SAMPLING THE STREAM LANDSCAPE: FACTORS INFLUENCING DETECTION AND DISTRIBUTION OF STREAM FISHES

By

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Stream fishes are among the most threatened species due to natural flow regime alterations and fragmented habitats stemming from both anthropogenic activities and climate change. A fundamental challenge of establishing stream fish-environment relationships is variable detection (the proportion of available individuals captured or the probability of detecting a species when present), which confounds perceived species distributions. My overarching goals were to improve detection estimates for common stream-fish sampling methods and identify multiscale factors related to stream-fish distributions in Ozark Highland streams. I used gear calibration to model tow-barge electrofishing detection among stream fishes across environmental conditions at multiple spatial scales. Multiple stream reach-scale variables were associated with stream-fish detection including water depth and clarity, emergent vegetation, and a discharge-proportion riffle interaction, where the magnitude of these relationships varied among species in relation to habitat use. Lithological characteristics of stream segments explained additional variation in stream-fish electrofishing detection. I compared snorkeling to tow-barge electrofishing to examine tendencies in the number of species detected and evaluated the efficacy of snorkeling for estimating stream-fish abundance. Electrofishing tended to detect more rare species than snorkeling. Snorkeling typically underestimated stream-fish abundance, particularly for cryptic species such as Green Sunfish and Rock Bass; however, snorkeling did provide informative population estimates for Smallmouth Bass. I improved the applicability of electrofishing for monitoring stream-dwelling Smallmouth Bass using a multinomial *N*-mixture model. Water clarity, effort, and a wetted channel width-water depth interaction explained variation in Smallmouth Bass electrofishing detection. Smallmouth Bass abundance estimates derived from the model agreed with baseline estimates via snorkeling. Empirical Bayes confidence intervals for Smallmouth Bass abundance from the model were more precise than unbiased Petersen mark-recapture estimates. Lastly, I examined stream fish-environment relationships at multiple spatial scales. Variation in stream-fish densities were associated with stream reach-scale groundwater contribution and stream segment-level lithology. Variation in stream-fish occurrence was associated with riffle-run-pool sequence area and stream reach-scale substrate size, groundwater contribution, and residual pool depth. My project demonstrates the complexity of stream-fish detection across environmental conditions, highlights the importance of multiscale approaches for examining stream-fish environment relationships, and contributes to advancements in stream-fish ecology and management.

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CHAPTER I

INTRODUCTION

PROBLEM STATEMENT

The loss of biodiversity in freshwater ecosystems is alarming. Freshwater fishes experienced the highest extinction rate among vertebrates in the twentieth century and the current extinction rate is estimated at eight species per decade (Burkhead 2012). To put the magnitude of the problem in perspective, consider that the background extinction rate for freshwater fishes was only one species per three million years. Stream fishes are perhaps the most threatened vertebrates due to major alterations of the natural flow regime (magnitude, timing, duration, rate of change, and frequency of streamflow; Poff et al. 1997) and fragmented habitats stemming from both anthropogenic activities (e.g., damming and water withdrawals) and climate change (e.g., shifting precipitation patterns and increased water temperatures). As interconnected linear systems, habitat perturbations can affect fish assemblages in both upstream and downstream directions (Meyers et al. 1992; Pringle 1997; Guenther and Spacie 2006). Despite increased awareness of the multifaceted threats to the persistence of stream fishes, the ability of stream systems to support aquatic life continues to decline (EPA 2013). Unfortunately, stream scientists often rely on reactive rather than predictive management because fish-environment relationships remain poorly understood.

Predictive stream-fish management and conservation strategies require both identifying factors related to species occurrence (e.g., environmental flow requirements and channel formation) and understanding how populations respond to changing environments (e.g., altered timing or magnitude of high-flow events). Although stream fishes appear to form structured, nonrandom assemblages across space and time (Jackson et al. 2001), interacting factors across multiple scales and dimensions (Ward 1989) present difficulty for explaining observed patterns. Furthermore, increased insight into why some life-history strategies succeed while other fail under certain environmental conditions may require improved conceptual, as well as methodological, approaches to stream fish ecology (Verberk et al. 2013). Effective sampling protocols are also integral to predictive stream-fish management and conservation as the timing, nature, and extent of sampling largely influences perceived patterns of species occurrence across the stream landscape (Jackson et al. 2001; Poff and Zimmerman 2010; Gwinn et al. 2016).

Variable detection (either the proportion of available individuals captured or the probability of detecting a species when present) among sampling methods, environmental conditions, and species confounds perceived patterns of fish distributions across the stream landscape. The failure to account for variable stream-fish detection can impede effective management and conservation, limit advancements in ecology, and result in wasted resources (i.e., time and money spent on sampling). Improved analytical approaches to address variable detection are increasingly prevalent in the ecological literature (e.g., Williams et al. 2002; MacKenzie et al. 2005; Royle et al. 2013). However, catch-per-unit-effort (CPUE) remains the most common metric used to evaluate and monitor stream fish assemblages (Gwinn et al. 2016), despite that it calculates only an indirect measure of abundance, relies on a typically untested and often unrealistic assumption of constant detection, and provides no insight into detection relationships among species. Standardizing sampling conditions (e.g., sampling only at baseflows) to support the use of CPUE may minimize variation in site-specific short-term stream-fish detection; however, this strategy severely limits the ability to identify regional long-term trends in populations because environmental conditions in streams vary extensively across space and time. Furthermore, identifying relationships between stream fishes and the environment (e.g., flow-ecology relationships), often fundamental research goals, are not possible when sampling is limited to a narrow range of environmental conditions. Standardized sampling also does not ensure constant detection among stream fishes (Price and Peterson 2010; McManamay et al. 2014), which can lead to misrepresentations of both assemblage structure and stream conditions (Seegert 2000; Price and Peterson 2010). For example, biotic integrity indices (e.g., IBI) that use the relative abundance of indicator fish species as a surrogate for stream water quality require standardized sampling; however, variation in detection among surveys and species is ignored.

THE STREAM LANDSCAPE

Stream landscapes are dynamic environments that operate across multiple spatial and temporal scales to form the physical habitat where aquatic organisms evolve, adapt, and interact (Southwood 1977; Gorman and Karr 1978; Zwick 1992). Streams are hierarchically structured, where characteristics at finer scales are largely constrained by coarser-scale factors (Frissell et al. 1986; Stevenson 1997). At the coarsest scale, the climate and geology of the surrounding valley (Hynes 1975), along with land use (the human element) and biogeography (Stevenson 1997), are ultimate determinants of stream ecosystems and dictate species pools within ecoregions (Poff 1997). Moving from ecoregions to progressively finer scales (i.e., watersheds to microhabitats), observed species assemblages in streams are a result of both local abiotic factors and physical characteristics of coarser scales. For example, species occurrence at the stream reach scale may reflect local characteristics (e.g., substrate and channel form), the flow regime of the stream segment (a length of stream between tributary confluences), and underlying lithology. Biotic factors play only a minimal role in structuring species assemblages in streams due to the high frequency of disturbance (e.g., extreme high and low flows), which essentially “resets” systems and limits population growth and competitive exclusion (Resh et al. 1988; Jackson et al. 2001; Peres-Neto 2004).

Spatial factors are also hypothesized to play an important role in structuring biotic assemblages in streams. One of the most influential contributions to stream ecology was the river continuum concept (RCC; Vannote et al. 1980), which provided a theoretical model to predict patterns in invertebrate assemblages across increasing stream order in relation to the processing of allochthonous organic matter (i.e., energy) and riparian characteristics (e.g., soil and vegetation type). Schlosser (1991) extended the basic principles of the RCC to a conceptual view of patterns in stream-fish assemblages, particularly in relation to land use, across the terrestrial-aquatic interface. As a simplistic model, the RCC falls short of providing an accurate realization of the stream landscape as a heterogeneous spatially-continuous mosaic (i.e., "riverscape"; Ward 1998; Fausch et al. 2002; Wiens 2002; Allan 2004). For example, spatial position relative to a tributary (Osbourne and Wiley 1992; Magalhaes et al. 2002), heterogeneity in local habitat characteristics along the downstream continuum (Fauch et al. 2002), and the characteristics of adjacent habitat (Jackson et al. 2001; Scheurer et al. 2003; Falke and Fausch 2010) can also influence the structure of stream-fish assemblages.

Incorporating traits into stream-fish research can both simplify complex datasets by grouping species based on shared characteristics and generalize patterns in assemblages across the stream landscape. Species traits are morphological, physiological, behavioral, and life-history characteristics. This definition of traits excludes habitat use because habitat preferences are the species-environment relationship researchers typically seek to explain through traits (Violle et al. 2007; Verberk et al. 2013). A trait-based approach to examine species distributions can both transcend taxonomy in generalizing environment relationships (Westoby et al. 1995; Verberk et al. 2013), increase the applicability of individual research efforts by providing useful comparisons among ecoregions (Winemiller 1991; Lamouroux et al. 2002; Stuart-Smith et al. 2013), and provide insight into the ecology of rare and poorly-studied species (Cornelissen et al. 2003; Poff et al. 2006; Frimpong and Angermeier 2010). Grouping species based on shared traits can both generalize relationships and improve statistical power by reducing data dimensionality (Goldstein and Meador 2004; Frimpong and Angermeier 2010; Verberk et al. 2013). Despite the profusion of trait-based research in the scientific literature, the establishment of trait-environment relationships has largely favored terrestrial plants and aquatic invertebrates (Verberk et al. 2013). Applications of trait-based approaches to improve stream-fish ecology and management do exist (e.g., Olden et al. 2008; Craven et al. 2010; Bergerot et al. 2015); however, overall success has been limited (Olden et al. 2010; Heino et al. 2013; Verberk et al. 2013) and the vast majority of species and systems remain unexplored (Frimpong and Angermeier 2010).

Although improved theoretical perspectives of the dynamic stream landscape have been invaluable to stream fish ecology, proper applications of these concepts to research and management are equally essential. The recognition of multiscale processes and the importance of the variety of physical factors required for species persistence has led to notable advancements in stream-fish ecology; however, much more progress by researchers is needed to meet the challenges ahead (Matthews 2010; Fausch 2010). Another step forward for stream-fish ecology would be increased implementation of study designs and analyses that accurately account for the hierarchical spatiotemporal nature of streams (Fausch et al. 2002; Wagner et al. 2006; Stewart-Koster et al. 2013) to better identify underlying factors related to observed assemblage patterns.

SAMPLING THE STREAM LANDSCAPE

Assessments of animal distributions are confounded by both species and environmental characteristics that influence variation in detection. Addressing detection when sampling stream fishes is particularly challenging because the stream environment varies considerably across both space and time and there is a wide range of variation in species characteristics. Wetted channel width, water depth, water clarity, ambient water conductivity, flow (i.e., water velocity and discharge), and instream structure are all common environmental factors that contribute to variation in stream-fish detection (Rabeni et al. 2009; Peterson and Paukert 2009); however; these factors have disparate relationships among gear types. Although dynamic instream characteristics primarily determine species-specific detection probabilities, relatively temporally-stable variables (e.g., substrate and bank formation) also contribute. In addition to size, both morphology and habitat use contribute to variable stream-fish detection (Peterson and Paukert 2009; Rabeni et al. 2009; McManamay et al. 2014).

The challenges of variable stream-fish detection have long been recognized and addressing the problem remains an evolving process. The first research efforts focused on stream-fish sampling methodologies were around 1950 (e.g., Funk 1949; Cleary and Greenbank 1954; Sullivan 1956). In fact, Cleary and Greenbank (1954) were perhaps the first authors to explicitly discuss the implications of variable detection on long-term stream-fish conservation and management and highlight the importance of efforts at the research level. Larimore (1961) was the first to demonstrate the substantial variation in electrofishing detection among species. A proliferation of research dedicated to stream-fish sampling methodologies occurred in the latter part of the twentieth century; however, the primary focus was often on comparing the numbers of fish captured between or among gear types (i.e., more fish was considered “better”; e.g., Dauble and Gray 1980; Layher and Maughan 1984; Dewey et al. 1989) than estimating detection probability (but see Peterson and Cederholm 1984; Slaney and Martin 1987; Rodgers et al.1992). Likely due to increased attention by ecologists in general, there has been considerable stream-fish research focused on variable detection in the twenty-first century (e.g., Bayley and Peterson 2001; Peterson et al. 2004; Hense et al. 2010); however, studies have been largely biased toward coldwater streams and salmonids (but see Peterson et al. 2001; Price and Peterson 2010; Brewer and Ellersieck 2011). For example, only 7% of published studies focused on detection have been performed in wadeable warmwater streams of the southern United States (Mollenhauer et al., unpublished data). Recently, there has also been increased emphasis on standardizing freshwater fish sampling protocols to improve the comparability of population estimates among sites and surveys, with Bonar et al. (2009) representing a major step forward for inland fisheries. Appropriate crew training and consistent methodologies are essential aspects of sound stream-fish monitoring protocols; however, standardization seldom results in constant detection. For example, assuming constant effort or identical stream environments (e.g., considering sampling conditions among sites comparable at baseflows) are not only unrealistic expectations, but also constrain both the development of strategic monitoring plans and the comparison of populations or assemblages across a large study area (see also Problem Statement). Despite notable progress in addressing stream-fish detection by researchers, the lack of major strides, particularly across different systems, has also constrained advancements in ecology (Brewer and Orth 2015; Gwinn et al. 2016). Continued progress in stream-fish sampling methodologies requires not only a broader examination and application of contemporary approaches to address the challenges of variable detection, but also expanding research efforts in poorly studied regions.

Improved analytical techniques for addressing detection are readily available to stream-fish scientists, but their use has been relatively minimal. One notable advancement for improving the reliability of animal population surveys was occupancy estimation and modeling (hereafter referred to as occupancy modeling; MacKenzie et al. 2005). Occupancy modeling provides a highly flexible framework that uses binary detection-nondetection data from spatially- or temporally-replicated surveys to independently model both species detection and species occupancy (i.e., the probability of presence) given detection probability, where both occupancy and detection can vary among sites and surveys as a function of covariates. Although originally designed for single-species single-season studies (MacKenzie et. al 2002), occupancy modeling has been extended to a wide range of applications including multiple seasons (MacKenzie et al. 2003), multiple species (MacKenzie et al 2004; Dorazio et al. 2010; Mihaljevic et al. 2015), and community metrics (Kéry and Royle 2008; Kéry et al. 2009; Dorazio et al. 2010). The use of occupancy models in stream-fish ecology have been relatively limited despite their broad applicability (but see Falke et al. 2010; Wagner et al. 2013; McManamay et al. 2014; Pregler et al. 2015; Peterson and Shea 2015). The general concept of occupancy modeling has been extended to models that accommodate count data to provide inference on both species occurrence and abundance (see also MacKenzie and Nichols 2004 for applications of occupancy models to infer abundance), thus providing a useful approach to survey common species (i.e., counts are typically > 0 across sites). For example, *N*-mixture models (Royle 2004a, Royle 2004b; Royle and Dorazio 2006) independently estimate site-level abundance and detection, where both parameters can vary among sites and surveys as a function of covariates (see also Webster et al. 2008). Multinomial *N*-mixture models (Royle 2004b; Royle and Dorazio 2006) are particularly attractive for stream-fish sampling because they accommodate common estimation methods with temporally-replicated surveys such as removal (known also as depletion) and mark-recapture (known also as capture-recapture); however, applications for stream fishes have been rare (but see Dorazio et al 2005; Coggins et al. 2011; Yard et al. 2011; Dodrill et al. 2015; Mollenhauer and Brewer 2016). Sampling-gear calibration is another method to estimate abundance while explicitly account for variable detection among stream fishes and across sampling conditions (e.g., Peterson and Rabeni 2001; Price and Peterson 2010; Brewer and Ellersieck 2011). Gear calibration requires establishing detection by repeat sampling across a range of environmental conditions using either known population sizes (e.g., marking or stocking) or comparisons with a previously calibrated gear (Peterson and Paukert 2009). Once a large number of fish have been sampled across the range of environmental conditions, a model can be developed to estimate detection for a single sampling event, which can be used to estimate site-specific abundance by adjusting raw catch data (Thompson and Seber 1994; Peterson and Paukert 2009). Effective gear calibration requires extensive sampling effort, which despite the long-term benefits for stream-fish monitoring, likely explains their limited examination in the scientific literature.

STUDY AREA

My field work was conducted in the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri. The Ozark Highlands ecoregion is characterized by oak-hickory forests, with valleys primarily converted to pasture (Woods et. al 2005). The dominant underlying lithology of the Ozark Highlands is cherty limestone; however, there is considerable variation, particularly near the ecotones. Many Ozark Highland streams are classified as groundwater dominated with clear water (but with substantial variation in water clarity under baseflows; Nigh and Schroeder 2002). Ozark Highland streams have diverse fish assemblages including black basses, sunfishes, minnows, darters, and suckers. The primary sportfish of interest in Ozark Highland streams is the Smallmouth Bass *Micropterus dolomieu*.

OBJECTIVES

The overarching goals of my dissertation were to (1) examine approaches that would improve estimates of detection probability and abundance using common stream-fish sampling methods and (2) identify multiscale factors related to stream-fish distributions in Ozark Highland streams. My project will both contribute to the advancement of stream ecology and improve predictive aspects of stream-fish ecology and management by applying current theory and contemporary analytical approaches. My specific objectives were:

Objective 1. Construct groups of Ozark Highland stream fishes based on morphological traits using a novel clustering approach.

Objective 2. Develop a multiscale multi-species electrofishing detection model for Ozark Highland stream fishes using sampling-gear calibration.

Objective 3. Compare tendencies in species detection for warmwater stream fishes between snorkeling and electrofishing and evaluate the effectiveness of snorkel counts to provide reliable warmwater stream-fish abundance estimates.

Objective 4. Develop an electrofishing detection model for Smallmouth Bass using a multinomial mark-recapture *N*-mixture model.

Objective 5. Identify multiscale factors associated with Ozark Highland stream-fish densities and occurrence, with an emphasis on intermediate-scale environmental factors.

For objective one, I evaluated the effectiveness of hierarchical clustering with multiscale bootstrapping to group members of a large assemblage of stream fishes based on morphology and identified traits that distinguished groups at multiple levels of the assemblage. The trait groups developed in objective one were integrated into objectives two, three, and five. For objective two, I modeled tow-barge electrofishing detection among multiple stream fishes across a range of environmental conditions at multiple spatial scales using a mixed (i.e. random effects) regression model. The performance of the electrofishing detection model was evaluated using both a cross validation and a comparison of abundance estimates with unbiased Petersen mark-recapture estimates. I also examined variation in electrofishing detection in relation to both morphological groups developed for objective one and differences in habitat use among stream fishes. For objective three, I assessed the utility of snorkel surveys for surveying warmwater stream fishes. In addition to a general comparison of species detection between snorkeling and tow-barge electrofishing, I used electrofishing catch data adjusted for variable detection to evaluate the reliability of unadjusted snorkel counts. The stream-fish detection model developed for chapter two was used to adjust electrofishing catch data. I also examined tendencies in snorkeling detection in relation to both morphological groups developed for objective one and differences in habitat use among stream fishes. For objective four, I developed a single-species multinomial *N*-mixture model to improve the applicability of mark-recapture electrofishing for Smallmouth Bass, where abundance estimates were derived using a hierarchical empirical Bayes estimator. The performance of the Smallmouth Bass electrofishing detection model was evaluated using baseline snorkel counts when appropriate for reliable estimates, unbiased Petersen mark-recapture estimates, and simulations. Lastly, objective five addressed the ecological components of my dissertation while incorporating objectives one and two. I used hierarchical models to identify factors related to both stream-fish densities and stream-fish occurrence at multiple spatial scales. Stream-fish density data was adjusted for variable detection using the electrofishing detection model developed in chapter 2. I also attempted to generalize relationships between stream-fish densities and environmental variables using both life-history traits and morphological groups developed in chapter one.

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CHAPTER II

MORPHOLOGICAL GROUPS OF STREAM FISHES OF THE OZARK HIGHLANDS

ABSTRACT

Ecologists commonly grouping species based on shared traits to simplify multi-species datasets. Hierarchical clustering, in particular, is well suited for grouping species based on trait combinations; however, determining where meaningful associations exist within the dendrogram is inherently challenging. Multiscale bootstrapping generates approximately unbiased *p*-values to provide a quantitative measure of strength for each nested cluster within the hierarchy. I used hierarchical clustering with multiscale bootstrapping to group Ozark Highland stream fishes based on combinations of morphological traits. The cluster analysis resulted in 53 significant groupings where significant clusters at finer scales of the species assemblage were nested within higher-level significant clusters throughout the dendrogram. A linear discriminant analysis confirmed group membership of species at multiple levels of the hierarchy. Variable loadings of discriminant functions indicated that similar combinations of body shape, swimming, and feeding traits distinguished groups of species at multiple levels of the hierarchy. The approach is not limited to either fishes or morphology and can be applied to both other organisms and alternative sets of species traits.

INTRODUCTION

A fundamental challenge of ecology is to reduce the complexity of multi-species data while retaining enough information to detect patterns both within and among assemblages. One common approach for simplifying multi-species datasets is to group species based on shared traits (e.g., reproductive and trophic guilds). Generalizing species relationships by grouping based on traits can reduce data dimensionally when studying large groups of species (Growns 2004; Frimpong and Angermeier 2010) and trait combinations can be represented by a single variable (e.g., trait group A, trait group B, etc.) to examine how traits relate to species distributions across an environmental gradient (e.g., Gross et al. 2007; Douma et al. 2012).

Transforming an ecological continuum to discrete classes is inherently arbitrary and resulting classifications are not necessarily related to underlying mechanisms of nature. Even the most widely accepted form of grouping in ecology, the species concept, is an artifact of anthropocentrism that arbitrarily groups individuals to provide a convenient basis to study natural phenomena (Allen and Hoekstra 2015). The uncertainties inherent to ecological grouping make diagnostic tests of group strength (i.e., the certainty of the relationship among group members based on chosen characteristics) challenging (Lyons et al. 2016). Therefore, analytical methods that provide quantitative assessments of group strength are invaluable to ecologists.

Hierarchical clustering, which merges objects based on a specified distance measure, results in a dendrogram that allows users to visualize groupings. A unique feature of hierarchical clustering is that groupings can simultaneously be visualized at multiple levels due to the nested structure of the analysis. However, the nested groupings resulting from hierarchical clustering also make identifying strong associations among objects contained in individual groupings difficult to identify. Common approaches used by ecologists to determine “good” groups when using hierarchical clustering include distance cut-offs based on branching in the dendrogram (e.g., Growns 2004; Dumay et al. 2004) and identifying levels of the dendrogram where "distinct" groupings occur (e.g., McCune 1988; Perry and McIntosh 1991); however, neither criteria explicitly provides a measure of group strength. Multiscale bootstrapping a method that improves the interpretation of hierarchical clustering (Suzuki and Shimodaira 2006). Hierarchical clustering with multiscale bootstrapping (HCMB) generates approximately unbiased probability values (AU *p*-values) at each node of the dendrogram for assessing the strength of resulting clusters, where high values suggest a significant association among objects contained within the cluster (Jackson et al. 2010). Multiscale bootstrapping provides a better approximation of classification error than traditional bootstrap probability values and does not require an assumption of multivariate normality (Efron et al. 1996; Shimodaira 2004).

HCMB has been widely applied in genetic research (e.g., Brambrink et al. 2006; Gaur et al. 2007; Ebert et al. 2009) and has also been effectively used for ecological questions (e.g., Jackson et al. 2010; Jouffray et al. 2015). HCMB is well suited for examining species trait questions. For example, Kelly and Motani (2015) used HCMB to identify drivers of trait convergence in marine tetrapods. HCMB also provides a means to examine how unique trait combinations define groups of species at various levels of the assemblage. The level of an assemblage to be examined may vary based on individual research questions for trait-based studies. Ecologists may be interested in either traits that broadly define groups of species across an entire assemblage or traits that are related to fine-scale habitat use (or both). For example, different trait combinations may explain differences in species abundances across an environmental gradient (e.g., Yan et al. 2013) versus local-scale microhabitat use (e.g., Lürig et al. 2016). For stream fishes, a recent study incorporated HCMB into a trait-based analysis of minnows (Burress et al. 2016); however, the authors examined only 15 species at a single level of the assemblage. Thus, further research is needed to assess the utility of HCMB for identifying trait associations among a large number of species at multiple scales. Accordingly, my objective was to use HCMB to develop trait groups from an assemblage of Ozark Highland stream fishes and identify trait combinations separating groups at multiple levels of the hierarchy (McCune and Grace 2002). Detection tendencies and ecological relationships both within and among groups are explored in other objectives (see Chapters 3, 4, and 6).

METHODS

An extensive number of fish traits can be used to characterize species (Frimpong and Angermeier 2010). I focused on morphological traits because (1) morphological characteristics provide a measurable value that can be applied to each species that is less subjective than nominal traits (e.g., guilds and behavioral categories), (2) basic knowledge about the ecology and life history of many stream fishes is lacking (Matthews 2010); therefore, the use of morphological traits allowed rare and poorly studied species to be included, (3) effectively grouping stream fishes based on morphology supports research efforts that further examine the functional importance of morphological traits, and (4) morphological characteristics represent continuous variables that are easily incorporated into a framework that examines trait combinations by allowing the range of a trait to be expressed across a species assemblage.

I measured external morphological traits (referred to hereafter as traits) on 92 stream fishes of the Ozark Highlands ecoregion, Oklahoma (Appendix 1). Traits were chosen that encompassed the overall morphology of an individual (e.g., body shape, swimming, and feeding; Appendix 2) and are hypothesized to be surrogates for ecological-niche relationships among stream fishes (Winemiller 1991; Gatz 1979). Fish specimens were acquired either via field sampling or from the Oklahoma State University Collection of Vertebrates. All specimens were preserved in 70% ethanol. I measured body traits using digital calipers (0.01 mm) on a minimum of 10 adult specimens for each species (Appendix 1). Traditional fin and body measurements using calipers remain an effective method to represent morphological variation among an assemblage of stream fishes (Franssen et al. 2014). Because fins were damaged on the vast majority of specimens due to sampling and storage, all fin measurements were obtained from digital photographs. I conducted an internet search to locate regional photographs that provided the best representation of "natural" fin positions (i.e., the spread of each fin during swimming) for each species. The majority of fin traits was obtained from FishBase (www.fishbase.org) and Fishes of Texas (www.fishesoftexas.org); however, the large number of species in our analysis required photographs to be obtained from a variety of sources and multiple photographs were sometimes required to provide comprehensive fin morphology for each species. Using online photographs to acquire fish traits is not uncommon (e.g., Clavel et al. 2013) as a comprehensive traits database is severely lacking for freshwater fishes (Frimpong and Angermeier 2010). I used the image-analysis program AnalyzingDigitalImages (Draft 2.0, 2011, Museum of Science, Boston, MA) for the fin measurements and to calculate body area for each species (rounded to nearest pixel). Each photograph was measured twice and the average value was used for a given trait.

Ecologically-relevant trait combinations can provide insight into relationships among species (Southwood 1977; Frimpong and Angermeier 2010; Verberk et al. 2013). Accordingly, I used certain trait values to calculate indices hypothesized to represent stream-fish morphology (Gatz 1979). These indices encompassed combinations of trait values and were used in the analyses in lieu of the associated point measurements. Both single traits and trait indices were used to represent the morphology of each species in the cluster analysis (Table 1).

Correlation between variables was expected due to the nature of the dataset. I examined the Pearson product-moment correlation coefficient for each pair of traits and removed certain traits to achieve $|r| \leq 0.70$. Because a certain trait was often correlated with multiple alternate traits, I was able to address correlations in the dataset while maintaining a comprehensive set of morphological fish traits.

Traits with considerable within-species variation may be less informative in detecting ecological relationships across an assemblage (McGill et al. 2006; Poff et al. 2006). Therefore, I performed a one-sided *F*-test (Freund et al. 2010) on the variance of each trait to confirm that interspecific variation was significantly higher than intraspecific variation ($\alpha < 0.05$). Because only one individual was used for each fin measurement, I was unable to test whether interspecific variation was higher than intraspecific variation for fin traits.

I converted traits to ratios using standard length, body area, or maximum body depth to adjust for differences in body size (Table 1 and Appendix 2). The mean of each trait ratio was used as a representative value for each species. An alternative to dividing by body size to adjust for non-isometric scaling among individuals is to regress each trait against body size and use the residuals in the analysis (e.g., Pease et al. 2012; Lundsgaard-Hansen et al. 2013). However, the lack of replication of fin measurements for each species resulted in a single individual representing fin traits for each species, which precluded the use of residuals. I was mindful to use only adults for the body traits, which minimized any intraspecific variation due to ontogenetic changes. I also used a standardized major axis to test for allometry in body traits in relation to standard length (Warton et al. 2006). The slopes for body traits ranged from 0.98 to 1.40, suggesting a reasonably proportional relationship with body size. Additionally, due to the large number of individuals used for the body measurements, I was constrained to assigning a single value for each trait per species (i.e., clustering nearly 2,000 individuals would make the dendrogram difficult to interpret). A mean residual rather than a mean trait ratio would still only represent the "average" individual and may not have accurately reflected variation among individuals within a species. Therefore, I chose to use ratios for both body and fin traits to maintain consistency.

I used the library "pvclust" (Suzuki and Shimodaira 2006) in the statistical software R (version 3.2.2, R Core Development Team, 2014) to construct groups of Ozark stream fishes based on morphological trait combinations (referred to hereafter as trait groups). Fish species were clustered using HCMB with the unweighted paired group with arithmetic means (UPGMA) linkage method based on chord distance. Chord distance is essentially a standardized Euclidian distance method and puts differently scaled variables on equal footing (Legendre and Gallagher 2001; McCune and Grace 2002; Austen et al. 2014). Data were resampled with 100,000 bootstraps to minimize standard error (SE) associated with AU *p*-values. Significant clusters were assessed using (AU *p*-value - SE) ≥ 0.95). My analyses could have resulted in 91 (i.e., *n* -1) significant clusters, which would suggest strong groupings of stream fishes based on combinations of morphological traits at all levels of the dendrogram. For simplicity, I chose significant groupings at three of levels of the hierarchy for further examination.

I used the library "MASS" (Venables and Ripley 2002) in the statistical software R to perform a linear discriminant analysis (LDA) on selected trait groups. A LDA identifies combinations of variables (i.e., discriminant functions) that provide the best possible separation between predefined groups by maximizing among-group variation relative to within-groups variation (McCune and Grace 2002). A preliminary examination of residual plots using multiple regression indicated that all traits used in the cluster analysis conformed to a normal error distribution; therefore, I did not perform any data transformations for the LDA. All traits were scaled such that the mean of each covariate was zero with a variance of one to improve the interpretation of variable loadings (i.e., variable importance).

RESULTS

Body traits varied more among species than among individuals within a species, although there was a high degree of correlation between several fin and body traits. The *F*-tests confirmed that interspecific variation was significantly higher than intraspecific variation for each body trait across all fish species. However, I removed seven traits from the dataset based on Pearson product-moment correlation coefficients prior to the cluster analysis (Table 1).

HCMB resulted in 90 clusters with 54 significant groupings (Figure 1). Standard errror was ≤ 0.01 for all AU *p*-values associated with each cluster (Figure 2). Only two species were not included in significant clusters. River Darter *Percina shumardi* clustered with Banded Sculpin *Cottus carolinae* (AU *p*-value = 0.94), suggesting that River Darter may be morphologically different from other darters (*Etheostoma* and *Percina*) despite the degree of relatedness.

First, I evaluated three significant clusters which comprised 90 stream fishes at the highest level of the hierarchy in the dendrogram (trait groups 1-3; Figure 1). Trait group 1 comprised darters, topminnows (*Fundulus*), and Western Mosquitofish *Gambusia affinis*. Trait group 2 comprised four genera of catfishes (Ictaluridae). Trait group 3 comprised the remainder of fishes, which were primarily suckers (Catostomidae), black basses and sunfishes (Centrarchidae), and minnows and chubs (Cyprinidae). The LDA for trait groups 1-3 resulted in 63.3% separation using the first discriminant function. A plot of the discriminant functions for trait groups 1-3 confirmed group membership for all species (Figure 3a). Traits with the highest loadings based on coefficients of the first discriminant function for trait groups 1-3 were mouth width, trunk length, caudal peduncle flatness, and dorsal fin aspect ratio (Table 2). Pectoral fin area and eye position were important for providing additional separation among species in trait groups 1-3 based on loadings of the coefficients for the second discriminant function.

I then evaluated 10 significant clusters that comprised 85 stream fishes at a lower level of the hierarchy in the dendrogram (trait groups A-J; Figure 1). Trait group H was identical to trait group 2 as it contained no significant nested clusters, suggesting that the traits I measured did not provide additional separation among catfishes. Most of the significant clusters contained “core” fish species that one would intuitively group together due to a high degree of relatedness or obvious similarity in morphology. However, the significant clusters at this level of the dendrogram also provided considerable diversity among member species to further evaluate the effectiveness of HCMB for generating trait groups. Multiple families were represented in two or more significant clusters and no significant clusters consisted of only a single genus. For example, suckers and black basses and sunfishes were represented in two significant clusters (trait groups A and D and trait groups E and G, respectively) with white basses (*Morone*) grouped with the black basses (*Micropterus*). Chubs and minnows were distributed among three significant clusters (trait groups B, C, and D) with Common Carp *Cyprinus carpio* grouped with two genera of suckers (*Ictiobus* and *Carpiodes).* Some significant clusters also included taxonomically-unique fishes that would provide difficulty in determining group membership based on morphology without analytical support. For example, Freshwater Drum *Aplodinotus grunniens,* the sole member of Sciaenidae in my species pool, grouped with Common Carp and suckers. Western Mosquitofish, the sole member of Poeciliidae in my species pool, grouped with topminnows (trait group J). The LDA for trait groups A-J resulted in 67.9% separation using the first two discriminant functions and 83.0% separation using the first three discriminant functions (Table 3). A plot of the first two discriminant functions confirmed group membership for trait groups A-E and trait groups G-J (Figure 3b). Trait group F, which comprised only two species, overlapped with trait group D, which suggested the traits I measured did not sufficiently separate Skipjack Herring *Alosa chrysochloris* and Gizzard Shad *Dorosoma cepedianum* at this level of the assemblage. Traits with the highest loadings based on coefficients of the first discriminant function for trait groups A-J were dorsal fin aspect ratio, pectoral fin area, flatness index, and caudal peduncle length. (Table 3); however, eye position mouth width, and trunk length had high loadings for the second and third discriminant functions, suggesting that these traits were important for providing additional separation among the fish species.

Significant clusters were nested within trait groups A-E, trait group G, and trait groups I and J (i.e., at lower levels of the dendrogram; Figure 1). For example, four significant clusters comprising 15 small-bodied cyprinids were nested within trait group C (trait groups C1-C4; Figure 1 and Figure 4). Small-bodied cyprinids are often challenging to distinguish based solely on external morphology and perceived phylogenetic relationships are often fluid. *Cyprinella* was split between two clusters (trait group C1 and C2) and *Notropis* was distributed among all four trait groups, suggesting that the traits I measured may better differentiate these species than phylogeny. I did not perform a LDA on trait groups C1-C4 because the species to trait ratio (15:15) was unfavorable for an accurate interpretation of variable loadings (McGarigal et al. 2002).

DISCUSSION

Groups of Ozark Highland stream fishes developed using HCMB suggested strong associations among species based on combinations of external morphological traits at various levels of the assemblage. Interestingly, similar body shape, feeding, and swimming traits distinguished significant clusters at two levels of the hierarchy in the dendrogram. Trunk length, measures of body flatness, eye position, mouth width, dorsal fin aspect ratio, and pectoral fin area had high coefficient loadings for both trait groups 1-3 and trait groups A-J. Unfortunately, a LDA would have been both unreliable and difficult to interpret for significant groupings at lower levels of the dendrogram due to the species-trait ratio; however, this also highlights an advantage of HCMB. A favorable trait-to-species ratio can be achieved using HCMB and, due to nested groups with associated AU *p*-values, significant groupings comprising a small number of species can be identified.

Arbitrariness in identifying groupings is an inherent aspect of hierarchical clustering (Romesburg 2011). However, criteria such as AU *p*-values that provide quantitative assessments of uncertainty in clusters to complement visual assessments of a dendrogram are superior when compared to relying solely on distance cut-offs. For trait-based studies, AU *p*-values both guide where separation may occur among species with similar trait combinations and provide a measure of the strength of the relationship between unique species and other members of an assemblage. Furthermore, assessing the strength of groupings at various levels of the dendrogram promotes flexibility to examine multiple questions related to trait relationships across a species assemblage.

I demonstrated how HCMB can identify trait combinations that distinguish species at various levels of an assemblage. Research that studies traits in combination, rather than individually, can help advance the predictive ability of community ecology (Lavorel and Garnier 2002; Villéger et al. 2008; Verberk et al. 2013). Individual traits may not be ecologically meaningful to a species unless taken in the context of its entire suite of characteristics and the size of certain traits (e.g., eye diameter) may represent an evolutionary choice to give up advantages of alternative variations. Examining how traits combine due to interactions and trade-offs during the course of a species evolution can enhance insight into mechanistic species-environment relationships (Verberk et al. 2013). For example, single traits of aquatic macroinvertebrates only described the habitat use of abundant species rather than revealing more complex community dynamics (Pilière et al. 2016). Additionally, four unique trait combinations explained the response of grassland plant species to land-use change (Gross et al. 2007).

Although phylogenetic relationships were apparent (and expected) in significant clusters, my results suggested that variation in the measured morphological traits among closely related species was an important factor in the observed trait groups. The influence of phylogeny was beyond the scope of this objective; however, isolating similarities and differences between closely related species can provide important insight into potential functional roles of traits (Westoby et al. 1995; Verberk et al. 2013). Because convergent evolution is such a common phenomenon in freshwater fishes (e.g., Muschick et al. 2012; Montaña and Winemiller 2013;

Furness et al. 2015) phylogenetic relationships among study species may be an asset to identifying key trait adaptations across coarse spatial and temporal scales. Of course, for many research questions phylogenic inertia remains an important consideration.

The trait groups I constructed using HCMB encompassed the holistic morphology of a species and comprised stream fishes that were similar in trait combinations. A further assessment of ecological relationships among species, while considering the traits removed due to high correlations, is required to determine if my results represent functional groups. For example, Freshwater Drum grouped with suckers and common does not necessarily indicate a functional relationship; however, Freshwater Drum have been shown to have ecological similarities with these fishes (Edsall 1967). A detailed evaluation of the functional importance of these trait groups is beyond the scope of the objective here, which was to group stream fishes based on similar morphology. Although I chose to focus on morphological fish traits, the approach described here is easily adaptable to both other species traits and different taxa.

Table 1. Description of the morphological traits used for hierarchical clustering with multiscale bootstrap resampling (HCMB) for 92 Ozark Highland stream fishes incorporating ecologically-important indices (see also Appendix 2 for complete definitions of individual traits). Asterisks indicate traits that were removed prior to the cluster analyses due to high correlations with other traits (See Methods for details).

