“EVALUATING SAMPLING METHODS AND INVESTIGATING DISTRIBUTION AND RICHNESS OF FISH AND AMPHIBIANS IN MISSOURI WETLANDS”

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled:

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ABSTRACT

Freshwater wetlands are some of the most imperiled ecosystems on the planet, meaning species that depend on them are at great risk. In Missouri, wetland filling and drainage for agriculture and the elimination and degradation of floodplain wetlands due to river management projects account for the destruction of >85% of the state's wetland area since the 1780's. River management projects, including damming, channelization, and levee construction, altered flow regimes and disrupted essential exchanges between the main channel and floodplain areas. Declines of wildlife populations spawned early conservation efforts aimed at protecting existing wetlands and replacing those already devastated. The Missouri Department of Conservation is bound by the state constitution to protect and manage the fish, forest, and wildlife resources of the state. As such, efforts to increase wetland area focused on restoring degraded and converted wetlands by establishing public wetland complexes where, in an attempt to restore ecological function, wetlands are actively managed with precise water level manipulation and soil disturbance. While management regimes have historically focused on providing habitat for waterbird species, these wetlands are home to variety of taxa, and the agency is working towards management that focuses on providing resources for a range of wetland dependent species, including fish and amphibians. Fish and amphibians are ideal taxa to monitor in wetlands because they are sensitive to the environment, so species presence and the general health of the population can be a direct reflection of wetland health and function. Development of efficient sampling procedures is essential for detecting fish and amphibian species using restored wetlands to investigate the distribution and richness of fish and amphibians. Investigating fish and amphibian species will give insight into the

conditions present in Missouri wetlands to help managers determine if management objectives are being met. The objectives of this study were to 1) investigate how sampling method and wetland habitat characteristics influence measures of species richness, 2) determine the sampling effort needed to detect wetland species, and 3) investigate the influence of wetland hydrology, within wetland conditions, and upland habitat on species richness and distribution. We evaluated four sampling methods in 29 wetlands across three regions in the state of Missouri during spring and summer, 2015-1016. Results suggest that a single method, a mini-fyke net, is able to identify the majority of fish and amphibian species in Missouri wetlands with minimal effort but that consistent detection of amphibians across seasons may require additional samples with minnow traps or dipnets. Species richness measures were influenced by method, water depth, distance from shore, and vegetation density, as well as spatial and temporal variables. In general, 6-7 samples with a mini-fyke net detected the majority of fish and amphibian species in a wetland. In addition to spatial and temporal variables, wetland hydrologic connectivity and managed water source were the main factors structuring the distribution and richness of fish and amphibian wetland taxa. This study enhances our understanding of factors influencing fish and amphibian use of restored wetlands and provides conservation practitioners with information to help select the most efficient and effective fish and amphibian sampling methods to meet monitoring objectives.

THESIS FORMAT

The first two chapters of this thesis were written as independent manuscripts intended for submission to peer-reviewed journals. Because of this, there is some overlap in content in the introduction and methods. Separate literature cited sections, tables, and figures follow each chapter. The third chapter is intended for use by resource professionals and is formatted as a handout. A single set of appendices was included for the entire thesis. I have chosen to use plural nouns throughout to include co-authors.

CHAPTER 1

A COMPARISON OF AQUATIC SAMPLING METHODS FOR SURVEYING FISH AND AMPHIBIANS IN RESTORED FLOODPLAIN WETLANDS

Julia G. Kamps, Craig. P. Paukert, Elisabeth B. Webb

ABSTRACT

Being able to monitor fish and amphibian species in restored wetland habitats is essential for effective management of these degraded systems and requires efficient and effective sampling methods. We sampled 29 wetlands across three ecoregions in Missouri to compare the effectiveness of four aquatic sampling gears (mini-fyke nets, minnow traps, dipnets, and seines) to detect fish and amphibian species. We evaluated the influence of method, temporal (spring and summer of 2015 and 2016) and regional variation, and sample site habitat on the number of fish and amphibian species detected and used a resampling procedures to determine the sampling effort needed to detect wetland fish and amphibian species richness by methods. Mini-fyke nets detected more fish species for both spring and summer sampling periods than other methods and captured 100% of all fish species detected across the entire study. The highest percentage of fish species detected by dipnets, minnow traps and seines in a season was 40, 51, and 52%, respectively. Mini-fyke nets caught a higher percentage of the total amphibian species caught in 3 of the 4 sampling periods, but mini-fyke nets, dipnets, and minnow traps all detected 13 of the total 15 amphibian species collected across the study. The number of fish species detected in a sample was affected by wetland fish species richness, season, year, region, and sample site characteristics including distance of the sample from shore, percent vegetation cover, and water depth. The number of amphibian species detected in

a sample was affected by amphibian species richness, year, region, and water depth at the sample site. In general, 6-7 mini-fyke nets will detect the majority of fish species in a wetland unit, but detecting amphibian species in any season will likely require a combination of 6-7 mini-fyke nets and dipnet or minnow trap samples. This information will be useful for establishing efficient and effective monitoring protocols to integrate the needs of fish and amphibian species into wetland management decisions.

INTRODUCTION

Although it is widely known that fish and amphibian species use floodplain wetlands for all or portions of their life cycles, these taxa are not always regularly monitored in these systems. In some areas of the United States, like Missouri, where wetland habitats have been severely degraded or lost entirely (Dahl 1990), the stimulus for wetland restoration was to provide habitat for waterfowl (Galat et al. 1998). These wetlands are frequently surveyed for waterbird species, and information gained from monitoring has guided wetland management practices for years (Galat et al. 1998). Unfortunately, these areas are under sampled when it comes to other, less obvious wetland taxa. Recently, the focus has shifted so that natural resource practitioners seek to manage for a wider range of wetland-dependent species, including fish and amphibians. In order to adequately meet the needs of fish and amphibian species, land managers must be able to evaluate how management actions affect these groups. The ability to survey and monitor the variety of taxa using floodplain wetland areas is essential to comprehensive management of these habitats and requires effective and efficient sampling methods.

Numerous studies have evaluated the effectiveness of different fish or amphibian sampling methods (Fago 1998; Buech and Egeland 2002*a*; Buech and Egeland 2002*b*; Neebling and Quist 2011), but few studies have considered the taxa together (Gunzburger 2007). Separate monitoring protocols have been suggested for each group (Heyer et al. 1994; Bonar et al. 2009), yet there are uncertainties about how successful techniques would be detecting both taxa. There is also uncertainty as to how effective many of the fish sampling methods would be in wetland habitats. While many studies comparing amphibian sampling protocol focus on wetland habitats, the majority of studies comparing fish sampling methods, and most established protocols, are focused on large, lentic water bodies (i.e. lakes and reservoirs) (Pierce et al. 1990; Bonar et al. 2000; Chow-Frasier et al. 2005; Ruetz et al. 2007; Fischer and Quist 2014), coastal/estuarine habitats (Layman and Smith 2001, Samarasin et al. 2017), or lotic systems (i.e. streams and rivers) (Neebling and Quist 2011; Schloesser et al. 2012). The literature is sparse and outdated for fish sampling protocols in wetlands or similar freshwater habitats (Khal 1963; Kushlan 1974; Freeman et al. 1984).

