foliage. "Dist" refers to total stream distance upstream from each sampling site. † = only qualitative conventional sampling performed.

Site ID	Lat./Long.	eDNA	EU Detected	EU Density (#/m^2)	Canopy	Distance (km)	Temp (ºC)
BFK_1	36.7623, -91.5286	0/4	N	0.00	0.0666	52.94	26.45
DC_2	36.4519, -91.2540	0/4	N	0.00	0.6958	32.59	28.1
EPR_8	36.9653, -91.9108	0/4	N	0.00	0.4982	66.12	24.72
EPR_16	36.8856, -91.7057	0/4	N	0.00	0.7079	354.49	17.61
EPR_23	36.7859, -91.5279	0/4	N	0.00	0.5791	792.05	20.69
EPR_24	36.7818, -91.4886	0/4	N	0.00	0.6100	886.35	20.67
EPR_25	36.7853, -91.4598	0/4	Y	0.42	0.0597	928.06	20.63
EPR_26	36.7833, -91.4290	1/4	Y	0.92	0.5276	959.07	20.62
EPR_27	36.7998, -91.3981	1/4	Y	1.42	0.1177	972.06	20.61
EPR_28	36.8137, -91.3619	2/4	Y	0.17	0.0241	1349.42	19.7
EPR_29	36.8019, -91.3367	1/4	Y	N/A	N/A	N/A	N/A
EPR_30	36.7857, -91.3157	4/4	Y	2.75	0.0545	1384.73	15.25
EPR_31	36.7752, -91.2805	3/4	Y	2.00	0.0016	1447.08	15.64
EPR_32	36.7525, -91.2594	2/4	Y	2.92	0.3197	1769.66	15.9
EPR_33	36.7449, -91.2371	1/4	Y	7.00	0.1050	1786.61	16.2
EPR_34	36.7297, -91.2129	4/4	Y	7.92	0.1507	1801.79	16.6
EPR_35	36.6888, -91.1969	4/4	Y	1.08	0.0129	1938.82	16.8
EPR_36	36.6599, -91.1893	4/4	Y	1.75	0.0016	1983.43	18.2
EPR_37	36.6467, -91.2008	3/4	Y	1.83	0.0016	2009.48	19.21
EPR_38	36.6246, -91.1804	2/4	Y	2.50	0.3613	2021.77	19.58
EPR_39	36.5871, -91.1791	3/4	Y	0.42	0.4141	2085.56	19.98
EPR_40	36.5564, -91.1861	4/4	Y	0.50	0.0016	2581.97	20.39
EPR_41	36.5299, -91.1957	3/4	Y	0.17	0.0709	2598.64	19.98
EPR_42	36.4885, -91.1716	2/4	Y	0.17	0.0857	2714.75	19.7
EPR_43	36.4559, -91.1728	1/4	Y	0.00	0.0016	2751.21	20
EPR_44	36.4366, -91.1477	2/4	Y	0.08	0.0016	2832.14	20.7
FRC_2	36.5879, -91.2513	0/4	N	0.00	0.4887	371.42	24.9
FRC_12	36.6036, -91.3979	0/4	N	0.00	0.4675	96.22	21.9
HC_1	36.6082, -91.1483	0/4	N	0.00	0.5844	19.72	22
HC_6†	36.8654, -91.2897	0/4	N	N/A	N/A	N/A	N/A
HC_10	36.7881 -91.2737	0/4	Y	0.25	0.6952	290.38	20.6
MC_1	36.5140, -91.2810	0/4	N	0.00	0.3330	20.24	19.11
MC_3	36.5004, -91.2187	0/4	N	0.00	0.2007	57.41	27.13
MF_3	36.7846, -91.7614	0/4	N	0.00	0.6849	47.95	24.71
MF_6	36.8138, -91.6802	0/4	N	0.00	0.7279	111.42	22.24
MF_7	36.7815, -91.5608	0/4	N	0.00	0.0302	197.44	23.5

Table B.1cont'd

Site ID	Lat./Long.	eDNA	EU Detected	EU Density (#/m^2)	Canopy	Distance (km)	Temp (ºC)
PC_3	36.6654, -91.3633	0/4	N	0.00	0.6464	67.15	23.9
PC_6	36.7017, -91.4015	0/4	N	0.00	0.0111	18.61	24.4
SPC_6	36.8321, -91.4571	0/4	N	0.00	0.6854	264.52	21.14