Trait	Definition
*Head depth	Vertical distance from dorsum to ventrum passing through the left pupil divided by standard length
*Head length	Horizontal distance from the anterior tip of the jaw to the posterior margin of opercular membrane divided by standard length
Flatness	Maximum body depth divided by body width
*Body depth below midline	Vertical distance along line of maximum body depth from the point of an imaginary perpendicular line coming from the pupil divided by standard length
Trunk length	Horizontal distance from the anterior tip of the jaw to point of maximum body depth divided by standard length
Caudal peduncle length	Horizontal distance from the anterior margin of the posterior base of the caudal fin to an imaginary vertical line from the posterior base of the anal fin divided by standard length
Caudal peduncle flatness	Caudal peduncle depth divided by caudal peduncle width
Eye position	Vertical distance from the center of the pupil to the ventrum divided by standard length
Eye diameter	Distance from between fleshy orbits of the eye along an anterior-posterior axis divided by standard length

Snout length	Distance from the pupil to the tip of the upper jaw with mouth shut divided by standard length
Mouth width	Horizontal distance across the mouth when fully open divided by standard length
*Mouth height	Vertical distance across the mouth when fully open divided by standard length
*Jaw length	Distance of the lower jaw mandible divided by standard length
*Dorsal fin area	Surface area of the dorsal fin divided by body area
Dorsal fin aspect ratio	Dorsal fin height divided by dorsal fin length
Caudal fin span	Maximum distance from the top edge of the caudal fin to the bottom edge of the caudal fin divided by maximum body depth
Caudal fin aspect ratio	Maximum distance from the top edge of the caudal fin to the bottom edge of the caudal fin squared divided by the surface area of the caudal fin
Anal fin area	Surface area of the anal fin divided by body area
Anal fin aspect ratio	Anal fin height divided by anal fin length
*Pelvic fin area	Surface area of the pelvic fin divided by body area
Pelvic fin aspect ratio	Pelvic fin height divided by pelvic fin length
Pectoral fin area	Surface area of the pectoral fin divided by body area
Pectoral fin aspect ratio	Pectoral fin height divided by pectoral fin length

Table 2. The first three discriminant functions resulting from a linear discriminant analysis (LDA) for 3 groups (trait groups 1-3) comprising 90 Ozark Highland stream fishes using external morphological traits (see also Figure 3). Groups were selected based on results of hierarchical clustering with multiscale bootstrapping (HCMB; Figure 1).

Trait	First discriminant function	Second discriminant Function
Trunk length	0.98	0.82
Caudal peduncle length	0.72	-0.09
Caudal peduncle flatness	-0.93	-0.20
Flatness	0.81	-0.26
Eye position	0.12	-1.02
Eye diameter	0.28	0.37
Mouth width	-1.11	0.07
Anal fin area	-0.47	0.24
Anal fin aspect ratio	0.25	-0.19
Pectoral fin area	-0.40	1.81
Pectoral fin aspect ratio	-0.35	-0.22
Dorsal fin aspect ratio	-0.93	-1.06
Pelvic fin aspect ratio	0.03	0.33
Caudal fin span	-0.22	-0.05
Caudal fin aspect ratio	0.55	0.04

Table 3. The first three discriminant functions resulting from a linear discriminant analysis (LDA) for 10 groups (trait groups A-J) comprising 85 Ozark Highland stream fishes using external morphological traits (see also Figure 3). Groups were selected based on results of hierarchical clustering with multiscale bootstrapping (HCMB; Figure 1).

Trait	First discriminant function	Second discriminant function	Third discriminant function
Trunk length	0.33	-0.27	1.17
Caudal peduncle length	0.93	-0.25	0.26
Caudal peduncle flatness	0.44	-0.13	-0.94
Flatness	-0.94	-1.42	-0.17
Eye position	-0.13	-1.54	-0.65
Eye diameter	-0.50	-0.13	-0.12
Mouth width	0.63	1.09	-0.83
Anal fin area	-0.22	0.81	-0.37
Anal fin aspect ratio	0.21	-0.36	0.65
Pectoral fin area	-1.95	1.38	0.78
Pectoral fin aspect ratio	0.75	0.17	-0.07
Dorsal fin aspect ratio	2.15	0.57	-0.65
Pelvic fin aspect ratio	-0.55	-0.06	0.09
Caudal fin span	0.21	0.37	0.01
Caudal fin aspect ratio	-0.17	-0.48	0.10

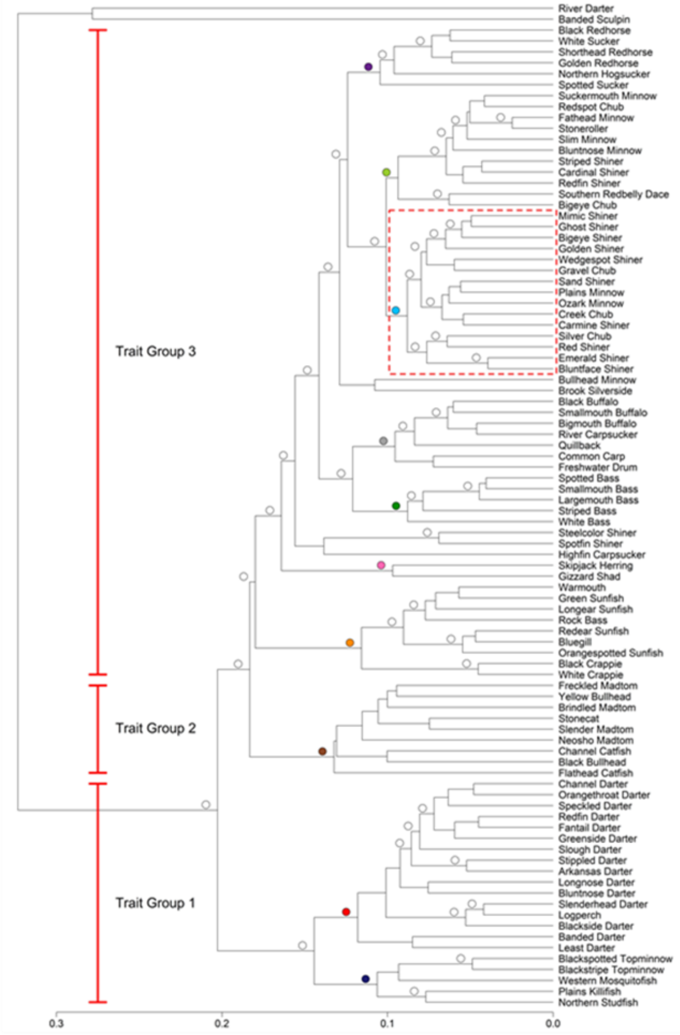

Figure 1. Results of hierarchical clustering with multiscale bootstrapping (HCMB) for 92 Ozark Highland stream fishes using external morphological traits (Table 1 and Appendix 2). Values at nodes of the dendrogram are approximately unbiased (AU) *p*-values. Standard error was ≤ 0.01 for all AU *p*-values (Figure 2). Significant clusters (*n* = 54) were identified as (AU *p*-value minus SE) ≥ 0.95 and are indicated by circles at nodes of the dendrogram. Vertical red lines with bars indicate species members of trait groups 1-3 comprising 90 species. Color-coded circles represents 10 significant clusters comprising 85 species (trait groups A through J), where purple is trait group A, light green is trait group B, light blue is trait group C, light gray is trait group D, dark green is trait group E, pink is trait group F, orange is trait group G, brown is trait group H, and red is trait group I, and dark blue is trait group J. The red box around trait group C highlights one example of significant nested clusters (trait groups C1-C4; Figure 4).

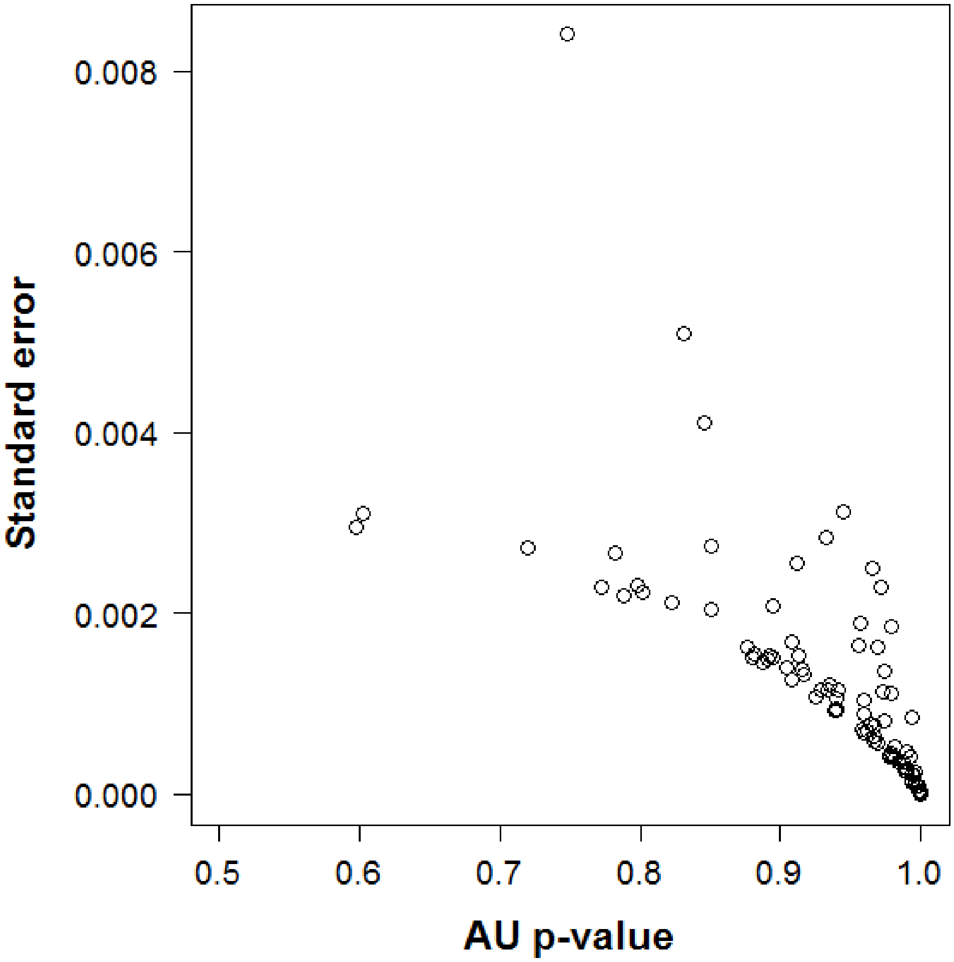

Figure 2. Standard error (y-axis) for approximately unbiased probability values (AU p-values; *x*-axis) associated with 90 clusters (circles) resulting from hierarchical clustering with multiscale bootstrapping (HCMB) for 92 Ozark Highland stream fishes based on external morphological traits (Table 1 and Appendix 2).

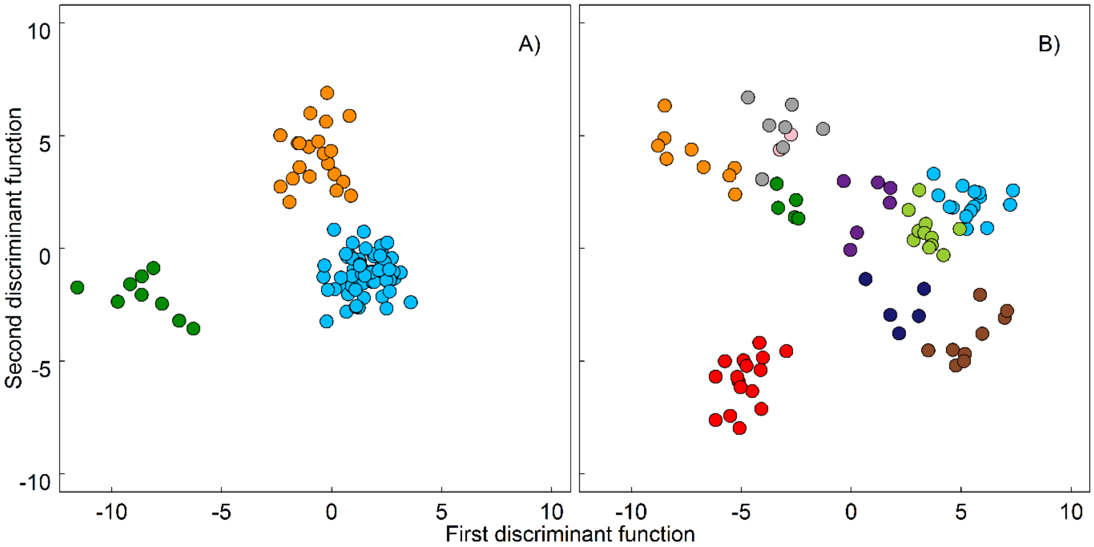

Figure 3. Scatterplot of the first two discriminant functions for trait Groups 1-3 and trait groups A-J resulting from hierarchical clustering with multiscale bootstrapping (HCMB) for 92 Ozark Highland stream fishes based on external morphological traits (Table 1 and Appendix 2). For Figure 3A, orange represents trait group 1, dark green represents trait group 2, and light blue represents trait group 3. Color coding for fish species in Figure 3B corresponds with Figure 1.

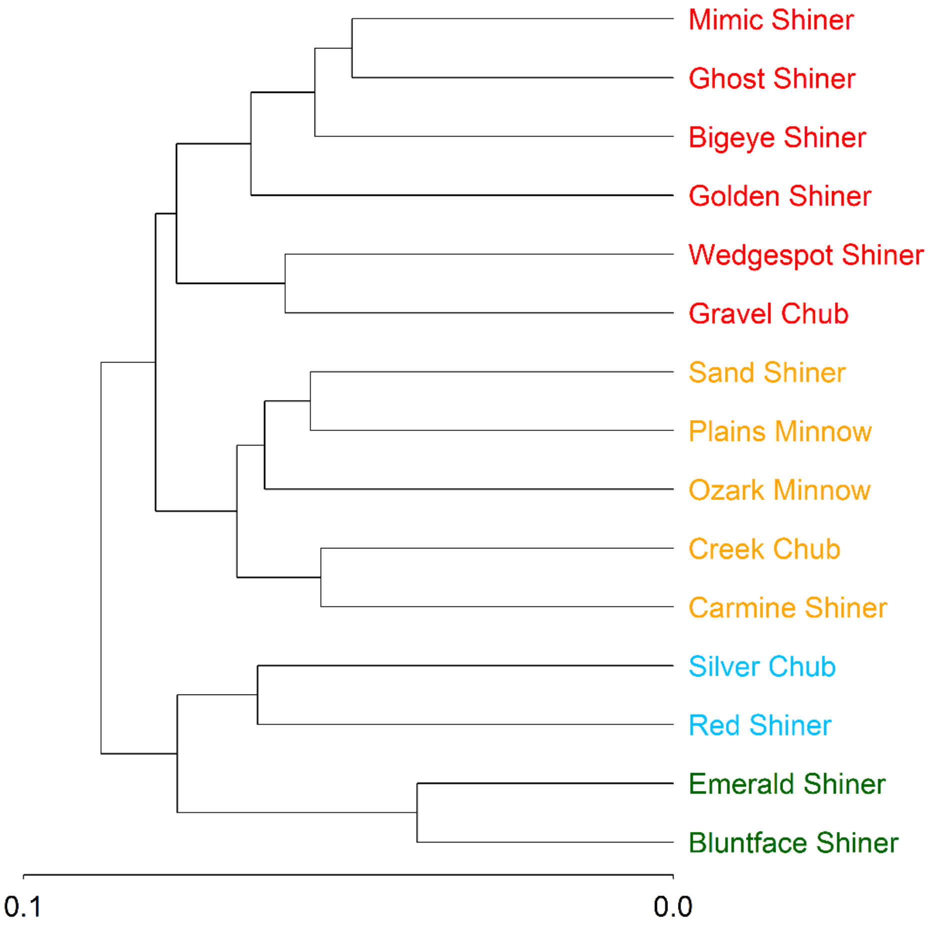

Figure 4. View of dendrogram for trait groups C1-C4. Color coding represents four significant clusters comprising 15 species nested within trait group C (Figure 1), where red is trait group C1, orange is trait group C2, blue is trait group C3, and green is trait group C4. Significant clusters were assessed using approximately unbiased (AU) *p*-values as (AU *p*-value minus SE) ≥ 0.95 based on results of hierarchical clustering with multiscale bootstrapping (HCMB) for 92 Ozark Highland stream fishes based on external morphological traits (Table 1 and Appendix 2).

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APPENDICES

Appendix 1. Common name and Latin name of 92 Ozark Highland stream fishes used for hierarchical clustering with multiscale bootstrapping (HCMB) to group species based on combinations of external morphological traits.

Common name	**Latin name**
Arkansas Darter	*Etheostoma cragini*
Banded Darter	*Etheostoma zonale*
Banded Sculpin	*Cottus carolinae*
Bigeye Chub	*Hybopsis amblops*
Bigeye Shiner	*Notropis boops*
Bigmouth Buffalo	*Ictiobus cyprinellus*
Black Buffalo	*Ictiobus niger*
Black Bullhead	*Ameiurus melas*
Black Crappie	*Pomoxis nigromaculatus*
Black Redhorse	*Moxostoma duquesnei*
Blackside Darter	*Percina maculata*
Blackspotted Topminnow	*Fundulus olivaceus*
Blackstripe Topminnow	*Fundulus notatus*
Bluegill	*Lepomis macrochirus*
Bluntface Shiner	*Cyprinella camura*
Bluntnose Darter	*Etheostoma chlorosomum*
Bluntnose Minnow	*Pimephales notatus*
Brindled Madtom	*Noturus miurus*
Brook Silverside	*Labidesthes sicculus*
Bullhead Minnow	*Pimephales vigilax*
Cardinal Shiner	*Luxilus cardinalis*
Carmine Shiner	*Notropis percobromus*
Channel Catfish	*Ictalurus punctatus*
Channel Darter	*Percina copelandi*
Common Carp	*Cyprinus carpio*
Creek Chub	*Semotilus atromaculatus*

Emerald Shiner	*Notropis atherinoides*
Fantail Darter	*Etheostoma flabellare*
Fathead Minnow	*Pimephales promelas*
Flathead Catfish	*Pylodictis olivaris*
Freckled Madtom	*Noturus nocturnus*
Freshwater Drum	*Aplodinotus grunniens*
Ghost Shiner	*Notropis buchanani*
Gizzard Shad	*Dorosoma cepedianum*
Golden Redhorse	*Moxostoma erythrurum*
Golden Shiner	*Notemigonus crysoleucas*
Gravel Chub	*Erimystax x-punctatus*
Green Sunfish	*Lepomis cyanellus*
Greenside Darter	*Etheostoma blennoides*
Highfin Carpsucker	*Carpiodes velifer*
Largemouth Bass	*Micropterus salmoides*
Least Darter	*Etheostoma microperca*
Logperch	*Percina caprodes*
Longear Sunfish	*Lepomis megalotis*
Longnose Darter	*Percina nasuta*
Mimic Shiner	*Notropis volucellus*
Neosho Madtom	*Noturus placidus*
Northern Hogsucker	*Hypentelium nigricans*
Northern Studfish	*Fundulus catenatus*
Orangespotted Sunfish	*Lepomis humilis*
Orangethroat Darter	*Etheostoma spectabile*
Ozark Minnow	*Notropis nubilus*
Plains Killifish	*Fundulus zebrinus*
Plains Topminnow	*Fundulus sciadicus*
Quillback	*Carpiodes cyprinus*
Red Shiner	*Cyprinella lutrensis*
Redear Sunfish	*Lepomis microlophus*
Redfin Darter	*Etheostoma whipplei*
Redfin Shiner	*Lythrurus umbratilis*

Redspot Chub	*Nocomis asper*
River Carpsucker	*Carpiodes carpio*
River Darter	*Percina shumardi*
Rock Bass	*Ambloplites rupestris*
Sand Shiner	*Notropis stramineus*
Shorthead Redhorse	*Moxostoma macrolepidotum*
Silver Chub	*Macrhybopsis storeriana*
Skipjack Herring	*Alosa chrysochloris*
Slender Madtom	*Noturus exilis*
Slenderhead Darter	*Percina phoxocephala*
Slim Minnow	*Pimephales tenellus*
Slough Darter	*Etheostoma gracile*
Smallmouth Bass	*Micropterus dolomieu*
Smallmouth Buffalo	*Ictiobus bubalus*
Southern Redbelly Dace	*Phoxinus erythrogaster*
Speckled Darter	*Etheostoma stigmaeum*
Spotfin Shiner	*Cyprinella spiloptera*
Spotted Bass	*Micropterus punctulatus*
Spotted Sucker	*Minytrema melanops*
Steelcolor Shiner	*Cyprinella whipplei*
Stippled Darter	*Etheostoma punctulatum*
Stonecat	*Noturus flavus*
Stoneroller	*Campostoma* spp.
Striped Bass	*Morone saxatilis*
Striped Shiner	*Luxilus chrysocephalus*
Suckermouth Minnow	*Phenacobius mirabilis*
Warmouth	*Lepomis gulosus*
Wedgespot Shiner	*Notropis greenei*
Western Mosquitofish	*Gambusia affinis*
White Bass	*Morone chrysops*
White Crappie	*Pomoxis annularis*
White Sucker	*Catostomus commersoni*
Yellow Bullhead	*Ameiurus natalis*

Appendix 2. Description of external morphological traits measured on 92 Ozark Highland stream fishes.

Trait	**Definition**
Standard length	Horizontal distance from the anterior tip of the jaw to the anterior margin of the posterior base of the caudal fin
Body area	Total surface area of the body excluding fins
Maximum body depth	Maximum vertical distance from dorsum to ventrum
Head depth	Vertical distance from dorsum to ventrum passing through the left pupil divided by standard length
Head length	Horizontal distance from the anterior tip of the jaw to the posterior margin of opercular membrane divided by standard length
Maximum body depth	Maximum body depth divided by standard length
Body depth below midline	Vertical distance along line of maximum body depth from the point of an imaginary perpendicular line coming from the pupil divided by standard length
Trunk length	Horizontal distance from the anterior tip of the jaw to point of maximum body depth divided by standard length
Body width	Maximum horizontal distance of body perpendicular to an imaginary vertical line at the point of maximum body depth divided by standard length
Caudal peduncle length	Horizontal distance from the anterior margin of the posterior base of the caudal fin to an imaginary vertical line from the posterior base of the anal fin divided by standard length
Caudal peduncle depth	Vertical distance from dorsum to ventrum of the caudal peduncle at midlength divided by standard length
Caudal peduncle width	Maximum horizontal distance of the caudal peduncle at midlength perpendicular to caudal peduncle depth measurement divided by standard length

Eye position	Vertical distance from the center of the pupil to the ventrum divided by standard length
Snout length	Distance from the pupil to the tip of the upper jaw with mouth shut divided by standard length
Dorsal fin area	Surface area of the dorsal fin divided by body area
Dorsal fin height	Maximum distance from the base to the tip of the dorsal fin divided by standard length
Dorsal fin length	Maximum distance from the anterior edge of the dorsal fin to the posterior edge of the dorsal fin divided by standard length
Caudal fin area	Surface area of the caudal fin divided by body area
Caudal fin span	Maximum distance from the top edge of the caudal fin to the bottom edge of the caudal fin divided by maximum body depth
Anal fin area	Surface area of the anal fin divided by body area
Anal fin length	Maximum distance from the base to the tip of the anal fin divided by standard length
Anal fin height	Maximum distance from the top edge of the anal fin to the bottom edge of the anal fin divided by standard length
Pelvic fin area	Surface area of the pelvic fin divided by body area
Pelvic fin length	Maximum distance from the base to the tip of the left pelvic fin divided by standard length
Pelvic fin height	Maximum distance from the top edge of the pelvic fin to the bottom edge of the pelvic fin divided by standard length
Pectoral fin area	Surface area of the pectoral fin divided by body area
Pectoral fin length	Maximum distance from the base to the tip of the left pectoral fin divided by standard length
Pectoral fin height	Maximum distance from the top edge of the pectoral fin to the bottom edge of the pectoral fin divided by standard length

CHAPTER III

A MULTISCALE MULTI-SPECIES ELECTROFISHING DETECTION MODEL FOR STREAM FISHES OF THE OZARK HIGHLANDS

ABSTRACT

Variable detection across sampling conditions and among species confounds perceived patterns of fish distributions across the stream landscape and presents challenges for research and management. Approaches to account for variable detection that strike a balance between complexity and practicality promote increased implementation by stream-fish scientists. I used a straight-forward gear calibration method to model tow-barge electrofishing capture probability among Ozark Highland stream fishes across environmental conditions at multiple spatial scales. A primary advantage of gear calibration is that it can be used to derive temporally- and spatially-comparable estimates of absolute abundance across a range of environmental conditions with equivalent sampling effort as catch-per-unit-effort. Multiple reach-scale variables were associated with variation in stream-fish detection including water depth and clarity, emergent vegetation, and a discharge-proportion riffle interaction. The magnitude of the relationship between detection probability and reach-scale variables also varied among species. Additionally, lithological characteristics of stream segments explained additional variation in detection. Multiple validation methods indicated good model performance. Average model bias and average root mean square error of detection probability based on a cross validation was -0.01 and 0.09, respectively. The model-predicted detection probability was contained in a binomial probability confidence interval for observed data 83% of the time. Species abundance estimates using model-predicted detection

probability were contained in the Petersen capture-recapture confidence intervals 86% of the time. Application of this relatively simple approach to model variation in stream-fish detection can both maximize available resources and promote advances in stream-fish ecology and management.

INTRODUCTION

Perceived fish distributions across the dynamic stream landscape are largely influenced by the timing and nature of sampling (Jackson et al. 2001; Poff and Zimmerman 2010; Gwinn et al. 2016). Variation in detection (the proportion of available individuals captured) among sampling methods, environmental conditions, and species hinders our ability to detect changes in stream-fish abundance and assemblage structure. The importance of addressing variable detection for sound management and conservation has long been recognized by stream-fish researchers (e.g., Cleary and Greenbank 1954; Larimore 1961). Despite improved analytical approaches to account for variable detection (e.g., Williams et al. 2002; MacKenzie et al. 2005; Royle et al. 2013), catch-per-unit-effort (CPUE) remains the most common metric used to evaluate and monitor stream-fish populations (Gwinn et al. 2016). Major limitations of CPUE include calculating only an indirect measure of abundance, relying on a typically untested and often unrealistic assumption of constant detection, and providing no insight about detection relationships among species.

Standardizing sampling conditions (e.g., sampling only at baseflows) to support the use of CPUE may minimize variation in site-specific, short-term detection. However, maintaining constant detection during long-term stream-fish monitoring or across a broad study area is challenging because environmental conditions in streams vary extensively across space and time, with interactions among environmental variables across varying scales further increasing the complexity (Jackson et al. 2001; Price and Peterson 2010; Gwinn et al. 2016). Furthermore, identifying relationships between stream fishes and the environment (e.g., flow-ecology relationships), often fundamental research and management goals, are not possible when sampling is limited to a narrow range of environmental conditions (Gwinn et al. 2016 provide a detailed discussion of the limitations of CPUE for stream-fish sampling).

One approach to account for variable detection when sampling stream-fish populations is gear calibration (Peterson and Rabeni 2001; Peterson and Paukert 2009). Gear calibration requires repeat sampling over a broad range of sampling conditions using either capture-recapture or comparisons with a previously calibrated gear. Although a substantial amount of effort and data are initially required for effective gear calibration, a model can be developed to explicitly account for variable detection. The detection model can be used to adjust catch data to an absolute abundance estimate based on site-specific conditions (Thompson and Seber 1994; Peterson and Paukert 2009). Therefore, the long-term benefit of gear calibration is temporally- and spatially-comparable abundance estimates with equivalent sampling effort as CPUE (i.e., a single sampling event). However, an inherent challenge of developing an effective detection model is capturing the complex relationships across environmental conditions and among species while providing an end product that remains practical for implementation into long-term monitoring or research.

My objective was to develop a practical, flexible, multiscale model to predict detection among stream fishes in wadeable warmwater streams across a range of environmental conditions using tow-barge electrofishing. A novel aspect of my approach was to incorporate spatial data to generalize coarse-scale lithology among sites. Differences in morphology can also contribute to variation in stream-fish detection (Peterson and Paukert 2009; Rabeni et al. 2009). Thus, I also evaluated electrofishing detection tendencies among morphological trait groups developed in Chapter 2. Previous research addressing stream-fish detection has been biased toward salmonids and my study represents a rare effort to model multi-species electrofishing detection in wadeable warmwater streams of the southern United States (see also Price and Peterson 2010). I detail an effective, yet straightforward, approach to model detection and adjust catch data that can be easily be implemented by stream-fish scientists in other systems or with other sampling gears.

METHODS

Study sites. - I sampled stream fishes in 34 stream reaches (referred to hereafter as sites) in the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri during summer 2014-2015 (Fig. 1). All sites were wadeable (i.e., most habitat was < 1 m deep; Rabeni et al. 2009) and comprised three to five riffle-run-pool sequences to characterize stream habitat. The sites provided both environmental variation and geographic diversity, with some streams located at the southern ecoregion boundary. I focused on nine species of Centrarchidae (Table 1) because they are both common and abundant in Ozark Highland streams, include popular sportfishes, and recover quickly from electrofishing with minimal mortality (Bardygula-Nonn et al. 1995; Dolan et al. 2002; Dolan and Miranda 2004). These stream fishes also comprised three significant morphological trait groups developed in Chapter 2 (trait groups A-C; Table 1).

Fish sampling. - I installed two sets of block-off nets at both the upstream and downstream end of each site to close the area to fish movement. Block-off nets were preferentially placed at shallow riffles to further inhibit fish movement (Peterson et al. 2004; Price and Peterson 2010). Either a low-water bridge at base flows or a dry riffle located at one end of the site provided an adequate fish barrier at a few sites and no block-off nets were installed.

On day one (marking day), I used both a tow-barge electrofisher (Midwest Lake Management, Polo, Missouri) and angling to establish marked populations of centrarchids. Marked fish were not released until the sampling crew had proceeded a minimum of one riffle-run-pool sequence upstream to minimize the probability of being recaptured (none were recaptured). Angling was used on marking day primarily to increase the number of marked Smallmouth Bass *Micropterus dolomieu* because detection is often low using electrofishing

(Lyons and Kanehl 1993; Heimbuch et al. 1997; Dauwalter and Fisher 2007), although other captured centrarchids were also included in the marked population. All captured centrarchids were identified to species, measured (1.0-mm TL), and marked with a caudal fin clip. The minimum size for fish was 50-mm TL for *Lepomis* and Rock Bass *Ambloplites rupestris* and 80-mm TL for *Micropterus*. The size restrictions excluded most age-0 fishes not recruited to electrofishing (McClendon and Rabeni 1986) and was also based on observed mortalities for very small centrarchids (Dolan and Miranda 2004; personal observations). Marked fish were released throughout the site and allowed to recover and redistribute for ~48 h. The time between electrofishing events was nearly double the commonly accepted guideline for system recovery (Peterson and Cederholm 1984). Fish injured during sampling or that showed signs of excessive stress were released outside of the blocked-off area.

I used several methods to assess delayed fish mortality and potential emigration. The blocked-off area was routinely inspected from the surface for dead marked centrarchids, which were removed from the study. The block-off nets and the area between them were inspected periodically for trapped or dead fish. The method used to inspect the area between the block nets was dependent on water depth (typical depth was < 0.3 m) and water clarity, where I used a visual inspection from the surface, snorkeling, or electrofishing. As an additional assessment of fish mortality, a snorkel pass was performed at sites with reasonable water clarity ($n = 22$) to identify dead centrarchids. I also evaluated fish movement at a subset of sites to provide insight into potential emigration in an instance where the block nets were ineffective. At three sites, I marked fish in the lower portion of the site with an upper caudal fin clip and fish in the upper portion of the site with a lower caudal fin clip, where the location of the fin clip was noted for recaptured centrarchids. At each of the three sites, the depth of the riffle that divided the lower and upper portion of the site was ≥ 0.3 m (i.e., deeper than the typical depth of riffles where block nets were set).

The recapture event (calibration day) consisted of two standardized removal electrofishing passes per riffle-run-pool sequence. The electrofishing crew comprised three people: one tow-barge operator (myself) armed with a hand net and two persons equipped with dip nets each operating one of the two anodes. I used pulsed direct current (DC), 60 Hz, and a 25% duty cycle for electrofishing. Voltage was adjusted to achieve a target power (W) that maintained a consistent electric field across levels of ambient water conductivity while minimizing electrofishing-induced injuries as described by Miranda (2009). During each electrofishing pass, we sampled areas ≥ 0.2-m deep, which excluded most riffle habitat, in an upstream direction with a zigzag pattern. Logistic constraints of the tow-barge made very shallow habitats difficult to sample effectively, although use of these habitats are uncommon by centrarchids (Probst et al. 1984; Schlosser 1987; Brewer 2013). Care was taken to thoroughly electrofish areas of structure (e.g., instream large wood, rootwads, and boulders). All captured centrarchids were identified to species and measured (1.0-mm TL). Unmarked fish data were later used for model validation.

Environmental measurements. - I measured environmental variables hypothesized to influence detection of stream fishes (see also Table 2). A conductivity pen (Myron L Company, Carlsbad, California; Model PT1) was used to measure water temperature (0.1ºC) and ambient water conductivity (µS/cm) at the downstream end of the site. Wetted channel width (1.0 m) and thalweg depth (0.1 m) were measured at 50-m transects. Stream discharge (0.01 m^3/s) was measured in a homogenous area of a run using the velocity-area method (Gordon et al. 2004). Water clarity (0.5 m) was measured as the horizontal distance an underwater observer could see a fish silhouette. I used the same fish silhouette at all sites to maintain consistency and it was designed to mimic the color, markings, and typical size (~200 mm) of Smallmouth Bass in the study streams. GPS coordinates were taken at both the upstream and downstream end of each site to estimate reach length (1.0 m). I also measured the length of each riffle (1.0 m) to calculate the proportion of riffle habitat. Both instream large wood and emergent vegetation were estimated as the length (1.0 m) and width (1.0 m) of each patch to calculate proportional coverage.

I used spatial data to group stream segments (hereafter referred to as segment) into categories based on geology and soils (hereafter referred to "geosoil" groups) as cherty limestone, cherty alluvium, stony alluvium, and shale. The geosoil categories provided surrogates for substrate and streambank characteristics (e.g., lithologic complexity and interstitial spaces). Segments were defined as a stream length between 3rd order and higher tributary confluences. GIS layers were obtained for both rock fragment type (Miller and White 1998; Pennsylvania State University 2008) and geology type (USGS 2005). Using ArcMap (version 10.2.1, ESRI, Red Lands, California), a 50-m buffer was generated around each segment, where the dominant rock fragment type and geology type were used to characterize each segment.

Electrofishing detection model. - I modeled variation in electrofishing detection among centrarchids across environmental conditions at multiple spatial scales using a generalized linear mixed model (GLMM). GLMMs are a flexible, powerful class of statistical models that allow for the inclusion of random effects, which broadens the scope of inference, accounts for a lack of independence among observations (i.e., pseudoreplication), and accommodates the multiscale (i.e., nested) structure of stream systems and unequal sample sizes common in ecological data (Wagner et al. 2006; Jamil et al. 2013). I implemented models using the package "lme4" (Bates et al. 2014) in the statistical software R (version 3.2.2, R Core Development Team, 2014). Detection was modeled as a Bernoulli process using a binomial error distribution, where recapture was a binary response variable (i.e., recaptured or not recaptured). I assigned recaptures by matching each recaptured fish to a marked fish for each species ± 5.0-mm TL to incorporate variation in fish size into the model. Individual recognition was not required from a modeling perspective (e.g., if 2 of 5 Smallmouth Bass ~200-mm TL were recaptured, the results of the model would be identical regardless of which two were assigned as recaptured); however, accurate species identification and measurements of fish length were critical for reliable results. The model can be written as

(1) $$\text{logit}(p_{ijk}) = \beta_0 + \mu_{0i} + \nu_{0j} + \gamma_{0k} + \beta_1 x_{ijk} + \beta_2 x_{ijk} + \beta_n x_{ijk} + \mu_1 x_j + \mu_n x_j$$

where p is estimated detection probability, β_0 is the grand mean intercept, μ is the random species intercept, ν is the random site intercept, γ is the random segment intercept, x is a variable corresponding to observation (fish) i at site j nested in segment k, and $\mu_i x_j$ is the random species by site-scale variable slope. Random effects were assumed normally distributed as $N(0,\tau^2)$, where τ is the population variance among levels of a random effect. All continuous variables were natural-log transformed due to asymmetry and standardized such that each variable had a mean of zero and a variance of one to improve interpretation of model coefficients and promote model convergence.

I fitted models using a tiered forward selection similar to Jamil et al. (2013). I chose forward selection because, due to the number of variables being examined, a backward selection would have resulted in convergence issues with the most complex models. Pearson's product moment coefficient (r) was calculated among site-scale variables to allow levels of correlation to be considered during the model-selection process. At each step, a variable was only included if it decreased Akaike information criterion (AIC; Burnham and Anderson 2001) by at least one. Remaining variables with $|r| \geq 0.50$ were eliminated as each site-scale variable was added to the model. A moderate level of correlation among variables is tolerable for predictive modeling and can actually improve predictive power of a model despite increasing the standard error of coefficients (Allison 1999). Also, multicollinearity among predictor variables does not affect measures of model fit using AIC with $|r| < 0.50$ (Burnham and Anderson 2001; Cohen et al. 2003). The null model contained both random effects (species, site, and segment) and TL as a fixed effect to account for the influence of fish size on capture probability prior to examining environmental variables. I considered 13 site-scale environmental variables for the random tier of the model-selection process (Table 2), where selected variables were added only as species-dependent random terms (i.e., each species had a random intercept and a random slope for each included site-scale variable). I did not anticipate ambient water conductivity would explain variability in electrofishing detection because electrofishing power was standardized; however, I included it in the set of site-scale variables to examine if the approach was effective. For the next tier, I added fixed effects for site-scale variables selected during the random tier, considered remaining site-scale variables, and examined all two-way interactions between site-scale variables. Lastly, I added the geosoil categories to the model as a segment-scale variable, where shale was the reference. Although variance components on a logit scale cannot be interpreted in the same fashion as Gaussian distributions (Nakagawa et al. 2013), I chose to perform a calculation to assess relative variation explained in the final model at both the site scale and segment scale as: $(\hat{\sigma}^2_{\text{null model}} - \hat{\sigma}^2_{\text{full model}}) / \hat{\sigma}^2_{\text{null model}}$, where $\hat{\sigma}$ is the variance component for each random effect (Wagner et al. 2006). A measure of total variation explained in the final model (i.e., R^2) would not have provided a useful assessment of the model's ability to predict the proportion of available individuals captured. The interpretation of R^2 would have been the ability of the final model to predict an individual observation (i.e., the probability of recapturing an individual fish; Mittlböck and Heinzl 2001) because recapture was a binary response variable.