Choosing the most appropriate sampling method can be difficult because of the large number of options available (Heyer et al. 1994; Bonar et al. 2009). When sampling amphibians, one also has to decide whether to target aquatic or terrestrial habitats. Many amphibian populations can be found in both aquatic and upland habitats, but monitoring commonly focuses on aquatic habitats because many amphibians spend a portion of their life cycle in the water and detection tends to be greater in these areas (Heyer et al. 1994). Common methods used to sample aquatic amphibians include dipnetting, funnel traps, breeding call surveys, egg mass surveys, seining, and visual encounter surveys (Heyer et

al. 1994). Techniques used to survey fish include light traps, seines, fyke or hoop nets, drop traps, electrofishing and gill nets (Bonar et al 2009). While many of these methods reflect equipment with similar designs, methods for fish and amphibian sampling do not always overlap.

Many studies comparing fish or amphibian sampling techniques have identified potential biases of sampling equipment (Freeman et al. 1984; Buech 2002*b*; Neebling and Quist 2011). Differences in method effectiveness may be linked to traits of individual species, design of the equipment, or a combination of the two. Larger, more mobile fish are more frequently caught in passive gear (e.g. mini-fyke nets and funnel traps) than in active methods (e.g. dipnets and seines) (Fago 1998; Gunzburger 2007). Lyons (1986) found seining to be most effective in detecting fish species that reside in the middle of the water column than those on or near the substrate. Gears with a smaller mesh size are more successful at detecting smaller individuals (Buech and Egeland 2002*a*; Ghioca and Smith 2007), and size selectivity of sampling methods can lead to different descriptions of species composition (Ruetz et al. 2007). In general, using active equipment requires more skill to ensure capture, while passive equipment relies more on the movement of the target taxa and the ability of the net to retain them once they are captured (Pope et al. 2009). Many studies suggest the inability for a single method to capture all species within each taxa and identify the need for further analysis of sampling techniques (Fago 1998; Buech and Egeland 2002*b*; Gunzburger 2007; Neebling and Quist 2011). The bias caused by certain sampling methods and traits of individual species make it essential that researchers identify the specific objectives of their sampling and choose gear appropriate to meet those objectives.

Species detection is also influenced by site-specific habitat factors. Chow-Frasier et al. (2006) found that fyke nets detected more fish species in poorer quality wetlands than higher quality wetlands based on a suite of physico-chemical, nutrient, and water clarity variables. The amount of vegetation in a sampling area can effect fish detection, and individual fish can go unrecorded if a large amount of vegetation is collected during sampling but not thoroughly inspected (Freeman et al. 1984). Vegetation, like algae, can clog nets with small mesh sizes, making sampling inefficient (Fellers and Freel 1995). Vegetation, woody debris, irregular substrate, and even waterbody morphology (e.g., steep shoreline slopes) may affect seining success (Fellers and Freel 1995, Pope et al. 2009). In addition, the location of the trap in the water body (e.g., nearshore vs offshore; different water depths) may affect detection rates and abundance of some fish species (Blaustein 1989; He and Lodge 1990). Understanding the specific habitat factors that influence detection and where specific sampling methods are likely to have the highest species detections can help managers maximize sampling efforts.

The timing and intensity of sampling can also impact species detection and species assemblage descriptions. The number of species detected generally increases with increasing sampling effort (e.g., number of samples or time spent sampling), but the relationship is typically only true to a certain point when it asymptotes, presumably because the majority of species have been detected (Buech and Egeland 2002*b*; Gunzburger 2007; Fischer and Paukert 2009; Samarasin et al. 2017). In a study in Wisconsin lakes, Fago (1989) determined that, on average, 14 mini-fyke nets were needed to detect all but one species detected overall by that method, whereas Buech and Egeland (2002*b*) found that one day of sampling in small forest ponds detected most

common amphibian species and additional days of sampling improved detection of less abundant species. As one might expect, it has been suggested that more samples are necessary to detect the majority of species in areas with higher species richness (Samarasin et al 2017). Timing of sampling can also influence detection. Within a 24 hour period, passive methods left overnight are more likely to collect a broader range of species than a method deployed only at night or during the day (Pope et al. 2009). Seasonality of sampling can greatly influence species detection probability, as some species only reside in areas during specific times of the year for activities like breeding and development (Turner et al. 1994; Killgore and Baker 1996; Smith et al. 2006). Sampling during summer or fall resulted in higher fish detections than spring sampling in Iowa lakes and reservoirs (Fischer and Quist 2014), and amphibian detection probabilities were higher during spring and summer in a study of the Okefenokee Swamp (Smith et al. 2006). If the objective is to document a rare species, or species with short breeding seasons or larval periods, detection is more likely if sampling is repeated and concentrated during times of the year when those species are known to use wetland habitats and conditions are suitable (Bishop et al. 2006; Gunzburger 2007; Farmer et al. 2009).

Persistence of freshwater fish and amphibian species will likely depend on developing a greater understanding of how these taxa use wetland ecosystems and applying this knowledge to preserve, restore, and manage wetlands to adequately meet the unique life-history needs of these species. Many of the professionals tasked with managing these complex ecosystems have limited time and resources available for sampling efforts, so knowing the most efficient and effective monitoring methods is

essential. The objectives of this study were to: 1) compare fish and amphibian species detection by four aquatic sampling methods, 2) evaluate the influence of habitat characteristics and sampling procedure (both temporal and geographic) on species detection within a sample, and 3) determine the sampling effort needed to detect fish and amphibians using different methods. Although study sites were located in Missouri, the results of this study are likely applicable to wetlands in other regions that exhibit similar habitat characteristics and fish or amphibian assemblages.

METHODS

Study area

This study was conducted on intensively managed, public wetland complexes located in five Conservation Areas (CA), managed by the Missouri Department of Conservation, and one National Wildlife Refuge (NWR), managed by the U.S. Fish and Wildlife Service. These study sites span three distinct ecoregions of Missouri, and are equally distributed among the Central Dissected Till Plains, Osage Plains, and Mississippi River Alluvial Basin ecoregions (Nigh and Schroeder 2002) . Specific study areas include: Duck Creek CA, Otter Slough CA, Schell Osage CA, Four Rivers CA, Fountain Grove CA, and Swan Lake NWR (Fig. 1). Water sources varied for each study site and included direct connection by overflow of creeks and rivers, ground water delivered by pump systems, precipitation, and water diverted from rivers or creeks through a series of wetland units or ditches. Levees and water control structures divided each of the study areas into separate wetland management units and allowed for precise control of water levels. Most moist-soil impoundments contained deep borrow ditches

alongside the levees that may hold water for most or all of the year (Fredrickson and Taylor 1982).

Habitat characteristics of managed wetlands vary by season, weather events, and management goals and capabilities (Fredrickson and Taylor 1982). Sampling was performed across a range of wetland depths, from 2 to 288 cm (Table 1). Inundated area of sampled wetlands ranged from 0.3 to 111.1 hectares (Table 1). Vegetation composition within seasonally flooded wetlands varied substantially among season and years, due to factors such as the timing of flood and drawdown events and natural weather conditions (Fredrickson and Taylor 1982). Naturally occurring vegetation was distributed along hydrologic gradients within wetland units, and coverage at sampling points within wetland units ranged from 0-100% (Table 1). Typical wetland flora included smartweeds (*Polygonom spp.*), beggarticks (*Bidens spp*.), barnyardgrasses (*Echinochloa spp.*), spike rushes (*Eleocharis spp.*), crabgrasses, and panic grasses (Fredrickson and Taylor 1982). Agricultural row crops, primarily corn (*Zea mays*), are commonly found in these publically managed wetlands, mainly to serve as a food resource for waterfowl (Fredrickson and Taylor 1982). While most managed wetlands are manipulated under similar management regimes, they differ in the level and degree of manipulation. Disturbance can be applied in several ways including extended inundation, prescribed burning and mechanical manipulation (Fredrickson and Taylor 1982). Mowing, tilling, and disking are all common methods of mechanical manipulation used in moist-soil management (Fredrickson and Taylor 1982).

Fish and amphibian sampling

We sampled 29 wetland units at 6 study areas in spring and summer across two

years; from 4 April to 30 May and 9 July to 19 August 2015 and 7 April to 12 May and 5 July to 27 August 2016. Sampling was performed seasonally to capture pre-drawdown conditions in the spring and pre-flooding conditions in the summer. We sampled four randomly selected wetland units with mini-fyke nets, minnow traps, dipnets, and seines at each study area during each season and sampled the same wetlands in both years if possible. Individual sampling sites within a wetland unit were stratified between two categories: areas within 20 m of the shoreline and all other flooded area within the unit. The area outside the 20 m nearshore area was divided into thirds, and the offshore sample was randomly located in one of the three sections according to a randomly generated list.

The initial sample site in a wetland was a location on the perimeter of the wetland determined by randomly selecting a compass degree between 1 and 360; the first sample was placed at that degree, within the first 20 m from shore, with a paired sample offshore. Selection of specific gear was done using a randomly generated list. Moving along the perimeter of a wetland, subsequent pairs of samples were placed at random distances from the previous nearshore sample according to the estimated perimeter of the wetland and the goal of collecting 10 samples using each sampling method per wetland (~40 samples/wetland). However, the number of samples within a wetland was dependent on the inundated size of the wetland at the time of sampling (N=7-36), and wetland area fluctuated greatly across seasons. In an effort to reduce autocorrelation between samples while maximizing the number of samples within each wetland unit, samples were located a minimum of 50 m apart. Exact distance needed to eliminate autocorrelation was unknown, but we chose a distance multiple times greater than distances documented by other researchers (Heyer et al. 1994; Buech and Egeland 2002*a*). At each sample site

observers recorded the date, study site, wetland unit, sample number, method, start/set time, end/collection time, site location (DD°, MM', SS.s"), and weather conditions.

Mini-fyke nets (60 by 120 cm frame, 7.6 m lead, and 0.32 cm mesh) were set by staking the lead from shore or other barrier within the wetland (e.g., hunting blind or downed trees) and extending the remainder of the net into the wetland. Minnow traps with a 0.32-cm mesh and 2.54-cm openings were attached to an anchored stake at randomly assigned sampling sites. Mini-fyke nets and minnow traps were set in mid-afternoon/evening and left overnight until the following day to include both diurnal and nocturnal species (Heyer et al. 1994; Bonar et al. 2009). Soak times ranged from 9-24 hours.

We used a 1.2 m x 4.5 m seine with 0.32-cm mesh at randomly selected sampling sites by fully extending the seine, one person holding each end, and walking with the seine for 6 m (Bonar et al. 2009). Dipnet sampling, with a 40 x 23 x 30.5 cm frame size and 0.32 cm mesh, consisted of 10-15 sweeps distributed across all available habitat at the randomly assigned sampling location (Heyer et al. 1994; Mensing et al. 1998; Hamer and Parris 2011). The dipnet was operated by bumping the net over the substrate, pulling the net towards the observer with each sweep.

All fish and amphibians captured were placed into aerated live wells and identified to species, enumerated and returned to the water. Additionally, fish total length (TL) was measured to the nearest 1 mm. If identification was impractical in the field, voucher specimens were collected and identified in the lab at the University of Missouri. To limit the number of individuals euthanized, unidentified individuals were grouped according to visual similarities and up to five voucher specimens were collected from

each group. If an individual was suspected to be rare, it was photographed to verify identification and promptly released. Voucher specimens were euthanized and preserved using a 10% formalin solution or 70% ethanol (Heyer et al. 1994; Kelsch and Shields 1996). All observers were trained in fish and amphibian identification prior to field sampling and fisheries biologists were consulted to help verify detections. All sampling was conducted under MDC permit #16717 and University of Missouri Animal Care and Use Committee permit #8211.

Sample site characteristics

Habitat characteristics were measured and recorded at each sampling site. Water depth (cm) was measured next to the mouth of minnow traps and mini-fyke nets and in the middle of the dipnet and seine sampling sites. Habitat complexity within 2 square meters of each sampling location was visually estimated and defined by the percentages of the area consisting of vegetation or open water. Vegetation was further described by estimating the proportion of the vegetation comprising categories similar to those described by Burne and Griffin (2005) with the addition of agriculture crops. Vegetation categories included: submergent vegetation (i.e. coontail and elodea), emergent vegetation (i.e. cattails, smartweed, and sedges), small woody vegetation (i.e. button bush and rose mallow), agricultural crops (i.e. corn), trees (i.e. willows) or other (i.e. floating vegetation like duckweed). Distance of the sampling location from the nearest shore (m) was measured using a range-finder or estimated by the observer. The inundated area of the wetland (ha) was verified using ArcGIS after visually estimating the extent of flooded area within wetland units at the time of sampling.

DATA ANALYSIS

We pooled species detections from all wetlands to summarize the number of unique and total species detected by each method across seasons and years. We considered the combined presence data from all samples in a wetland to represent the total number of species available for detection in that wetland unit. We used a Kruskal-Wallis rank sum test, a non-parametric test that allows for comparison of more than two groups of non-normally distributed data, to assess whether median fish total length (TL) differed among our four sampling methods. To determine differences in fish TL between specific methods we used a pairwise Wilcoxon signed-rank test with correction for multiple comparisons. We used generalized linear models to test the effects of sample site habitat conditions, temporal variables, and method on the number of species detected in each sample. Models were fit using the zeroinfl function (Zeileis et al. 2008) in the "pscl" package (Jackman 2017) which was able to incorporate the over-dispersion and excess zeros found in the data (Su and He 2013). We included combinations of covariates thought to explain the factors influencing the number of species caught in each sample, as well as interactions between the method being used and sample site habitat covariates (Table 2). Covariates included the overall fish or amphibian species richness of the wetland unit [SpRich], sampling method [Method], water depth [Depth], percentage of sample site that was vegetated [Veg], inundated area of the wetland [WetArea], distance of the sample from shore [Dist], region [Region], season [Season], and year [Year] (Table 1). Because zero-inflated models have two design matrices (one for the count component and one for zero component) we attempted to include the same covariates in both components of each model. All fish models included the same parameters in the count

and zero component of the model. For certain combinations of variables in the amphibian data, the number of observations (i.e. number of samples detecting > 4 species in the OP region) could be low or even zero, and led to convergence problems when estimating the parameter estimates for those regression terms. Therefore, we simplified amphibian models and included only sampling method as a covariate in the zero component of the model. We ranked models using AIC (Burnham and Anderson 2002) and using the most supported model, we predicted the number of fish or amphibian species collected in a sample across the ranges of significant covariates while holding all other covariates constant at the mean and averaging across season, region, and year. We considered a covariate to be significant when the 95% confidence interval did not overlap zero. All modeling was done in program R (R Core Team 2015).