Reported estimates of detection probability for validation methods and model interpretation were back transformed. Standard error was first estimated with the delta method (Oehlert 1992) using the package "msm" (Jackson 2011) in the statistical software R. Both the detection estimate and standard error were then back transformed from the logit scale using calculations described by Jørgensen and Pedersen (1998).

The final model was evaluated using both a cross-validation method and a comparison of model-predicted fish abundance to a widely used population estimation method with known reliability when assumptions were met. These approaches assessed model performance, site-level bias, and the general applicability of the model for reliable stream-fish abundance estimates. I performed a 10-fold cross validation, where data were randomly split into 10 groups of three to four sites (~10% of the sites). At each cross-validation step, I modeled the remaining data and assessed model bias and accuracy based on observed recapture proportions at each site not included in the model for species with ≥ 20 marked individuals and model-predicted detection probabilities. I assessed bias as the average of predicted detection probability minus observed recapture proportion across all cross-validation steps. I assessed accuracy as root mean square error (RMSE; see Appendix 1). Values of fish size and environmental variables were used to calculate species-specific capture probabilities based on linear combinations of model coefficients. Species-specific detection was calculated individually for 25-mm TL size classes using median fish size, where the weighted average was used to represent overall detection probability. Because detection was modeled as a Bernoulli process, I also calculated 95% confidence intervals for species-specific observed recapture proportions at sites not included in the model using the exact Clopper-Pearson method (Freund et al. 2010) and evaluated how often predicted detection probabilities were contained in the intervals. Although the binomial probability confidence intervals did not account for individual variation in detection (e.g., fish size), it provided a more restrictive test of the model (i.e., confidence intervals that introduced individual variation in detection would have been wider). Additionally, I used catch data from calibration day at each site to calculate Petersen capture-recapture estimates with the Chapman (1954) bias correction (hereafter referred to as Petersen estimates) when criteria were met for unbiased estimates as defined by Ricker (1975):

$$\hat{N} = \{(M + 1)\,(C + 1) / (R + 1)\} - 1, \quad (2)$$

where $\hat{N}$ is the population estimate, M is the number of fish marked during the capture event, C is the number of fish captured during the recapture event, and R is the number of recaptured fish that were marked. I used the package “Rcapture” (Baillargeon and Rivest 2007) in the statistical software R to calculate the Petersen estimates. I compared the Petersen estimates to species- and site-specific abundance estimates derived from the final model using catch data from calibration day. Detection probability was calculated without the random site intercept and used to adjust catch data as $\hat{N} = c / \hat{q}$, where $\hat{q}$ is the species- and site-specific estimated detection probability, and c is the species- and site-specific number of individuals captured on calibration day (Thompson and Seber 1994; Peterson and Paukert 2009). I then evaluated how often $\hat{N}$ was contained in 95% confidence intervals for the Petersen estimates calculated as: $\hat{N} \pm z_{\alpha/2}(\mathrm{SE})$, where we used a bias-corrected SE (Seber 1970). Although Petersen estimates do not require an assumption of constant detection between capture and recapture events (Williams et al. 2002), the population estimator does assume constant among individual during an event. The assumption of constant detection among individuals was not strictly met, particularly due to variation in fish size; however, the Petersen estimates still provided a useful supplemental method to assess model performance. Additionally, data collected on calibration day was used to calculate both the recapture proportions and the Petersen estimates, which may have somewhat biased the comparisons. However, the modeled detection probability estimates are a reflection of all data collected (i.e., all 34 sites), whereas the Petersen estimates only reflect site-specific recapture proportions.

RESULTS

Fish sampling and environmental measurements. - I marked 17,123 centrarchids across a range of site- and segment-level environmental conditions (Table 1 and Table 2). As expected, *Micropterus* size was greater and more variable than other centrarchids and Rock Bass tended to be larger than *Lepomis*. Site-scale environmental variables varied considerably with the exception of instream large wood and water temperature (Table 2). Sites were distributed among 20 segments and approximately half were classified as cherty limestone.

Assessments of delayed mortality and emigration suggested that both were trivial to the results of the study. No dead centrarchids were discovered during the snorkel passes and only one was found in the block nets. Less than 0.05% of the marked centrachids (*n* =69) were discovered dead during routine surface inspections. No trapped live centrarchids were discovered in the block nets or in the area between them. Only 4 fish marked in the lower portion of the site were captured in the upper portion (2 Longear Sunfish and 2 Smallmouth Bass and only 3 fish found in the upper portion of the site were captured in the lower portion (2 Longear Sunfish and 1 Green Sunfish) at the three sites where I evaluated movement.

Electrofishing detection model. - In addition to fish length, I identified six site-scale environmental characteristics and the segment-scale geosoils category as variables that were related to variation in electrofishing detection (Table 2). As expected, there was a strong positive relationship between detection and fish length (Table 3). The site-scale variables included in the model provided a comprehensive characterization of stream sampling conditions, where the most correlated variables were water depth and discharge (r = 0.48). Relative variation explained at the site scale was 0.93 (remaining variance ± SD: 0.04 ± 0.20). Detection was higher for all geosoil categories relative to shale but the effect was more pronounced (i.e., greater positive slope) for stony alluvium. Relative variation explained at the segment scale was 0.96 (remaining variance ± SD: 0.02 ± 0.15).

Proportion of emergent vegetation, water clarity, and width-depth ratio were site-scale variables modeled as fixed effects only, where estimated detection decreased with increasing levels of each of these stream habitat characteristics (Table 3). The model-selection process also identified a discharge-proportion riffle interaction term that was included as a fixed effect, where the magnitude of the interaction was the same among species after accounting for random components. An examination of the discharge-proportion riffle interaction indicated a sharp increase in detection at lower flows, with the magnitude of the relationship greater at high proportions of riffle habitat (Figure 2).

The random tier of the model-selection process identified discharge, proportion of riffle, and water depth as site-scale variables that improved model fit; therefore, I included species-specific random slopes in the model (Table 3 and Table 4). Detection tendencies among species were not related to similar morphology (i.e., no tendencies among the three trait groups were apparent based on a visual examination). Detection decreased with increasing discharge across species; however, the magnitude of the relationship was considerably variable and most pronounced for Smallmouth Bass. For example, Smallmouth Bass had higher detection at lower flow and lower detection at higher flow when compared to other species (Figure 3). Conversely, there was little influence of discharge on Green Sunfish *Lepomis cyanellus* detection, which resulted in much higher detection than other species at higher flows. There was generally a negative relationship between detection and water depth; however, this relationship was also variable among species (Table 4). For example, there was virtually no relationship between water depth and Longear Sunfish *Lepomis megalotis* detection (Figure 4). Alternatively, there was a strong negative relationship between water depth and Smallmouth Bass detection, which resulted in higher detection in shallower conditions and lower detection in deeper conditions when compared to other species. The relationship between detection and riffle habitat was also variable among species (Table 4). For example, Bluegill *Lepomis macrochirus* detection increased sharply with higher proportions of riffle when compared to other species (Figure 5). Interestingly, Rock Bass detection decreased with higher proportions of riffle with other site-scale variables held constant. Similar to other species, Rock Bass detection increased as flow decreased and was higher at lower flows with higher proportions of riffle. However, Rock Bass detection actually increased at higher flows with higher proportions of riffle.

The validation methods indicated that the model typically provided reliable detection estimates with minimal site-level bias. Average model bias based on the cross validation was -0.01, which suggests the model did not tend to overestimate or underestimate detection probability (*n* = 126; Table 5). Average model bias for species with > 10 observations was highest for Bluegill (-0.03) and lowest for Green Sunfish (0.00), Longear Sunfish (0.00), and Rock Bass (0.00). Average RMSE based on the cross validation was 0.09 (*n* = 126; Table 5; Appendix 1). Average RMSE for species with > 10 observations was highest for Smallmouth Bass (0.12) and lowest for Longear Sunfish (0.07). The model-predicted detection probability was contained in the binomial probability confidence interval for observed data 83% of the time. Predicted detection was contained in the binomial probability confidence interval for observed data the highest percent of the time for Rock Bass (85%) and the lowest percent of the time for Smallmouth Bass (72%) for species with > 10 observations. Species abundance estimates using model-predicted detection probability were contained in the Petersen confidence interval 86% of the time (*n* = 106; Table 5). Predicted species abundance was contained in the Petersen confidence interval the highest percent of the time for Green Sunfish and Longear Sunfish (85%) and the lowest percent of the time for Smallmouth Bass (75%) for species with > 10 observations. There was no evidence that the model-predicted detection estimates were not reliable for species with fewer observations. The average RMSE was within levels observed for species with larger sample size for Largemouth Bass *Micropterus salmoides*, Redear Sunfish *Lepomis microlophus*, and Warmouth *Lepomis gulosus*. Also, both predicted detection and abundances for these three species were contained in respective confidence intervals with a similar frequency as species with a larger sample size. I was unable to provide any evaluations of model-predicted detection for Spotted Bass *Micropterus punctulatus* due to sample size limitations. Data collected for Spotted Bass still provided information to the model; however, its estimated detection largely reflected the overall intercept and slopes in the model due to the small sample size.

DISCUSSION

I detailed an effective, yet straight-forward, approach to model detection among stream fishes at multiple spatial scales with practical applications for ecology and management. A major advantage of this approach is that, after an initial intensive data collection period, subsequent sampling requires the same sampling effort as CPUE, while data can be adjusted for variable detection. Additionally, standardization of environmental conditions is not required and catch data can be adjusted for an absolute, rather than relative, abundance estimate. Thus, this approach promotes both improved long-term, datasets for stream-fish management and conservation and the establishment of species-environment relationships. Site- and species-specific detection probability estimates can be easily derived from model coefficients in a spreadsheet using values for environmental variables and fish size. Although I chose to model detection of stream-dwelling centrarchids using tow-barge electrofishing, the gear calibration and modeling methods are easily adaptable to other fish species and sampling gear types.

The multiscale structure of the model allowed detection to vary as functions of both reach- and segment-scale characteristics. A novel aspect of my study was using spatial data to generalize lithological characteristics among sites, which reduces field effort and increases the spatial extent of the model's applicability. For example, sites across a broad spatial extent may sometimes share reach-scale characteristics but differ in underlying geology and soils that can also contribute to variation in stream-fish detection.

My findings also highlight the complexity of variable detection across the dynamic stream landscape. Low flows resulted in very shallow (sometimes dry) riffles and sharp increases in detection, particularly with high proportions of riffle habitat. The disconnected riffle-run-pool sequences not only eliminated potential fish movement across deeper riffle areas, but may have altered centrachid habitat use. For example, Hafs et al. (2010) found that Smallmouth Bass tended to congregate in shallow areas at low flows, presumably due to altered foraging strategies. The combination of less available run and pool habitat and the occupancy of shallow areas at low flows potentially explains the sharp increase in detection at sites with high proportions of riffle (i.e., fish were highly susceptible to electrofishing by being concentrated in shallow areas).

I also identified species-dependent detection tendencies for closely-related fishes that were associated with habitat use and behavior rather than morphology. For example, the lack of a relationship between Green Sunfish detection and discharge was presumably due to their sit-and-wait feeding strategy resulting in an affinity for structure in calmer stream areas (Werner and Hall 1977; Stuber et al. 1982). The average-size Smallmouth Bass (200 mm) in my study streams tended to occupy open water (Probst et al. 1984; personal observations during snorkeling), which lowered detection at higher flows and deeper conditions. Conversely, Longear Sunfish typically occupy shallow pools and runs (Laughlin and Werner 1980; Bietz 1981); therefore, increased availability of deep water habitats did not decrease detection. The relationship between Rock Bass detection and the proportion of riffle is curious. I found that, under low-flow conditions, Rock Bass detection increased with higher proportions of riffle habitat (similar to other species). Conversely, under higher-flow conditions, Rock Bass detection decreased with increasing riffle habitat. Rock Bass have been associated with shallow cover (Probst et al. 1984; Grossman et al. 1995); however, little is known about their habitat use or behavior under higher-flow conditions (i.e., use of refuge habitat, feeding behavior, etc.). My findings suggest Rock Bass response to variable flows may be an area of research interest.

The inclusion of random effects in the model increased the robustness of the detection estimates, accommodated both the nested structure of the data and the variability in sample size, and promoted general flexibility in study design. In a GLMM, each coefficient for a random effect is pulled towards a common value that is a reflection of information compiled across all levels of the random effect (shrinkage estimates; Pinheiro and Bates 2000). For example, treating site as a random effect (i.e., modeling the variance) resulted in random intercepts that represented a compromise between site-specific fit and the overall mean intercept. Additionally, treating species as a random effect was both a superior and more statistically-appropriate approach than fitting separate generalized linear models (GLMs) for each species because (1) species-specific fit was presumably improved and estimated error was more likely more accurate due to the shrinkage estimates (Jamil et al. 2013) and (2) a usable single-species model for species with smaller samples size would have been impossible, where as a random effect these species have a degree of robustness. A multi-species GLM that treated species as a fixed categorical effect would have been unlikely to converge if many species were included due to the additional parameters and unequal sample sizes. As a random effect, model performance actually benefited from the inclusion of additional species as it improved the robustness of the variance component. Also, additional species can seamlessly be added to the model if management objectives change or the existing model is incorporated into a new research project.

Gear calibration results in a model that uses data collected across all sites to explicitly account for variable detection; therefore, this approach has advantages over other abundance estimation methods available to stream-fish scientists. For example, "traditional" mark-recapture (e.g., Carle and Strub 1978) and removal (e.g., Zippin 1958; Manly and Seber 1973) do account for variable detection. However, these population estimators do not provide information about specific factors influencing variation in detection and a reduction in sampling effort over time is not possible. Gear calibration ultimately allows for catch data from a single sampling event to be adjusted for detection once sufficient data have been acquired (see Introduction). Modeling detection across sites also minimizes wasted data and resources. For example, I was unable to calculate unbiased Petersen estimates when assumptions were not met. Detection estimates are a reflection of data collected across all sites with approaches such as gear calibration (see also Dorazio et al. 2005; Price and Peterson 2010; Gwinn et al. 2011), which reduces bias in site-specific estimates and promotes increased reliability in all collected data. Additionally, when model-derived estimates were considerably different than the observed recapture proportion and the Petersen estimate, the discrepancy could be related to unusually high or low recapture rates. For example, Rock Bass recapture proportion was curiously high at Five-mile Creek (0.55), while Longear Sunfish recapture proportion was unexplainably low at Lost Creek (0.03). It is reasonable to assume that the model-derived estimates better reflected "true" detection (i.e., based on trends across all sites) on occasions where recapture proportions were atypical, which also supports that the model performance results are conservative. Of course, unusual events and unexplained heterogeneity are inherent to the nature of sampling and no modeling approach will result in accurate detection probability estimates 100 percent of the time.

The relationships that I identified between stream-fish detection and environmental variables may not be analogous to other systems, species, or sampling gears. For example, variation in stream-fish detection has been attributed to the amount of instream large wood (Kruse et al. 1998; Rosenberger and Dunham 2005; Price and Peterson 2010) but it likely did not vary enough in my study streams to contribute to variability among sites or species. Also, seining can have different detection relationships with environmental variables than electrofishing (Price and Peterson 2010; Pregler et al. 2015). Stream-fish researchers and managers might benefit from measuring a comprehensive suite of environmental variables they hypothesize will influence detection rather than solely relying on results of other studies, particularly those involving dissimilar species or conducted in different ecoregions.

My study highlights the advantages of implementing contemporary approaches to stream-fish sampling. Despite increased prevalence in the ecological literature of abundance estimation methods that address variable detection, CPUE remains the dominant metric used by stream-fish scientists (Gwinn et al. 2016). Potential reasons variable detection is often not considered during stream-fish sampling include the statistical complexity of available options and increased field effort. For example, approaches to model detection with potentially more precise estimators than a GLMM do exist (e.g., Webster et al. 2008; Price and Peterson 2010); however, the increased statistical complexity may not be a desirable trade-off with practicality for many stream managers and researchers. I used a relatively simple modeling approach that effectively identified tendencies in stream-fish detection across varying environmental conditions and among species. In addition to the ability to reduce sampling effort over time, the environmental variables were either measured on site in ~2 h or were available via spatial data. Approaches that strike a balance between complexity and applicability can result in increased implementation of stream-fish studies and monitoring efforts that both account for variable detection and sample across a broader range of conditions. I believe application of the approach presented here both maximizes available resources and promotes advances in stream-fish ecology and management.

Table 1. Summary of fish species marked in 34 stream reaches in the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri during summer 2014-2015 to develop a detection model for tow-barge electrofishing. Morphological groups A-C correspond to trait groups developed in Chapter 1 (see Figure 1 of Chapter 1).

Common name	Scientific name	Morphological group	Number marked	Mean TL ± SD (mm)	Total length range (mm)
Bluegill	*Lepomis macrochirus*	B	1,904	94 ± 25	50 - 215
Green Sunfish	*Lepomis cyanellus*	A	2,503	106 ± 29	50 - 207
Longear Sunfish	*Lepomis megalotis*	A	8,595	94 ± 21	50 - 196
Redear Sunfish	*Lepomis microlophus*	B	158	85 ± 24	50 - 186
Rock Bass	*Ambloplites rupestris*	A	2,032	128 ± 37	50 - 286
Warmouth	*Lepomis gulosus*	A	240	113 ± 23	52 - 182
Largemouth Bass	*Micropterus salmoides*	C	513	175 ± 73	80 - 460
Smallmouth Bass	*Micropterus dolomieu*	C	1,150	200 ± 61	80 - 404
Spotted Bass	*Micropterus punctulatus*	C	28	165 ± 67	90 - 360

Table 2. Site-scale variables to characterize 34 stream reaches in the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri during summer 2014-2015 to develop a detection model for tow-barge electrofishing. Asterisks indicate site-scale variables included in the model using the model-selection process described in the text.

Variable	Definition	Mean ± SD	Range
Cross-sectional area (m^2)	Mean of wetted width times thalweg depth measured at 50 m transects	13.45 ± 6.52	5 - 39
*Discharge (m^3/s)	Mean of three replicates in a homogenous area of a run	1.86 ± 2.11	0.09 - 8.52
Proportion cover	Percent emergent vegetation plus percent instream large wood	0.06 ± 0.06	0.00 - 0.28
*Proportion emergent vegetation	Total area divided by sampling area	0.03 ± 0.06	0.00 - 0.25
Proportion instream large wood	Total area divided by sampling area	0.03 ± 0.02	0.00 - 0.10
*Proportion riffle	Total length divided by reach length	0.22 ± 0.09	0.07 - 0.42
Pool depth (m)	Mean maximum pool depth	1.35 ± 0.33	0.7 - 2.5
*Water clarity (m)	Horizontal distance an underwater observer could see fish silhouette	3.25 ± 1.53	1.0 - 8.5
Water conductivity (µS/cm)	Ambient water conductivity measured at the downstream end of reach	284.23 ± 80.35	160 - 510
*Water depth (m)	Mean thalweg depth measured at 50 m transects	0.82 ± 0.17	0.5 - 1.3
Water temperature (°C)	Measured at downstream end of the reach	21.60 ± 2.64	16.1 - 25.7
Wetted channel width (m)	Mean wetted width measured at 50 m transects	15.11 ± 4.73	9 -32
*Width-depth ratio	Mean wetted width of reach divided by mean thalweg depth of reach	18.69 ± 5.12	10 - 34

Table 3. Coefficients for fixed effects from a generalized linear mixed model developed to model two-pass tow-barge electrofishing detection for stream fishes of the Ozark Highlands ecoregion using the model-selection method described in the text (see Methods and Table 2 for a full description of variables). Model coeffiecients are reported on a logit scale. All continuous variables were standardized such that each variable had a mean of zero and a standard deviation of one, where the model intercept estimates detection at mean conditions and coefficients for continuous variables represent a unit change of one standard deviation. Geosoil is the only categorical variable, where shale is the reference category. Asterisks indicate site-scale variables that were also modeled as species-dependent random terms (see Table 4).

Parameter	Coefficient ± SE
Intercept	-1.77 ± 0.21
Fish length	0.31 ± 0.02
*Discharge	-0.25 ± 0.10
Proportion emergent vegetation	-0.26 ± 0.07
*Proportion riffle	0.04 ± 0.07
Water clarity	-0.23 ± 0.06
*Water depth	-0.10 ± 0.09
Width-depth ratio	-0.12 ± 0.07
Discharge x proportion riffle	-0.14 ± 0.06
Geosoil (Cherty alluvium)	0.51 ± 0.23
Geosoil (Stony alluvium)	1.16 ± 0.28
Geosoil (Cherty limestone)	0.52 ± 0.21

Table 4. Species-dependent coefficients from a generalized linear mixed model developed to model tow-barge electrofishing detection for stream fishes of the Ozark Highlands ecoregion using the model-selection method described in the text. The row labeled all provides the grand intercept and slopes for the model.

Species	Intercept	Discharge	Water depth	Proportion riffle
All	-1.77 ± 0.21	-0.25 ± 0.10	-0.10 ± 0.09	0.04 ± 0.07
Bluegill	-1.58 ± 0.06	-0.25 ± 0.06	-0.05 ± 0.03	0.11 ± 0.06
Green Sunfish	-1.54 ± 0.06	-0.06 ± 0.05	-0.11 ± 0.03	0.03 ± 0.05
Largemouth Bass	-1.94 ± 0.09	-0.34 ± 0.08	-0.11 ± 0.05	0.02 ± 0.08
Longear Sunfish	-1.66 ± 0.05	-0.27 ± 0.04	0.02 ± 0.02	0.08 ± 0.04
Redear Sunfish	-1.62 ± 0.14	-0.29 ± 0.11	0.01 ± 0.06	0.10 ± 0.10
Rock Bass	-2.00 ± 0.07	-0.16 ± 0.06	-0.13 ± 0.04	-0.10 ± 0.06
Smallmouth Bass	-2.25 ± 0.08	-0.37 ± 0.06	-0.40 ± 0.04	-0.04 ± 0.07
Spotted Bass	-1.88 ± 0.20	-0.27 ± 0.12	-0.12 ± 0.08	0.01 ± 0.11
Warmouth	-1.42 ± 0.11	-0.26 ± 0.09	0.06 ± 0.05	0.15 ± 0.10

Table 5. Results of performance evaluations of a generalized linear mixed model to predict tow-barge electrofishing detection of stream fishes using both a 10-fold cross validation to compare model-predicted detection to observed recapture proportions and model-predicted abundances to Petersen capture-recapture estimates. Bias was calculated as the predicted detection probability minus the observed recapture proportion. Root mean square error (RMSE) was calculated as $\sqrt{(\sum(\text{observed recapture proportion} - \text{predicted detection probability})2)\ /n}$,where *n* is the number of observations used for the cross validation (see also Appendix 1). CI is a 95% confidence interval (see Methods for a full description of calculations).

Species	Average bias	Average RMSE	Percent model-predicted recapture proportion in binomial probability CI	Percent model-predicted abundance in Petersen CI
All	-0.01	0.09	83% (104 of 126)	86% (91 of 106)
Bluegill	-0.03	0.09	84% (16 of 19)	88% (15 of 17)
Green Sunfish	0.00	0.08	78% (18 of 23)	89% (17 of 19)
Largemouth Bass	-0.02	0.03	100% (10 of 10)	90% (9 of 10)
Longear Sunfish	0.00	0.07	80% (24 of 30)	89% (23 of 26)
Redear Sunfish	-0.08	0.08	100% (2 of 2)	50% (1 of 2)
Rock Bass	0.00	0.10	85% (17 of 20)	88% (14 of 16)
Smallmouth Bass	0.01	0.12	72% (13 of 18)	75% (9 of 12)
Warmouth	-0.05	0.11	100% (4 of 4)	75% (3 of 4)

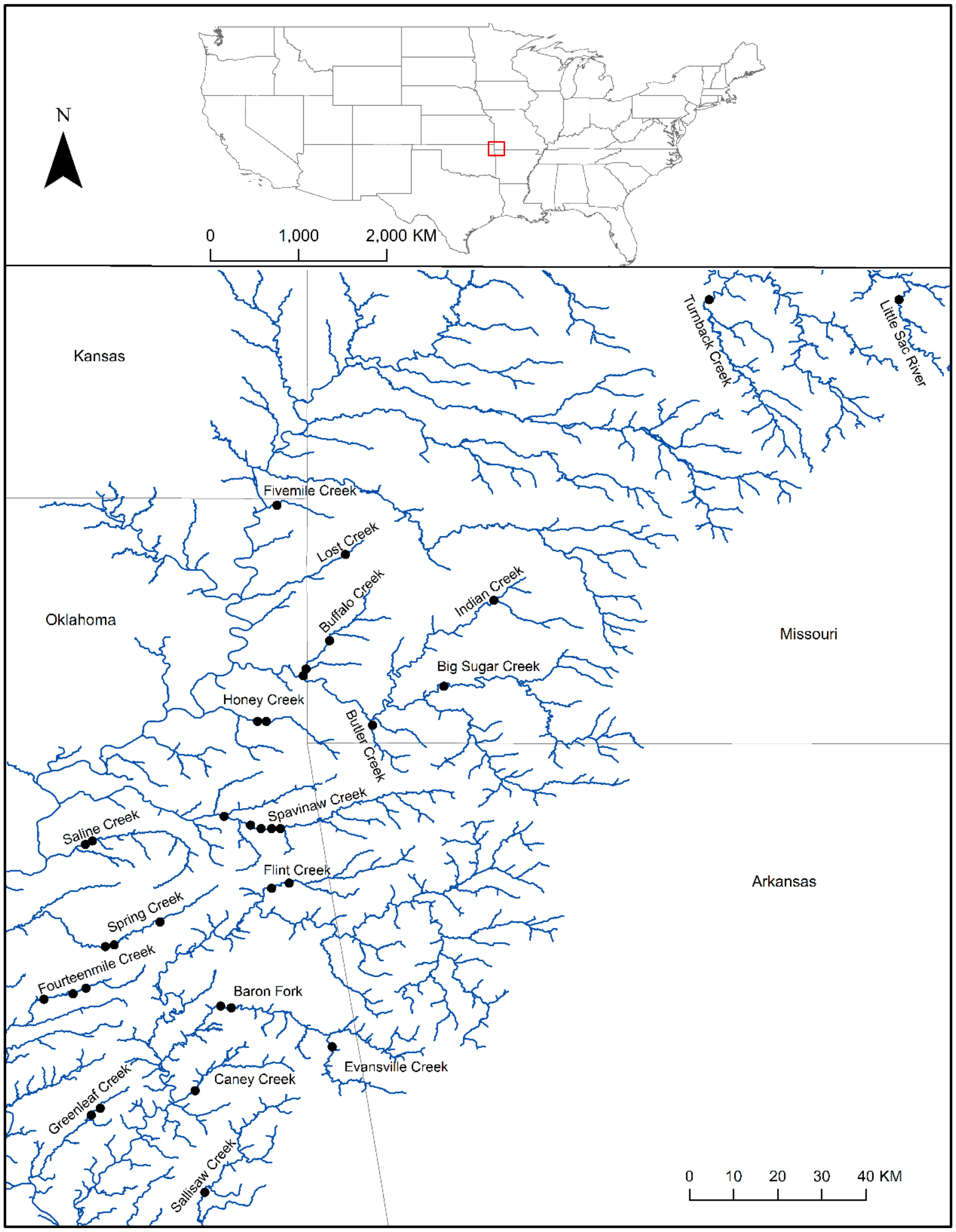

Figure 1. Study sites in the Ozark Highland ecoregion of northeast Oklahoma and southwest Missouri. Black dots represent the 34 stream reaches sampled during summer 2014-2015 to develop a detection model for tow-barge electrofishing.

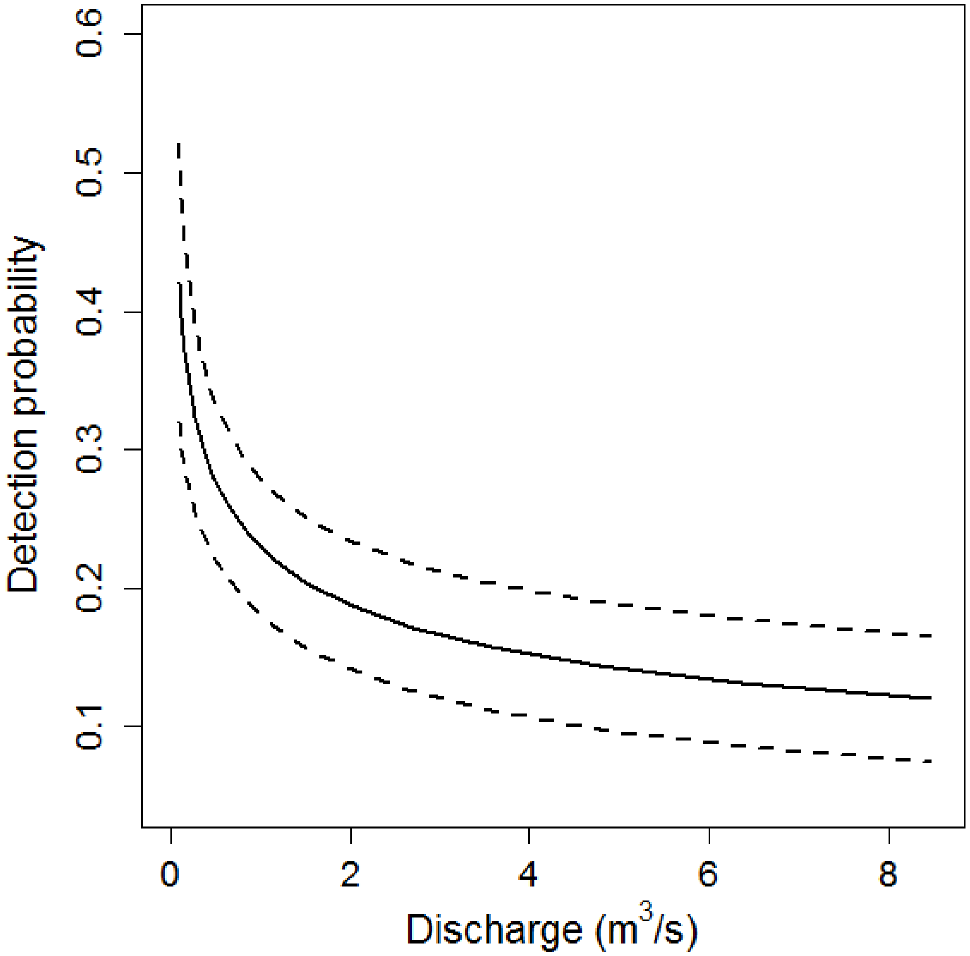

Figure 2. Relationship between detection and discharge with respect to riffle habitat using estimates derived a generalized linear mixed model. The x-axis represents values of discharge from -2 to 2 standard deviations. Proportion of riffle habitat was held at 1 standard deviation. Other site-scale variables included in the model were held at mean values and the geosoil category was cherty limestone. Detection was calculated for the average-sized Longear Sunfish using species-specific random terms (see Table 4), although the magnitude of the discharge-proportion riffle interaction was the same among species. Dashed lines are 95% confidence intervals.

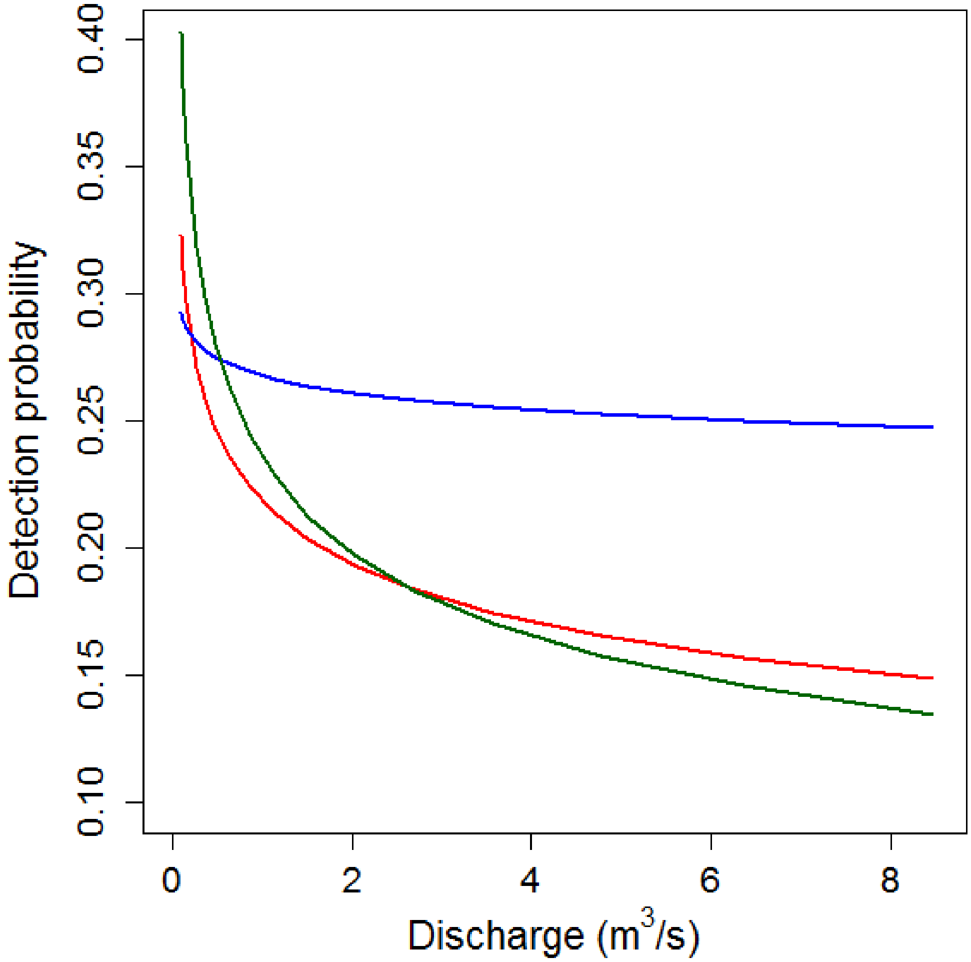

Figure 3. Example of the relationship between detection and discharge among stream fishes using estimates derived from a generalized linear mixed model. Species-specific detection was obtained using random terms (see Table 4), where red represents the average-size Bluegill, blue represents the average-size Green Sunfish, and green represents the average-size Smallmouth Bass. The x-axis represents values of discharge from -2 to 2 standard deviations. Other site-scale variables included in the model were held at mean values and the geosoil category was cherty limestone. I did not report standard error in the figure to improve visualization.

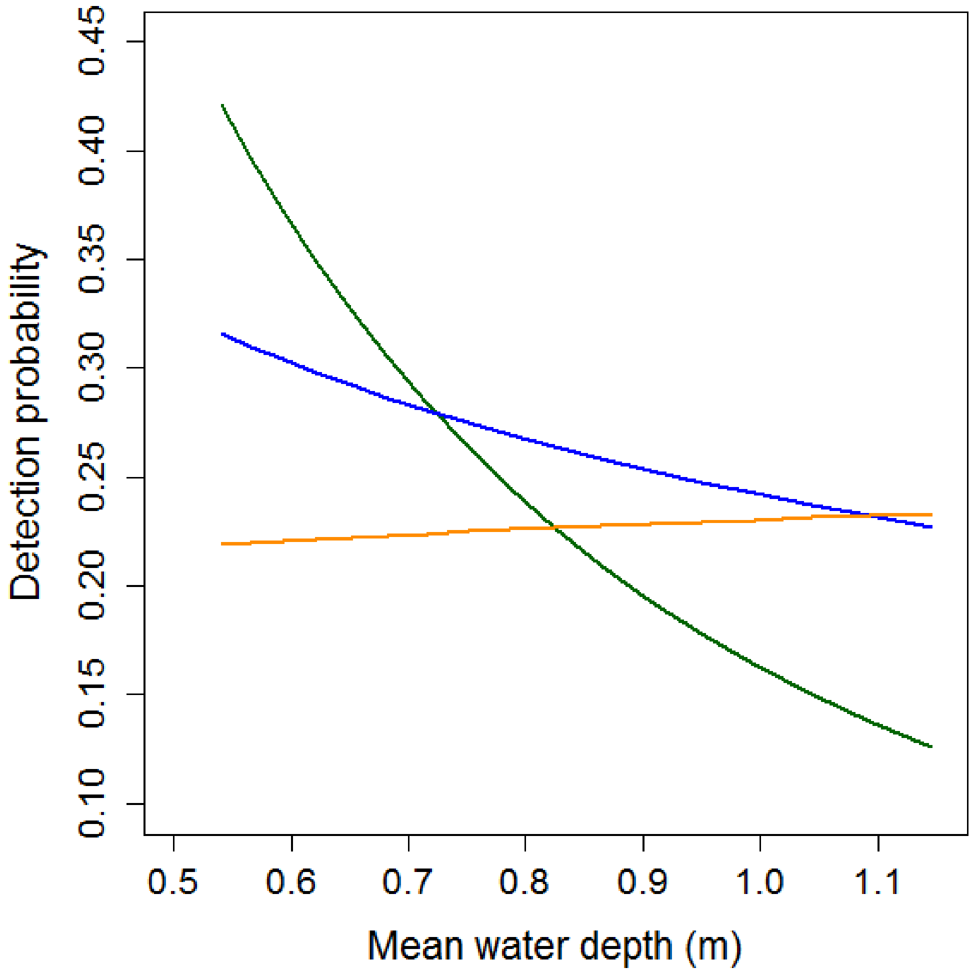

Figure 4. Example of the relationship between detection and water depth among stream fishes using estimates derived from a generalized linear mixed model. Species-specific capture probability was obtained using random terms (see Table 4), where green represents the average-size Smallmouth Bass, blue represents the average-size Green Sunfish, and orange represents the average-size Longear Sunfish. The x-axis represents values of mean water depth from -2 to 2 standard deviations. Other site-scale variables included in the model were held at mean values and the geosoil category was cherty limestone. I did not report standard error in the figure to improve visualization.