To evaluate the influence of season and sampling effort on species detection we constructed species accumulation curves for each sampling method using the specaccum function in package "Vegan" (Oksanen et al. 2015) and program R (R Core Team 2015). This functions estimates mean species accumulation based on number of samples and calculates standard deviation from random permutations of the data, or sub-sampling without replacement. We ran 1000 permutations, excluding data where fewer than 2 species were detected in a wetland, and averaged species accumulation across all wetland units sampled within each seasonal period. We used these curves to determine the mean percentage of species detected in a wetland with increasing numbers of samples with each method (Fischer and Paukert 2009; Neebling and Quist 2011).

RESULTS

Over the two year period we sampled 29 wetlands and collected 2,176 samples.

Due to differences in wetland conditions, the number of samples collected varied by method across seasons and years (Table 3). In 2015 we collected 1,178 samples and in 2016 we collected 998 samples. In 2015 we collected 692 samples in the spring and 486 in the summer. In 2016 we collected 633 samples in the spring and 365 in the summer. Between 2015 and 2016, we detected a total of 15 amphibian and 54 fish species across all study areas (Appendix A, Table 1 and 2). Five of the 54 fish species were considered Missouri species of Conservation Concern (SOCC) including bantam sunfish (*Lepomis symmetricus*), brown bullhead (*Ameiurus nebulosus*), flier (*Centrarchus macropterus*), lake chubsucker (*Erimyzon sucetta*), and starhead topminnow (*Fundulus dispar*). We detected both adult and juvenile individuals of both taxa.

Wetland species richness varied spatially and temporally across both taxa. Fish species richness ranged between 0 to 24 species among individual wetlands (Table 1), and total species detected in a season ranged from 34 to 48 (Table 4). Amphibian species richness ranged between 0 to 11 among individual wetlands (Table 1), and overall detection by season ranged from 8 to 13 species (Table 4). Overall, we detected differing numbers of fish species in different seasons, but detected the same number of amphibian species during both sampling seasons each year (Table 4). We captured 213,553 total individuals comprised of 64,158 individual amphibians and 149,395 individual fish and number of individuals caught varied widely by season (Table 5). We caught greater numbers of fish in summer seasons and greater numbers of amphibians in spring seasons (Table 5).

Species detection by method

Mini-fyke nets consistently caught greater numbers of fish species in both seasons

than the other sampling methods (Table 4). Mini-fyke nets detected 100% of total species detected in three of the four sampling periods and 91% of the total species detected in the other (summer of 2016). Dipnets, minnow traps or seines did not capture more than 52% of the total fish species pool detected within a sampling season. The percentage of fish species detected by dipnets (21-40%) and seines (26-52%) fluctuated across seasons while minnow traps were more consistent (48-51%). Across all seasons and years, mini-fyke nets detected all 54 fish species while dipnets, minnow traps and seines detected 22, 30, and 31 species, respectively.

Mini-fyke nets were the only method to detect unique fish species in all seasons, capturing between 14 and 18 unique species, depending on year and season (Table 4). Nineteen fish species were detected by all four methods across both years, and 17 species were detected only by mini-fyke net sampling. Of the five SOCC species detected, four (Bantam pygmy sunfish, Flier, Lake chubsucker, and Starhead topminnow) were detected by all sampling methods, while one species (Brown bullhead) was only detected by mini-fyke nets and minnow traps. The Kruskal-Wallis rank sum test determined there were differences in the median size of fish captured by different methods ($P<0.001$). Fish captured in mini-fyke nets (median TL=100 mm, SE=4.0 mm) were significantly larger than fish captured by minnow traps (median TL=45 mm, SE=1.7 mm), whereas fish captured by dipnets (median TL=30 mm, SE=0.8 mm), were similar in length to fish captured by seines (median TL=25 mm, SE=1.1 mm) (Fig. 2; Wilcoxon signed rank test, $P<0.001$).

The number of amphibian species detected by each method varied by season and year (Table 4). Mini-fyke nets detected the greatest percentage of amphibian species in

three out of four sampling periods, catching ≥85 percent of total species detected. Seines consistently detected the lowest number of species in each season (25-62%) whereas dipnets detected between 75-77% of species detected in three out of the four seasons and 38% of the total species in the other. Minnow traps caught between 50-85% of the species detected each season. However, sample size varied by method across seasons and years (Table 3), and counts of overall species detected during a season did not account for sample size. Mini-fyke nets and seines detected the most and least, respectively, number of the species while having the lowest or second lowest number of samples each season (Table 3). There were similar numbers of dipnet and minnow trap samples within each season (Table 3).

Three gears detected unique amphibian species throughout the study (Table 4). Mini-fyke nets detected unique species in three out of the four sampling periods, dipnets detected unique species in two sampling periods, and minnow traps detected unique species in one sampling period. Seines never detected any unique amphibian species. Over the entire two year study period, dipnets, mini-fyke nets, and minnow traps each detected 13 of the 15 total amphibian species, while seines detected nine. Seven amphibian species were detected by all four methods. One amphibian species, Plains leopard frog, was only detected in mini-fyke net samples.

Factors affecting number of species caught in a sample

The number of fish species caught in a sample was best explained by the global model (Table 6). The most significant parameters predicting the number of fish species caught were fish species richness of the wetland, region, season, year, and interactions between method and water depth, distance of the sample from shore and percent of

vegetation covering the sample site, but the degree of relationship of the latter three depended upon the method used (Table 7). The number of fish species detected increased with fish species richness (Fig. 3a). In addition, region MRAV had greater numbers of species detected compared to OP or CDTP, more species were detected in summer, and less in 2016.

To determine the effect of depth, distance, and vegetation on number of species caught with each method we ran the global model separately for each method and used these models to predict number of fish species caught in a sample across the range of significant variables. The number of species caught increased with water depth with seines, but not the other gears (Fig 3b). Fewer species were detected with increasing distance from shore across all methods, but was most pronounced for dipnets and mini-fyke nets (Fig. 3c). Samples at sites with greater vegetation caught fewer species with dipnets but greater species with minnow traps (Fig. 3d). However, no significant relationships were evident between vegetation and species collected for mini-fyke nets and seines (Fig. 3d).

Similar to the fish data, the Global model ranked as the top model for predicting the number of amphibian species caught in a sample, and no other model came within 2 AIC units (Table 6). Significant parameters within this model were the amphibian species richness of the wetland, sampling method, the water depth at the sample site, region, and year (Table 7). The number of species collected was higher in 2016 than 2015, and sampling in the Osage plains region yielded fewer species detected per sample. The 95% confidence intervals of the interaction terms between method and sample site habitat characteristics all overlapped zero. Because no interactions were significant, all methods

were similarly influenced by the effects of water depth on amphibian species detection. The number of species caught increased with amphibian species richness (Fig. 4a) and decreased with greater water depth at the sample site (Fig. 4b). Therefore, amphibian species richness and water depth were the primary drivers in determining the number of species collected.

Sampling effort

On average, each number of mini-fyke net samples caught a higher percentage of total fish species detected than the other methods during every sampling season (Fig. 5). A single mini-fyke net sample caught an average of 50.2-57.4% of the total fish species detected in a wetland across all four seasons, whereas a single sample of the other three methods detected between 3.0-24.7% of the total species. With 2 to 3 samples, mini-fyke nets detected approximately 75% of total fish species. Mini-fyke nets were able to detect an overall average of 100% of the total species in a wetland with seven samples in three of four seasons (Fig. 5a, c, and d). Even with as many as ten samples, no other method was able to detect greater than an average of 70% of total fish species, and most did not detect greater than 40% overall.

There was a greater range in the percentage of amphibian species detected by different methods with varying levels of sampling effort. Mini-fyke nets detected a greater average percentage of the total species with fewer samples than all other methods in both spring sampling seasons (Fig. 6a and 6b), collecting a total average of 100% of species detected with 7-8 samples. Mini-fyke nets collected a lower total percentage of species during summer seasons than minnow traps (Fig. 6c) or dipnets (Fig. 6d), but were generally more efficient with fewer samples. With nine or ten samples, minnow traps

detected 100% of species in two of the four sampling periods (Fig. 6b and 6c) but lower percentages in other sampling seasons (48.7% and 70.7%) (Fig.6a and 6d). Dipnets were also inconsistent across sampling seasons, detecting between 58.3 and 100% of species with a maximum sampling effort of ten samples. With 7 samples, seines were able to detect as much as 88.8% of the total species detected in a wetland (Fig. 6b) but were left out of amphibian analysis in summer 2016 due to low sample numbers and the inability to detect more than one species in a wetland.

DISCUSSION

Our findings suggest that a single method, a mini-fyke net, was able to detect the majority of fish and amphibian species found in Missouri wetlands with minimal effort, but consistent detection of amphibians across seasons may require additional samples with minnow traps or dipnets. Differences in taxa and heterogeneous conditions exhibited by these wetlands throughout the year and across years, likely contributed to variation in method effectiveness. Our results indicate that with a few sampling gears and a day or two of effort, wetland managers can gain insight into the fish and amphibian species using wetland areas to make more informed management decisions. While this study was conducted in the state of Missouri, this information is likely applicable across a range of wetland types with comparable habitat characteristics and similar species assemblages.Mini-fyke nets were able to detect the majority of, if not all, fish species during seasonal sampling periods, suggesting a single method sampling approach is sufficient for surveying fish in wetlands. Our results are supported by Henning et al. (2007) who anecdotally determined fyke nets (compared to electrofishing, seining, or minnow traps) were the preferred method for estimating fish abundance and

richness in floodplain wetlands in Washington. Similar to our results, fyke nets detected greater numbers of individual fish, fish species, and unique fish species compared to a pop net method in a study by Mazumder et al. (2005). Seines were the least effective fish sampling method in our study, and similar results were seen by Freeman et al. (1984). Gunzburger (2007) detected similar numbers of fish species with minnow traps, and dipnets but determined there were differences in catch efficiency of specific species among methods. Many of the other methodological studies evaluating fish sampling methods in wetlands, or similar vegetated habitats, focus on drop traps, making comparison with our study difficult (Kushlan 1974; Kushlan 1981; Chick et al. 1992; Jordan et al. 1997).

Differences in type (i.e. passive vs active) and design (i.e. size) of sampling methods might have contributed to differences in species detected by different methods. We found differences in the number of species detected by passive and active sampling methods, and we would have greatly underestimated fish species richness if only using data from active sampling methods, which is similar to Layman and Smith (2001). Dissimilar catch rates by passive and active methods may be partially influenced by differing behavioral traits of individual species. Fago (1998) found that more mobile fish are likely to be captured in passive methods, while more sedentary fish are more susceptible to capture by active methods. Many fish move from river channel habitats into floodplain wetlands seasonally or during specific life stages (Copp and Penza 1988; Junk et al. 1989; Burgess et al. 2013), and this active movement may make them more susceptible to passive sampling methods. Passive methods, like mini-fyke nets and minnow traps, were used during both day and night while active methods, like seines and

dipnets, are typically only used during the day. Differences in temporal usage of active vs. passive methods may lead to differences in species detected as some species are more active at night (Pflieger 1975). Gritters (1994) suggested that differences in the timing of passive and active methods contributed to differences in effectiveness of seines and mini-fyke nets in a Mississippi River slough. These trends were also seen in studies done in lake habitats by Fago (1998) and Fisher and Quist (2014). In addition to differences in passive and active methods, the size and dimensions of individual nets may contribute to variation in catch efficiency as this hypothesis is well documented by sampling guidelines and scientific studies (Laarman and Ryckman 1982; Bonar et al. 2009; Portt et al. 2006). Mini-fyke nets had the largest dimensions of all sampling methods and may have been more successful at detecting greater numbers of species because they were able to catch fish across a greater range of body sizes (Fig. 2). Mini-fyke nets caught large-bodied riverine species that may have been using the wetlands as breeding and foraging areas (e.g., freshwater drum) and smaller, young-of-year fish that may have been using wetlands as refuge from volatile river conditions and as nursery areas (e.g. juvenile crappie and sunfish) (Kilgore and Baker 1996; Zeug and Winemiller 2007). Smaller methods like a minnow trap may have only detected a portion of the species present, excluding individuals too large to enter the trap (Bloom 1976). While some studies attribute size selectivity of methods to varying mesh size (Shultz 2005), all methods used in this study had the same mesh size.

Even with reduced sampling during some seasons (Table 3), mini-fyke nets were still able to detect the majority of fish species and more species than any other method (Table 4). Low water levels and dense vegetation during summer reduced wetland sizes

and restricted the number of sampling locations within a wetland, particularly for mini-fyke nets and seines that require deeper and more open water to be effective. In an attempt to deploy multiple samples of all four methods and maintain spacing of at least 50 m, we had to compromise between the number of methods deployed and the number of samples of each method. In general, trends indicate that even with increased samples, seines are unlikely to detect similar numbers of species as a mini-fyke net. These findings suggest that our methods are applicable in wetlands across a range of hydrologic conditions, including low water levels during dry years.

Detection of amphibian species was more similar among methods in most seasons than fish. While the mini-fyke net detected more species in most seasons, no method detected all of the species in any season, supporting the suggestion that multiple methods may be required for best explaining overall amphibian species assemblages (Heyer et al. 1994; Buech and Egeland 2002*b*; Gunzburger 2007). Dipnets, mini-fyke nets, and minnow traps each detected a unique species, so a combination of these methods might produce the best overall description of the amphibian species present. In Missouri private wetlands, dipnets and minnow traps were deemed appropriate to detect amphibian species (Mengel 2010), further suggesting multiple gears may be needed to assess wetland communities. Our results are complimented by Buech and Egeland (2002*b*) and Gunzburger (2007) who suggested a combination of methods (dipnets, minnow traps and breeding call surveys) was the most effective way to assess species community composition. While breeding call surveys may be effective for detecting amphibian species, we only evaluated methods capable of detecting both fish and amphibians, eliminating aural surveys as a practical option. Seines consistently detected the lowest

percentage of species caught in a season, indicating that it is not a useful sampling method for describing amphibian species assemblages. However, this analysis did not take number of samples into account and some methods might have detected more species overall simply because more samples were taken.

The number of fish species detected in a sample of any method was influenced by season and year. We suspect this was likely due to fluctuations in wetland condition and number of species present. We detected greater numbers of fish species in 2015 than 2016 and greater numbers of individuals in summer of 2015 than any other season. Summer sampling may lead to more fish species detected per sample simply because there are more individuals available to catch (Royle and Nichols 2003). We detected a higher number of individual fish during summer sampling seasons than spring sampling seasons, even though we were not able to set as many samples. Our modeling indicated that higher overall species richness of a wetland contributed to more species detected per sample, and high species detection in summer of 2015 may have disproportionately skewed model results. Inconsistencies in seasonal detections in our study were similar to findings by Fischer and Quist (2014) who determined that lake fish detections were higher during summer or fall rather than in spring. Haynes et al. (2013) found day and year both contributed to differences in species detections. Variation in number of species detected in a sample across years and seasons might be explained by differences in observed hydrologic patterns. Many study sites experienced frequent flooding in 2015, and summer sampling often followed recent flood events (Appendix C, Fig.1 and 2), which may have introduced additional species to wetland units, increasing the number of species detected. This hypothesis is supported by studies that have documented fish

moving into wetlands during flood events (Burgess et al. 2013) and increased abundance of fish in wetlands with increased river-wetland connections (Beesley et al. 2012). In contrast, many wetland managers had to alter flooding regimes in 2016 to keep wetland water levels low to accommodate Lidar imaging coverage. This may have contributed to fewer species detected during this year overall and in individual samples.