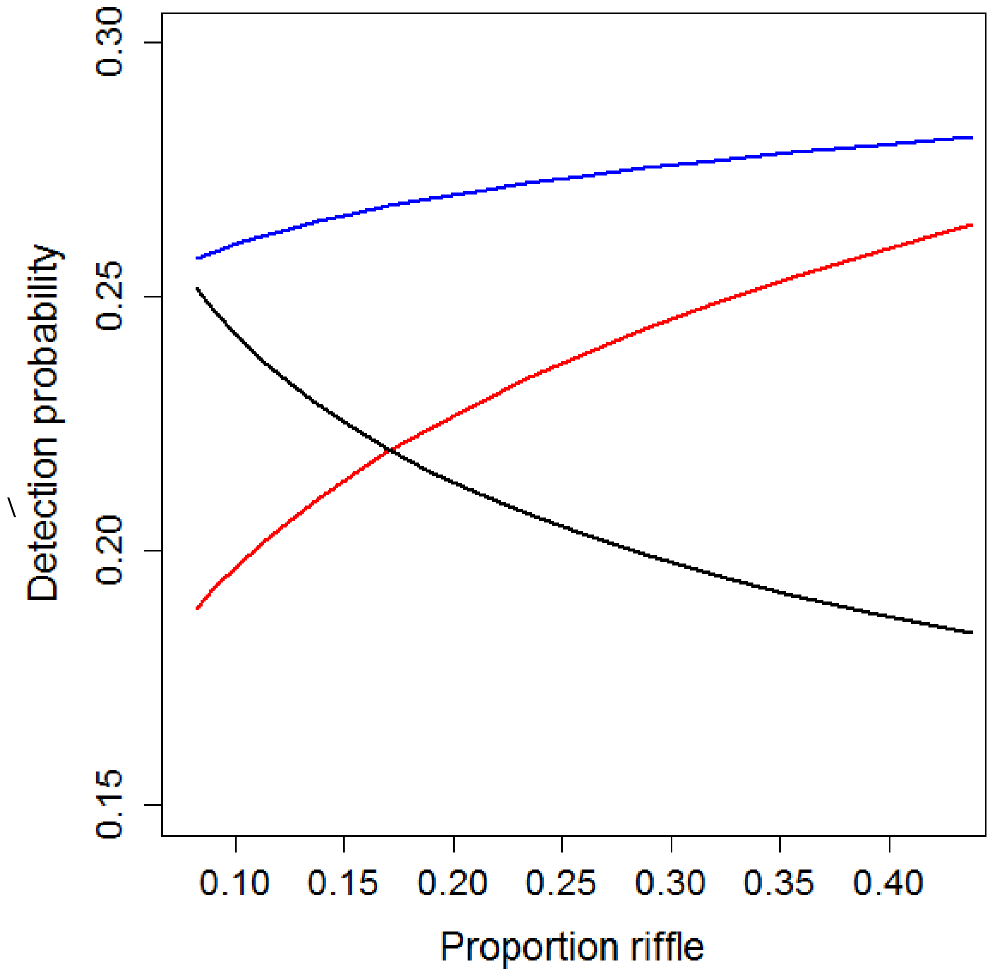

Figure 5. Example of the relationship between detection and proportion of riffle habitat among stream fishes using estimates derived from a generalized linear mixed model. Species-specific capture probability was obtained using random terms (see Table 4), the red the average-size Bluegill, blue represents the average-size Green Sunfish, and black represents the average-size Rock Bass. The x-axis represents values of proportion riffle from -2 to 2 standard deviations. Other site-scale variables included in the model were held at mean values and the geosoil category was cherty limestone. I did not report standard error in the figure to improve visualization.

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APPENDICES

Appendix 1. Results of a 10-fold cross validation for a generalized linear mixed model to predict tow-barge electrofishing detecion for Ozark Highland stream fishes, where data were randomly split the data into 10 groups of three to four sites (~10% of the sites). For each cross-validation step, the remaining data were modeled and estimated detection was compared to observed recapture proportions (see Methods for details). Root mean square error (RMSE) was calculated as $\sqrt{(\sum(\text{observed recapture proportion} - \text{predicted detection probability})2)/n}$,where n is the number of observations used for the cross validation.

Cross validation	RMSE
One	0.101
Two	0.116
Three	0.083
Four	0.082
Five	0.135
Six	0.113
Seven	0.104
Eight	0.085
Nine	0.080
Ten	0.086
Mean	0.098

CHAPTER IV

AN EVALUATION OF SNORKELING FOR WARMWATER STREAM FISH DETECTION AND ABUNDANCE ESTIMATES

ABSTRACT

The continued evaluation and comparison of stream-fish sampling methods are essential to inform managers when developing long-term monitoring protocols. Snorkeling has potential advantages relative to other stream-fish sampling methods including minimal intrusion, cost effectiveness, and increased detection in deep areas. However, applicability of snorkeling to monitor fishes in warmwater stream has not been adequately researched. I compared snorkeling to tow-barge electrofishing to examine tendencies in the number of Ozark stream fish species detected and evaluated the reliability of snorkel counts to provide informative stream-fish abundance estimates. Electrofishing tended to more effectively detect rarer species than snorkeling, although using both sampling methods sometimes increased the number of stream fishes encountered. Snorkel counts typically underestimated stream-fish abundance; however, they did often provide informative population estimates for black basses, particularly Smallmouth Bass. The effectiveness of snorkeling was attributable to differences in behavioral traits and habitat use, where snorkel counts for cryptic stream fishes (e.g., Green Sunfish and Rock Bass) grossly underestimated population size. My findings suggest that snorkeling may be a useful supplemental stream-fish sampling method in Ozark streams for certain objectives and species. However, managers may consider implementing sampling designs that account for variable

detection across environmental conditions and among species if snorkeling is used as a primary method for long-term stream-fish monitoring.

INTRODUCTION

The continued evaluation and comparison of fish sampling methods are essential aspects of progressive stream ecology and management. In particular, there is considerable value in examining both the general applicability and tendencies in detection of different stream-fish sampling methods among systems and species. A comprehensive understanding of fish sampling method options promotes informed decisions by managers when developing monitoring protocols. Bonar et al. (2009) represents a substantial effort to provide a detailed summary of available methods for sampling fishes in inland freshwater systems. However, Bonar et al. (2009) fails to deliver an all-inclusive overview of stream-fish sampling methods, with no discussion of the applicability of snorkel surveys in warmwater streams.

Although electrofishing and seining are the most common stream-fish sampling methods (Rabeni et al. 2009), snorkel surveys are also sometimes used to both identify species presence and estimate abundance, particularly in coldwater streams. Snorkeling is relatively cost effective (e.g., typically requires only a mask, snorkel, and wetsuit), minimally intrusive, and often has higher detection in deeper stream areas relative to other stream-fish sampling methods (Bonneau et al. 1995; Dunham et al. 2009; Thurow et al. 2012). However, the applicability of snorkeling is severely limited by water clarity and time of day because stream-fish detection is extremely low in poor visibility (Dunham et al. 2009). Additionally, discriminating between similar-looking stream fishes underwater is a challenging aspect of snorkeling (Dauwalter et al. 2005; Rieman et al. 2006) and may be a limiting factor for certain objectives. The requirements of crew members also differ for snorkeling compared to other stream-fish sampling methods. In addition to adequate training being critical for replicable snorkeling protocols, all crew members must be comfortable navigating underwater structure, adept at swimming in current, and tolerant of underwater thermal conditions (Bozek and Rahel 1991; Dunham et al. 2009). Effective communication among snorkelers is also essential to minimize double counting of fish. A major limitation of relying solely on snorkel surveys for stream-fish management is that demographic information (i.e., age and growth) is not easily attained. For example, estimating fish size accurately enough to establish year classes is challenging with snorkeling and structures for aging (e.g., scales and otoliths) are difficult (if not impossible) to obtain using snorkeling (Dunham et al. 2009).

Environmental conditions that contribute to variable electrofishing detection (e.g., flow and water conductivity) often have little influence on variation in snorkeling detection (Schill and Griffith 1984; Bonneau et al. 1995); however, certain environmental variables and stream-fish characteristics (i.e., species traits and habitat use) are important to consider. McManamay et al. (2014) showed that species detection using snorkeling varied considerably among an assemblage of stream fishes. In addition to poor visibility, excessive structure or interstitial spaces (Mullner et al. 1998; Wildman and Neuman 2003) and cryptic coloration or behavior (Bozek and Rahel 1991; Korman et al. 2010; Macnaughton et al. 2014) can substantially reduce stream-fish snorkeling detection. Additional environmental variables that can result in variable detection when snorkeling include surface glare (Bozek and Rahel 1991), water depth (Schill and Griffith 1984; Brewer and Ellersieck 2011), and lithology (Ensign et al. 1995; Albanese et al. 2011). Information from repeat surveys across varying environmental conditions can be used to estimate species detection (e.g., occupancy modeling; McManamay et al. 2014) if the objective is species occurrence or species richness. However, common population estimation methods that account for variable detection (e.g., capture-recapture and removal) are challenging for stream-fish snorkeling because individuals are usually not physically captured (i.e., only visually observed; but see Dorazio et al. 2005; Jordan et al. 2008 for removal examples). Capture-recapture abundance estimates can be derived from snorkel counts if an alternate sampling method is used to capture and mark fish; however, this approach is only effective when the mark is highly visible (Bonneau et al. 1995), the surveys are conducted in exceptional water clarity (Zubik and Frayley 1988), or target species are gregarious (e.g., age-0 Smallmouth Bass *Micropterus dolomieu*; Brewer and Ellersieck 2011), where these factors are not mutually exclusive. In addition, markings must not be too visible or large that they result in considerable variability in snorkeling detection between marked and unmarked fish, which would violate a basic assumption of capture-recapture estimation. For example, extremely colorful tags may increase the detectability of marked fish relative to unmarked fish and heavy tags may alter marked fish behavior.

Given the inherent challenges of using abundance estimation methods with snorkeling, point counts are often used to characterize stream-fish populations (Dunham et al. 2009). Point counts provide informative abundance estimates (i.e., useful for establishing baseline populations or identifying major changes in population size) in ideal environmental conditions (i.e., good water clarity) and with certain species (e.g., non-cryptic or open water fishes). For example, both Hillman et al. (1992) and Bonneau et al. (1995) found snorkeling detection probability was ~70% for non-cryptic fishes in clear-water streams, with > 90% detection when fish were found in groups of < 40 individuals (Hillman et al. 1992).

Studies that have evaluated snorkel surveys largely favor coldwater streams (e.g., Zubrick and Fraley 1988; Mullner et al. 1998; Wildman and Neumann 2003; Thurow et al. 2006, Macnaughton et al. 2014), which is likely due to both the general bias of stream-fish sampling research towards salmonids and the clear conditions of many spring-fed coldwater streams. However, clear water is also common to warmwater streams. Many Ozark Highland streams are characterized by clear water with excellent underwater visibility during dry weather periods (Nigh and Schroeder 2002). Nevertheless, relatively few studies have examined the applicability of snorkeling for effective fish monitoring in warmwater streams (but see McManamay et al.

2014; Weaver et al. 2014; Hain et al. 2016). Additionally, I am not aware of any studies in Ozark Highland streams that have evaluated snorkeling stream-fish detection for multiple species (see Dauwalter et al. 2007; Brewer and Ellersieck 2011 for Smallmouth Bass examples) or compared snorkeling detection to other stream-fish sampling methods. Accordingly, my objectives were to: (1) compare the number of stream fishes encountered between a single snorkel pass and a single tow-barge electrofishing pass, (2) evaluate the efficacy of a single snorkel pass for providing informative abundance estimates for common stream-dwelling centrarchids, and (3) relate tendencies in snorkeling detection and abundance estimates among stream fishes to species traits and habitat use.

METHODS

I sampled stream fishes in 23 reaches that each comprised three to five riffle-run-pool sequences to characterize habitat (referred to hereafter as sites) in 19 streams of the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri from late spring to early fall 2014-2015 (Appendix 1). Data collection largely corresponded to streams sampled for Chapter 3 (see study area and Figure 1 of Chapter 3). I also sampled two additional sites on Spavinaw Creek and one additional site on Baron Fork, Buffalo Creek, Evansville Creek, and Spring Creek in 2014. I considered two centrachid species rare to Ozark Highland streams (Orangespotted Sunfish *Lepomis humilis* and White Crappie *Pomoxis annularis*) in 2014-2015 and eight additional stream fishes in 2015 for the species detection objective, where I defined species detection as observing at least one individual of a species. Detection tendencies among certain species were considered visually with respect to four significant morphological groups from Chapter 1 (trait groups A-D; Appendix 2), where trait groups A-C comprised centrarchids and trait group D comprised several Catostomids (suckers). I also visually examined abundance estimates among trait groups A-C.

I installed two sets of block-off nets at both the upstream and downstream ends of each site to ensure a closed system during the sampling period. Block-off nets were preferentially placed at shallow riffles to further inhibit fish movement (Peterson et al. 2004; Price and Peterson 2010). Either a low-water bridge at base flows or a dry riffle located at one end of the site provided an adequate fish barrier at a few sites and no block-off nets were installed.

I performed snorkel surveys ~24 h prior to the electrofishing event. Three persons were typically used for the snorkel surveys; however, only two snorkelers were used in stream areas where wetted channel width was < 10 m. The snorkel surveys occurred ~24 h after marking day to allow the system to recover (Peterson and Cederholm 1984) for sites that overlapped with sampling described in Chapter 3 (see Chapter 3 methods). All crew members were trained in snorkeling protocols and participated in "practice" surveys with experienced snorkelers. Horizontal water clarity was ≥ 3.0 m at all sites that were snorkeled. The water clarity criterion is consistent with other stream-fish snorkeling studies (e.g., Schill and Griffith 1984) and also coincides with the minimum distance between crew members and a fish before identification. The opportunity to snorkel was somewhat limited, particularly in 2015, due to periodic heavy rainfall that resulted in extended periods of poor water clarity. We snorkeled areas ≥ 0.2-m deep in a slow upstream direction while avoiding sudden movements and carefully inspected areas of structure (e.g., searched for fish under logs and between boulders). Each snorkeler maintained a designated lane and stayed approximately in line laterally with other crew members. In general, snorkeling lanes with higher amounts of structure were narrower and snorkeling lanes with mostly open water were wider. Snorkelers maintained communication with each other to minimize double counting of individual fish. For snorkel counts (i.e., abundance estimates), *Micropterus* estimated to be ≥ 80-mm TL and *Lepomis* and Rock Bass ≥ 50-mm TL were tallied on an underwater wrist cuff when they either passed or were passed by the snorkeler. We used fish silhouettes and rocks of known sizes to confirm the ability of crew members to recognize the fish-size cutoffs underwater. All fish sizes were considered for species detection, where snorkelers noted the presence of a species on their wrist cuff. Redhorses (*Moxostoma* spp.) were only considered at the genus level because species are difficult to distinguish underwater. Similarly, snorkelers did not differentiate between Largemouth Bass *Micropterus salmoides* and Spotted Bass *Micropterus punctatus* because confident identification underwater is challenging; however, electrofishing data suggested that the vast majority of these individuals at my sites were Largemouth Bass.

I used a combination of data from calibration day in 2014-2015 (see Chapter 3 methods), the six additional sites sampled in 2014, and from marking day in 2015 (see Chapter 3 methods). Electrofishing protocols at the six additional sites in 2014 were identical to methods described in Chapter 3, where I performed two standardized electrofishing removal passes per riffle-run-pool sequence. I used data from the first electrofishing pass on calibration day in 2014 and from the first electrofishing pass on marking day in 2015 to evaluate species detection. I chose to perform the detection surveys in 2015 on marking day due to observed mortalities using tow-barge electrofishing for many of the fishes added to the species detection objective (e.g., cyprinids and suckers). Fish-size cutoffs for both abundance estimates and species detection were identical to those described for snorkeling. I only considered species detection at Spring Creek3 and Spring Creek4 (i.e., we did not perform snorkel counts for any species).

I (1) compared single-pass tow-barge electrofishing and single-pass snorkeling based on both the number and identity of species detected, (2) evaluated if the number of stream fishes encountered increased using both snorkeling and electrofishing compared to each sampling method individually, (3) and assessed the effectiveness of a single snorkel pass for abundance estimates of common centrarchids. I derived electrofishing abundance estimates adjusted for variable detection using the model developed in Chapter 3, where the abundance estimates were used to evaluate the snorkel counts. Model-predicted electrofishing detection estimates were calculated using fish size and reach- and segment-scale environmental data with methods described in Chapter 3. Standard error for model-predicted capture probabilities were estimated with the delta method (Oehlert 1992) using the package "msm" (Jackson 2011) in the statistical software R (version 3.2.2, R Core Development Team, 2014). Both the model-predicted detection estimates and standard errors were backtransformed using calculations described by Jørgensen and Pedersen (1998). I then calculated 95% confidence intervals for model-predicted detection as: $\hat{q} \pm z_{\alpha/2}(\mathrm{SE})$, where $\hat{q}$ is the back transformed species- and site-specific detection estimate. The range of abundances from the electrofishing data were calculated using both the lower and upper the confidence intervals of the detection estimates as $\hat{N} = c / \hat{q}$ where $\hat{N}$ is the species- and site-specific abundance estimate, c is the species- and site-specific catch, and $\hat{q}$ corresponds to the lower and upper bounds of the back transformed species- and site-specific detection 95% confidence interval (Thompson and Seber 1994; Peterson and Paukert 2009).

RESULTS

Single-pass tow-barge electrofishing tended to detect more stream fish species than a single snorkel pass, although more species were sometimes encountered using both methods. Electrofishing detected more species than snorkeling at 17 of 23 sites (Table 1). Electrofishing typically resulted in the detection of one or two additional stream fishes but did result in the detection of three additional species at both Butler Creek and Caney Creek and four additional species at Buffalo Creek3. Snorkeling detected more species than electrofishing only at Evansville Creek2 (both White Crappie and Largemouth Bass). One additional species was encountered at four sites using both electrofishing and snorkeling.

Species detection tendencies were not apparent based on morphology. There was no apparent tendency in species detection between trait groups A and B but rather among stream fishes of each group based on a visual examination. Electrofishing tended to more effectively detect less common centrarchids than snorkeling. Warmouth *Lepomis gulosus* was detected at six additional sites using electrofishing and I also detected Orangespotted Sunfish at two sites (Table 1). Conversely, I failed to detect Bluegill and Rock Bass using snorkeling at only one site where these species were detected using electrofishing. Both Smallmouth Bass and Longear Sunfish were either detected at a site using both electrofishing and snorkeling or not detected by either sampling method. Similar to centrarchids, I detected the less common White Sucker *Catostomus commersoni* more often using electrofishing than snorkeling among suckers (detected at five sites not detected with snorkeling). Redhorses and Northern Hogsucker *Hypentelium nigricans* were either detected using both electrofishing and snorkeling at a site or not detected by either sampling method.

Fish counts for common centrarchids obtained from a single snorkel pass tended to underestimate stream-fish abundance (severely for most species in trait group A) when compared to tow-barge electrofishing abundance estimates adjusted for variable detection; however, snorkeling often provided informative abundance estimates for black basses (trait group C). Smallmouth Bass snorkel counts were contained in the electrofishing abundance range at around 50 percent of the sites (10 of 21) and within 20 percent of the lower end of the range at two additional sites (Table 2). Additionally, Smallmouth Bass snorkel counts were less than 50 percent of the lower end of the electrofishing abundance range at only 14-mile Creek1 and 14-mile Creek2. Although less frequent than Smallmouth Bass, snorkel counts often provided informative abundance estimates for Largemouth Bass-Spotted Bass as they were either contained or within 20 percent of the electrofishing range at nine sites. The reliability of snorkel counts to provide informative abundance estimates was either inconsistent or extremely ineffective for other common centrarchids, which comprised one member of trait group A both members of trait group B. Snorkel counts were either contained or within 20 percent of the electrofishing range at five sites for Bluegill (trait group B), seven sites for Longear Sunfish (trait group A), and two sites for Redear Sunfish *Lepomis microlophus* (trait group B). Snorkel counts were not within 50 percent of the lower end of the electrofishing abundance range at any sites and more typically greater than 90 percent lower for the other three members of trait group A (Green Sunfish, Warmouth, and Rock Bass).

DISCUSSION

A single snorkel pass tended to both detect fewer species than tow-barge electrofishing and often considerably underestimated abundance for common Ozark Highland centrarchids recruited to both sampling methods; however, employing both approaches sometimes increased the number of species encountered. Both Albanese et al. (2011) and Chamberland et al. (2014) found that snorkeling detected more warmwater stream fishes than electrofishing, although both of these studies used backpack electrofishing. Backpack electrofishing is considered much less efficient (i.e., lower detection in comparable conditions) than tow-barge electrofishing (Rabeni et al. 2009). My results are similar to McManamay et al. (2014) who showed that using electrofishing in addition to snorkeling increased the number of species detected in warmwater streams, where the authors found that estimated species richness was 13% higher with a dual-method approach. The tendency of snorkel surveys to greatly underestimate stream fish-abundance is also consistent with other studies in warmwater streams. For example, Weaver et al. (2014) found that snorkeling detection across an assemblage of warmwater stream fishes was only ~0.15. Brewer and Ellersieck (2011) showed that age-0 Smallmouth Bass detection in warmwater streams was highly variable and as low as ~0.20.

Species detection tendencies were evident among stream fishes and were related to fish densities rather than morphology. Snorkeling consistently detected stream fishes usually found in high abundance when present (e.g., Longear Sunfish, Redspot Chub *Nocomis asper*, and Northern Hogsucker) but often failed to detect species typically found in low abundance that were detected at sites using tow-barge electrofishing (e.g., White Sucker and Warmouth). Interestingly, snorkeling was reliable for detecting more common centrarchids even when abundance was grossly underestimated by snorkel counts. For example, species detection was reasonable for Green Sunfish and effective for Rock Bass (18 of 23 sites and 22 of 23 sites when detected using electrofishing, respectively).

The reliability of stream-fish abundance estimates from snorkeling were related to the morphological groups but, in general, could better be explained by other characteristics. Snorkeling grossly underestimated abundance for three of the four members of trait group A, which could be related to behavioral traits and habitat use. For example, the extremely low Rock Bass snorkel counts relative to electrofishing abundance estimates can be attributed to both their cryptic coloration (Casterlin and Reynolds 1979) and the tendency to occupy cover (Probst et al. 1984; Grossman et al. 1995) and interstitial spaces (personal observations). Similarly, Green Sunfish tend to occupy structure (Werner and Hall 1977; Stuber et al. 1982) and submerged vegetation (personal observations), which likely contributed to their severe underestimation of abundance using snorkeling. Longear Sunfish are less cryptic than other members of trait group A and share characteristics with Bluegill (trait group B) and my findings do not suggest that a single snorkel pass could consistently be used to provide informative abundance estimates for either species. Longear Sunfish and Bluegill were typically observed in areas outside of structure during snorkeling; however, both species exhibit social behavior and grouping (Witt and Marzolf 1954; Colgan et al. 1979; Bietz 1981; Dugatkin and Wilson 1992), which can inhibit accurate snorkel counts (Heggenes et al. 1990; Hillman et al. 1992). Black basses (trait group C), particularly Smallmouth Bass, were the only species where snorkel counts often provided informative abundance estimates, which suggests that a single snorkel pass can be used to identify major changes in local populations or provide baseline estimates in Ozark streams. Trait group C was also the only group where snorkeling detection tendencies were similar among member species, thus suggesting an association with morphology. Black basses also exhibit behavioral traits that promote higher snorkeling detection because they are non-cryptic and were typically found in groups of < 5 fish. The effectiveness of snorkel counts to estimate Largemouth Bass-Spotted Bass abundance was slightly less consistent than Smallmouth Bass, possibly due to different habitat use. Largemouth Bass, which were much more common than Spotted Bass at my sites, are associated with areas of slow-moving water with submerged vegetation (Durocher et al. 1984; Brown and Maceina 2002). A large section of Spavinaw Creek4, where Largemouth Bass abundance was severely underestimated using snorkeling, was a heavily vegetated backwater that was challenging to snorkel effectively (and prime snake habitat for an added bonus). Conversely, typical-sized Smallmouth Bass in Ozark Highland streams (i.e., 200-220 mm) tended to occupy open water areas of pool and runs (Probst et al. 1984; personal observations), which likely promoted accurate snorkel counts. Larger Smallmouth Bass were often found in structure but were much easier to discover than Green Sunfish and Rock Bass because they occupied crevices big enough to be accessed by snorkelers.

My findings support that, for certain management objectives, single-pass snorkel counts can be an effective sampling method for black basses in “typical” Ozark Highland streams. Snorkel counts generally provided informative Smallmouth Bass abundance estimates; however, they did substantially underestimate abundance at some sites. In particular, Smallmouth Bass detection was low at sites located near the southern boundary of the Ozark Highlands ecoregion (e.g., 14-mile Creek). Sites near the ecotone resembled streams of the Boston Mountains ecoregion with different underlying lithology (Woods et al. 2005), which increased countershading and inaccessible interstitial spaces during snorkeling. Snorkeling offers a cost-effective approach to identify major changes in site-specific black bass abundance. For example, snorkeling could provide rankings of low and high Smallmouth Bass abundance in “typical” Ozark Highland streams given good visibility. The desired precision and accuracy of snorkel counts is largely related to management objectives. However, using snorkeling as a primary sampling method limits the ability to detect and understand spatial and temporal trends in black bass populations, particularly if age structure is an objective. If snorkeling is deemed a desirable sampling method option, stream managers may consider implementing approaches that provide insight on variable detection to better understand and quantify uncertainty in snorkel counts.

My study only considered one benthic stream fish for snorkeling species detection (Banded Sculpin) and none for snorkeling abundance estimates. Snorkeling has been shown to be an effective fish sampling method for some benthic species in warmwater streams (e.g., Ensign et al. 1995; Dorazio et al. 2005; Jordan et al. 2008). In fact, Hain et al. (2016) found that point counts were similar to capture-recapture abundance estimates for a benthic fish (Oʻopu Nākea *Awaous stamineus*) in Hawaiian streams. I found Banded Sculpin snorkeling detection to be reasonable (detected at 5 of 8 sites when detected with electrofishing). However, more research is needed to determine the applicability of snorkel surveys for both Banded Sculpin and other benthic fishes in Ozark Highland streams (e.g., *Etheostoma* and *Percina*).

Stream conditions vary considerably across space and time; therefore, both short-term studies (e.g., a single season) and studies conducted across a limited spatial extent (e.g., a few streams) may not accurately reflect potential variability in fish snorkeling detection. In addition to inaccurate species-specific abundance estimates, the use of snorkeling for multi-species studies without considering variable detection (e.g., Matthews and Marsh-Matthews 2006) may lead to false inferences about stream-fish composition. Because applications of removal and capture-recapture estimation methods are limited for stream-fish snorkeling (see Introduction), a more intensive examination of alternative approaches by researchers in warmwater streams is needed. One option is to account for variable stream-fish snorkeling detection is dual sampling. For example, Weaver et al. (2014) calibrated snorkel counts using abundance estimates obtained from a prepositioned areal electrofisher. Spatially-replicated counts (e.g. Royle 2004) are an approach to account for variable detection without requiring either a second gear type or physical capture that remains largely unexplored for warmwater stream-fish snorkeling. For example, Webster et al. (2008) developed a model to estimate juvenile Coho Salmon *Oncorhynchus kisutch* abundance using repeat snorkel counts at sites, where detection varied as a function of covariates. Sighting-based sampling methods (e.g., Royle et al. 2004; Koneff et al. 2008) that account for variable detection using covariates also have applications to stream-fish snorkeling.

Snorkeling has useful applications in warmwater streams as a fish sampling method to detect species presence and monitor populations for certain species. In addition to potentially improving estimates of species richness, a sampling protocol that uses both snorkeling and electrofishing results in increased flexibility. For example, managers could use snorkeling in non-wadeable streams inaccessible with a boat electrofisher given that a subset of sites are sampled using both approaches to examine differences in detection. Unfortunately, the overall advantages and trade-offs of snorkeling to sample fishes in warmwater streams are poorly understood. My results suggest that snorkeling detection is highly variable among stream fishes and inappropriate for estimating the abundance of cryptic species. Given the inherent advantages of snorkeling as a stream-fish sampling method (e.g., minimal cost and intrusion), more research is needed to better understand factors contributing to variable detection and to better inform managers concerning the long-term applicability of snorkel surveys in warmwater streams.

Table 1. Comparison of the number of species detected using single-pass electrofishing and single-pass snorkeling for 19 stream fishes at 23 sites in the Ozark Highlands ecoregion (see Appendix 2 to interpret site codes). Columns 2-20 provide records of species detection at each site (see Appendix 1 to interpret two-letter species codes), where E indicates electrofishing, S indicates snorkeling, zero indicates detection, and one indicates non-detection. Species records with an NA correspond to sites sampled in 2014, where less species were considered. The last column provides both the number of species detected with each sampling method at each site and the total number of species detected at each site, where the number in the parentheses is the total number of species detected considering both sampling methods.

Site	BS E/S	BG E/S	CC E/S	GS E/S	LB E/S	LS E/S	MX E/S	NH E/S	NS E/S	OS E/S	RS E/S	RB E/S	RC E/S	SB E/S	SS E/S	WA E/S	WC E/S	WS E/S	Total detected E/S (Both)
BAFO2	NA	0/0	NA	1/1	1/1	1/1	NA	NA	NA	0/0	0/0	1/1	NA	1/1	NA	0/0	0/0	NA	5/5 (6)
BISU1	1/0	1/1	0/0	1/1	1/1	1/1	1/1	1/1	1/1	0/0	1/1	1/1	1/1	1/1	0/0	0/0	0/0	1/1	13/12 (13)
BUFF1	NA	1/1	NA	1/1	1/1	1/1	NA	NA	NA	0/0	1/0	1/1	NA	1/1	NA	1/1	0/0	NA	8/7 (8)
BUFF2	NA	1/1	NA	1/1	1/1	1/1	NA	NA	NA	1/0	0/1	1/1	NA	1/1	NA	1/0	0/0	NA	8/7 9)
BUFF3	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/0	0/0	0/0	1/1	1/1	1/1	0/0	1/0	1/0	1/0	15/11 (15)
BUFF4	1/0	1/1	0/0	1/1	1/1	1/1	1/1	1/1	0/0	0/0	1/1	1/1	1/1	1/1	0/0	1/1	0/0	1/0	13/11 (13)
BUTL1	1/0	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/0	1/1	1/1	1/1	1/1	0/0	1/0	0/0	1/0	15/12 (15)
CANE1	1/1	1/1	0/0	1/1	1/1	1/1	1/1	1/1	0/0	0/0	1/0	1/1	1/1	1/1	1/0	1/1	0/0	1/0	14/11 (14)
EVAN1	NA	1/1	NA	1/1	1/1	1/1	NA	NA	NA	0/0	0/0	1/1	NA	1/1	NA	0/0	0/0	NA	6/6 (6)
EVAN2	NA	1/1	NA	1/1	0/1	1/1	NA	NA	NA	0/0	0/0	1/1	NA	1/1	NA	0/0	0/1	NA	5/7 (7)
FIVE1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	0/0	1/0	1/1	1/0	1/1	0/0	1/0	0/0	0/0	13/11 (14)
FLIN2	NA	1/1	NA	1/1	1/1	1/1	NA	NA	NA	0/0	1/1	1/1	NA	1/1	NA	0/0	0/0	NA	7/7 (7)

FOUR1	NA	1/1	NA	1/1	1/1	1/1	NA	NA	NA	1/0	0/0	1/1	NA	1/1	NA	1/0	0/0	NA	8/6 (8)
FOUR2	NA	1/1	NA	1/1	1/1	1/1	NA	NA	NA	0/0	1/0	1/1	NA	1/1	NA	0/0	0/0	NA	7/6 (7)
SALI1	NA	1/1	NA	1/0	1/0	1/1	NA	NA	NA	0/0	0/0	1/1	NA	1/1	NA	0/0	0/0	NA	6/4 (6)
SPAV1	NA	1/1	NA	1/1	1/1	1/1	NA	NA	NA	0/0	0/0	1/1	NA	1/1	NA	0/0	0/0	NA	6/6 (6)
SPAV3	NA	1/1	NA	1/0	1/1	1/1	NA	NA	NA	0/0	0/0	1/1	NA	1/1	NA	0/0	0/0	NA	6/5 (6)
SPAV4	NA	1/1	NA	1/0	1/1	1/1	NA	NA	NA	0/0	1/1	1/0	NA	1/1	NA	1/0	0/0	NA	8/6 (9)
SPAV5	NA	1/1	NA	1/0	1/1	1/1	NA	NA	NA	0/0	1/0	1/1	NA	1/1	NA	0/0	0/0	NA	7/6 (8)
SPRI1	NA	0/0	NA	1/1	0/0	0/0	NA	NA	NA	0/0	0/0	1/1	NA	1/1	NA	0/0	0/0	NA	3/3 (3)
SPRI2	NA	1/0	NA	1/1	1/0	0/0	NA	NA	NA	0/0	0/0	1/1	NA	1/1	NA	0/0	0/0	NA	5/3 (5)
SPRI3	1/1	1/1	1/1	1/1	1/1	0/0	1/1	1/1	0/0	0/0	0/0	1/1	1/1	1/1	0/0	1/1	0/0	1/0	12/11 (12)
SPRI4	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/0	0/0	0/0	1/1	1/1	1/1	0/0	1/0	0/0	1/0	13/11 (13)

Table 2. Comparison of snorkel counts and tow-barge electrofishing catch data adjusted for detection for 9 stream fishes at 21 sites in the Ozark Highlands ecoregion (see Appendix 2 to interpret site codes), where data for Largemouth Bass and Spotted Bass were combined. Columns 2-8 provide snorkel counts and a range of abundance estimates at each site, where E indicates electrofishing and S indicates snorkeling. The range of abundance estimates were derived from the electrofishing detection model in Chapter 3 using 95% confidence intervals of estimated detection (see Methods for details).

Site	Bluegill E/S	Green Sunfish E/S	Largemouth-Spotted Bass E/S	Longear Sunfish E/S	Redear Sunfish E/S	Rock Bass E/S	Smallmouth Bass E/S	Warmouth E/S
BAFO2	(0)/0	(15-26)/1	(99-169)/59	(371-661)/182	(0)/0	(81-146)/3	(124-216)/73	(0)/0
BISU1	(30-71)/130	(97-187)/44	(23-48)/42	(976-2048)/819	(16-29)/17	(112-231)/14	(106-242)/138	(0)/0
BUFF1	(146-212)/25	(242-354)/3	(21-32)/17	(398-589)/121	(2-3)/0	(70-108)/1	(176-250)/139	(67-93)/6
BUFF2	(186-296)/149	(182-291)/5	(22-31)/6	(538-883)/374	(0)/1	(100-167)/11	(356-601)/192	(79-122)/0
BUFF3	(54-120)/30	(283-617)/7	(3-7)/2	(57-122)/26	(0)/0	(71-165)/1	(84-207)/86	(3-6)/0
BUFF4	(289-502)/125	(165-285)/7	(176-319)/69	(482-841)/231	(15-26)/6	(45-83)/7	(103-206)/59	(223-361)/8
BUTL1	(273-431)/207	(378-573)/15	(16-24)/17	(596-917)/597	(8-12)/1	(154-235)/6	(185-288)/244	(6-9)/0
CANE1	(315-553)/140	(259-441)/11	(140-255)/41	(889-1641)/424	(5-9)/0	(8-15)/1	(16-28)/18	(56-95)/8
EVAN1	(22-35)/27	(104-179)/2	(3-4)/5	(749-1201)/344	(0)/0	(77-147)/7	(87-162)/66	(0)/0
EVAN2	(16-29)/81	(122-213)/2	(0)/10	(428-720)/500	(100-100)/0	(29-56)/18	(126-234)/115	(0)/0
FIVE1	(263-491)/192	(176-324)/3	(9-17)/26	(197-354)/264	(3-6)/0	(91-172)/4	(47-91)/72	(4-6)/0
FLIN2	(10-15)/4	(41-62)/1	(11-17)/9	(1903-2956)/686	(3-5)/2	(794-1217)/184	(206-319)/208	(0)/0
FOUR1	(193-399)/83	(362-764)/22	(50-107)/47	(2062-4363)/536	(0)/0	(60-122)/1	(165-347)/59	(9-19)/0
FOUR2	(34-57)/4	(262-439)/5	(32-51)/14	(665-1154)/142	(2-4)/0	(11-19)/17	(152-219)/66	(0)/0
SALI1	(30-51)/16	(37-66)/0	(6-10)/0	(271-491)/216	(0)/0	(297-539)/23	(119-202)/156	(0)/0

SPAV1	(19-33)/12	(21-37)/4	(8-14)/12	(126-239)/416	(0)/0	(479-846)/94	(288-555)/205	(0)/0
SPAV3	(49-75)/16	(56-88)/0	(7-11)/3	(240-375)/278	(0)0	(576-908)/114	(255-411)/167	(0)/0
SPAV4	(1991-5868)/603	(160-457)/0	(506-1484)/102	(415-1245)/153	(879-2616)/143	(117-346)/0	(35-112)/36	(83-233)/0
SPAV5	(126-271)/54	(37-77)/0	(7-15)/7	(162-366)/127	(6-13)/0	(128-292)/9	(117-302)/196	(0)/0
SPRI1	(0)/0	(13-24)/1	(0)/0	(0)/0	(0)/0	(182-352)/53	(229-445)/247	(0)/0
SPRI2	(6-10)/0	(22-37)/4	(4-7)/0	(0)/0	(0)/0	(425-776)/11	(168-291)/146	(0)/0

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APPENDICES

Appendix 1. Sites and site code for 23 stream reaches in 19 streams of the Ozark Highlands ecoregion sampled to evaluate tow-barge electrofishing and snorkeling for species detection and evaluate snorkeling to provide reliable stream fish abundance estimates (See Figure 1 in Chapter 3 for site locations).

Site code	**Site**
BAFO2	Baron Fork2
BISU1	Big Sugar Creek
BUFF1	Buffalo Creek1
BUFF2	Buffalo Creek2
BUFF3	Buffalo Creek3
BUFF4	Buffalo Creek4
BUTL1	Butler Creek
CANE1	Caney Creek
EVAN1	Evansville Creek1
EVAN2	Evansville Creek2
FIVE1	Five-mile Creek
FLIN2	Flint Creek2
FOUR1	14-mile Creek1
FOUR2	14-mile Creek2
SALI1	Saline Creek1
SPAV1	Spavinaw Creek1
SPAV3	Spavinaw Creek3
SPAV4	Spavinaw Creek4
SPAV5	Spavinaw Creek5
SPRI1	Spring Creek1
SPRI2	Spring Creek2
SPRI3	Spring Creek3
SPRI4	Spring Creek4

Appendix 2. Species code, common name, and Latin name of 19 stream fishes of the Ozark Highlands ecoregion considered for an evaluation of tow-barge electrofishing and snorkeling for species detection and an evaluation of snorkeling to provide reliable stream fish abundance estimates. Species with no asterisk were considered for both stream fish abundance and detection. Species with a single asterisk were considered only for species detection in both 2014 and 2015. Species with a double asterisk were considered only for species detection in 2015 only.