Site specific habitat characteristics impacted the number of fish species detected but effects often varied depending upon the method. Unexpectedly, we found the number of species detected in a seine sample increased with water depth, but we saw no significant trends with other methods. Explanations for this trend were not immediately clear but may be related to the volume of water sampled by a seine at different depths. Presumably, a greater volume of water is sampled at a depth of 100 cm than a depth of 10 cm, and sampling a greater area is likely to detect more fish. Dewey et al. (1989) similarly hypothesized that the amount of area sampled may have contributed to differences in fish collections by seines and pop nets. Fish exhibit preferences for specific microhabitat conditions, including different depths (Baltz et al. 1993), so sampling at greater depths could mean sampling a wider range of microhabitats, increasing the number of species detected. Even with increased species detection in deeper water, seines did not detect as many species as a mini-fyke net.

We saw opposite relationships between amount of vegetation and number of species caught with different sampling methods and hypothesize that contradictory effects might be based on the passive vs active nature of different nets. Dipnets, which works by sweeping through the water, caught fewer species with increasing vegetation density while minnow traps, a stationary trap, caught greater number of species with

increasing vegetation. In areas with dense vegetation and higher habitat complexity, nets can frequently become snagged, potentially allowing individuals to escape and decrease the number of species detected (Pierce et al. 1990; Parsley et al. 1989; Lyon 1986). Denser vegetation may have prevented dipnet sweeps from coming in contact with the substrate (as is recommended procedure), enabling individuals to evade capture or escape (Heyer et al. 1994; Fellers and Freel 1995). Similar to our findings, Pierce et al. (1990) recorded negative relationships between increased vegetation and fish capture efficiency with seines and attributed it to the inability to drag a net across rough substrate. Dipnetting in dense vegetation often resulted in large amounts of vegetation and debris in the net and some individuals may have been removed with the debris and not recorded. A study by Freeman et al. (1984) supports this hypothesis as they recovered 17% of fish post-sampling while sorting detritus caught in dipnets in a laboratory. We may have seen the opposite trend with minnow traps because they are small and easily nestle into vegetation (Portt et al. 2006). Cover-seeking species are especially susceptible to capture in passive sampling methods (Hubert 1996), and increased vegetation surrounding minnow trap samples may have attracted these species and increased the likelihood of those species entering the trap for refuge. This hypothesis is supported by studies like that by Layman and Smith (2001) that found fish species that aggregate in structurally complex habitats were attracted to the refuge created by a minnow trap. Contrary to our findings, other studies have seen no or negative impacts of vegetation on minnow trap efficiency (Blaustein 1989; Dupuch 2011). We might not have seen an impact of vegetation on mini-fyke net or seine performance because setting a mini-fyke net sometimes involved flattening the vegetation at the sample site, altering the natural

vegetation cover, and areas with very thick vegetation are known to cause issues when seining (Pierce et al. 1990; Parsley et al. 1989; Lyon 1986) and may have been inadvertently avoided by the observers.

While we only had significant parameter estimates with mini-fyke nets and dipnets, trends indicate that greater numbers of fish species are detected in samples closer to shore. This relationship could be attributed to fish microhabitat preferences, movement patterns, or observer disturbance. Ecotone habitats, like those found on the edge of our study wetlands, may exhibit greater habitat complexity (Chapman et al. 1996) and greater species richness (Tews et al. 2003), increasing the likelihood of capturing more species per sample closer to shore. Similar to our findings, Peterson and Turner (1994) and Baltz et al. (1993) recorded high fish density and species richness near the water's edge in Louisiana marsh habitats. Fyke nets are especially effective at capturing fish species that tend to travel along shorelines (Hubert 1996), so mini-fyke nets set in areas away from shore may have missed these species and captured fewer species overall. Mini-fyke nets samples set further from shore, in the interior of the wetland, were frequently set from raised duck blind mounds or downed trees. Compared to the impervious barrier created by continuous shorelines, these determinate structures might allow fish species to more easily evade capture by simply going around the structure and not entering the net. Furthermore, sampling at a greater distance from shore may have been less successful due to disturbance by researchers. Although we attempted to limit disturbance as we moved throughout wetland units, walking to the interior of units may have caused individuals to flee sample areas as we approached or altered microhabitat conditions (i.e. flattened vegetation), causing individuals to avoid these areas.

Water depth at the sample site was the only variable that had a significant effect on the number of amphibian species detected in a sample across all methods suggesting that managers sampling for amphibians may need to consider wetland depth during their survey efforts. Many species of amphibians prefer to breed in shallow, ephemeral wetland areas (Babbitt and Tanner 2000; Semlitsch 2000) and studies have found higher amphibian richness in wetlands containing shallow littoral zones (Porej and Hetherington 2005). Amphibians are known to alter their behavior and habitat choice in water containing fish (Kats and Sih 1992; Hopey and Petranka 1994; Hecnar and M'Closkey 1997), and fish may have collected in deep borrow pits within wetlands. Amphibians may have thus congregated in the shallower areas of these wetland units, leading us to detect fewer species in samples located in deeper water.

A total of 6-7 samples with a mini-fyke net is likely to detect the majority of fish and amphibian species in a wetland, but additional minnow trap or dipnet samples may be necessary to detect maximum numbers of amphibian species during dry conditions. Trebitz et al. (2009) determined a similar number of sample sites were necessary in a study using electrofishing to sample fish in coastal wetlands. Studies done in lakes and impoundments indicate more mini-fyke net samples (10-20) or a combination of mini-fyke nets and several other methods are required to characterizes fish assemblages and detect >90% of species (Fago 1988; Fisher and Quist 2014). Species accumulation rates of minnow traps, seines, and dipnets in our study suggest that 10 samples is not adequate to detect the majority of fish species within a wetland and that those sampling methods would never detect the high number of species detected by mini-fyke nets even with increased sampling effort. This contrasts with results found by Gunzburger (2007) who

determined fish species detection leveled off after 7-8 samples using dipnets and minnow traps, and Samarasin et al (2017) who determined that 97 seine samples were required to detect 90% of fish species in wetlands. In our study, seines never detected 90% of species and trends indicate they could not have detected that percentage, even with more samples. Minnow traps and dipnets each detected the greatest overall percentage of amphibian species during one summer sampling period, but curves for mini-fyke nets were similar and they detected the greatest number during both spring periods with fewer samples. Mini-fyke nets were able to detect almost all amphibian species in a wetland with 7-8 samples during spring sampling periods, while minnow traps and dipnets detected greater percentages during summer seasons with 9 and 7 samples, respectively. This was similar to results seen by Gunzburger (2007) who found that 10 samples of dipnets and minnow traps are likely to detect most wetland amphibian species. Seines never detected the greatest percentage of species and is likely not an ideal survey method for vegetated wetlands. Decreased water levels within wetland unit during the summer season prevented us from deploying as many mini-fyke net or seine samples. With fewer samples, we may not have been able to set enough samples to detect the majority of amphibian species. If sampling is restricted to times when water levels are low, dipnets and minnow traps may be more effective sampling methods than a method like a mini-fyke net, which requires deeper water.

MANAGEMENT IMPLICATIONS

Our study suggests that mini-fyke nets are the most effective method for detecting fish species and likely a combination of mini-fyke nets and another method are most effective for detecting amphibian species, especially under certain conditions (e.g., low

water levels or dense vegetation) that restrict the use of some methods. Our research was based on the idea that managers often have limited time and resources to dedicate to sampling efforts and may not be able to use multiple sampling methods, as suggested by most protocols. Our results recognized the strengths and weakness of different sampling methods and identified the sampling intensity necessary in managed wetlands, thus providing managers and researchers with better information on the capabilities and limitations of various levels of sampling effort. If a manager was specifically trying to sample fish, our results indicate that sampling close to shore is more effective than sampling in the interior of wetland units, which also likely creates less disturbance within the wetland and is less time and labor intensive. Vegetation and water depth did not seem to impact fish species detection by mini-fyke nets indicating that mini-fyke nets should be effective fish sampling methods across a range of wetland conditions. We detected more species overall and in individual samples during the summer season suggesting that sampling during the summer is likely the most efficient time to sample fish species.

If amphibians are the target of sampling efforts, our research suggests that samples may be directed towards areas with shallower water depth to detect the greatest number of species per sample, regardless of season. While mini-fyke nets generally detected the most species each season and more species with each sample, it may be beneficial to use more than one sampling method to accurately describe a more complete assemblage of amphibian species in a wetland unit. Minnow traps and dipnets detected similar number of species across seasons and required similar effort to detect the majority of species in a wetland. Methods reviewed in this study would likely work well in

conjunction with other amphibian survey techniques, such as aural surveys, that specifically target adult amphibians (Heyer 1994).

A combination of mini-fyke nets and another method (either minnow trap or dipnets) will likely produce the most accurate description of both fish and amphibian species present in a wetland unit. Avoiding areas with extremely dense vegetation or deep water may improve species detection of both taxa. Sampling closer to shore will decrease the effort required by managers without any cost to results. Depending on the objectives of monitoring efforts, sampling during summer may be sufficient for detecting the highest number of species, but sampling across multiple seasons will likely give greater insight into which species are using a wetland, their habitat preferences, and how they may respond to management actions. This study helps reduce uncertainty regarding the extent of sampling needed and recognizes the advantages and drawbacks of using individual sampling methods to monitor fish and amphibian taxa. Our research serves as a necessary step in answering future, wetland-related fish and amphibian research questions.

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TABLES

Table 1. Descriptive statistics of sample site characteristics and landscape scale factors for study of relationships between fish and amphibian species detection in Missouri wetlands, 2015-2016.

Covariate	Abbreviation	Mean	Min	Max	SD
Amphibian species richness	AmphSpRich	2.8	0	11	1.99
Fish species richness	FishSpRich	10.4	0	24	6.09
Vegetation cover (%)	Veg	12.5	0	100	16.4
Distance from shore (m)	Dist	23.3	0	300	42.4
Water depth (cm)	Depth	33.9	3	288	21.3
Wetland area (ha)	WetArea	18.8	0.2	111.1	23.3
Region (CDTP/OP/MRAV)	Region	--	--	--	--
Season (Spring/Summer)	Season	--	--	--	--
Year (2015/2016)	Year	--	--	--	--

Table 2. Models and covariates for zero-inflated regression models for number of fish and amphibians caught in a sample. All amphibian models include only "Method" in the zero component of the model. Fish models include the same variables in the count and zero component of the models.

Model type	Model Name	Variables in count component
Fish models	Null	FishSpRich
	Method	FishSpRich, Method
	Region	FishSpRich, Method, Region, WetArea
	Temporal	FishSpRich, Method, Season, Year
	Interaction	FishSpRich, Method, Depth, Veg, Dist, Method*Depth, Method*Veg, Method*Dist
	Global	FishSpRich, Method, Depth, Veg, Dist, Region, Season, Year, Method*Depth, Method*Veg, Method*Dist
Amphibian models	Null	AmphSpRich
	Method	AmphSpRich, Method
	Region	AmphSpRich, Method, Region, WetArea
	Temporal	AmphSpRich, Method, Season, Year
	Interaction	AmphSpRich, Method, Depth, Veg, Dist, Method*Depth, Method*Veg, Method*Dist
	Global	AmphSpRich, Method, Depth, Veg, Dist, Region, Season, Year, Method*Depth, Method*Veg, Method*Dist

Table 3. Total number of samples taken with each sampling method during spring and summer sampling seasons, 2015-2016, across six study sites in the state of Missouri, USA.

	2015		2016	
Method	Spring	Summer	Spring	Summer
Dipnet	182	171	168	121
Mini-fyke net	132	75	144	76
Minnow trap	221	173	164	121
Seine	157	67	157	47

Table 4. Total number of species detected, percent of total species detected (in parenthesis), and number of unique species of fish and amphibians detected by each sampling method during spring and summer of 2015 and 2016, across six study sites in the state of Missouri, USA.

	2015				2016			
	Spring		Summer		Spring		Summer	
	Species	Unique	Species	Unique	Species	Unique	Species	Unique
Fish								
Dipnet	14 (40)	0	18 (38)	0	8 (21)	0	12 (35)	0
Mini-fyke net	35 (100)	14	48 (100)	18	38 (100)	16	31 (91)	18
Minnow trap	18 (51)	0	23 (48)	0	19 (50)	0	17 (50)	0
Seine	18 (51)	0	25 (52)	0	13 (34)	0	9 (26)	0
Total	35 (100)	14	48 (100)	18	38 (100)	16	34 (100)	18
Amphibians								
Dipnet	10 (77)	0	10 (77)	0	3 (38)	1	6 (75)	2
Mini-fyke net	11 (85)	2	12 (92)	1	7 (88)	3	5 (63)	0
Minnow trap	9 (69)	0	11 (85)	0	4 (50)	0	6 (75)	1
Seine	8 (62)	0	6 (46)	0	2 (25)	0	2 (25)	0
Total	13 (100)	2	13 (100)	1	8 (100)	4	8 (100)	3

Table 5. Number of individual fish and amphibians detected during spring and summer sampling seasons in a study of 29 Missouri wetlands during 2015 and 2016.

	2015		2016	
Taxa	Spring	Summer	Spring	Summer
Fish	30,245	73,731	10,789	34,630
Amphibians	21,609	14,757	25,389	2,403

Table 6. Number of parameters (k), delta AICc (ΔAICc), Akaike's weight (wt), and log likelihood for zero-inflated regression models predicting number of species caught in a sample in wetlands across the state of Missouri, 2015-2016.

Model type	**Model name**	**k**	**ΔAICc**	**wt**	**logLik**
Fish models	Global	44	0.00	1.00	-2445.61
	Temporal	14	86.89	0.00	-2520.11
	Interaction	16	119.61	0.00	-2534.43
	Method	34	128.48	0.00	-2520.32
	Region	10	193.01	0.00	-2577.23
	Null	4	2694.09	0.00	-3833.82
Amphibian models	Global	27	0.00	1.00	-1391.21
	Temporal	12	15.09	0.00	-1414.16
	Interaction	22	32.56	0.00	-1412.66
	Method	10	36.17	0.00	-1426.73
	Region	13	36.45	0.00	-1423.82
	Null	5	311.90	0.00	-1569.64

Table 7. Parameter estimates (Estimate), standard errors (SE), P values (P), and 95% confidence intervals upper (UCL and LCL) of the count component of top ranked regression models predicting the number of fish and amphibian species detected in a sample in Missouri wetlands. Significant estimates are shown in bold.