Species code	Common name	Latin name	Trait group
MX	**Redhorses	*Moxostoma* spp.	D
MH	**Northern Hogsucker	*Hypentelium nigricans*	D
SS	**Spotted Sucker	*Minytrema melanops*	D
WS	**White Sucker	*Catostomus commersoni*	D
BB	Largemouth Bass	*Micropterus salmoides*	C
BB	Spotted Bass	*Micropterus punctulatus*	C
SB	Smallmouth Bass	*Micropterus dolomieu*	C
BG	Bluegill	*Lepomis macrochirus*	B
GF	Green Sunfish	*Lepomis cyanellus*	A
LS	Longear Sunfish	*Lepomis megalotis*	A
OS	*Orangespotted Sunfish	*Lepomis humilis*	NA
RS	Redear Sunfish	*Lepomis microlophus*	B
RB	Rock Bass	*Ambloplites rupestris*	A
WH	Warmouth	*Lepomis gulosus*	A
WC	*White Crappie	*Pomoxis annularis*	NA
RC	**Redspot Chub	*Nocomis asper*	NA
CC	**Creek Chub	*Semotilus atromaculatus*	NA
NS	**Northern Studfish	*Fundulus catenatus*	NA
BS	**Banded Sculpin	*Cottus carolinae*	NA

CHAPTER V

USING A MULTINOMIAL *N*-MIXTURE MODEL TO IMPROVE THE APPLICABILITY OF ELECTROFISHING FOR MONITORING STREAM-DWELLING SMALLMOUTH BASS

ABSTRACT

Despite major advancements in accounting for variable detection when surveying animal populations, contemporary approaches remain largely ignored by stream-fish scientists and catch-per-unit-effort remains the most commonly used metric. One notable advancement for addressing the challenges of variable detection are multinomial *N*-mixture models; however, the applicability of these models for stream-fish sampling has not been adequately evaluated. Multinomial *N*-mixture models use a flexible hierarchical framework to model detection across sites as a function of covariates. My objective was to examine the use of multinomial *N*-mixture models to improve the applicability of electrofishing for estimating Smallmouth Bass *Micropterus dolomieu* abundance. I sampled Smallmouth Bass populations using tow-barge electrofishing across a range of environmental conditions in Ozark Highland streams. The covariates water clarity, wetted channel width, water depth and effort explained variation in Smallmouth Bass electrofishing detection. Smallmouth Bass abundance estimates derived from the top model consistently agreed with baseline snorkel counts. Additionally, confidence intervals obtained from the final model using an empirical Bayes estimator were consistently more precise than unbiased Petersen capture-recapture estimates. Simulations that evaluated the performance of multinomial *N*-mixture models supported their use for monitoring and managing stream-dwelling Smallmouth Bass populations. I demonstrated how the application of a contemporary population estimation

can be a viable alternative to CPUE but while using similar sampling methods. In addition to improving the reliability of population estimates, hierarchical models allow for absolute abundance estimates to be calculated across a range of conditions, thus promoting the establishment of species-environment relationships and advancements in stream-fish ecology.

INTRODUCTION

Variability in detection confounds perceived patterns of fish populations across the landscape (Peterson and Paukert 2009). The failure to account for variable detection can hinder effective sportfish management (Price and Peterson 2010) and rare species conservation (Dorazio et al. 2005). Stream-fish populations present unique challenges for addressing variable detection due to highly dynamic environments (Jackson et al. 2001; Poff and Zimmerman 2010). Standardizing environmental conditions (e.g., sampling only at base flows) to maintain constant stream fish detection among surveys is not only challenging, but often impractical for meeting management and conservation objectives. For example, surveying across a wide range of environmental conditions is essential for establishing stream fish-environment relationships that set the foundation of environmental-flow standards (Gwinn et al. 2016).

Examples of how variable detection can influence stream-fish management and conservation decisions are pervasive in the fisheries literature. Sammons (2014) showed that seasonal variation in electrofishing detection influenced abundance estimates for lotic centrachid sportfishes. Suspected decline of the Brindle Shiner *Notropis bifrenatus* in northeastern streams of the U.S. was attributed to varying detection between sampling gears across environmental conditions rather than local extirpations (Pregler et al. 2015). An additional challenge for reliable stream-fish sampling data is the considerable variability in species traits (e.g., morphology, behavior, and mobility) that results in different tendencies in detection among streams fishes (Rabeni et al. 2009; Peterson and Paukert 2009). Variable detection among fishes confounds assessments of instream habitat restoration to improve species diversity (Price and Peterson 2010) and hinders accurate interpretations of assemblage-level stream-fish monitoring efforts (McManamay et al. 2014). In fact, the validity of Biotic Integrity Indices (e.g., IBI) that use certain stream fishes as surrogates for water quality have come under scrutiny for ignoring variable detection (e.g., Seegert 2000; Price and Peterson 2010). More recently, Gwinn et al. (2016) illustrated how not accounting for variable detection hinders the establishment of meaningful environmental flow standards for stream fishes. Improved and highly flexible approaches to address variable detection are increasingly common in the ecological literature (e.g., Williams et al. 2002; MacKenzie et al. 2005; Royle et al. 2013). Despite the numerous options available to the stream fish scientist, the lack of widespread implementation of these contemporary approaches to account for variable detection has impeded progress in both ecology and management (Brewer and Orth 2015; Gwinn et al. 2016).

The Smallmouth Bass *Micropterus dolomieu*, a stream fish of recreational and ecological value (Brewer and Orth 2015), provides an applied example of how the lack of progressive monitoring approaches has prevented improved insight into life-history characteristics and demographics and the development of long-term management strategies. Although electrofishing is the most common, and often most practical, stream-fish sampling method (Rabeni et al. 2009), its use for studying and monitoring local Smallmouth Bass populations is challenging. Long-standing issues using electrofishing to monitor Smallmouth Bass populations are prevalent in the grey literature. For example, Lyons and Kanehl (1993) conducted extensive Smallmouth Bass surveys using electrofishing and determined that both removal (e.g., Zippin 1958; Carle and Strub 1978) and capture-recapture (e.g., Manly and Seber 1973) estimation methods were generally inappropriate for estimating the abundance of stream-dwelling populations because they fail to meet model assumptions. In particular, Lyons and Kanehl (1993) demonstrated that highly variable detection among removal passes and low Smallmouth Bass capture rates (i.e., low detection) resulted in biased abundance estimates, inflated variance, and wasted resources (i.e., time and money spent on surveying that does not result in usable data). Low detection when sampling Smallmouth Bass in streams using electrofishing is also supported by peer-reviewed studies (e.g., Heimbuch et al. 1997; Dauwalter and Fisher 2007; Hense et al. 2010). The failure to successfully apply electrofishing to population estimation methods that account for variable detection has led to a reliance on catch-per-unit-effort (CPUE) for ecological information and management decisions pertaining to Smallmouth Bass (Brewer and Orth 2015). For example, a review of the biology and ecology of genetically-distinct Smallmouth Bass stream populations (Brewer and Long 2015) relied primarily on data collected via CPUE electrofishing. In addition to providing only an indirect measure of abundance, CPUE assumes constant detection across time and space, which is often unrealistic in stream environments (Price and Peterson 2010; Gwinn et al. 2016). Thus, the usefulness of CPUE data for long-term datasets or large study areas is limited. An examination of agency reports shows how variable electrofishing detection confounds the results of statewide long-term monitoring efforts of lotic Smallmouth Bass populations using CPUE (e.g., Meneau 2010). It is surprising that contemporary approaches have rarely been applied to improve the applicability of electrofishing for population estimation methods given the importance of Smallmouth Bass. Although Dauwalter and Fisher (2007) developed an electrofishing detection model that provided absolute abundance estimates of stream-dwelling Smallmouth Bass populations, the authors surveyed only two streams located in different ecoregions. Additionally, Dauwalter and Fisher (2007) used channel units (e.g., individual pools, backwaters, etc.) to define the 28 “sites”, which not only provided a misleading sample size, but was also too fine a spatial scale to be practical for comparable estimates of Smallmouth Bass abundance among distinct populations.

One notable advancement in addressing the challenges of variable detection when sampling animal populations is a class of models known as multinomial *N*-mixture (Royle 2004;

Dorazio et al. 2005; Royle and Dorazio 2006). Multinomial *N*-mixture models use a flexible hierarchical framework to independently estimate both abundance and detection probability of spatially-distinct subpopulations as a function of covariates, where detection can vary among both sites and surveys. In contrast to similar models that predict species occupancy by reducing counts to binary detection-nondetection data (see MacKenzie et al. 2005), multinomial *N*-mixture models provide inference on both species occurrence and abundance (Royle and Dorazio 2006). Thus, multinomial *N*-mixture models are applicable for common species (i.e., counts are typically > 0 across sites), where the primary focus is typically to estimate variation in local abundance. Multinomial *N*-mixture models also accommodate temporally-replicated counts at sites, which makes them applicable for common fish population estimation methods such as removal and capture-recapture (Royle 2004). The hierarchical structure of multinomial *N*-mixture models enables an empirical Bayes approach (Carlin and Louis 2000) to estimate abundance across spatially-distinct sites. Empirical Bayes estimates, unlike removal and capture-recapture approaches that calculate abundance at each site separately, provide site-specific abundance estimates that are a reflection of data collected across all sites (i.e., the sites "borrow" information; Dorazio et al. 2005; Royle and Dorazio 2006). The dependency among datasets in the hierarchical multinomial *N*-mixture model framework not only improves the precision of confidence intervals but also reduces bias and improves the estimability of abundance at sites with sparse or insufficient data (e.g., low detection or sample size) given adequate data are available at some sites; therefore, all data are informative (i.e., no wasted resources).

There are multiple approaches that can be used to increase confidence in the reliability of abundance estimates. One option is to provide a comparison using results from another sampling method associated with high detection probability. Snorkel counts can provide informative minimum population estimates (i.e., a reliable baseline) to compare with abundance estimates obtained via other methods given adequate water clarity in Ozark Highland streams (see Chapter

4). Another option for assessing the reliability of abundance estimates is to compare the results to an estimation method with known reliability. For example, the traditional Petersen capture-recapture estimator (hereafter referred to as Petersen capture-recapture) remains a viable approach for estimating stream-fish abundance given that assumptions are met for unbiased estimates (e.g., adequate sample size and recapture rate and minimal individual detection variability; Lockwood and Schneider 2000; Rosenberger and Dunham 2005).

Despite the applicability of multinomial *N*-mixture models to common fish estimation methods, their use for stream fishes has been relatively rare in the scientific literature. Coggins et al. (2011), Yard et al. (2011), and Dodrill et al. (2015) used multinomial *N*-mixture models for boat electrofishing surveys in the Colorado River basin. Korman et al. (2016) demonstrated the advantages of multinomial *N*-mixture models for multiple gear sampling designs. I am not aware of any capture-recapture applications for electrofishing in wadeable warmwater streams (but see Dorazio et al. 2005 for a snorkeling removal example) or for estimating Smallmouth Bass populations. Accordingly, my objective was to use a multinomial *N*-mixture capture-recapture model (hereafter referred to as multinomial capture-recapture model) to improve the applicability of electrofishing for estimating the abundance of Smallmouth Bass > age 1 in wadeable streams. Although Smallmouth Bass were included in the multi-species detection model in Chapter 3, it was also important to use a single-species approach to examine variation in electrofishing detection given their value as a sportfish. Site-specific detection probability using the generalized linear mixed model represents a trade-off with estimates across all species (see Chapter 3 methods for details) and certain management objectives may involve targeting only Smallmouth Bass during sampling. I performed tow-barge electrofishing surveys across a range of environmental conditions to identify variables that influenced variation in Smallmouth Bass detection. As a basis of comparison with my abundance estimates derived from the multinomial capture-recapture model, I also conducted snorkel surveys at a subset of sites where water clarity was ideal. Snorkeling is an appropriate method to survey Smallmouth Bass in clear, warmwater streams (Brewer and Ellersieck 2011; Chapter 4). Lastly, I compared Smallmouth Bass abundance estimates derived from the multinomial capture-recapture model to Petersen capture-recapture when assumptions were met for unbiased estimates. I used two approaches for comparisons because a wide range of environmental conditions and fish densities across the study site was expected.

METHODS

Study sites. - I sampled > age-1 Smallmouth Bass in 25 stream reaches that each comprised three to five riffle-run-pool sequences to characterize habitat (referred to hereafter as sites) in the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri (Figure 1). Among the sites, the mean ± SD water temperature was 21.5± 2.7 ºC and the mean ± SD ambient water conductivity was 276 ± 68 µS/cm. The sites represented spatially-distinct subpopulations of Smallmouth Bass that were demographically closed during the sample event with mixing of individuals permitted over longer time periods, which is consistent with assumptions of multinomial *N*-mixture models (Royle 2004; Dorazio et al. 2005).

Environmental measurements. - At each site, I measured environmental variables hypothesized to influence electrofishing detection of Smallmouth Bass. Wetted width (1.0 m) and thalweg depth (0.1 m) were measured at 50-m transects to calculate mean wetted channel width and mean depth. Stream discharge (0.01 m3/s) was measured in a homogenous area of a run using the velocity-area method (Gordon et al. 2004), where I reported the average of three replicates. Water clarity (0.5 m) was measured as the horizontal distance an underwater observer could see a fish silhouette. I designed my fish silhouette to mimic the color, markings, and typical size (~200 mm) of Smallmouth Bass in my study streams (Dunham et al. 2009). I also estimated the total sampling area at each site by subtracting the summed length of riffles from total reach length and multiplying by the mean wetted channel width. Environmental measurements are reported as mean ± SD.

Fish sampling. –I installed two sets of block-off nets at both the upstream and downstream ends of each site to ensure a closed system during the sampling event. Block-off nets were preferentially placed at shallow riffles further inhibit fish movement (Peterson et al. 2004; Price and Peterson 2010). Either a low-water bridge at low flow or a dry riffle located at one end of the site provided an adequate fish barrier at a few sites and no block-off nets were installed.

I sampled Smallmouth Bass subpopulations over a three-day period. On day one (hereafter referred to as capture day), I used a tow-barge electrofisher (Midwest Lake Management, Polo, Missouri) to establish marked populations of Smallmouth Bass. All Smallmouth Bass were measured and fish < 80 mm TL were excluded from the study. My minimum fish size excluded most age-0 Smallmouth Bass and was also based on both observed mortalities and lack of recapture via electrofishing of Smallmouth Bass < 80 mm TL. In addition to size, age-0 Smallmouth Bass were easily recognizable due to prominent tri-colored tails. I used pulsed direct current (DC), 60 Hz, and a 25% duty cycle for electrofishing. Voltage was adjusted based on ambient water conductivity (ranging from 300-400 V) to maintain a target power that maintained a consistent electric field across levels of ambient water conductivity while minimizing electrofishing-induced injuries as described by Miranda (2009). The electrofishing crew consisted of three people: one tow-barge operator (myself) armed with a hand net and two persons equipped with dip nets each operating one of the two anodes. I electrofished areas ≥ 0.2 m deep in an upstream direction with a zigzag pattern. The depth limitation of the tow-barge electrofisher excluded most riffle areas, but use of this habitat by Smallmouth Bass > 80 mm TL is very uncommon (Brewer 2013; unpublished snorkel data from the Oklahoma Department of Wildlife Conservation). Care was taken to thoroughly electrofish areas with structure (e.g., large wood, rootwads, and boulders). A minimum of two electrofishing passes were performed per riffle-run-pool sequence, although additional passes were performed at some sites to increase the marked population of Smallmouth Bass for Petersen capture-recapture estimates. Electrofishing time was recorded at each site to estimate variation in effort both among sites and between capture and recapture events, where electrofishing effort was calculated as electrofishing time divided by sampling area. I marked captured Smallmouth Bass with an upper caudal fin clip. Marked Smallmouth Bass were released throughout the site and allowed to recover and redistribute for ~24 h prior to snorkeling and ~ 48 h prior to the electrofishing recapture event to allow the system to fully recover (Peterson and Cederholm 1984). Smallmouth Bass injured during the sampling episode or that showed signs of excessive stress were released outside of the blocked-off area. I inspected the block-off nets and the area between them periodically for trapped or dead Smallmouth Bass.

I conducted snorkel counts of Smallmouth Bass subpopulations on day two of the sampling event at 13 sites to provide a coarse estimate of abundance, where the protocol was identical to methods described in Chapter 4. Snorkel surveys were only performed when horizontal water clarity was ≥ 3.0. I also did not use snorkeling data at sites located at the southern ecoregion boundary (Evansville Creek and 14-mile Creek) of the Ozark Highlands due to evidence of low detection (see Chapter 4). I inspected the blocked-off area for dead Smallmouth Bass prior to the snorkel surveys and mortalities were removed from the marked population. Smallmouth Bass estimated to be ≥ 80-mm TL with no prominent tri-colored tail were recorded on an underwater wrist cuff when they either passed or were passed by the snorkeler. I also instructed snorkelers to collect dead Smallmouth Bass for an additional method to estimate delayed mortality.

The electrofishing recapture event for the Smallmouth Bass subpopulations were conducted on day three of the sample. The electrofishing procedure was identical to capture day, with the exception that only two passes were performed for each riffle-run-pool sequence at each site. Prior to sampling, I again inspected the blocked-off area for dead Smallmouth Bass and mortalities were removed from the marked population. I recorded both marked and unmarked Smallmouth Bass ≥ 80 mm TL. Fish counts are reported as mean ± SD.

Subpopulation estimates. - I developed multinomial capture-recapture models using the package "unmarked" (Fiske and Chandler 2011) in the statistical software R (version 3.2.2, R Core Development Team, 2014). Specifically, I used the function "*gmultmix*" with a single primary period, which fits a generalized form of the multinomial *N*-mixture model described by Royle (2004) and assumes a closed system during the capture-recapture event at each site. In the multinomial *N*-mixture model framework, capture-recapture data collected at a set of sites can be used to model variation in both abundance and detection probability, where site-specific abundance N is treated as a latent variable with a discrete distribution (Chandler 2015). I specified a negative-binomial error distribution, which introduces a dispersion parameter to the model, due to evidence of overdispersion in the dataset. Overdispersed subpopulations of animals are a common phenomenon because spatial randomness is uncommon in distributions (Dorazio et al. 2005). Following Chandler (2015), the multinomial capture-recapture model is written as:

(1) $N_i \sim \text{Negative binomial}(\theta, \lambda)$

$Y_i | N_i \sim \text{Multinomial}\{N_i, \pi(p)\}$,

where θ is the overdispersion parameter, λ is the estimated number of individuals at each site i, Y_i is a vector of counts at each site representing the three possible capture histories for the capture and recapture events $H \in (11, 10, 01)$, and $\pi(p)$ is a function that converts detection probability p to multinomial cell probabilities as:

(2) $\pi\ (p) = \{p^2, p(1 - p), (1 - p)p\}$,

where the probability of not capturing an individual is $H = 00$ is $(1 – p)^2$.. Site-specific Smallmouth Bass abundance was modeled using a log-link function as:

(3) $\log(\lambda_i) = \beta_0$.

I did not include covariates to explain variation in abundance because the objective was solely to model Smallmouth Bass abundance (i.e., my focus was on "how many fish were there" rather than "why"); however, their inclusion is straight forward (see Fiske and Chandler 2011; Chandler 2015). Detection probability was modeled using a logit-link function as

(4) $\text{logit}(p_{ij}) = \alpha_0 + \alpha_1 v_{ij} + \alpha_2 v_{ij} + \alpha_n v_{ij}$,

where v_{ij} is a detection covariate corresponding to survey j at site i.

I fitted a candidate set of 12 multinomial capture-recapture models with varying complexity (Table 1). An effort detection covariate was included in every model to account for variation in electrofishing intensity among surveys. I used the detection covariates mean wetted channel width, mean water depth, discharge, and water clarity in the candidate models to characterize environmental variation in survey conditions, where discharge was natural-log transformed due to a right-skewed distribution. An examination correlations among detection covariates indicated that the Pearson product-moment correlation coefficient (r) was $> |0.50|$ between discharge and water depth ($r = 0.51$), thus these covariates did not co-occur in any candidate models. Water temperature was not included in the candidate models because it did not vary considerably among sites (see study area). The candidate models were ranked using Akaike information criterion corrected for small sample size (AICc; Burnham and Anderson 2002), where site was the sample size. The number of sites represents the most conservative estimate of sample size for multinomial N-mixture models. The number of residuals in the model corresponds to the number of vector counts across sites (here $n = 75$) and is a more accurate, yet less conservative, estimate of sample size. I used AICc to compare the top-ranked model to a model that also included a conductivity detection covariate to assess if water conductivity influenced Smallmouth Bass electrofishing detection despite standardizing power. Fish size is also an important consideration when estimating electrofishing detection (Peterson and Paukert 2009; Price and Peterson 2010); however, I did not anticipate it would influence our abundance estimates because mean Smallmouth Bass TL did not vary considerably among sites (200 ± 28 mm). To evaluate this expectation, I used AICc to compare the top-ranked model to a model that also included a fish size detection covariate, where I used mean Smallmouth Bass TL to represent each site. Lastly, I used AICc to compare the top-ranked model to a model that also included a categorical survey event detection covariate (i.e., capture and recapture) to confirm that included covariates adequately accounted for variation in detection between capture and recapture events. All detection covariates were scaled such that each had a mean of zero and a standard deviation of one to both promote model convergence and simplify interpretation of coefficients.

I assessed fit of the top model using both a visual examination of residuals and a calculation of c-hat (an estimate of overdispersion where c-hat > 1 suggests overdispersion). I used a Chi-squared test as described by MacKenzie and Bailey (2004) with 10,000 bootstraps for the calculation of c-hat. I also calculated 95% confidence intervals for coefficients in the top-ranked model using a profile likelihood method (see Fiske and Chandler 2011).

I calculated site-specific detection and abundance estimates for the Smallmouth Bass subpopulations from the estimation methods. I derived cumulative detection probability and abundance at each site using the top-ranked multinomial capture-recapture model. Cumulative detection probability was calculated as the sum of the multinomial cell probabilities at each site and is interpreted as the proportion of available individuals detected across the capture and recapture events. Empirical Bayes calculations were used for both multinomial capture-recapture abundance estimates and 95% confidence intervals. I calculated Petersen capture-recapture estimates with the Chapman (1954) bias correction (hereafter referred to as Petersen capture-recapture) using the library “Rcapture” (Baillargeon and Rivest 2007) in the statistical software R as

(5) $\hat{N} = \{(M + 1)\,(C + 1) / (R + 1)\} - 1,$

where $\hat{N}$ is the population estimate, M is the number of Smallmouth Bass marked during the capture event, C is the number of Smallmouth Bass captured during the recapture event, and R is the number of recaptured Smallmouth Bass that were marked. I only calculated Petersen capture-recapture at sites where assumptions were met for unbiased population estimates outlined by Lyons and Kanehl (1993): (1) At least 20 fish were marked, (2) At least 5 fish were recaptured, and (3) At least 15% of the number of fish captured during the recapture event were marked. We calculated 95% confidence intervals for site-specific Petersen capture-recapture estimates as $\hat{N} \pm z_{\alpha/2}$(SE), where we used a bias-corrected SE (Seber 1970). I compared estimates of Smallmouth Bass abundance derived from empirical Bayes calculations to both snorkel counts and Petersen capture-recapture estimates.

I further assessed the utility of snorkeling and Petersen estimates to evaluate Smallmouth Bass abundance estimates by comparing them to removal estimates. My dataset also allowed for two-pass electrofishing removal estimates to be calculated (Appendix 1). Reliable two-pass removal estimates depend on declining capture between passes, reasonable “depletion” of individuals, and higher detection relative to capture-recapture (Lockwood and Schneider 2000). My data and field observations suggested these essential two-pass removal assumptions were seldom (if ever) met; therefore, I expected that Smallmouth Bass abundance estimates using two-pass removal, even in the multinomial *N*-mixture model framework, would tend to overestimate electrofishing detection (thus underestimating abundance). I developed a multinomial two-pass removal *N*-mixture model (hereafter referred to as multinomial removal model) as described for the multinomial capture-recapture model (Appendix 2) and compared the snorkel counts and Petersen estimates to Smallmouth Bass abundance estimates derived from the top-ranked model.

I also performed simulations using various levels of detection and abundance to assess the robustness of the multinomial capture-recapture model. I performed simulations under 18 scenarios (1000 runs per scenario) with combinations of four levels of mean abundance, detection probability and standard deviation of detection probability (i.e., unexplained variation), and both 25 and 50 sites (Table 2). I chose the scenarios to reflect the number of sites used in the model and Smallmouth Bass abundance estimates typical to south-central streams. I used a level of average detection and standard error similar to estimates from the top-ranked multinomial capture-recapture model, as well as simulations with lower levels of detection and higher unexplained variation in detection. I also doubled the number of sites under some scenarios, particularly those with very low detection or very high unexplained heterogeneity, to assess how increasing sample size affected multinomial capture-recapture model performance. The multinomial capture-recapture model was assessed based on bias in estimated abundance and root mean square error (RMSE). I also calculated a coefficient of variation (CV) for RMSE as (RMSE/mean estimated abundance)*100 to improve the interpretation of precision for varying levels of abundance.

RESULTS

Environmental measurements. - I sampled Smallmouth Bass subpopulations across a range of environmental conditions (Table 3). Mean wetted channel width varied among sites from 9-18 m (14 ± 3 m) and mean thalweg depth varied among sites from 0.5-1.1 m (0.8 ± 0.1 m). Discharge and water clarity varied the most among sites, ranging from 0.091-5.81 m3/s (1.50 ± 1.43 m3/s) and 1.5-7.0 m (3.5 ± 1.3 m), respectively. Electrofishing effort varied both among surveys, ranging from 0.013 -0.053 min/m^2 (0.033 ± 0.011 min/m^2), and between capture and recapture events (mean of 0.036 min/m^2 and 0.030 min/m^2, respectively).

Fish sampling. – Monitoring of the study area provided evidence that I maintained a closed system during the surveys and that delayed mortality of Smallmouth Bass due to capture and handling was trivial. No Smallmouth Bass (living or dead) were found in the block-off nets or in the area between them. Only three dead marked Smallmouth Bass were found during routine inspections of the site (one fish at Buffalo Creek2 and two fish at 14 Mile Creek2).

The number of marked Smallmouth Bass, the proportion of fish recaptured, and the number of fish encountered during snorkeling were highly variable among sites. The number of Smallmouth Bass marked at a site ranged from 8 fish at Caney Creek to 120 fish at 14 Mile Creek2 (39 ± 30 fish; Table 3). The proportion of Smallmouth Bass recaptured at a site ranged from 0.00 at Flint Creek1 to 0.57 at 14 Mile Creek2 (0.24 ± 0.15). Baseline population estimates of Smallmouth Bass obtained using snorkel counts ranged from 18 fish at Caney Creek to 247 fish at Spring Creek1 (127 ± 75 fish).

Subpopulation estimates. – A top-ranked multinomial capture-recapture model was evident and included the detection covariates electrofishing effort, water clarity, and a mean wetted channel width and mean water depth interaction (AICc = -7184.09; Table 1 and Table 4). There was no evidence that either water conductivity or mean fish size influenced Smallmouth Bass detection among sites when these covariates were added to the top-ranked model (AICc = -7179.95 and -7179.35, respectively). A comparison of the top-ranked model to a model that also included a categorical survey event detection covariate provided evidence that the detection covariates adequately explained variation in detection probability between capture and recapture events (AICc = -7180.41). Site-specific cumulative detection of Smallmouth Bass varied considerably from 0.23 at Big Sugar Creek to 0.84 at 14-mile Creek3 (0.45 ± 0.15; Table 3). Estimated detection probability at mean levels of covariates for a single survey was 0.25 ± 0.02 (Table 3). Smallmouth Bass detection increased with both increased electrofishing effort and increased water clarity (Table 4; Figure 2). The interaction term in the model indicated that the relationship between Smallmouth Bass detection and both wetted channel width and water depth varied at different levels of these covariates. To interpret the interaction term, I predicted Smallmouth Bass detection probability at various levels of mean wetted channel width and mean water depth using linear combinations of model coefficients. Detection decreased sharply as mean water depth increased in narrower surveying conditions; however, the magnitude of the relationship diminished at higher levels of depth (Figure 3a). Conversely, there was only a slight negative relationship between detection and water depth in wider surveying conditions (Figure 3b). Similarly, detection increased with mean wetted channel width in shallower surveying conditions (Figure 3c), with virtually no relationship between detection and wetted width in deeper surveying conditions (Figure 3d). The interaction between mean water depth and mean wetted channel width on Smallmouth Bass detection probability indicated that the influence of each of the covariates was more pronounced at lower levels of the alternate covariate, there was no influence of wetted width in deep conditions, and detection (although low) no longer decreased considerably at high levels of width and depth (i.e., very wide and very deep). The estimate of c-hat from the Chi-squared test (c-hat < 1) did not indicate overdispersion in the model. A plot of predicted versus fitted residuals ($n = 75$) also suggested adequate model fit (i.e., no evidence of heteroscedasticity).

A comparison of the empirical Bayes calculations derived from the multinomial capture-recapture model to secondary methods increased confidence in the reliability of the estimates. Although I only met assumptions for unbiased Petersen capture-recapture at 11 of 25 sites, the estimates were in general agreement with the empirical Bayes estimates and the 95% confidence intervals overlapped at every site (Table 3). However, the range of the confidence intervals for the empirical Bayes estimates were more precise at every site compared to the Petersen capture-recapture confidence intervals. The width of the confidence intervals for empirical Bayes and Petersen capture-recapture was 48 ± 16 fish and 109 ± 83 fish, respectively, at sites where both estimates were available. There was a similar level of precision for empirical Bayes confidence intervals calculated across all sites, where the width of the interval ranged from 14 to 87 fish (49 ± 20 fish). The empirical Bayes confidence intervals contained the snorkel count at 8 of 13 sites. The empirical Bayes confidence intervals exceeded the Smallmouth Bass snorkel count at four sites; however, the snorkel count was within 9 fish of the lower bound of the confidence interval at two sites (Buffalo Creek3 and Caney Creek) and within 16 fish of the lower bound of the confidence interval at Buffalo Creek1. The Smallmouth Bass snorkel count exceeded the empirical Bayes confidence interval only at Spring Creek1. The three approaches compared favorably at the sites where I could compare the empirical Bayes abundance estimate to both snorkel counts and Petersen capture-recapture (Buffalo Creek1, Butler Creek, Spring Creek1, and Spring Creek2). I could not compare Smallmouth Bass abundance estimates derived from the multinomial capture-recapture model to secondary methods at only five sites.

As expected, estimated cumulative Smallmouth Bass detection for the top-ranked multinomial removal model (Appendix 3) was higher (often by 20-30%) than the top-ranked multinomial capture-recapture model at 24 of 25 sites (Appendix 1). More importantly, both snorkel counts and Petersen capture-recapture estimates supported my expectation that the multinomial removal model would consistently underestimate Smallmouth Bass abundance. The snorkel counts were contained in the empirical Bayes confidence intervals derived from the multinomial removal model at only 2 of 13 sites where I was able to make the comparison. The snorkel count was more than double the empirical Bayes estimates at the other 11 sites, where the counts were > 50 fish higher than the upper bound of the confidence interval at seven sites and > 100 fish higher at four sites. The snorkel count was over double the value of the upper bound of the empirical Bayes confidence interval at five sites. The empirical Bayes confidence intervals derived from the multinomial removal model overlapped with Petersen confidence intervals at only 3 of 11 sites where I was able to make the comparison. The lower bound of the Petersen confidence interval was higher than the upper bound of the empirical Bayes confidence interval at the other eight sites, where the difference was > 50 fish at three sites.

Simulations indicated that the multinomial capture-recapture model performed well under conditions similar to my model estimates. Bias and precision was acceptable for meeting typical stream-dwelling Smallmouth Bass management objectives and with other levels of abundance, detection, and unexplained variation in detection reasonable for Smallmouth Bass tow-barge electrofishing scenarios in Ozark Highland streams (assuming that covariates related to variable detection could be identified). However, the model performed poorly with both considerably lower detection and higher unexplained variation in detection. Estimated abundance and RMSE with scenarios similar to my model-estimated average detection probability (0.25) and unexplained variation (0.05) were -5 fish and 25 fish, respectively (Table 2). Bias was -7 fish and RMSE was 31 fish with slightly lower detection probability (0.20). Bias in estimated abundance and RMSE were also reasonable at a lower level of detection (-12 fish and 40 fish, respectively, with detection probability of 0.15); however, bias increased and RMSE decreased considerably at very low levels of detection (-60 fish and 96 fish, respectively, with detection probability of 0.05). Bias in estimated abundance and RMSE were also reasonable with double the unexplained heterogeneity of my model estimate (0.10) with a detection probability of 0.25 (bias of-18 fish and RMSE of 44 fish). A “breaking point” of the multinomial capture-recapture model was identified at a detection probability of 0.20 and unexplained variation of 0.10 (bias of -25 fish and RMSE 54 fish), where model performance diminished substantially with increasing levels of unexplained variation. For example, bias in estimated abundance and RMSE with a detection probability of 0.20 and unexplained variation of 0.15 were -66 fish and 89 fish, respectively. Not surprisingly, model performance was exceptionally poor with a combination of low detection and high unexplained heterogeneity. The performance of the multinomial capture-recapture model did not appear sensitive to varying levels of abundance or number of sites. Bias in estimated abundance was < 7 fish and the CV of RMSE was ≤ 30% for abundances ranging from 35-185 fish with a detection probability of 0.25 and unexplained heterogeneity of 0.05. Model results were virtually identical for scenarios simulated with both 25 and 50 sites.

DISCUSSION

I used a highly flexible hierarchical population estimation model to address a long-standing stream-fish management issue. Dynamic stream environments present a widespread challenge for both effective stream-fish monitoring and quality research due to variable detection among surveys. The application of electrofishing, the most common stream-fish sampling method, to population estimation methods has been particularly challenging for Smallmouth Bass due to both highly variable and often low detection. The modeling approach presented here effectively addresses concerns raised by Lyons and Kanehl (1993) regarding the use of capture-recapture electrofishing to estimate Smallmouth Bass abundance in streams. The covariates included in the multinomial capture-recapture model effectively accounted for variable Smallmouth Bass detection across a range of electrofishing effort, environmental conditions, and fish densities. Abundance estimates for spatially-distinct Smallmouth Bass populations derived from my model compared favorably to both baseline estimates obtained via snorkel counts and unbiased Peterson capture-recapture estimates. Additionally, the confidence intervals derived using an empirical Bayes estimator were much more precise than the Petersen capture-recapture confidence intervals. The increased precision of the empirical Bayes confidence intervals was due to the hierarchical framework of multinomial *N*-mixture models introducing a dependency among datasets (i.e., sites are models simultaneously using data collected across all sites), which also improves the reliability of abundance estimates at sites with deficient data (Dorazio et al. 2005; Royle and Dorazio 2006). Dorazio et al. (2005) presented similar results using multinomial *N*-mixture models for sampling Okaloosa Darter *Etheostoma okaloosae* populations with a removal estimation method, where confidence intervals were consistently more precise than those derived from an approach that modeled each site individually. Although Dauwalter and Fisher (2007) proposed a model to address the challenges of Smallmouth Bass electrofishing detection, the authors failed to provide stream managers with a usable approach due to both an extremely limited number of streams (*n* = 2; each in a different ecoregion) and the modeling of detection at a spatial scale far too fine to be practical for comparing local abundance estimates across a study area (i.e., managers typically do not sample individual channel units for comparison across sites). Conversely, I modeled the abundance of Smallmouth Bass subpopulations in reaches of 15 streams across a large study area with similar geologic and climate constraints (i.e., a single ecoregion). Thus, I demonstrated a broadly applicable approach to monitor stream-fish populations at an ecologically-meaningful scale (i.e., representative habitat directly related to variation in local abundance). Although I focused on stream-dwelling Smallmouth Bass using electrofishing, the approach detailed here is easily adaptable to other stream fishes and gear types.

Multiple environmental covariates explained variation in Smallmouth Bass detection probability. I observed a positive relationship between detection and water clarity, which contradicts findings of studies that examined backpack electrofishing detection of stream fishes (e.g., Price and Peterson 2010). A common behavioral response of Smallmouth Bass during the electrofishing surveys was to evade capture by moving in a downstream direction around sampling crews, even if initially pushed upstream. The reduced detection with reduced water clarity was presumably due to Smallmouth Bass shocked behind the anode operators going unseen by the tow-barge operator, thus suggesting the operator netting fish under clear conditions increased detection probability. I also identified wetted channel width and water depth, which together characterize the cross-sectional survey area in a stream, as factors contributing to variable Smallmouth Bass electrofishing detection. The observed interaction of wetted channel width and water depth highlights both the complexity of factors that contribute to variable electrofishing detection in streams and the need to survey across a wide range of environmental conditions. Although I identified specific environmental covariates that explained Smallmouth Bass electrofishing detection probability among the study sites, many environmental factors affect stream-fish detection and the magnitude of influence varies considerably among both systems and species (Peterson et al. 2004; Hense et al. 2010; Price and Peterson 2010). Thus, stream-fish managers might benefit from measuring a comprehensive suite of environmental covariates they hypothesize will influence detection rather than solely relying on results of other studies, particularly those involving dissimilar species or conducted in different ecoregions.

Hierarchical population estimation approaches, such as multinomial *N*-mixture models, offer many advantages to stream-fish managers relative to both CPUE and population estimation methods that model each site separately; however, there are trade-offs that should be considered. The primary advantages of all population estimation methods over CPUE are the ability to calculate a direct measure of abundance and account for variable detection across environmental conditions. Accounting for variable detection across time and space is of particular importance in dynamic streams systems because standardizing environmental conditions is often unrealistic. Adjusting for variable detection allows stream-fish abundance estimates to be comparable over long time periods across broad study areas. For example, long-term statewide stream-fish monitoring is a common agency objective and relying on CPUE data can hinder both identifying trends in populations and refining management strategies (see Introduction). The ability to estimate abundance across variable conditions at greater temporal scales also promotes the establishment of stream fish-environment relationships, which are essential to both ecology and management. Hierarchical population estimation methods also have other advantages. In addition to decreased uncertainty in Smallmouth Bass abundance estimates (i.e., narrower confidence intervals) compared to Petersen capture-recapture, the multinomial capture-recapture model provided reliable population estimates at sites with deficient data. I would have only been able to calculate unbiased Smallmouth Bass abundance estimates at less than half of the sites using Petersen capture-recapture. For long-term stream-fish monitoring, the failure to obtain usable data at all sites results in both lost information and wasted resources. However, adequate data at some sites (e.g., reasonable sample size and detection) is required for hierarchical population estimation methods to be effective (Dorazio et al. 2005). Requirements of multinomial *N*-mixture models do include collecting environmental covariate information at each site and repeat surveys (i.e., both a capture and recapture event or additional removal passes), which necessitates extra time and labor. However, both the hierarchical framework and the use of covariates to explicitly model detection for each survey event enables an optional application of multinomial capture-recapture models that is not possible with capture-recapture methods that both model each site separately and only implicitly account for variable detection. Once covariates that influence detection are well established, a site-specific detection probability from a single survey (i.e., identical survey effort to CPUE) can be derived from the multinomial *N*-mixture model to adjust catch data to an absolute abundance estimate (see Thompson and Seber 1994; Peterson and Paukert 2009). I identified relationships between Smallmouth Bass electrofishing detection and environmental covariates with a reasonable number of sites. The increased statistical complexity of multinomial *N*-mixture models is another important consideration. However, necessary R code is well described in both the "unmarked" manual (Fiske et al. 2015) and related literature (e.g., Fiske and Chandler 2014; Chandler 2015; Fiske and Chandler 2015). Multinomial *N*-mixture models also have the additional flexibility to incorporate covariates that explain variation in abundance independent of detection. The metapopulation structure of multinomial *N*-mixture models views local abundance as a random process and allow users to focus on examining variation in fish populations at a spatial scale often of interest to stream researchers and managers (i.e., the reach) independent of detection. Thus, another advantage of multinomial *N*-mixture models is that the pooling of surveys for small populations (i.e., scaling up), where site-level information on abundance and detection can be lost, is not necessary (Royle 2004).

My findings also highlight the advantage of using secondary methods to increase confidence in the reliability of abundance estimates (see also Rosenberger and Dunham 2005). Baseline snorkel counts and unbiased Petersen capture-recapture estimates, both independently and in conjunction, supported Smallmouth Bass abundance estimates derived from the multinomial capture-recapture model. Petersen capture-recapture confidence intervals were consistently in general agreement with the empirical Bayes estimates, thus supporting the Smallmouth Bass abundance estimates at sites where I was able to make the comparison. The snorkel surveys provided a coarse comparison for the empirical Bayes estimates at sites where unbiased Petersen capture-recapture estimates were not obtainable. For example, the Smallmouth Bass snorkel count at Caney Creek supported a low abundance rather than suggesting a detection probability lower than what was estimated by the multinomial capture-recapture model. Whereas at Big Sugar Creek, the Smallmouth Bass snorkel count supported the low detection estimated by the multinomial capture-recapture model rather than low abundance. All three approaches were in general agreement at sites where such a comparison was possible, which provided weighted evidence to support the reliability of the empirical Bayes estimates. The secondary methods supporting the expectation that a multinomial electrofishing removal model would underestimate Smallmouth Bass abundance provided further support of their usefulness to assess the reliability of the multinomial capture-recapture estimates.

Although the simulations supported the use of a multinomial capture-recapture model to survey Smallmouth Bass populations in Ozark Highland streams using tow-barge electrofishing, they also identified scenarios where the models may be inappropriate. Bias in estimated abundance and precision were acceptable for promoting effective monitoring of stream-dwelling Smallmouth Bass under conditions similar to the multinomial capture-recapture estimates and were well within reported estimates of uncertainty (e.g., the precision of the empirical Bayes confidence intervals). Bias and precision were also satisfactory under less optimal conditions (i.e., decreased detection and increased unexplained heterogeneity) realistic to Smallmouth Bass tow-barge electrofishing in south-central streams. For example, an inherent bias of 10-20 Smallmouth Bass and precision of 20-40 Smallmouth Bass is reasonable for stream managers because changes in abundance at these levels would likely not trigger management actions, particularly when considering year-to-year variation in mortality rates and movement between stream reaches. However, these levels of bias and precision may be not desirable for monitoring other stream fishes (e.g., species of conservation concern where identifying even very slight changes in population sizes may be critical). Multinomial capture-recapture model performance was poor under scenarios with much lower detection and much higher unexplained heterogeneity. Reevaluating the sampling method may be a consideration when average detection probability is extremely low (i.e., < 0.10) and approaches that only evaluate species presence-absence (e.g., occupancy modeling) may be more appropriate when low detection is a function of low abundance. The simulations also further highlight the importance of identifying factors (i.e., covariates) that account for variable detection. Multinomial capture-recapture model performance decreased substantially and greatly underestimated abundance with high levels of unexplained heterogeneity in detection (see also Webster et al. 2008 for similar findings using a repeated-count model). Furthermore, model diagnostics to assess fit are essential for reliable detection estimates (here c-hat and residual plots suggested adequate model fit). Overdispersion results in an underestimation of standard error around model coefficients (i.e., higher unexplained heterogeneity in detection than estimated by the model). Thus, multinomial *N*-mixture models may be inappropriate for stream fishes with very low detection, when variation in detection is difficult to explain through covariates, or when subtle changes in populations are critical for effective management and conservation.

One limitation of multinomial *N*-mixture models (common to most population estimation methods; but see Ford et al. 2012), is the inability to account for variation in detection among individuals (Chandler 2015). Veech et al. (2016) showed that when detection probability is < 0.50 nonrandom individual variation (e.g., behavior) may result in erroneous abundance estimates using *N*-mixture models. Two primary sources of individual variation in detection for single-species electrofishing are fish size and a “trap response” (i.e., variation in detection between marked and unmarked individuals during the recapture event). My results support that neither fish size nor a trap response influenced estimated Smallmouth Bass detection. Mean Smallmouth Bass TL did not vary considerably among sites and the inclusion of a fish size covariate did not improve model fit. Additionally, the mean and variation of Smallmouth Bass TL at my sites were consistent with other studies in the Ozark Highlands ecoregion (e.g., Brewer and Long 2015), suggesting that the results are applicable at sites within the study area that were not surveyed. I also found no evidence of considerable remaining variation in detection between capture and recapture events, which precludes a trap response. If a trap response is suspected, the influence on detection can be addressed by modifying the capture-recapture function in “unmarked” by introducing a behavioral covariate to the capture histories (see Chandler 2015).

I demonstrated how a contemporary population estimation method can be a viable alternative to CPUE but while using similar sampling methods. I argue that the long-term benefits of hierarchical population estimation methods greatly outweigh the additional effort and learning curve. Although standardized sampling is an important aspect of sound fisheries research and management (Bonar et al. 2009), attempting to maintain equal stream-fish detection across space and time by replicating environmental conditions is often an unrealistic expectation that constrains progressive ecology and management. Additionally, I concur with Gwinn et al. (2016) that examining relationships between stream fishes and the environment (e.g., seasonal changes in populations and flow-ecology relationships) is fundamental to advancing science and requires surveying across a broad range of conditions. Flexible contemporary approaches, such as multinomial *N*-mixture models, that account for variable detection and promote both strategic and flexible monitoring protocols are readily available to stream-fish scientists.

Table 1. Results from 12 candidate multinomial capture-recapture models fitted with a negative-binomial error distribution to estimate site-specific detection abundance and detection probability for Smallmouth Bass using tow-barge electrofishing in 25 stream reaches of the Ozarks Highlands in northeast Oklahoma and southwest Missouri from July to October 2014-2015. In the model set, λ is latent abundance, p is the estimated detection probability, effort is electrofishing effort, width is average wetted width., Q is discharge, depth is average thalweg depth, clarity is horizontal water clarity and θ is the overdispersion parameter. K is the number of the parameters in each model, AICc is the Akaike information criterion score for the model corrected for small sample size, and w_i is the relative support for the model.

Model	K	Log-likelihood	AICc	Δ AICc	w_i
λ, p(effort + width * depth + clarity), θ	8	3604.54	-7184.09	0.00	0.95
λ, p(effort + width + depth + clarity), θ	7	3598.67	-7176.75	7.34	0.02
λ, p(effort + width * depth), θ	7	3598.34	-7176.09	8.00	0.02
λ, p(effort + width + depth), θ	6	3595.29	-7173.91	10.18	0.01
λ, p(effort + depth), θ	5	3591.39	-7169.61	14.48	0.00
λ, p(effort + width * Q + clarity), θ	8	3594.18	-7163.36	20.73	0.00
λ, p(effort + width + Q + clarity), θ	7	3591.58	-7162.58	21.51	0.00
λ, p(effort + width + Q), θ	6	3587.67	-7158.68	25.41	0.00
λ, p(effort + width + Q), θ	7	3588.97	-7157.35	25.41	0.00
λ, p(effort + Q), θ	5	3883.79	-7154.42	29.67	0.00
λ, p(effort + width), θ	5	3572.88	-7132.61	51.48	0.00
λ, p(effort), θ	4	3562.54	-7115.08	69.01	0.00

Table 2. Results of simulations to assess bias, root mean square error (RMSE), and coefficient of variation (CV) of RMSE fo a capture-recapture multinomial *N*-mixture models under 18 different scenarios (1000 runs per scenario) with varying levels of number of sites, mean abundance, detection probability, and standard deviation (SD) of detection probability. CV of RMSE was calculated as (RMSE/mean estimated abundance)*100. Mean abundance SD was 20 for all simulations.

Test	Number of sites	Mean abundance	Detection probability	Detection probability SD	Bias in estimated abundance	RMSE	CV of RMSE
1	25	135	0.25	0.05	-4.6	24.7	19%
2	25	85	0.25	0.05	-3.1	17.7	22%
3	25	35	0.25	0.05	-1.0	10.3	30%
4	25	185	0.25	0.25	-6.3	32.4	18%
5	25	135	0.20	0.05	-6.8	30.7	24%
6	25	135	0.15	0.05	-11.7	39.6	32%
7	25	135	0.05	0.05	-59.7	96.1	126%
8	25	135	0.25	0.10	-17.5	43.8	37%
9	25	135	0.25	0.15	-33.8	61.7	61%
10	25	135	0.25	0.25	-65.9	89.3	130%
11	25	135	0.20	0.10	-25.3	54.4	50%
12	25	135	0.15	0.10	-39.1	69.6	72%
13	25	135	0.05	0.20	-124.1	132.3	1256%
14	50	135	0.25	0.05	-4.8	24.8	19%
15	50	135	0.05	0.05	-63.3	93.5	131%
16	50	135	0.25	0.25	-67.0	90.0	133%
17	50	135	0.20	0.10	-26.4	55.1	51%
18	50	135	0.05	0.20	-126.4	132.4	1553%

Table 3. Summary of snorkel surveys and capture-recapture estimates for 25 Smallmouth Bass subpopulations in streams of the Ozarks Highlands, where *M* is the total number of fish captured during the capture event, *C* is the total number of fish captured during the recapture event (both marked and unmarked), recap is the proportion of marked fish recaptured, and cumulative detection is the estimated proportion of Smallmouth Bass captured across both capture and recapture events and was calculated as the sum of the multinomial cell probabilities. Cumulative detection, multinomial abundance estimates, and multinomial 95% confidence intervals (CI) were derived from a multinomial negative-binomial-mixture model. Petersen abundance estimates were calculated using Peterson capture-recapture with the Chapman (1954) bias correction. Petersen 95% CI were calculated with a bias-corrected SE (Seber 1970) as $\hat{N} \pm z_{\alpha/2}(\text{SE})$. NA’s for snorkel count indicate sites that were not surveyed due to insufficient water clarity. NA’s for Petersen abundance estimates and Petersen 95% CI indicate sites where assumptions for unbiased estimates were not met as described by Lyons and Kanehl (1993).

Site	*M*	*C*	Recap	Cumulative detection	Snorkel count	Multinomial abundance estimate	Petersen abundance estimate	Multinomial 95% CI	Petersen 95% CI
Baron Fork1	71	37	0.13	0.46	NA	214	299	185 - 246	147 - 451
Baron Fork2	16	11	0.38	0.54	NA	41	NA	30 - 53	NA
Big Sugar Creek	24	11	0.08	0.23	138	144	NA	105 - 188	NA
Buffalo Creek1	79	96	0.47	0.75	153	183	204	169 - 199	167 - 241
Buffalo Creek2	11	17	0.09	0.32	85	86	NA	62 - 114	NA
Buffalo Creek3	15	17	0.07	0.35	59	91	NA	68 - 118	NA
Butler Creek	64	60	0.36	0.43	244	232	164	200 - 267	123 - 204
Caney Creek	8	6	0.13	0.45	18	31	NA	21 - 45	NA
Evansville Creek	70	44	0.36	0.52	NA	170	128	147 - 195	101 - 155
Five-mile Creek	15	13	0.20	0.50	61	52	NA	39 - 67	NA

Flint Creek1	14	21	0.00	0.33	NA	107	NA	81 - 137	NA
Flint Creek2	28	45	0.18	0.30	208	224	NA	183 - 270	NA
14-mile Creek1	32	40	0.25	0.40	NA	159	150	131 - 190	75 - 224
14-mile Creek2	36	25	0.14	0.51	NA	109	162	91 - 130	57 - 268
14-mile Creek3	120	97	0.57	0.84	NA	176	173	166 - 188	157 - 188
Honey Creek1	59	50	0.42	0.62	NA	135	117	118 - 153	93 - 141
Honey Creek2	12	12	0.17	0.30	NA	75	NA	52 - 103	NA
Indian Creek	14	19	0.21	0.31	NA	97	NA	72 - 128	NA
Lost Creek	9	4	0.22	0.62	NA	19	NA	13 - 27	NA
Saline Creek	48	29	0.04	0.40	156	186	NA	156 - 220	NA
Spavinaw Creek1	63	59	0.13	0.40	167	282	NA	244 - 323	NA
Spavinaw Creek2	41	32	0.34	0.44	NA	133	92	110 - 160	64 - 120
Spring Creek1	29	39	0.31	0.33	247	176	120	142 - 214	66 - 174
Spring Creek2	85	84	0.40	0.62	225	215	207	194 - 238	167 - 248
Spring Creek3	17	18	0.47	0.40	84	69	NA	51 - 90	NA

Table 4. Coefficients from a multinomial negative-binomial-mixture capture-recapture model to estimate the abundance of 25 Smallmouth Bass subpopulations in streams of the Ozarks Highlands surveyed using tow-barge electrofishing. The model was chosen using AICc from a set of 12 candidate models that incorporated the detection covariates discharge, mean wetted width, mean depth, water clarity, and electrofishing effort. The model had a high level of support (AICc weight = 0.95; Supplemental Table S1). Detection covariates were reported on a logit scale and were standardized such that the intercept estimates detection at mean values and the coefficients represent a unit change of one SD. The 95% confidence intervals (CI) were calculated using a profile likelihood method (see Fiske and Chandler 2011).

Parameter	Estimate ± SE	Lower 95% CI	Upper 95% CI
Latent abundance	136 ± 17.6	4.66	5.18
Detection - intercept	-1.08 ± 0.08	-1.24	-0.92
Detection - water clarity	0.24 ± 0.07	0.11	0.36
Detection – electrofishing effort	0.28 ± 0.06	0.17	0.39
Detection – mean wetted width	-0.13 ± 0.09	-0.30	0.04
Detection – mean depth	-0.39 ± 0.07	-0.52	-0.25
Detection – width x depth	0.21 ± 0.06	0.09	0.33
Overdispersion	3.01 ± 0.88	0.49	1.65

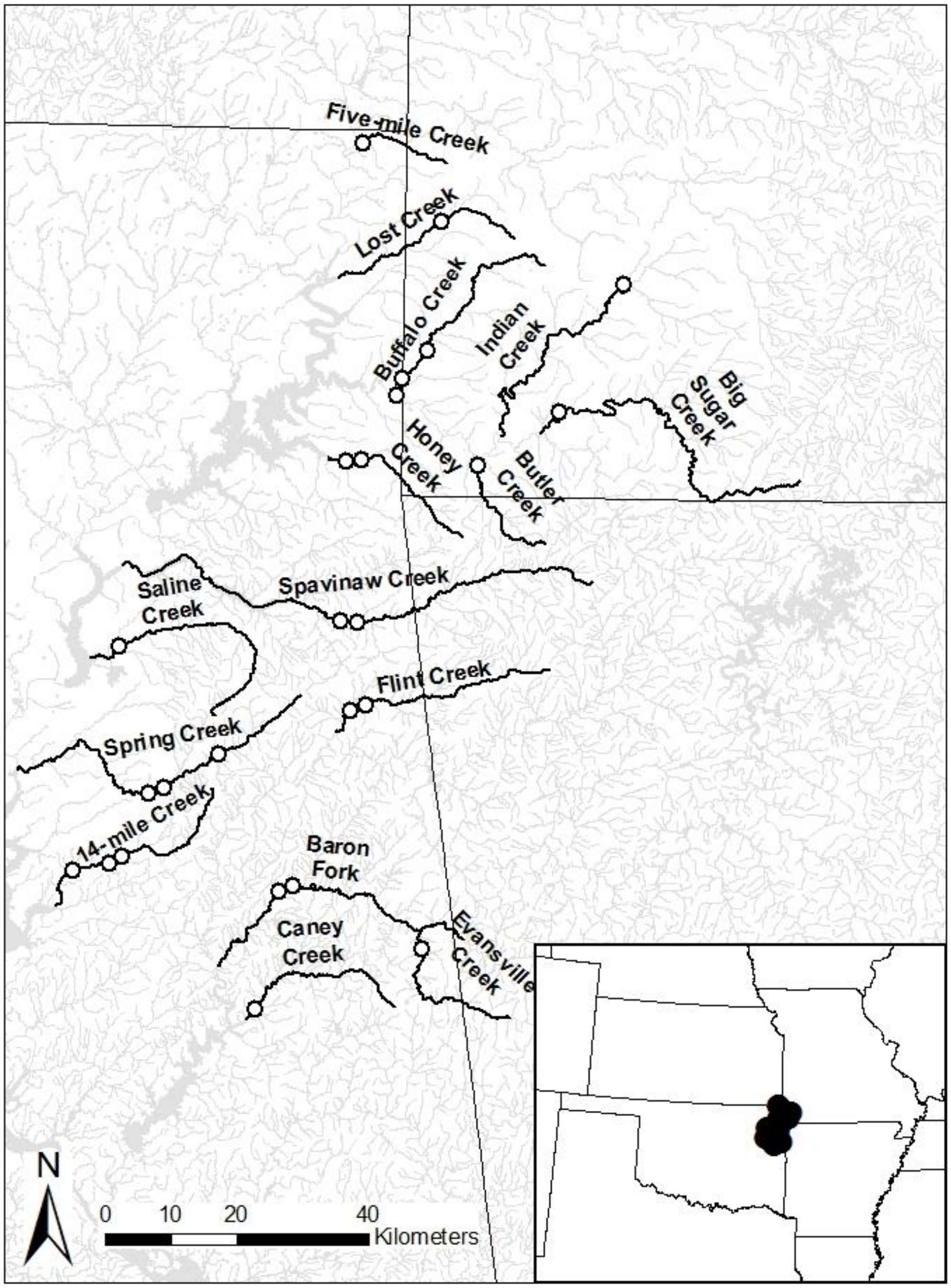

Figure 1. Location of 25 stream reaches of the Ozarks Highlands in northeast Oklahoma and southwest Missouri where spatially-distinct Smallmouth Bass subpopulations were surveyed using tow-barge electrofishing and snorkeling from July to October 2014-2015.

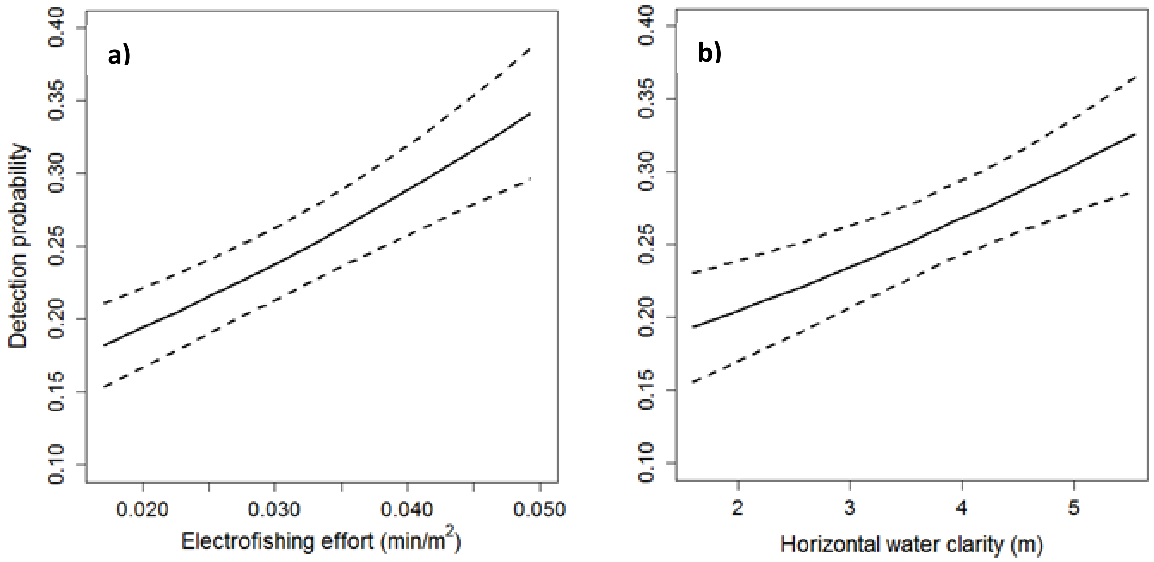

Figure 2. The relationship between Smallmouth Bass detection and increasing electrofishing effort (a) and water clarity (b) in Ozarks Highland streams. Estimates of detection probability were derived from a multinomial negative-binomial capture-recapture model with mean wetted channel width and mean depth held at mean sampling levels. Dashed lines indicate 95% confidence intervals.

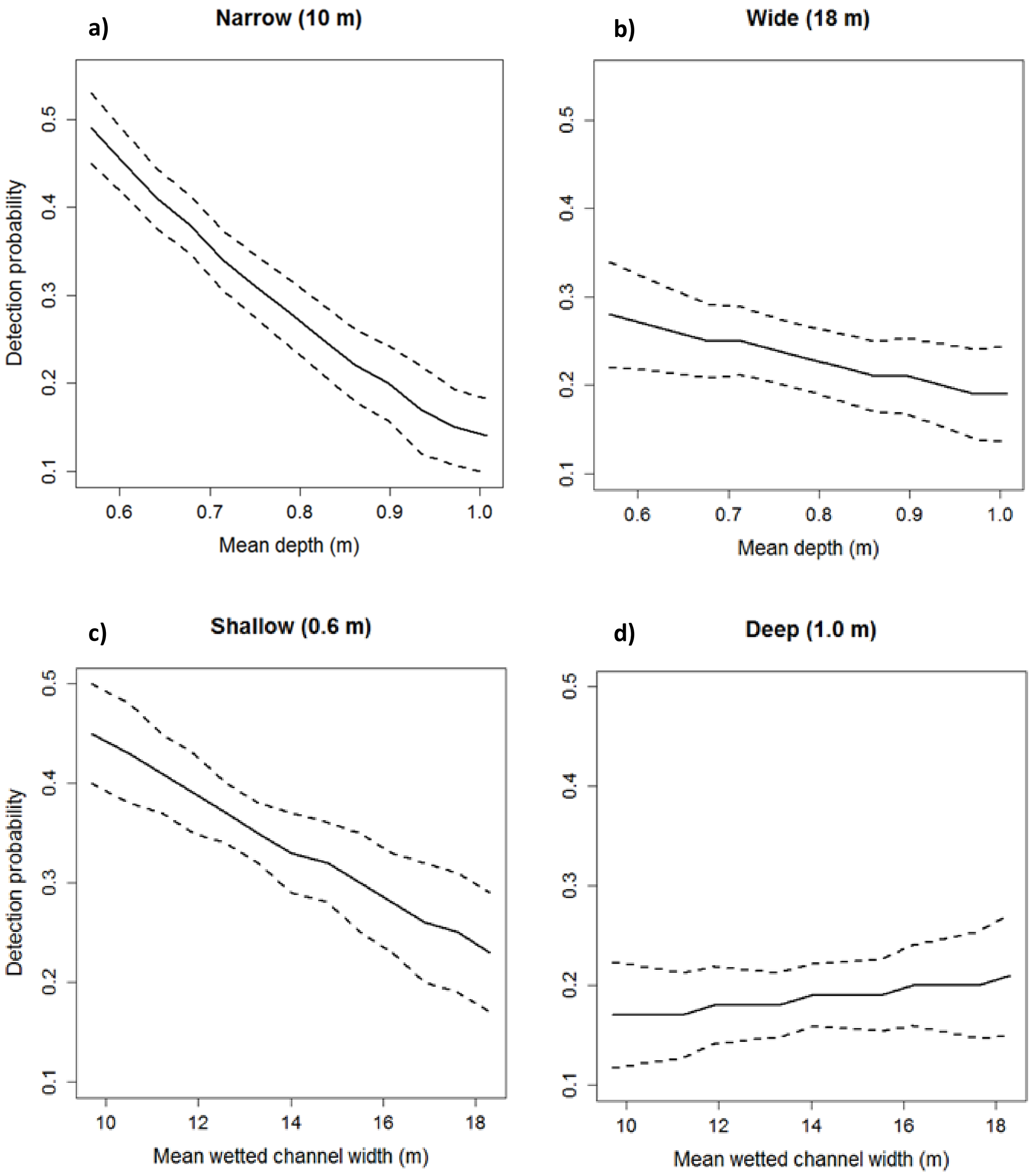

Figure 3. Interaction of mean wetted channel width and mean depth on Smallmouth Bass electrofishing detection probability in Ozark Highland streams. Estimates of detection probability were derived from a multinomial negative-binomial capture-recapture model with electrofishing effort and water clarity held at mean sampling levels. Narrow and wide represent values of mean wetted width of -1 and +1 SD, respectively Shallow and deep represent values of mean depth of -1 and +1 SD, respectively. Dashed lines indicate 95% confidence intervals.

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APPENDICES

Appendix 1. Summary and comparison of Smallmouth Bass tow-barge electrofishing abundance estimates derived from a multinomial negative-binomial-mixture removal model for 25 subpopulations in streams of the Ozarks Highlands, where pass 1 is the number of fish captured on removal pass 1 and pass 2 is the number of fish captured on removal pass 2 ,and cumulative detection is the estimated proportion of Smallmouth Bass captured across both capture and recapture events and was calculated as the sum of the multinomial cell probabilities. Petersen abundance estimates were calculated using Peterson capture-recapture with the Chapman (1954) bias correction. Petersen 95% CI were calculated with a bias-corrected SE (Seber 1970) as $\hat{N} \pm z\alpha/2(\text{SE})$. NA's for Petersen abundance estimates and Petersen 95% CI indicate sites where assumptions for unbiased estimates were not met as described by Lyons and Kanehl (1993).

Site	Pass 1	Pass 2	Removal cumulative detection	Capture-recapture cumulative detection	Snorkel count	Removal 95% CI	Petersen 95% CI
Baron Fork4	28	9	0.77	0.46	NA	41 - 56	147 - 451
Baron Fork5	13	5	0.85	0.54	NA	18 - 26	NA
Big Sugar Creek2	17	11	0.25	0.23	138	76 - 141	NA
Buffalo Creek5	79	18	0.90	0.75	153	102 - 115	167 - 241
Buffalo Creek6	9	1	0.50	0.32	85	14 - 32	NA
Buffalo Creek7	7	8	0.28	0.35	59	34 - 77	NA
Butler Creek2	43	32	0.65	0.43	244	101 - 131	123 - 204
Caney Creek2	4	4	0.69	0.45	18	9 - 16	NA
Evansville Creek3	28	16	0.69	0.52	NA	54 - 75	101 - 155
Five-mile Creek2	6	6	0.78	0.50	61	12 - 21	NA

Flint Creek3	12	9	0.55	0.33	NA	29 - 51	NA
Flint Creek4	23	22	0.55	0.30	208	67 - 98	NA
14-mile Creek4	26	14	0.69	0.40	NA	49 - 69	75 - 224
14-mile Creek5	29	8	0.78	0.51	NA	41 - 56	57 - 268
14-mile Creek6	78	19	0.95	0.84	NA	98 - 106	157 - 188
Honey Creek3	30	16	0.81	0.62	NA	50 - 64	93 - 141
Honey Creek4	8	5	0.36	0.30	NA	24 - 55	NA
Indian Creek2	10	4	0.45	0.31	NA	22 - 46	NA
Lost Creek2	7	2	0.81	0.62	NA	9 - 16	NA
Saline Creek3	19	10	0.73	0.40	156	33 - 48	NA
Spavinaw Creek8	30	29	0.55	0.40	167	89 - 125	NA
Spavinaw Creek9	32	10	0.55	0.44	NA	62 - 92	64 - 120
Spring Creek5	23	16	0.50	0.33	247	62 - 95	66 - 174
Spring Creek6	47	38	0.69	0.62	225	109 - 137	167 - 248
Spring Creek7	11	6	0.69	0.40	84	19 - 33	NA

Appendix 2. Results from 12 candidate multinomial removal models fitted with a negative-binomial error distribution to estimate site-specific detection abundance and detection probability for Smallmouth Bass using tow-barge electrofishing in 25 stream reaches of the Ozarks Highlands in northeast Oklahoma and southwest Missouri from July to October 2014-2015. In the model set, λ is latent abundance, *p* is the estimated detection probability, effort is electrofishing effort, width is average wetted width., Q is discharge, depth is average thalweg depth, clarity is horizontal water clarity and θ is the overdispersion parameter. K is the number of the parameters in each model, AICc is the Akaike information criterion score for the model corrected for small sample size, and w_i is the relative support for the model.

Model	K	Log-likelihood	AICc	ΔAICc	w_i
λ, *p*(width + depth), θ	5	2044.57	-4075.97	0.00	0.51
λ, *p*(width * depth), θ	6	2044.97	-4073.28	2.69	0.13
λ, *p*(width * depth + clarity), θ	6	2044.93	-4073.20	2.77	0.13
λ, *p*(width * Q), θ	6	2044.76	-4072.84	3.13	0.11
λ, *p*(width + Q), θ	5	2042.28	-4071.41	4.56	0.05
λ, *p*(depth), θ	4	2039.89	-4069.78	6.19	0.02
λ, *p*(width + depth + clarity), θ	7	2045.11	-4069.64	6.33	0.02
λ, *p*(width * Q + clarity), θ	7	2044.81	-4069.02	6.95	0.02
λ, *p*(width + Q + clarity), θ	6	2042.56	-4068.46	7.51	0.01
λ, *p*(width), θ	4	2038.38	-4066.76	9.22	0.01
λ, *p*(Q), θ	4	2036.71	-4063.41	12.56	0.00
λ, *p*, θ	3	2028.66	-4050.17	25.80	0.00

Appendix 3. Coefficients from a multinomial negative-binomial-mixture removal model to estimate the abundance of 25 Smallmouth Bass subpopulations in streams of the Ozarks Highlands surveyed using tow-barge electrofishing. The model was chosen using AICc from a set of 12 candidate models that incorporated the detection covariates discharge, mean wetted width, mean depth, water clarity, and electrofishing effort. The model had a high level of support (AICc weight = 0.51; Appendix 1). Detection covariates were reported on a logit scale and were standardized such that the intercept estimates detection at mean values and the coefficients represent a unit change of one SD. The 95% confidence intervals (CI) were calculated using a profile likelihood method (see Fiske and Chandler 2011). The estimate of c-hat for the model from the Chi-squared test (c-hat =1.02) did not indicate overdispersion in the model. A plot of predicted versus fitted residuals (*n* = 50) also suggested adequate model fit (i.e., no evidence of heteroscedasticity).

Parameter	Estimate ± SE	Lower 95% CI	Upper 95% CI
Latent abundance	59 ± 9.2	3.79	4.41
Detection - intercept	-0.36 ± 0.17	-0.70	-0.05
Detection – mean wetted width	-0.48 ± 0.14	-0.79	-0.17
Detection – mean depth	-0.47 ± 0.16	-0.75	-0.21
Overdispersion	2.61 ± 0.76	0.35	1.51

CHAPTER VI

MULTISCALE FACTORS INFLUENCING THE DISTRIBUTION OF OZARK HIGHLAND STREAM FISHES

ABSTRACT

Stream-fish assemblages are structured by complex, multiscale abiotic processes, which makes identifying relationships with environmental and spatial factors challenging. The intermediate scale (i.e., stream reaches and stream segments) is of particular importance to both research and management because it largely reflects the life-history characteristics of stream fishes. Furthermore, management actions at the intermediate scale are more feasible than at coarser scales. I used a hierarchical modeling approach to examine multiscale environmental and spatial factors associated with the distribution of Ozark Highland stream fishes. I collected density and occurrence data for Ozark Highland stream fishes using tow-barge electrofishing, where densities were adjusted for variable detection. Variation in sunfish densities were associated with both reach-scale groundwater contribution and segment-level lithology. Variation in stream-fish occurrence was associated with the area of riffle-run-pool sequences and reach-scale substrate size, groundwater contribution, residual pool depth. The modeled relationship between sunfish densities and relative groundwater contribution explained notable observed trends for Longear Sunfish *Lepomis megalotis* and Rock Bass *Ambloplites rupestris*. My results also suggest that variation in sunfish densities associated with underlying lithology, rather than land use or spatial factors. The occurrence of Redspot Chub *Nocomis asper*, a stream-fish species

of conservation concern, was associated with higher reach-scale groundwater contribution, which supports their perceived reliance on spring flow. Intermediate-scale environmental factors were also associated with the occurrence of Northern Studfish *Fundulus catenatus* and Smallmouth Bass *Micropterus dolomieu*. My findings demonstrate both the importance of using appropriate analytical approaches to identify multiscale relationships between stream-fish distributions and environmental variables and how different stream-fish environmental relationships may emerge when viewed from different scales. In addition to promoting advances in stream-fish ecology, a multiscale perspective, with an emphasis on intermediate scales, is essential to successful long-term stream-fish conservation and management.

INTRODUCTION

Stream fishes are hypothesized to form structured, non-random assemblages (Jackson et al. 2001); however, relationships among species with environmental and spatial factors remain poorly understood (Fausch 2010; Matthews 2010; Winemiller 2010). Our limited understanding of stream fish-environment relationships is largely due to the inherent challenges of studying dynamic, hierarchically-structured stream systems, where observed assemblages across the landscape are a result of complex, multiscale abiotic processes (Jackson et al. 2001; Fausch et al. 2002; Lowe et al. 2006; see also Chapter 1). For example, lithology and climate are hypothesized to be primary determinants of biotic assemblages in streams (Hynes 1975; Frissell et al. 1986), but local environmental characteristics (e.g., channel formation and instream cover) also contribute to variation in species abundance and occurrence (Frissell et al. 1986; Stevenson 1997). Stream-fish assemblages are also a reflection of complex spatial factors. The structure of biotic assemblages is theorized to change in a predictable fashion along a downstream continuum (Vannote et al. 1980); however, heterogeneity in local habitat and spatial position in the stream network (e.g., distance to tributaries) can lead to variation within downstream trends (i.e., variability around a common theme; Osborne and Wiley 1992; Fausch et al. 2002). Land use (the human element) confounds species-environment relationships by interacting with variables across multiple scales (Allan et al. 1997; Stevenson 1997; Allan 2004). Anthropogenic activities also alter the spatial dynamics of the stream landscape. For example, impoundments disrupt the downstream continuum and obstruct connectivity within the stream network, which can alter both the nature of biotic assemblages (Guenther and Spacie 2006; Falke and Gido 2006) and population dynamics (e.g., local colonization and extinction; Pringle 1997; Pringle 2003).

Each length of stream has unique geomorphic characteristics and channel patterns (e.g., channel dimensions, substrate, and channel unit diversity; Leopold 1994) that result in highly diverse instream environments at intermediate scales (e.g., stream reaches and segments). Life-history characteristics of stream fishes are largely a reflection of these intermediate-scale environmental factors (Fausch et al. 2002). However, stream-fish research at intermediate scales is generally lacking as studies have tended to focus on either very coarse scales (e.g., ecoregions and watersheds) or very fine scales (e.g., channel units and microhabitats; Fausch et al. 2002; Falke and Fausch 2010). Fausch et al. (2002) provide a detailed discussion on the importance of examining species-environment relationships at intermediate scales to promote advancements in stream-fish ecology and their assertions are supported by empirical studies. For example, Brewer et al. (2007) found significant relationships between Smallmouth Bass *Micropterus dolomieu* populations and segment-scale soil characteristics. Brassy Minnow *Hybognathus hankinsoni* occurrence was associated with pool characteristics at intermediate scales (Scheurer et al. 2003). Walters et al. (2003) determined that reach-scale geomorphology was the primary factor associated with patterns in stream-fish composition. Flow dynamics at intermediate scales can also influence stream-fish distributions. Differential declines among salmonids have been associated with the timing and magnitude of flows at the segment scale (Wenger et al. 2010). Peterson and Shea (2014) found that the duration of reach-scale low-flow events explained variation in metademographic rates among an assemblage of stream fishes. Additionally, groundwater has profound effects on the instream environment including stabilizing water temperature, providing areas of thermal refuge, increasing productivity, and influencing riparian characteristics (Stanford and Ward 1993). Groundwater contribution can influence both the occurrence and abundance of stream fishes (Power et al. 1999) and reach-scale variation in groundwater contribution has been associated with patterns in assemblages (Wang et al. 2003).

Understanding species-environment relationships at intermediate scales is also essential for effective stream-fish management and conservation. The influence of reach- and segment-scale characteristics on the life history and ecology of stream fishes constrains management efforts at finer scales. For example, instream habitat enhancements (e.g., the addition of large wood) at the channel unit- or microhabitat-scale would be unlikely to improve the population status of target stream fishes if coarser-scale environmental factors (e.g., available pool habitat across a stream reach) were not adequate. Additionally, managing the stream environment at intermediate scales is more feasible than at coarser scales. Channel unit diversity, geomorphology, land use, and flow regime can be manipulated at the reach- and segment- scale, whereas factors at the watershed-scale (although important considerations for effective long-term stream-fish management) are difficult (e.g., land use) or impossible (e.g., lithology) to address.

My primary objectives were to use a hierarchical modeling approach to identify factors at multiple spatial scales that explained (1) variation in sunfish densities, where stream reaches (defined as multiple riffle-run-pool sequences) were nested within stream segments (defined as a length of stream between 3rd order and higher tributary confluences) and (2) the occurrence of characteristic Ozark stream fishes, where riffle-run-pool sequences (hereafter referred to as channel unit sequences), were nested within stream reaches. I also examined sunfish trait-environment relationships using variables that were associated with variation in densities (see Chapter 1). I developed parsimonious models using a comprehensive set of environmental and spatial variables hypothesized to structure stream-fish assemblages.

METHODS

Stream-fish assemblage - Characteristic stream fishes of the Ozark Highlands ecoregion include multiple species of black basses (*Micropterus*), sunfishes (e.g., *Lepomis, Ambloplites,* and *Pomoxis*), suckers (Catostomidae), large-bodied cyprinids (e.g., Creek Chub *Semotilus atromaculatus* and Redspot Chub *Nocomis asper),* and sculpins (*Cottus*). Redspot Chub and redhorses (*Moxostoma* spp.) are among stream fishes of conservation concern in Oklahoma Ozark Highland streams (ODWC 2005). Redspot Chub are also a protected species in Kansas streams (KDWPT 2016). In addition to black basses (particularly Smallmouth Bass *Micropterus dolomieu*), many sunfishes are popular recreational fishes. Sunfishes also play important functional roles in stream ecosystems as both top-level predators and prey. Longear Sunfish *Lepomis megalotis*, Green Sunfish *Lepomis cyanellus* Bluegill *Lepomis macrochirus*, and Rock Bass *Ambloplites rupestris* are common to Ozark Highland streams with varying degrees of abundance (Pflieger 1997; Miller and Robison 2004). Redear Sunfish *Lepomis microlophus* and Warmouth *Lepomis gulosus* are less common sunfishes in Ozark Highland streams, but are locally abundant. Despite their recreational and ecological importance, few studies have examined factors related to variation in abundance among stream-dwelling sunfishes (but see Rabeni and Jacobson 1993; Peterson and Rabeni 2001), particularly across multiple spatial scales.

I estimated stream fish-density and occurrence with tow-barge electrofishing using methods described in Chapter 3. I sampled six common Ozark Highland sunfishes at 40 stream reaches nested within 20 streams segments. (Table 1; Appendix 1, Appendix 2, and Appendix 3). Sampling coincided with streams surveyed in Chapter 3 and Chapter 4 in 2014-2015 (see Figure 1 of Chapter 3). Catch data were adjusted using the electrofishing detection model developed in Chapter 3 as $\hat{N} = c / \hat{q}$, where $\hat{N}$ is the species- and site-specific estimated abundance, $\hat{q}$ is the species- and site-specific estimated detection, and c is the species- and site-specific number of individuals captured. (Thompson and Seber 1994; Peterson and Paukert 2009). I converted the sunfish abundances to site-specific densities as abundance divided by reach length (fish/m) because the length of stream reaches was variable. I used a removal design to establish stream-fish occurrence at 80 channel unit sequences nested within 22 stream reaches in 2015 (Table 2; Appendix 2). Stream fishes were considered present in a channel unit sequence once encountered and absent if not encountered during four electrofishing passes. A removal design was necessary due to a dependency among electrofishing passes (e.g., multiple individuals of a species were often shocked during a pass and the time between passes did not allow for system recovery), which limited information about detection among stream fishes and channel unit sequences. Subadults and adults were considered separately for Smallmouth Bass and Largemouth Bass *Micropterus salmoides*. I used 250-mm TL as the cutoff between subadults and adults for both Smallmouth Bass and Largemouth Bass, which is consistent with both age-3 Smallmouth Bass in Ozark Highland streams (Brewer and Long 2015) and the minimum size for mature Largemouth Bass (Claassen 2015). I did not consider subadults and adults separately for Spotted Bass *Micropterus punctulatus* because their life history is poorly understood (Churchill and Bettoli 2015) and individuals large enough likely to have been adults were only encountered at four channel unit sequences. Individuals < 80 mm for all black basses were considered age 0 and not included in the study because they are not readily recruited to electrofishing (see Chapter 3 methods). Redhorses were considered only at the genus level because accurate species identification is difficult in the field. I did not consider Redspot Chub for channel unit sequences surveyed in Turnback Creek and Little Sac River because these streams are located outside of their native range (Pflieger 1997).

Sunfish traits. - I used sunfish morphological and life-history characteristics to examine trait-environment relationships (Table 1). Each sunfish species was assigned to a morphological group (trait group A and B) based on groups developed in Chapter 2. Longevity and age at maturation were acquired from “Fishes of Texas” (http://www.fishesoftexas.org/home/) to promote the regional accuracy of sunfish trait information.

Environmental and spatial variables- I characterized instream environmental variation among channel unit sequences. Wetted channel width (1.0 m) and thalweg depth (0.1 m) were measured at 50-m transects unless the channel unit sequence was < 150 m (allowing for ± 5 m GPS error). For shorter channel unit sequences, I measured wetted channel width and thalweg depth near the upstream end of the run, near deepest area of the pool, and near the downstream end the pool. Wetted channel width and thalweg depth measurements were averaged for each channel unit sequence, where I also used the mean values to calculate a wetted width-depth ratio. GPS coordinates were recorded at both the upstream and downstream end of each channel unit sequence to estimate length (1.0 m). The area of each channel unit sequence (1.0 m^2) was estimated as length multiplied by mean wetted channel width. I also measured the length of each riffle (1.0 m) to calculate the proportion of riffle habitat as riffle length divided by channel unit sequence area. The length (1.0 m) and width (1.0 m) of each patch of instream large wood and emergent vegetation were estimated to calculate area (1.0 m^2). I calculated proportion of instream large wood, emergent vegetation, and total cover (i.e., instream large wood plus emergent vegetation) as the area of each divided by channel unit sequence area.

I characterized spatial factors for each stream reach and stream segment. I used distance to the nearest downstream impoundment to characterize the spatial location of each stream reach. Using ArcMap (version 10.2.1, ESRI, Red Lands, California), locations of stream reaches were georeferenced to 1:100,000 National Hydrography Dataset (NHD) flowlines (http://nhd.usgs.gov/data.html). Locations of impoundments were acquired from the National Anthropogenic Barrier Dataset (https://www.sciencebase.gov/catalog/item/56a7f9dce4b0b28f1184dabd/), the Oklahoma Dams Inventory (http://www.owrb.ok.gov/hazard/dam/dams.php), or the Missouri Dams Inventory (https://www.gisinventory.net/GISI-26268-MO-2014-Dams-SHP-Dam-Inventory.html). The stream distance from the upstream end of the stream reach to the upstream edge of the nearest downstream impoundment (1.0 km) was calculated manually in ArcMap. Downstream link magnitude (D-link; Osborne and Wiley 1992), which incorporates both stream size and the size of the next downstream confluence to provide a measure of spatial location within the stream network, was calculated for each stream segment using ArcMap spatial analyst tools. Connectivity was a binary variable, where I assigned a one to a stream segment if a higher order downstream tributary occurred before an impoundment based on NHD flowlines. Because my sites were distributed from the southern boundary of the Ozark Highlands ecoregion to locations considerably further northeast, I also estimated the distance of the downstream end of each stream segment from the ecoregion centroid to account for variability in stream-fish assemblages due to spatial position relative to the ecoregion interior. Using ArcMap, the Ozark Highlands ecoregion centroid was determined based on Level III polygon geometry (https://archive.epa.gov/wed/ecoregions/web/html/level_iii_iv-2.html) and the Euclidean distance to the centroid (1.0 km) was measured manually from the downstream end of each stream segment.

I also characterized instream environmental variation among stream reaches. I calculated D50 from pebble counts to characterize substrate size. (Gordon et al. 2004). Fifty “rocks” were collected haphazardly from three transitional zones at each stream reach (e.g., runs and downstream areas of pools). I measured the intermediate diameter (1.0 m) of each “rock” except for sand and silt, which were distinguished using texture. I used 0.25 mm to represent sand and 0.05 mm to represent silt, which is consistent with average grain size on the Wentworth scale. I measured residual pool depth (a measure of pool depth independent of discharge) to characterize pool habitat. Residual pool depth was calculated for three pools at each stream reach as the difference in depth between the deepest point of a pool and the crest of the downstream riffle (0.1

m; Lisle 1987), where pools were chosen haphazardly at stream reaches with > 3 channel unit sequences. I calculated a bankfull width-depth ratio (Gordon et al. 2004) to characterize channel formation. In three transitional zones of each stream reach, I established bankfull using bank slope (i.e., a flat area adjacent to an abrupt slope towards the floodplain), point bars, deposition of fine sediment, and exposed root masses in undercut banks as indicators. I measured bankfull width (1.0 m) and bankfull depth (0.01 m), where depth was measured at 5.0-m intervals along a transect. I used the average D50, residual pool depth, and bankfull width-depth ratio to represent each stream reach. I calculated the proportion of riffle habitat. GPS coordinates were recorded at both the upstream and downstream ends of the reach to estimate length (1.0 m) and proportion of riffle habitat was estimated as the summed length of riffles divided by reach length. The length (1.0 m) and width (1.0 m) of each patch of instream large wood and emergent vegetation were estimated and proportional coverage was calculated in relation to reach area, where reach area was calculated as length multiplied by mean wetted channel width (see Chapter 3 methods). I used water temperature variation as a surrogate for relative groundwater contribution. Two water temperature loggers (HOBO ProV2, Onset, Bourne, Massachusetts) were deployed at stream reaches in a pool area ~ 1.0-m deep for ~4 weeks. When stream reaches were located within 0.5 stream kilometers, the same loggers were used to characterize each reach. I used the mean water temperature and mean standard deviation between the two loggers to calculate a coefficient of variation (CV). The time period water temperature was recorded varied among reaches, where ~70% of the loggers were active during January-February and the remaining loggers were active during April-May. I scaled the CV for each set of stream reaches by subtracting the mean CV and dividing by the standard deviation to improve comparability, where the resulting z-scores were used to represent relative groundwater contribution.

Lastly, I characterized variation in environmental characteristics among stream segments. The sinuosity of each stream segment was calculated manually in ArcMap as stream length (1.0

m) divided by valley length (1.0 m). GIS layers were obtained for rock fragment type (http://www.soilinfo.psu.edu/), geology (https://gdg.sc.egov.usda.gov/), and land use (http://www.mrlc.gov/nlcd2011.php). I used the percentage of agriculture and development, the dominant rock fragment type, and the dominant geology type within a 500-m buffer to characterize each stream segment. Geosoil categories for the stream segments (see also Chapter 3 methods) were developed based on rock fragment type and geology type as cherty limestone, cherty alluvium, cherty dolostone, stony alluvium, and shale. I calculated two-year flood magnitude to characterize the flow regime among stream segments. For ungaged stream segments, I predicted two-year flood magnitude based on the relationship between upstream drainage area and two-year flood magnitude at gaged hydrologically-similar segments with USGS gages. I defined a hydrologically-similar stream segment as being within both the "boundaries" of my study area and the range of drainage areas. Two-year flood magnitude for gaged stream segments was estimated with the Bulletin 17B method (IACWD 1982) using the online program PeakFQ (http://water.usgs.gov/software/PeakFQ/). Drainage area estimates for gaged and ungaged stream segments were obtained from USGS records and the NHD, respectively. Drainage area for a subset of the gaged stream segments was calculated using ArcMap to confirm consistency between USGS records and the NHD. I modeled the relationship between drainage area and two-year flood magnitude using ordinary-least-squares linear regression in the statistical software R (version 3.2.2, R Core Development Team, 2014), where the resulting equation was used to predict two-year flood magnitude at ungaged stream segments (Appendix 4). Two-year flood magnitude was log10 transformed to improve linearity and promote homoscedastic variance.

Stream-fish density and occurrence models. – I used a linear mixed model to examine reach- and stream segment-scale factors influencing both variation in sunfish densities and trait-environment relationships. Sunfish density was natural-log transformed due to evidence of heteroscedastic variance. The natural log-transformation of sunfish density is also justifiable ecologically because it diminishes the influence of extremely high densities in the analysis. D50, proportion emergent vegetation, proportion instream large wood, proportion total cover, distance to dam, and two-year flood magnitude were also natural-log transformed due to skewness. Shale was the reference for the geosoil categories. I included both stream reach and stream segment as random effects, where reaches were nested within segments. Because stream-fish occurrence can also vary considerably across temporal scales (Jackson et al. 2001), I incorporated both a year (2014 and 2015) and season random effect to account for temporal variability among the sampling events, where season was defined as late spring (May-June), summer (July-August), and early fall (September-October). Stream fish was also treated as a random effect, where included environmental and spatial variables in the model had species-dependent terms (i.e., both slopes and intercepts varied among species). The trait-environment relationship was modeled as an interaction term (see Jamil et al. 2013). The model can be written as:

$$(1) \qquad (y_{ijk}) = b_0 + \mu_{0i} + \nu_{0j} + \gamma_{0k} + \Omega_{0l} + \Phi_{0m} + z_{ijklm} + (\beta_1 x_{ijklm} + \mu_1 x_{jklm}) + (\beta_n x_{ijklm} + \mu_n x_{jklm}) + (\varepsilon_1 a_{ijklm} + \mu_1 a_{jklm}) + (\varepsilon_n a_{ijklm} + \mu_n a_{jklm}) + \alpha_1 zx_{ijklm} + \alpha_n zx_{ijklm},$$

where y is natural-log transformed sunfish density, b_0 is the grand mean intercept, μ is the random stream-fish intercept, ν is the random stream reach intercept, γ is the random stream segment intercept, Ω is the random season intercept, Φ is the random year intercept, z is a sunfish trait corresponding to observation i at reach j nested in segment k in season l of year m, x is an environmental variable corresponding to observation i at reach j nested in segment k in season l of year m, a is a spatial variable corresponding to observation i at reach j nested in segment k in season l of year m, $\mu_i x_{jklm}$ is the random species by environmental variable slope, $\mu_i a_{jklm}$ is the random species by spatial variable slope, and zx_{ijklm} is the sunfish trait-environment interaction term. Random effects were assumed normally distributed as $N(0,\tau^2)$, where τ is the population variance among levels of a random effect.

I used a generalized linear mixed model (GLMM) with a binomial error distribution to examine channel unit sequence- and reach-scale factors influencing variation in the occurrence of stream fishes, where occurrence was a binary response variable and a one indicated the stream fish was encountered in a channel unit sequence. Water depth, wetted channel width, width-depth ratio, channel unit sequence area and length, D50, distance to dam, and proportion cover, instream large wood, and emergent vegetation (both channel unit sequence and reach) were natural-log transformed due to asymmetry. I included both channel unit sequence and stream reach as random effects. I also incorporated a season random effect to account for temporal variability as described for the sunfish density model (only data collected in 2015 was used so a random year effect was not necessary). Stream fish was again treated as a random effect, where included environmental and spatial variables in the model had stream fish-dependent terms. The model can be written as:

$$\text{(2)} \quad \text{logit}(y_{ijk}) = b_0 + \mu_{0i} + \omega_{0j} + \nu_{0jk} + \Omega_{0l} + (\beta_1 x_{ijkl} + \mu_1 x_{jkl}) + (\beta_n x_{ijkl} + \mu_n x_{jkl}) + (\varepsilon_1 a_{ijkl} + \mu_1 a_{jkl}) + (\varepsilon_n a_{ijkl} + \mu_n a_{jkl}),$$

where y is the probability of stream-fish occurrence, b_0 is the grand mean intercept, μ is the random species intercept, ω is the random channel unit sequence intercept, ν is the random stream reach intercept, Ω is the random season intercept, x is an environmental variable corresponding to observation i at channel unit sequence j nested in reach k in season l, a is a spatial variable corresponding to observation i at channel unit sequence j nested in reach k in season l, $\mu_i x_{jkl}$ is the random species by environmental variable slope, $\mu_i a_{jklm}$ is the random species by spatial variable slope. Random effects were assumed normally distributed as $N(0,\tau^2)$, where τ is the population variance among levels of a random effect.

I used a tiered forward model selection similar to Jamil et al. (2013) for both the sunfish density model and stream-fish occurrence model. A forward selection is ideal for mixed models when many variables are hypothesized to be important because it results in a parsimonious solution and overcomes convergence issues with the most complex models using a backward selection. I calculated the Pearson's product moment coefficient (*r*) between variables prior to the model-selection process to allow levels of correlation to be considered. As variables were added to the models, remaining variables with $|r| \geq 0.28$ (Graham 2003) were eliminated. I was more conservative with levels of correlation between variables than the detection models (see Chapter 3 and Chapter 5 methods) to minimize confounded relationships between variables when interpreting model coefficients. The null models contained only the random intercepts. I considered environmental and spatial variables only as stream fish-dependent random terms during the first tier. In addition to individual variables, I also assessed interactions between percentage of agriculture and development and environmental variables for pairs of variables that were not correlated. The variable or interaction term that most decreased Akaike information criterion (by at least one) adjusted for small sample size (AICc; Burnham and Anderson 2001) was added to models at each step. The first tier of the model selection process stopped when no variables or interaction terms further decreased AICc and I then included fixed effects for selected environmental and spatial variables.

I conducted an additional model selection tier to consider trait-interaction terms using selected environmental variables for the sunfish density model. Longevity and age at maturation were highly correlated ($r = 0.72$). Thus, I only considered morphology and age at maturation as sunfish traits. An environmental-trait interaction term was only retained in the final model if it decreased AICc by at least one.

I assessed the variation in sunfish density and stream-fish occurrence explained by the models. Using the "MuMIn" package (Bartoń 2016) in the statistical software R, I also calculated conditional R^2 (variation explained by both fixed effects and random factors) for both final models (Nakagawa and Schielzeth 2013; Johnson 2014) and compared them to the conditional R^2

for null models to assess how much variation in sunfish density and stream-fish occurrence, respectively, was explained by included spatial and environmental variables. I did not calculate marginal R^2, which assesses the variation explained only by fixed effects because I was only interested in specific relationships among stream fishes rather than general relationships across species. For example, coefficients for fixed effects in the sunfish model explain relationships between sunfish densities and variables regardless of species identity, which was not a study objective. I developed the models using the package "lme4" (Bates et al. 2014) in the statistical software R. All continuous variables were standardized such that each had a mean of zero and a variance of one to improve interpretation of model coefficients and promote model convergence.

RESULTS

Stream-fish assemblage. **-** Sunfish density among stream reaches was highly variable (Table 1). On average, Longear Sunfish had the highest and most variable densities among stream reaches. Warmouth and Redear Sunfish had the lowest densities among stream reaches. Densities were similar for Bluegill, Rock Bass, and Green Sunfish among stream reaches, although Bluegill and Green Sunfish densities were considerably more variable.

The occurrence of stream fishes among channel unit sequences was also considerably variable (Table 2). Redspot Chub, Longear Sunfish, and Green Sunfish were the most common stream fishes (encountered in ~ 90% of the channel unit sequences) and Spotted Sucker *Minytrema melanops* and White Crappie *Pomoxis annularis* were the least common (encountered in ~10% of the channel unit sequences). Both subadult Largemouth Bass and subadult Smallmouth Bass were more common than adults and were encountered in a similar proportion of channel unit sequences (~80%). Adult Smallmouth Bass were more common than adult Largemouth Bass (encountered in ~60% and ~30% of the channel unit sequences, respectively). Banded Sculpin *Cottus carolinae*, Bluegill, Northern Hogsucker *Hypentelium* *nigricans*, Redhorses, and Rock Bass were relatively common (i.e., encountered in > 50 % of the channel unit sequences), whereas Creek Chub, Northern Studfish, *Fundulus catenatus*, Redear Sunfish, Spotted Bass, Warmouth, and White Sucker *Catostomus commersonii* were less common. Orangespotted Sunfish *Lepomis humilis* were not encountered in any channel unit sequence.

Sunfish traits. – Trait characteristics were not considerably variable among sunfishes. Bluegill and Redear Sunfish (trait group A) were more similar in morphology than other sunfishes (Table 1). Age at maturation and longevity among sunfishes ranged from 1-3 y and 3-7 y, respectively. Longear Sunfish and Rock Bass tend to live longer and reproduce at a later age than other sunfishes. Bluegill and Green Sunfish mature at a younger age, whereas Warmouth was the shortest-lived sunfish.

Environmental and spatial variables. Channel unit sequence-, reach-, and segment-scale variables provided considerable environmental and spatial variability to examine relationships with both sunfish densities and stream-fish occurrence. All instream environmental variables used to characterize channel unit sequences were highly variable (Table 3). Values for instream environmental variables and distance to downstream impoundment were similar for all stream reaches (i.e., 2014 and 2015; Table 4; Appendix 2) and for reaches surveyed only in 2015 (Table 5; Appendix 2). Water temperature variation was the most variable environmental characteristic among stream reaches. Stream reaches tended to be located close to impoundments (i.e., ~10-20 km), although some were considerably closer (< 5 km; e.g., Saline Creek1, Spavinaw Creek2, and Caney Creek1) or further (> 50 km; Flint Creek1, Flint Creek2; Appendix 2). The proportion of agriculture and development varied considerably among stream segments, with Greenleaf Creek1 having a particularly low proportion (0.05) and Turnback Creek1, Saline Creek1, Lost Creek1, Honey Creek1, and 14-mile Creek1 having much higher proportions (~0.70; Table 6; Appendix 3).). Stream segments were generally a similar distance from the Ozark Highlands ecoregions centroid (~ 200-250 km); however, Greenleaf Creek1 and Sallisaw Creek1 were located further (> 280 km) than other segments and Little Sac River1 and Turnback Creek1 were considerably closer (~100 km). D-link was a considerably variable spatial characteristic among stream segments and approximately half of the segments (11 of 20) were upstream of a higher order tributary.

Stream-fish density and occurrence models. - I identified one reach-scale environmental characteristic and one segment-scale environmental characteristic associated with variation in sunfish densities; however, the model-selection process did not identify any spatial or trait-environment relationships. Reach-scale water temperature variation and the segment-scale geosoil category were included in the final sunfish density model (Table 7). Estimated densities of Longear Sunfish, Bluegill, and Green Sunfish increased with increasing reach-scale water temperature variation (thus decreasing relative groundwater influence); however, the magnitude of the relationship was much stronger (i.e., greater positive slope) for Longear Sunfish (Table 7; Figure 1). Estimated Rock Bass and Warmouth densities decreased with increasing reach-scale water temperature variation, with the relationship much more pronounced for Rock Bass. There was virtually no relationship between Redear Sunfish densities and reach-scale water temperature variation (Table 7). Relative to shale, increased Longear Sunfish and Green Sunfish densities were associated with stream segments classified as stony alluvium (Figure 2). Higher Bluegill, Redear Sunfish, and Warmouth densities were associated with shale relative to other segment-scale geosoil groups, although the magnitude of the relationship was difficult to interpret for Warmouth due to very low estimated densities in all geosoil groups. Estimated Rock Bass densities increased with cherty alluvium and cherty limestone relative to shale, whereas decreased densities at the segment scale were associated with stony alluvium (Table 7).

My final model explained a moderate amount of total variation in sunfish densities and remaining variation due to temporal factors was minimal. Remaining variation in sunfish densities due to random temporal factors was minimal (remaining variance ± SD: 0.03 ± 0.18 and 0.02 ± 0.14 for season and year, respectively), although sunfish densities tended to be slightly lower at reaches sampled in season one (May and June) and reaches sampled in 2014. Remaining variation in sunfish densities was higher at the stream segment scale than the stream reach scale (remaining variance ± SD: 0.51 ± 0.71 and 0.31 ± 0.56, respectively). Conditional R^2 for the null model and final model was 0.63 and 0.80, respectively. Thus, reach-scale water temperature variation and segment-scale geosoil explained 17% of the variation in sunfish densities among stream reaches.

I identified one channel unit sequence-scale characteristic and three reach-scale environmental characteristics associated with variation in the stream-fish occurrence. Identical to the sunfish density model, the model-selection process identified reach-scale water temperature variation as a factor related to Ozark Highland stream-fish distributions. Channel unit sequence area and both reach-scale D50 and residual pool depth were also included in the final model (Table 8).

The probability of occurrence increased for all stream fishes with increasing channel unit sequence area; however, the magnitude of the relationship varied. For example, Northern Studfish was much more likely to occur in channel unit sequences with greater area, where occurrence probability was much higher (~0.80 with other variables at mean levels; Figure 2). Spotted Sucker and White Crappie were also more likely to occur in channel unit sequences with greater area relative to other stream fishes (Table 8). Conversely, the probability of Redspot Chub occurrence had virtually no relationship with increasing channel unit sequence area (Figure 2).

The relationship between occurrence and reach-scale substrate size varied among stream fishes. For example, the probability of adult Largemouth Bass occurrence increased sharply with increasing substrate size (Figure 3). Subadult Largemouth Bass, Spotted Bass, and Warmouth were more likely to occur in channel unit sequences nested in reaches with larger substrate relative to other stream fishes (Table 8). Conversely, both subadult and adult Smallmouth Bass were less likely to occur as reach-scale substrate size increased, although the magnitude of the relationship was stronger for subadults. Rock Bass was the only sunfish with a negative relationship with larger reach-scale substrate, where occurrence probability decreased sharply with increasing D50 (Table 8; Figure 3). Northern Studfish occurrence probability also decreased considerably in channel unit sequences nested in stream reaches with larger substrate (Table 8). There was virtually no relationship with reach-scale substrate size and occurrence probability for some stream fishes (e.g., Bluegill, redhorses, and Spotted Sucker). The occurrence of Banded Sculpin, a benthic species, was also not associated with reach-scale substrate size (Figure 3).

The occurrence of most stream fishes among channel unit sequences was associated with reach-scale water temperature variation. For example, the occurrence probability of Redspot Chub, a very common species across my sites, decreased sharply with increasing reach-scale water temperature variation (thus decreasing reach-scale relative groundwater contribution; Figure 4). Although the relationship was weaker for Banded Sculpin and Northern Hogsucker, these stream fishes were also less likely to occur in channel unit sequences nested in stream reaches with greater water temperature variation (Table 8). Among black basses, the probability of occurrence for both subadult and adult Smallmouth Bass declined with increasing reach-scale water temperature variation, but increased for Spotted Bass and both subadult and adult Largemouth Bass. The relationship between reach-scale water temperature variation and sunfish occurrence, with the exception of Redear Sunfish, was similar to relationships with densities. Green Sunfish, Longear Sunfish, Bluegill, and Redear Sunfish occurrence was positively associated with greater water temperature variation, whereas Rock Bass and Warmouth showed opposite relationships (Table 8; Figure 4).

The relationship between occurrence among channel unit sequences and reach-scale residual pool depth was not as variable as reach-scale substrate size and water temperature variation, although there were notable relationships among stream fishes. Green Sunfish and Longear Sunfish were more likely to occur in channel unit sequences nested in stream reaches with deeper residual pools (Table 8; Figure 5). Conversely, occurrence probability for Northern Studfish, White Sucker, and Rock Bass was higher in reaches with shallower residual pools. Among black basses, increased occurrence of Spotted Bass and both subadult and adult Largemouth Bass was associated with stream reaches with deeper residual pools, whereas occurrence probability for both subadult and adult Smallmouth Bass decreased with increasing with residual pool depth (Table 8). There was virtually no relationship between reach-scale residual pool depth and occurrence probability for redhorses and White Crappie.

My final model explained a respectable amount of total variation in stream-fish occurrence and remaining variation was somewhat due to temporal factors. There was more remaining temporal variation in stream-fish occurrence (remaining variance among seasons ± SD: 0.37 ± 0.50) than at the channel unit sequence scale and stream reach scale, where overall stream-fish occurrence was lower in season one (May-June). Remaining variation in stream-fish occurrence was higher at the channel unit sequence scale than the stream-reach scale (remaining variance ± SD: 0.25 ± 0.50 and 0.12 ± 0.35, respectively). Conditional R^2 for the null model and final model was 0.50 and 0.79, respectively. Thus, channel unit sequence area, reach-scale substrate size, reach-scale water temperature variation, and reach-scale residual pool depth explained 29% of the variation in stream-fish occurrence among channel unit sequences.

DISCUSSION

My findings highlight the advantages of using a hierarchical approach to identify multiscale abiotic factors associated with the distribution of stream fishes. I identified relationships between Ozark Highland stream fishes and environmental variables at three spatial scales. Multiple intermediate-scale (i.e., stream reach and segment scale) environmental variables explained variation in either sunfish densities or stream-fish occurrence (or both). Variation in sunfish densities was associated with both reach-scale relative groundwater contribution and segment-scale lithology, which provides insight into environmental relationships at scales that both reflect their life history and are relevant to long-term management. Variation in the occurrence of stream fishes, including both sportfishes and species of conservation interest, was explained to some degree by channel unit sequence area but was also associated with multiple intermediate-scale environmental characteristics (reach-scale relative groundwater contribution, reach scale-substrate size, and reach-scale residual pool depth).

Relationships with relative groundwater contribution based on water temperature variability were similar for both sunfish densities and occurrence; however, this reach-scale environmental characteristic was most strongly associated with variation in Longear Sunfish and Rock Bass densities. Both Longear Sunfish and Rock Bass were unusually rare in some reaches, which can somewhat be explained by model results. For example, reaches in lower Spring Creek (Spring Creek1, Spring Creek2, and Spring Creek3), which had high relative groundwater contribution, were void of Longear Sunfish (with low sunfish densities in general), but had high densities of Rock Bass. However, Longear Sunfish were found at moderate densities at a reach of Spring Creek ~15 km upstream (Spring Creek4), which had considerably lower relative groundwater contribution than reaches of lower Spring Creek. A similar trend in densities was observed at stream reaches nested in the upper segment of Spavinaw Creek (Spavinaw Creek1), which also had high relative groundwater contribution, where Longear Sunfish were found at relatively low densities and Rock Bass were found at high densities. Conversely, reaches of Little Sac River and Greenleaf Creek, which had relatively low groundwater contribution were void of Rock Bass, but one could walk across Longear Sunfish. Presumably, the relationship between groundwater contribution and variation in sunfish densities was associated with thermal requirements and preferences (e.g., reaches with more groundwater contribution would have less extremes in water temperature). For example, the “FishTraits” database reports a lower thermal tolerance for Rock Bass (29° C) than other sunfishes considered here (33° C). However, groundwater has numerous influences on instream processes (see Introduction) and it is difficult to speculate on functional relationships based solely on empirical observations, especially given the considerable unexplained variation in sunfish densities. The relationship between groundwater contribution and Rock Bass and Longear Sunfish densities may also have been somewhat confounded with differing relationships between the species with water turbidity. Water clarity is high at both Spavinaw Creek and Spring Creek under baseflows, whereas Little Sac River is consistently turbid (personal observations); however, I did not quantify water turbidity at baseflows among stream reaches.

Notable observed trends in sunfish densities can also be somewhat explained by the modeled relationship with segment-scale lithology. Longear Sunfish and Green Sunfish densities were very high at Sallisaw Creek, which was classified as stony alluvium, but Rock Bass densities were low. However, the relationship between Longear Sunfish, Green Sunfish, and Rock Bass densities with lithology may be correlated with water turbidity because Sallisaw Creek is relatively turbid under baseflows (personal observation). The relationship between shale and both Bluegill and Redear Sunfish densities is particularly interesting. I observed the highest densities of Bluegill and Redear Sunfish in the lower stream segment of Spavinaw Creek (Spavinaw Creek2), which was classified as shale, where nested stream reaches were located ~1 km from an impoundment. The model results suggest that high Redear Sunfish and Bluegill densities in lower Spavinaw Creek were associated with lithology rather than spatial position, which is supported by much lower densities at other stream reaches in close proximity of impoundments (e.g. Saline Creek1, Saline Creek2, and Honey Creek1).

My findings also provided insight into multiscale factors associated with the occurrence of both a stream-fish species of conservation concern and less common Ozark stream fishes across my study area. Reach-scale relative groundwater contribution was associated with the occurrence of both Redspot Chub and Banded Sculpin among channel unit sequences. The strong negative relationship between Redspot Chub occurrence and decreasing groundwater contribution supports the perceived importance of spring flow for their persistence (Seilheimer and Fisher 2010; KDWPT 2016). My finding that Banded Sculpin occurrence was not associated with reach-scale substrate size is consistent with other research. Burr et al. (2001) found that Banded Sculpin were more common in cave streams, but occurred across a range of substrate sizes. Northern Studfish was not associated with groundwater contribution; however, their occurrence was strongly associated with larger channel unit sequence area, smaller reach-scale substrate, and shallower reach-scale residual pools. The relationship between Northern Studfish and environmental factors is noteworthy because they were relatively uncommon across my study area, but fairly abundant when encountered. Although Northern Studfish is a poorly studied species, my findings are consistent with research at a finer scale. Northern Studfish have been associated with shallower pools and sand-gravel substrate at the channel-unit scale (Lonzarich et al. 2000). Lonzarich et al. (2000) also documented considerable diurnal movement for Northern Studfish, which may explain their increased occurrence in larger channel unit sequences (i.e., more room to mingle). The increased occurrence of White Crappie and Spotted Sucker (very rare species in my study area) in larger channel unit sequences is likely related to their tendency to occupy large rivers (Pflieger 1997) and also suggests they select habitat in smaller streams at a finer scale (i.e., no reach-scale variables that I measured were associate with the occurrence of either species). The positive relationship between the occurrence of Spotted Bass, a rare black bass species across my study area, and both deeper reach-scale residual pools and lower reach-scale groundwater contribution was not surprising. Spotted Bass are associated with warmer streams and commonly inhabit reservoirs (Warren 2009). Although Spotted Bass is associated with sand-gravel (Churchill and Bettoli 2015), my results suggest a relationship with larger substrate at the reach-scale. For example, Spotted Bass was encountered at reaches of Little Sac River and Turnback Creek, which despite having a notable amounts of fines (i.e., sand and silt), had larger substrate on average. The modeled relationship between Spotted Bass occurrence and substrate size may also be confounded with water turbidity. Spotted Bass is also associated with turbid water (Warren 2009) and stream reaches of Turnback Creek and Little Sac River, where they were encountered, were very turbid at baseflows (personal observation). However, Spotted Bass was also encountered at a reach of Caney Creek, which also had larger substrate but is clear under baseflows (personal observation).

My findings concerning Smallmouth Bass, a stream fish of both ecological and recreational value, demonstrate how different stream fish-environment relationships (similar to Spotted Bass; see above) can emerge when viewed at different scales. McClendon and Rabeni (1987) found that Smallmouth Bass in Ozark Highland streams were associated with larger substrate at the channel-unit scale, whereas my results indicated that increased occurrence among channel unit sequences (for both subadults and adults) was associated with smaller reach-scale substrate. Thus, Smallmouth Bass may be associated with larger substrate from a finer-scale perspective; however, they may tend to occupy stream reaches with relatively smaller substrate. The nature of the relationship between Smallmouth Bass and substrate size is perhaps related to crayfish, which are an important forage base for Smallmouth Bass (Brewer and Orth 2015). Ringed Crayfish *Orconectes neglectus neglectus*, a common species in my study area (Morehouse and Tobler 2013), is associated with gravel (i.e., smaller) substrate (Pflieger 1996). Also, Ringed Crayfish tended to be found in higher abundance at sites with relatively lower D50 (i.e., 20-25 mm; unpublished data), which is consistent with gravel on the Wentworth scale. However, the relationship between Smallmouth Bass and substrate size may also be confounded with water turbidity because stream reaches with larger substrate (e.g., Turnback Creek1 and Little Sac

River1) tended to have higher turbidity. Increased occurrence of both subadult and adult Smallmouth Bass among channel unit sequences was also associated with shallower reach-scale residual pools. Increased subadult Smallmouth Bass occurrence in reaches with shallower residual pool depth was not surprising because fish > 250-mm TL in Ozark Highland streams tend to occupy runs and shallower pool areas (Probst et al. 1984; personal observations). However, the increased occurrence of adult Smallmouth Bass, contrary to Largemouth Bass, in reaches with shallower residual pools is interesting. Other studies have also found reach-scale relationships between Smallmouth Bass and pool habitat. For example, Sowa and Rabeni (1995), Dauwalter et al. (2007), and Brewer (2013a) showed that increased Smallmouth Bass densities were associated with stream reaches with lower proportions of pool habitat, presumably due to increased crayfish biomass. The increased occurrence of both subadult and adult Smallmouth Bass with increasing reach-scale relative groundwater contribution is consistent with Brewer (2013b) who found that > age-0 Smallmouth Bass were associated with areas of higher spring flow at both the channel unit- and segment-scale.

I did not identify any relationships between stream-fish distributions and land use or spatial factors. Sunfish densities were not associated with segment-scale agriculture and development as either an individual or interaction effect. However, my findings do not discount the importance of land use on stream-fish distributions, particularly at coarser scales. For example, relationships may have emerged if I had examined variation in densities among stream fishes (preferably with the inclusion of additional species) in relation to watershed-scale land use; however, this was both not a study objective and could not be adequately addressed given both my dataset and the spatial extent of the study area. Despite variability in spatial factors among both stream reaches and steam segments, I also did not identify any relationships with either sunfish densities or stream-fish occurrence. Nevertheless, it was fundamentally important to incorporate spatial variables into the analyses to promote improved interpretation of relationships with environmental factors among stream fishes.

The failure to establish sunfish trait-environment relationships was not surprising. Stream fish-trait relationships with groundwater contribution would likely be a function of thermal preferences, rather than reproductive or morphological traits; however, variation in thermal tolerances among sunfishes were not adequate to examine in a trait-based context (see above). Lithology has a profound effect on the instream environment, including influencing forage bases for fishes (e.g., invertebrate populations; Neff and Jackson 2012). Morphological traits related to feeding strategies could certainly be associated with lithology, although relationships would be more likely to emerge when examining a larger group of stream fishes with more morphological variation.

I used a coarse method to estimate relative groundwater contribution that was not without its limitations; however, it still provided a useful way to characterize stream reaches. The most notable caveat of my approach is that water temperature loggers need to be deployed at stream reaches during the same time period to provide direct comparisons of relative groundwater contribution. Another consideration is that, although air temperature variation was the same for each set of loggers, canopy cover and solar radiation may have varied somewhat among stream reaches in the spring. However, the z-scores derived to improve comparably between the two sets of loggers provided a general ranking of stream reaches appropriate to address my study objectives. The exact ordering of stream reaches may have differed if all the loggers were deployed during the same period (preferably in the winter), although it is unlikely this would have drastically altered findings related to sunfish densities or stream-fish occurrence.

Although variable detection can be estimated for a removal design (MacKenzie and Royle 2005), the nature of my dataset prevented an informative assessment. Most commonly, stream fishes were encountered either during the first or second electrofishing pass or not all, which provided limited information about species detection. My data also suggested that additional removal passes would have rarely resulted in the encounter of additional stream fishes or provided more insight into species detection (e.g., only ~3% of the encounters occurred on the fourth electrofishing pass). Snorkeling surveys were also performed at a subset of channel unit sequences (*n* = 30) ~24 h after the electrofishing passes when water clarity was adequate and no additional stream fishes were encountered. Thus, my data and field observations support that the electrofishing effort was adequate to establish stream-fish occurrence among channel unit sequences, although it represented naïve occurrence.

Despite considerable research efforts, stream fishes continue to decline at alarming rates and species-environment relationships remain poorly understood (see also both Introduction and Chapter 1). A critical aspect of establishing empirical relationships between species and the environment are analytical methods that accommodate the hierarchical nature common to stream-fish datasets. Ignoring the hierarchy (i.e., nested structure) common to stream-fish datasets when performing analyses can lead to false inferences and represents a form of pseudoreplication (Wagner et al. 2006; Stewart-Koster et al. 2013). For example, examining finer scale-observations (e.g., channel unit- or reach-scale population assessments) in relation to watershed-scale characteristics without including grouping factors (i.e., random effects) to accommodate the nested study design is both fundamentally inappropriate and can result in spurious conclusions. By incorporating random effects (i.e., using a mixed model), I found that remaining variation in sunfish densities in the final model was higher among segments than reaches and a traditional regression model (i.e., ordinary least squares and generalized linear models) would have ignored this coarser-scale variation. Furthermore, simply including multiscale fixed effects in a traditional regression model (i.e., they are not nested) does not account for hierarchically-structured data and confounds results. The inclusion of a seasonal random effect in the stream-fish occurrence model identified additional variation at different temporal scales. Also, the variance partitioning (i.e., grouping factors) in mixed models provides not only an assessment of remaining variation for each random effect, but also allows for an evaluation of the total variation accounted for among observations. For example, the conditional R^2 for both the final sunfish density model and stream-fish occurrence model indicated that I accounted for (albeit not explained) ~80% of the variation among observations, which lends support to the validity of my findings. There are also advantages of using mixed models to analyze multi-species datasets, where species is also treated as a random effect (see both Chapter 3 Methods and Discussion). In fact, treating species as a random effect is advisable even when only a few species are considered. When there are only a few levels of a random effect, mixed model performance is at a minimum equal to using a categorical fixed effect in a traditional regression model and a reference category is not needed, thus simplifying interpretation (Gelman and Hill 2007). A trade-off of modeling multiple species simultaneously using a mixed model is that the random components (i.e., intercepts and slopes) represent a compromise between species-specific fit and overall model fit (shrinkage estimates; Pinheiro and Bates 2000); therefore, different relationships may emerge if a single-species model is used (here the explicit objective was to examine variation among species). Although mixed models may lack the elegance of Canonical Correspondence Analysis (Palmer 1993), they are an effective, flexible approach to identify species-environment relationships, where the magnitude of relationships can be both visualized and quantified. Additionally, a mixed model is superior to ordination for both predictive applications and accommodating the inherent pseudoreplication and unequal sample sizes common to ecological datasets (Jamil et al 2013).

Conservation and management implications. - A multiscale perspective is essential to the long-term success of stream-fish conservation and management. The consideration of coarser-scale factors that constrain stream-fish distributions at finer scales promotes both the efficient use of resources and sound decisions. For example, the likelihood of channel-unit scale habitat enhancements to improve stream-fish population status is greatly increased when coarser-scale factors (e.g., land use and flow regime) are favorable for target species. The effective manipulation of instream habitat is also constrained by coarser-scale factors. For example, restoring groundwater flow in streams is plausible, but its success largely depends on watershed-scale land use (Kasahara and Hill 2006a; Kasahara and Hill 2006b). Furthermore, life-history characteristics of stream fishes are often a reflection of factors at multiple scales and habitat use may be primarily due to intermediate-scale factors (Fausch et al. 2002). For example, I found Northern Studfish occurrence was associated with larger channel unit sequences, but also multiple reach-scale factors. Similarly, my findings support other studies that suggest Smallmouth Bass habitat associations may differ when viewed from the intermediate scale. Thus, managers may consider addressing multiple scales simultaneously to maximize success, with a particular emphasis on intermediate-scale characteristics.

Table 1. Mean density and trait characteristics of six stream fish species sampled using tow-barge electrofishing at 40 stream reaches in the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri in 2014-2015. Trait groups are morphological groups developed in Chapter 2. Age at maturation and longevity reflected both male and female individuals.

Common name	Latin name	Mean density (fish/m) ± SD	Trait group	Longevity (yr)	Age at maturation (yr)
Bluegill	*Lepomis macrochirus*	0.48 ± 0.80	A	5	1
Green Sunfish	*Lepomis cyanellus*	0.48 ± 0.70	B	5	1
Longear Sunfish	*Lepomis megalotis*	1.77 ± 2.18	B	6	3
Redear Sunfish	*Lepomis microlophus*	0.07 ± 0.27	A	5	2
Rock Bass	*Ambloplites rupestris*	0.35 ± 0.31	B	7	3
Warmouth	*Lepomis gulosus*	0.06 ± 0.12	B	3	1

Table 2. Twenty-one stream fishes of the Ozark Highlands ecoregion included in an examination of stream-fish occurrence using both channel unit sequence- and stream reach-scale variables.

Common name	Latin name	Proportion of occurrence
Banded Sculpin	*Cottus carolinae*	0.74
Bluegill	*Lepomis macrochirus*	0.79
Creek Chub	*Semotilus atromaculatus*	0.45
Green Sunfish	*Lepomis cyanellus*	0.88
Largemouth Bass (subadult)	*Micropterus salmoides*	0.70
Largemouth Bass (adult)	*Micropterus salmoides*	0.28
Longear Sunfish	*Lepomis megalotis*	0.88
Northern Hogsucker	*Hypentelium nigricans*	0.78
Northern Studfish	*Fundulus catenatus*	0.23
Redhorses	*Moxostoma* spp.	0.66
Redear Sunfish	*Lepomis microlophus*	0.31
Redspot Chub	*Nocomis asper*	0.87
Rock Bass	*Ambloplites rupestris*	0.74
Smallmouth Bass (subadult)	*Micropterus dolomieu*	0.79
Smallmouth Bass (adult)	*Micropterus dolomieu*	0.58
Spotted Bass	*Micropterus punctulatus*	0.18
Spotted Sucker	*Minytrema melanops*	0.09
Warmouth	*Lepomis gulosus*	0.38
White Crappie	*Pomoxis annularis*	0.10
White Sucker	*Catostomus commersoni*	0.18

Table 3. Continuous variables to characterize 80 channel unit sequences in the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri. See Methods for a detailed description of variables.

Variable	Minimum	Maximum	Mean ± SD
Mean water depth (m)	0.23	1.55	0.81 ± 0.28
Mean wetted channel width (m)	4.00	34.50	13.42 ± 6.25
Wetted width-depth ratio	4.55	49.17	17.75 ± 8.48
Proportion emergent vegetation	0.00	0.31	0.03 ± 0.05
Proportion instream large wood	0.00	0.40	0.04 ± 0.06
Proportion total cover	0.00	0.40	0.06 ± 0.08
Proportion riffle	0.02	0.68	0.26 ± 0.16
Area (m^2)	75.00	12938.00	2422.96 ± 2433.16
Length (m)	15.00	456.00	154.53 ± 99.71

Table 4. Continuous variables to characterize 40 stream reaches in the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri surveyed in 2014-2015. Relative groundwater contribution is reported as a z-score. See Methods for a detailed description of variables.

Variable	Minimum	Maximum	Mean ± SD
Bankfull width-depth ratio	17.00	72.00	45.90 ± 15.40
D50 (mm)	20.00	131.00	34.63 ± 23.25
Water temperature variation	-1.37	1.87	-0.32 ± 0.89
Proportion riffle	0.07	0.42	0.23 ± 0.09
Residual pool depth (m)	0.50	2.00	1.23 ± 0.36
Proportion emergent vegetation	0.00	0.25	0.04 ± 0.05
Proportion instream large wood	0.00	0.10	0.04 ± 0.02
Proportion total cover	0.00	0.28	0.07 ± 0.05
Distance to impoundment (km)	1.00	86.00	21.88 ± 22.19

Table 5. Continuous variables to characterize 22 stream reaches in the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri surveyed in 2015 using tow-barge electrofishing. Relative groundwater contribution is reported as a z-score. See Methods for a detailed description of variables.

Variable	Minimum	Maximum	Mean ± SD
Bankfull width-depth ratio	17.00	67.00	38.59 ± 14.37
D50 (mm)	20.00	131.00	38.11 ± 27.41
Water temperature variation	-1.37	1.87	-0.10 ± 0.92
Proportion riffle	0.12	0.42	0.24 ± 0.09
Residual pool depth (m)	0.50	1.70	1.09 ± 0.35
Proportion emergent vegetation	0.00	0.21	0.03 ± 0.05
Proportion instream large wood	0.00	0.10	0.04 ± 0.02
Proportion total cover	0.00	0.23	0.06 ± 0.05
Distance to impoundment (km)	2.00	71.00	18.35 ± 15.94

Table 6. Continuous variables that characterized 20 stream segments in the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri. See Methods for a detailed description of variables.

Variable	Minimum	Maximum	Mean ± SD
D-link	4.00	325.00	108.93 ± 100.75
Proportion agriculture and development	0.05	0.71	0.49 ± 0.18
Distance to ecoregion centroid (km)	92.00	285.00	230.20 ± 41.04
Sinuosity	1.09	1.73	1.35 ± 0.19
Two-year flood magnitude (m^3/sec)	87.00	355.00	160.93 ± 79.02

Table 7. Fixed effects and species-dependent coefficients with standard error from a linear mixed model to identify environmental and spatial factors related to variation in sunfish density, where sunfish density was natural-log transformed. All continuous variables were standardized such that each had a mean of zero and a standard deviation of one, where the model intercept estimates sunfish density at mean conditions and coefficients for continuous variables represent a unit change of one standard deviation. Geosoil is categorical, where shale is the reference category. Water temperature variation was measured as a surrogate for relative groundwater contribution, where a negative coefficient represents a positive relationship with increased groundwater contribution.

Effect	Intercept	Reach water temperature variation	Segment geosoil (Cherty alluvium)	Segment geosoil (Stony alluvium)	Segment geosoil (Cherty limestone)	Segment geosoil (Cherty dolostone)
Fixed	-1.88 ± 0.85	0.17 ± 0.39	-1.34 ± 1.08	0.52 ± 1.18	-0.77 ± 1.10	-0.82 ± 1.00
Bluegill	-1.04 ± 0.43	0.51 ± 0.21	-1.77 ± 0.55	0.79 ± 0.62	-0.96 ± 0.54	-0.66 ± 0.24
Green Sunfish	-1.11 ± 0.43	0.67 ± 0.21	-1.09 ± 0.55	1.40 ± 0.62	-0.16 ± 0.54	-0.22 ± 0.24
Longear Sunfish	-0.53 ± 0.43	1.13 ± 0.21	-0.77 ± 0.55	2.33 ± 0.62	0.48 ± 0.54	0.32 ± 0.24
Redear Sunfish	-2.13 ± 0.43	-0.01 ± 0.21	-3.94 ± 0.55	-0.38 ± 0.62	-3.65 ± 0.54	-2.72 ± 0.24
Rock Bass	-2.99 ± 0.43	-1.03 ± 0.21	1.60 ± 0.55	-1.29 ± 0.62	1.65 ± 0.54	0.55 ± 0.24
Warmouth	-3.49 ± 0.43	-0.26 ± 0.21	-2.09 ± 0.55	0.25 ± 0.62	-1.95 ± 0.54	-2.20 ± 0.24

Table 8. Fixed effects and species-dependent coefficients with standard error from a multiscale generalized linear mixed model to identify environmental and spatial factors related to variation in occurrence of 20 stream fishes of the Ozark Highlands ecoregion Stream-fish occurrence was a binary variable and the probability of stream-fish occurrence is reported on a logit scale. All variables were standardized such that each had a mean of zero and a standard deviation of one, where the model intercept estimated the probability of stream-fish occurrence at mean conditions and coefficients for continuous variables represent a unit change of one standard deviation. Water temperature variation was measured as a surrogate for relative groundwater contribution, where a negative coefficient represents a positive relationship with increased groundwater contribution.

Effect	Intercept	Channel unit sequence area (m^2)	Reach D50 (mm)	Reach water temperature variation	Reach residual pool depth (m)
Fixed	-0.01 ± 0.56	0.92 ± 0.13	0.03 ± 0.18	0.04 ± 0.24	-0.04 ± 0.15
Banded Sculpin	1.13 ± 0.38	0.69 ± 0.14	-0.09 ± 0.26	-0.67 ± 0.26	-0.14 ± 0.15
Bluegill	1.36 ± 0.38	0.95 ± 0.16	0.16 ± 0.27	0.64 ± 0.30	0.17 ± 0.15
Creek Chub	-0.39 ± 0.36	1.09 ± 0.14	-0.24 ± 0.22	0.27 ± 0.25	-0.15 ± 0.14
Green Sunfish	2.12 ± 0.44	0.88 ± 0.17	0.36 ± 0.32	0.91 ± 0.39	0.38 ± 0.19
Largemouth Bass (subadult)	0.86 ± 0.36	0.69 ± 0.15	0.68 ± 0.27	0.36 ± 0.27	0.38 ± 0.15
Largemouth Bass (adult)	-1.27 ± 0.38	0.77 ± 0.15	0.70 ± 0.25	0.29 ± 0.26	0.29 ± 0.14
Longear Sunfish	2.15 ± 0.45	0.86 ± 0.17	0.41 ± 0.32	0.93 ± 0.40	0.41 ± 0.19
Northern Hogsucker	1.37 ± 0.39	0.81 ± 0.14	-0.27 ± 0.26	-0.51 ± 0.26	-0.21 ± 0.15
Northern Studfish	-1.83 ± 0.42	1.38 ± 0.17	-0.69 ± 0.30	0.10 ± 0.30	-0.53 ± 0.18
Redear Sunfish	-1.09 ± 0.37	1.03 ± 0.14	0.25 ± 0.23	0.66 ± 0.26	0.14 ± 0.14
Redhorses	0.62 ± 0.36	0.92 ± 0.14	0.11 ± 0.24	0.29 ± 0.25	0.07 ± 0.14

Redspot Chub	2.78 ± 0.62	0.35 ± 0.20	-0.46 ± 0.40	-1.87 ± 0.43	-0.38 ± 0.23
Rock Bass	1.41 ± 0.42	0.81 ± 0.14	-0.83 ± 0.28	-1.06 ± 0.31	-0.51 ± 0.17
Smallmouth Bass (subadult)	1.55 ± 0.41	0.86 ± 0.15	-0.51 ± 0.27	-0.53 ± 0.27	-0.31 ± 0.16
Smallmouth Bass (adult)	0.19 ± 0.36	0.93 ± 0.14	-0.35 ± 0.24	-0.53 ± 0.27	-0.34 ± 0.15
Spotted Bass	-2.15 ± 0.44	0.97 ± 0.16	0.76 ± 0.26	0.74 ± 0.29	0.30 ± 0.16
Spotted Sucker	-2.89 ± 0.50	1.24 ± 0.18	0.13 ± 0.28	0.49 ± 0.34	-0.13 ± 0.17
Warmouth	-0.70 ± 0.36	0.56 ± 0.14	0.65 ± 0.24	-0.30 ± 0.27	0.23 ± 0.14
White Crappie	-2.91 ± 0.51	1.30 ± 0.17	0.25 ± 0.28	0.80 ± 0.33	-0.04 ± 0.17
White Sucker	-2.10 ± 0.43	1.13 ± 0.17	-0.25 ± 0.27	-0.23 ± 0.35	-0.38 ± 0.17

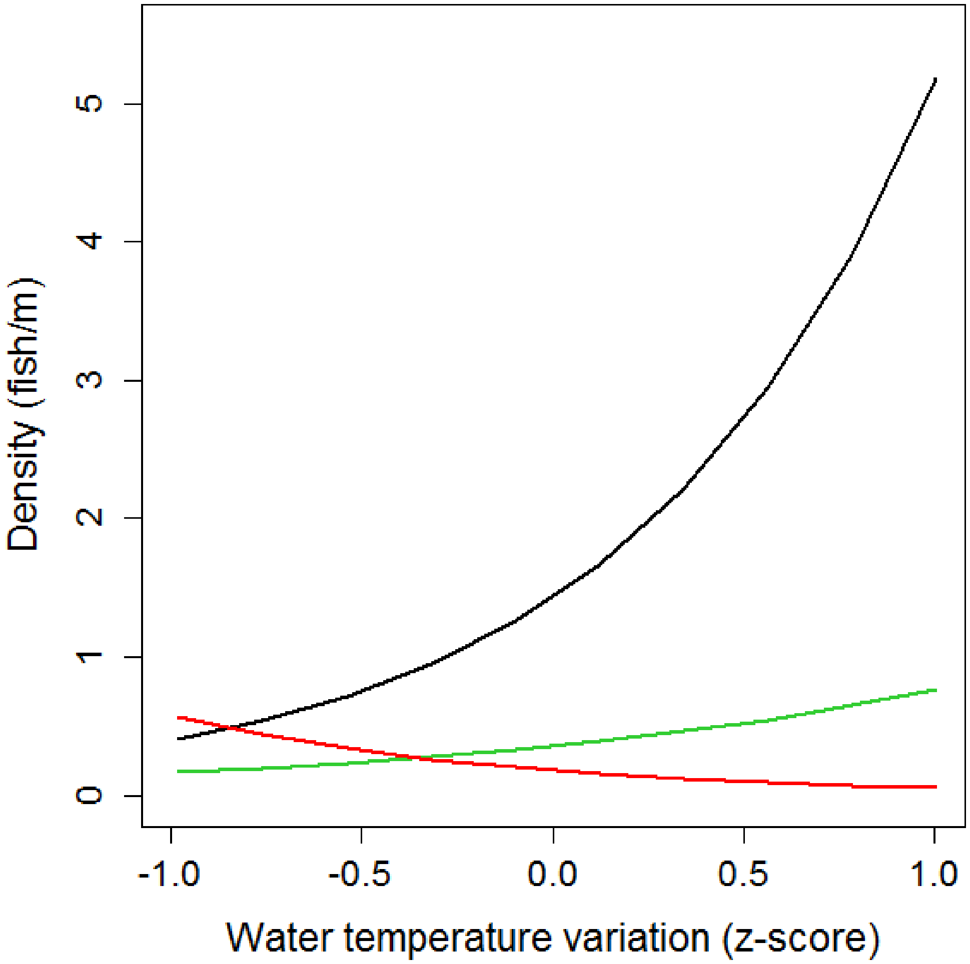

Figure 1. The relationship between fish density and relative groundwater contribution for three sunfishes, where black represents Longear Sunfish, light green represents Green Sunfish, and red represents Rock Bass. Estimates were derived from a linear mixed model, where the geosoil category was cherty limestone. Water temperature variation (x-axis) was used as a surrogate for groundwater contribution where more variation represents less groundwater contribution and (reported as a z-score).

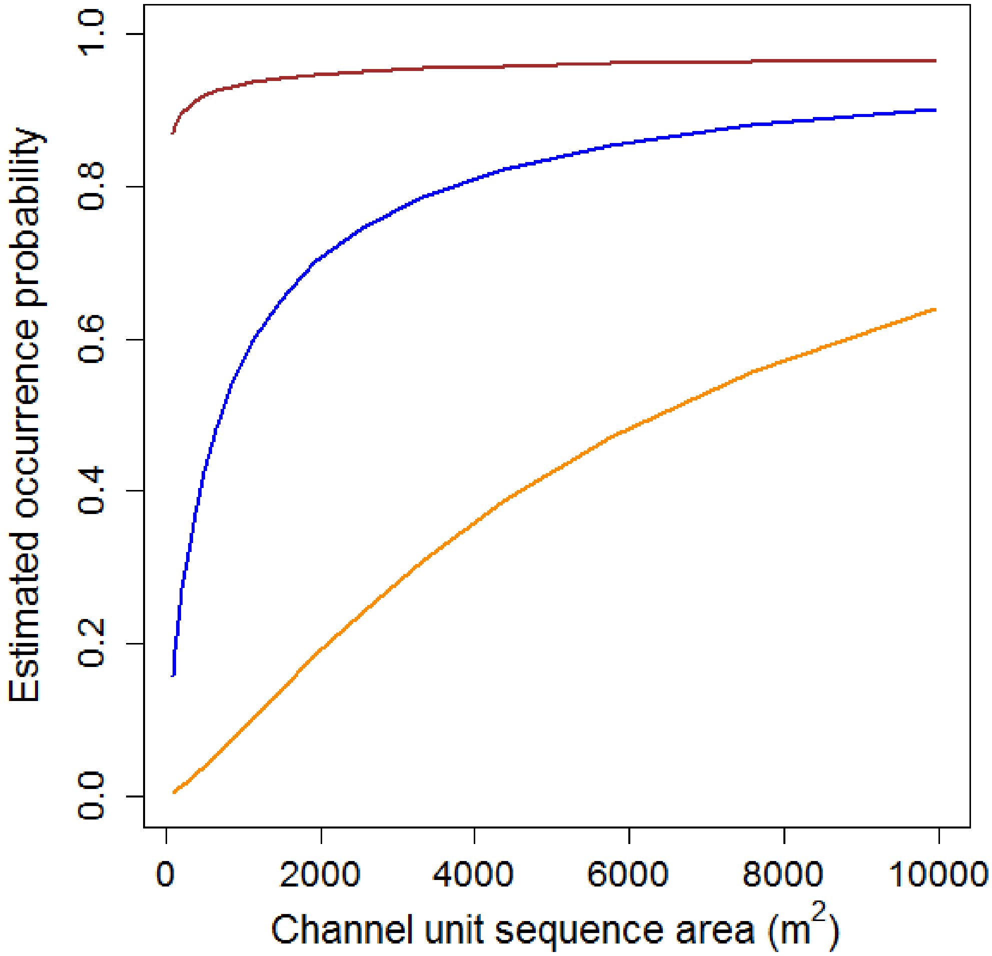

Figure 2. The relationship between occurrence and channel unit sequence area for three stream fishes, where brown represents Redspot Chub, blue represents Northern Studfish, and orange represents redhorses. Estimates of stream-fish occurrence were derived from a generalized linear mixed model, where other variables were held at mean levels.

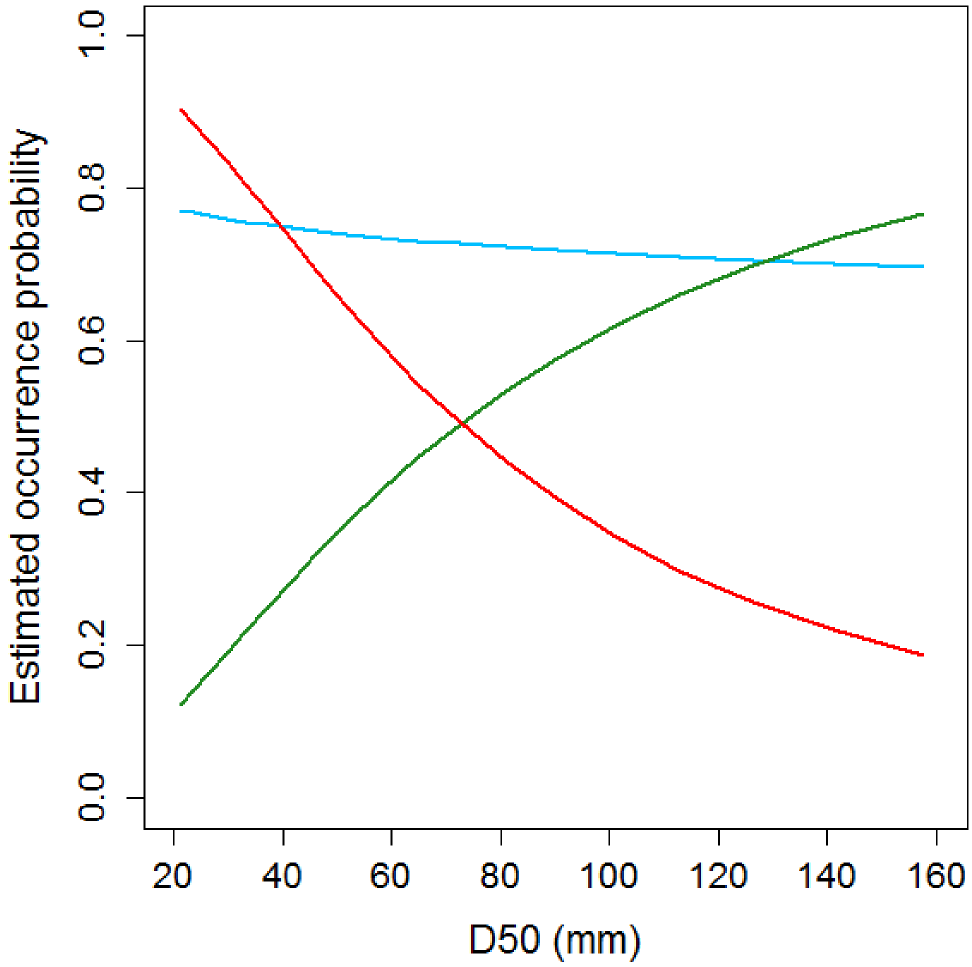

Figure 3. The relationship between substrate size and occurrence for three stream fishes, where light blue represents Banded Sculpin, dark green represents adult Largemouth Bass, and red represents Rock Bass. Estimates of stream-fish occurrence were derived from a generalized linear mixed model, where other variables were held at mean levels.

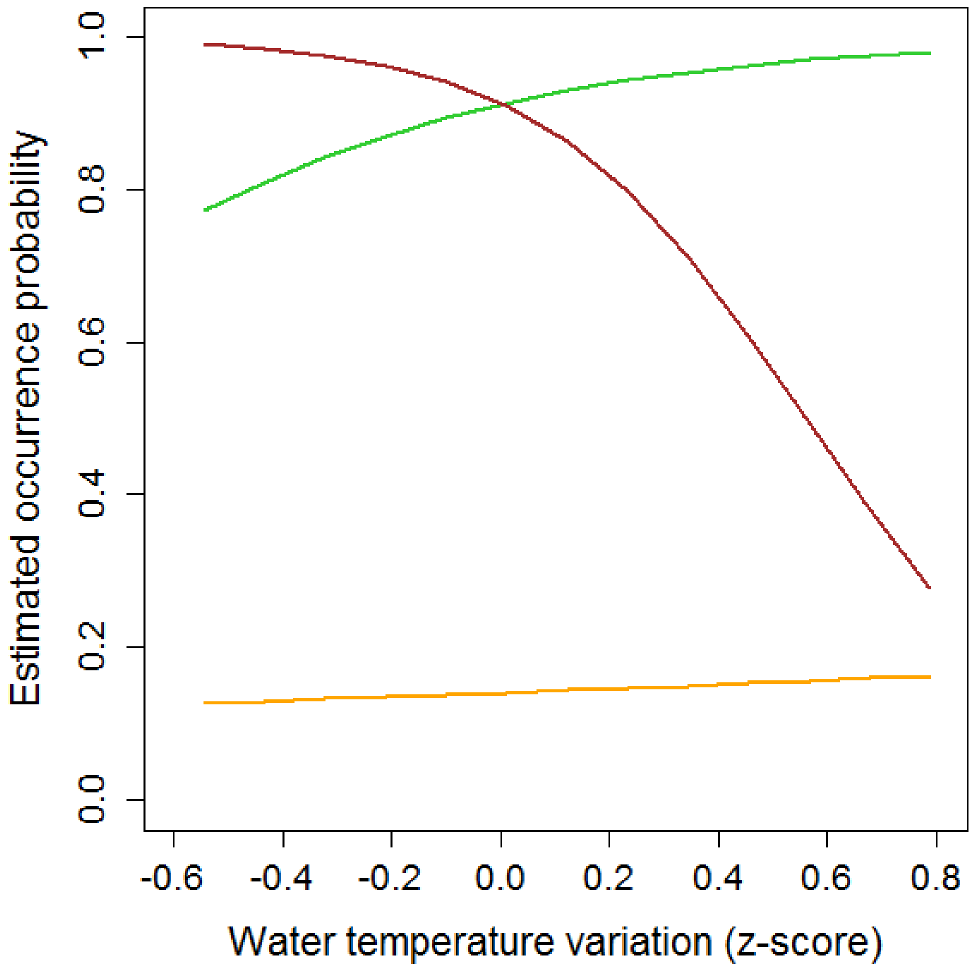

Figure 4. The relationship between relative groundwater contribution and occurrence for three stream fishes, where orange represents Northern Studfish, brown represents Redspot Chub, and light green represents green sunfish. Estimates were derived from a generalized linear mixed model, where other variables were held at mean levels. Water temperature variation (x-axis) was used as a surrogate for groundwater contribution, where more variation represents less groundwater contribution (reported as a z-score).

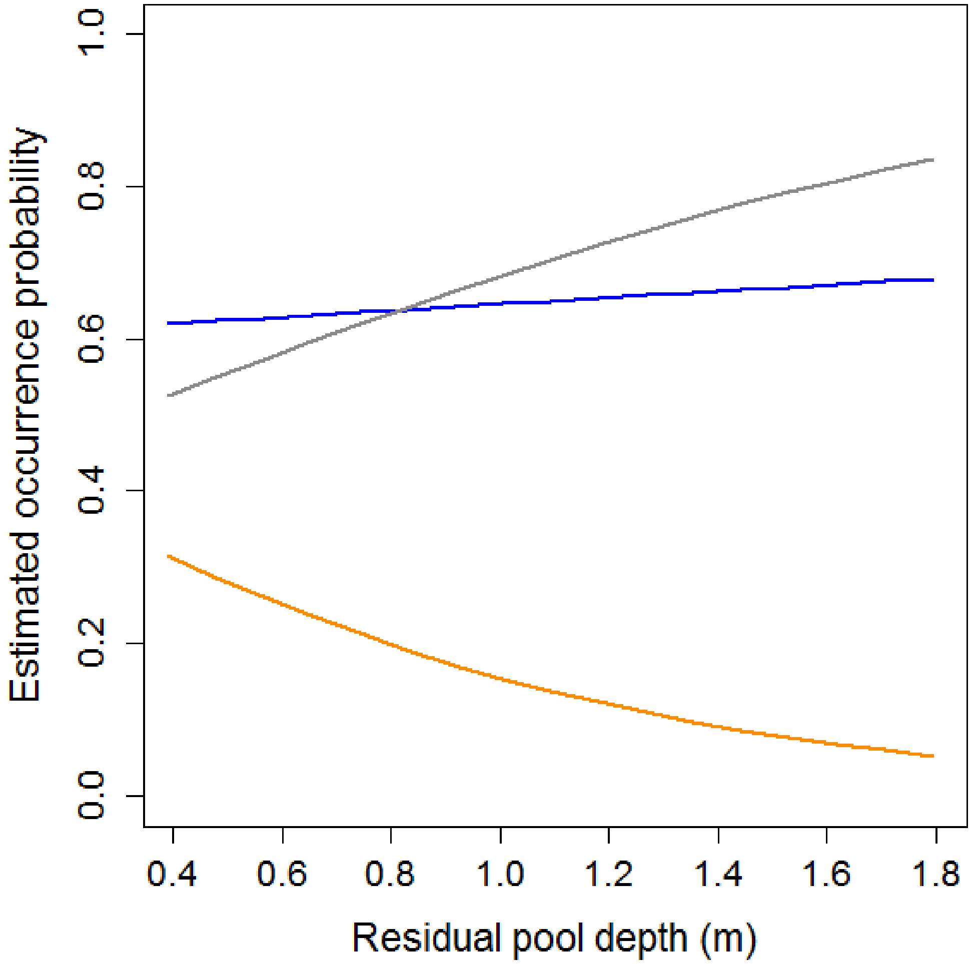

Figure 5. The relationship between residual pool and occurrence for three stream fishes, where blue represents redhorses, gray represents subadult Largemouth Bass, and orange represents Northern Studfish. Estimates were derived from a generalized linear mixed model, where other variables were held at mean levels.

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APPENDICES

Appendix 1. Year and season of sampling and associated stream segments for 40 stream reaches surveyed using tow-barge electrofishing in the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri in 2014-2015, where season one is May-June, season two is July-August, season three is September-October.

Reach	Year	Season	Segment
Baron Fork1	2014	2	Baron Fork1
Baron Fork2	2014	3	Baron Fork1
Baron Fork3	2015	2	Baron Fork1
Big Sugar Creek1	2015	2	Big Sugar Creek1
Buffalo Creek1	2014	2	Buffalo Creek1
Buffalo Creek2	2014	3	Buffalo Creek1
Buffalo Creek3	2015	2	Buffalo Creek1
Buffalo Creek4	2015	2	Buffalo Creek1
Butler Creek1	2015	2	Butler Creek1
Caney Creek1	2015	2	Caney Creek1
Evansville Creek1	2014	2	Evansville Creek1
Evansville Creek2	2014	3	Evansville Creek1
Five-mile Creek1	2015	2	Five-mile Creek1
Flint Creek1	2014	1	Flint Creek1
Flint Creek2	2014	2	Flint Creek1
14-mile Creek1	2014	2	14-mile Creek1
14-mile Creek2	2014	2	14-mile Creek1
14-mile Creek3	2015	2	14-mile Creek1
Greenleaf Creek1	2015	1	Greenleaf Creek1
Greenleaf Creek2	2015	1	Greenleaf Creek1
Honey Creek1	2015	1	Honey Creek1
Honey Creek2	2015	2	Honey Creek1
Indian Creek1	2015	2	Indian Creek1
Little Sac River1	2015	1	Little Sac River1
Lost Creek1	2015	2	Lost Creek1
Saline Creek1	2014	2	Saline Creek1

Saline Creek2	2015	2	Saline Creek1
Sallisaw Creek1	2015	2	Sallisaw Creek1
Spavinaw Creek1	2014	1	Spavinaw Creek1
Spavinaw Creek2	2014	2	Spavinaw Creek2
Spavinaw Creek3	2014	2	Spavinaw Creek1
Spavinaw Creek4	2014	3	Spavinaw Creek2
Spavinaw Creek5	2014	3	Spavinaw Creek1
Spavinaw Creek6	2015	1	Spavinaw Creek1
Spavinaw Creek7	2015	3	Spavinaw Creek1
Spring Creek1	2014	2	Spring Creek1
Spring Creek2	2014	3	Spring Creek1
Spring Creek3	2015	3	Spring Creek1
Spring Creek4	2015	3	Spring Creek1
Turnback Creek1	2015	1	Turnback Creek1

Appendix 2. Environmental and spatial variables for 40 stream reaches surveyed using tow-barge electrofishing in the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri in 2014-2015. See Methods for a complete description of variables.

Reach	Distance to impoundment (km)	D50 (mm)	Bankfull width (m)	Residual pool (m)	Proportion riffle	Relative groundwater contribution	Proportion emergent vegetation	Proportion instream large wood
Baron Fork1	23	34	66	2.0	16	-0.59	0.02	0.07
Baron Fork2	23	34	66	2.0	16	-0.59	0.02	0.07
Baron Fork3	24	34	66	1.5	18	-0.59	0.04	0.04
Big Sugar Creek1	35	25	40	1.5	14	-0.83	0.05	0.03
Buffalo Creek1	4	25	42	1.4	29	-0.83	0.01	0.09
Buffalo Creek2	4	25	42	1.4	29	-0.83	0.01	0.09
Buffalo Creek3	14	27	29	0.5	42	-0.59	0.00	0.01
Buffalo Creek4	5	25	42	1.4	30	-0.83	0.00	0.04
Butler Creek1	17	20	42	1.3	14	-0.46	0.00	0.03
Caney Creek1	2	131	49	0.8	23	-0.46	0.00	0.01
Evansville Creek1	54	30	72	1.6	38	0.23	0.03	0.02
Evansville Creek2	54	30	72	1.6	38	0.23	0.03	0.02
Five-mile Creek1	27	29	39	1.2	33	1.48	0.00	0.01
Flint Creek1	86	23	56	1.0	16	-0.30	0.03	0.02
Flint Creek2	85	23	56	1.2	8	-0.30	0.03	0.02
14-mile Creek1	7	23	49	1.5	7	0.45	0.25	0.03
14-mile Creek2	14	21	35	1.0	24	0.45	0.01	0.04

14-mile Creek3	15	21	35	1.0	27	0.45	0.01	0.04
Greenleaf Creek1	14	37	17	1.3	28	1.74	0.00	0.00
Greenleaf Creek2	13	37	17	1.3	18	1.87	0.00	0.00
Honey Creek1	4	38	33	0.7	20	0.19	0.01	0.01
Honey Creek2	3	38	22	0.6	15	0.19	0.00	0.10
Indian Creek1	71	33	46	0.8	28	-0.33	0.00	0.02
Little Sac River1	32	46	36	0.8	21	1.80	0.00	0.01
Lost Creek1	15	26	23	0.9	42	-0.33	0.03	0.05
Saline Creek1	2	37	50	0.7	15	-0.85	0.01	0.02
Saline Creek2	3	37	57	0.8	30	-0.85	0.07	0.04
Sallisaw Creek1	21	41	42	1.1	13	0.71	0.21	0.02
Spavinaw Creek1	9	27	39	1.6	22	-1.24	0.00	0.05
Spavinaw Creek2	1	25	43	1.3	18	-0.83	0.10	0.01
Spavinaw Creek3	10	25	58	1.6	22	-1.24	0.00	0.03
Spavinaw Creek4	1	27	43	1.3	18	-0.83	0.10	0.01
Spavinaw Creek5	9	25	39	1.6	22	-1.24	0.00	0.05
Spavinaw Creek6	10	25	58	1.6	18	-1.24	0.00	0.01
Spavinaw Creek7	8	25	59	1.2	12	-1.24	0.00	0.05
Spring Creek1	34	29	67	1.1	26	-1.37	0.05	0.02
Spring Creek2	34	29	67	1.1	26	-1.37	0.05	0.02
Spring Creek3	34	29	67	1.1	21	-1.37	0.00	0.03
Spring Creek4	47	29	26	1.7	29	-0.20	0.01	0.01

Turnback Creek1	7	131	29	0.9	18	0.75	0.01	0.03

Appendix 3. Environmental and spatial variables for 20 stream segments surveyed using tow-barge electrofishing in the Ozark Highlands ecoregion of northeast Oklahoma and southwest Missouri in 2014-2015, where a one for connectivity indicates the segment was located upstream of a higher order tributary. See Methods for a complete description of variables and Appendix 1 for associated reaches.

Segment	Geosoil	Distance to centroid (km)	Proportion agriculture-development	D-link	Connectivity	Sinuosity	Two-year flood magnitude (m3/sec)
Baron Fork1	Cherty alluvium	250	0.60	219	1	1.36	355
Big Sugar Creek1	Cherty dolostone	183	0.50	90	1	1.62	305
Buffalo Creek1	Cherty limestone	208	0.50	301	1	1.23	139
Butler Creek1	Cherty dolostone	197	0.56	280	1	1.19	97
Caney Creek1	Stony alluvium	266	0.63	69	0	1.21	158
Evansville Creek1	Cherty alluvium	240	0.55	34	1	1.62	109
Five-mile Creek1	Cherty alluvium	203	0.33	4	1	1.24	90
Flint Creek1	Cherty limestone	228	0.43	50	1	1.31	129
14-mile Creek1	Cherty alluvium	279	0.67	32	0	1.38	107
Greenleaf Creek1	Shale	285	0.05	12	1	1.15	87
Honey Creek1	Cherty limestone	215	0.66	14	0	1.29	105
Indian Creek1	Cherty limestone	168	0.53	17	1	1.57	142
Little Sac River1	Cherty dolostone	92	0.62	178	0	1.73	144
Lost Creek1	Cherty limestone	193	0.71	5	1	1.10	97
Saline Creek1	Cherty limestone	259	0.68	44	1	1.55	118
Sallisaw Creek1	Stony alluvium	281	0.21	91	0	1.41	150

Spavinaw Creek1	Cherty limestone	225	0.26	97	0	1.26	180
Spavinaw Creek2	Shale	231	0.37	162	0	1.10	290
Spring Creek1	Cherty alluvium	261	0.50	56	0	1.51	115
Turnback Creek1	Cherty dolostone	125	0.67	325	0	1.51	276

Appendix 4. Results of ordinary-least-squares regression modeling the relationship between drainage area and two-year flood interval for hydrologically-similar Ozark Highland stream segments ($R^2 = 0.69$). Two-year flood interval was log10 transformed.

Coefficients	Estimate	SE	*p*-value
Intercept	7.93	0.15	< 0.01
Drainage area (mile^2)	0.01	0.01	< 0.01

VITA

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