Model	Parameter	Estimate	S.E.	*p*-value	LCL	UCL
Fish	(Intercept)	**-0.22**	0.09	0.016	-0.41	-0.04
	FishSpRich	**0.30**	0.02	0.000	0.25	0.34
	MethodMF	**2.02**	0.09	0.000	1.84	2.20
	MethodMT	**0.29**	0.10	0.004	0.09	0.48
	MethodS	**0.56**	0.12	0.000	0.33	0.79
	Depth	**0.21**	0.09	0.029	0.02	0.39
	Veg	**-0.35**	0.08	0.000	-0.52	-0.19
	Dist	**-0.27**	0.11	0.020	-0.49	-0.04
	RegionMRAV	**0.17**	0.05	0.000	0.07	0.28
	RegionOP	0.06	0.05	0.165	-0.03	0.15
	WetArea	-0.01	0.03	0.683	-0.06	0.04
	Season:Summer	**0.18**	0.05	0.000	0.09	0.28
	Year:2016	**-0.09**	0.04	0.014	-0.16	-0.02
	MethosMF:Depth	**-0.20**	0.10	0.041	-0.39	-0.01
	MethodMT:Depth	-0.15	0.12	0.181	-0.38	0.07
	MethodS:Depth	0.06	0.12	0.599	-0.17	0.30
	MethosMF:Veg	**0.26**	0.09	0.003	0.09	0.43
	MethodMT:Veg	**0.44**	0.09	0.000	0.26	0.63
	MethodS:Veg	0.15	0.15	0.306	-0.14	0.45
	MethosMF:Dist	**0.24**	0.12	0.041	0.01	0.47
	MethodMT:Dist	0.21	0.14	0.140	-0.07	0.48
	MethodS:Dist	0.16	0.14	1.260	-0.12	0.43
Amphibians	(Intercept)	**-1.05**	0.13	0.000	-1.30	-0.79

AmphSpRich	**0.30**	0.03	0.000	0.22	0.37
MethodMF	**0.61**	0.14	0.000	0.33	0.88
MethodMT	**0.39**	0.13	0.002	0.12	0.65
MethodS	0.39	0.21	0.073	-0.03	0.81
Depth	**-0.30**	0.13	0.017	-0.55	-0.04
Veg	0.05	0.07	0.405	-0.09	0.19
Dist	-0.11	0.09	0.228	-0.29	0.07
RegionMRAV	0.17	0.09	0.073	-0.02	0.36
RegionOP	**-0.23**	0.11	0.032	-0.44	-0.01
WetArea	0.023	0.05	0.665	-0.08	0.12
Season:Summer	0.04	0.07	0.665	-0.10	0.18
Year:2016	**-0.48**	0.07	0.000	-0.63	-0.32
MethosMF:Depth	0.16	0.14	0.261	-0.12	0.44
MethodMT:Depth	0.22	0.15	0.143	-0.08	0.52
MethodS:Depth	0.17	0.19	0.363	-0.21	0.55
MethosMF:Veg	-0.11	0.08	0.206	-0.28	0.06
MethodMT:Veg	0.10	0.08	0.224	-0.06	0.26
MethodS:Veg	0.03	0.22	0.869	-0.40	0.46
MethosMF:Dist	0.11	0.10	0.289	-0.09	0.31
MethodMT:Dist	0.04	0.12	0.721	-0.19	0.27
MethodS:Dist	-0.22	0.18	0.208	-0.57	0.13
Log(theta)	17.81	16.80	0.289	-15.13	50.75

FIGURES

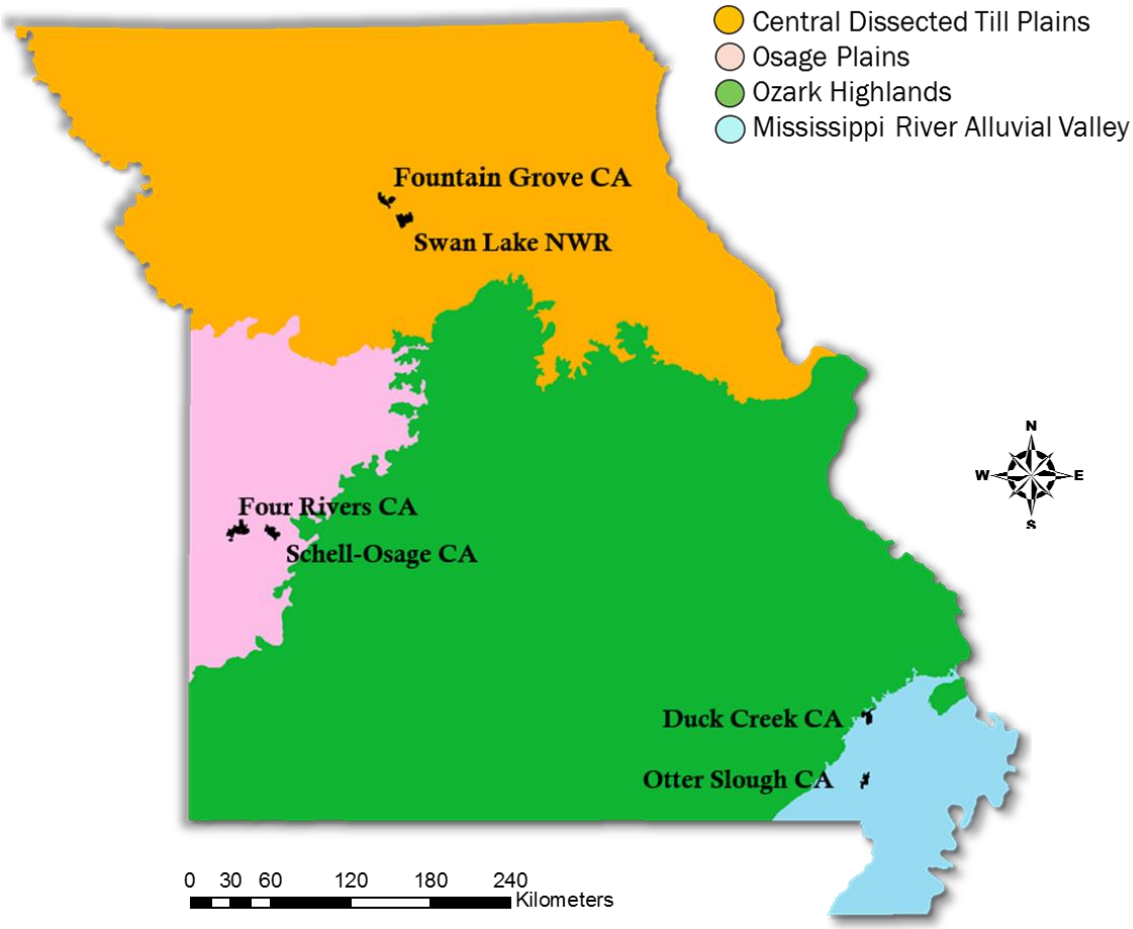

Fig. 1 Map of six study area and ecoregions within the state of Missouri sampled from May 2015 – August 2016. (CA= Conservation Area, NWR= National Wildlife Refuge)

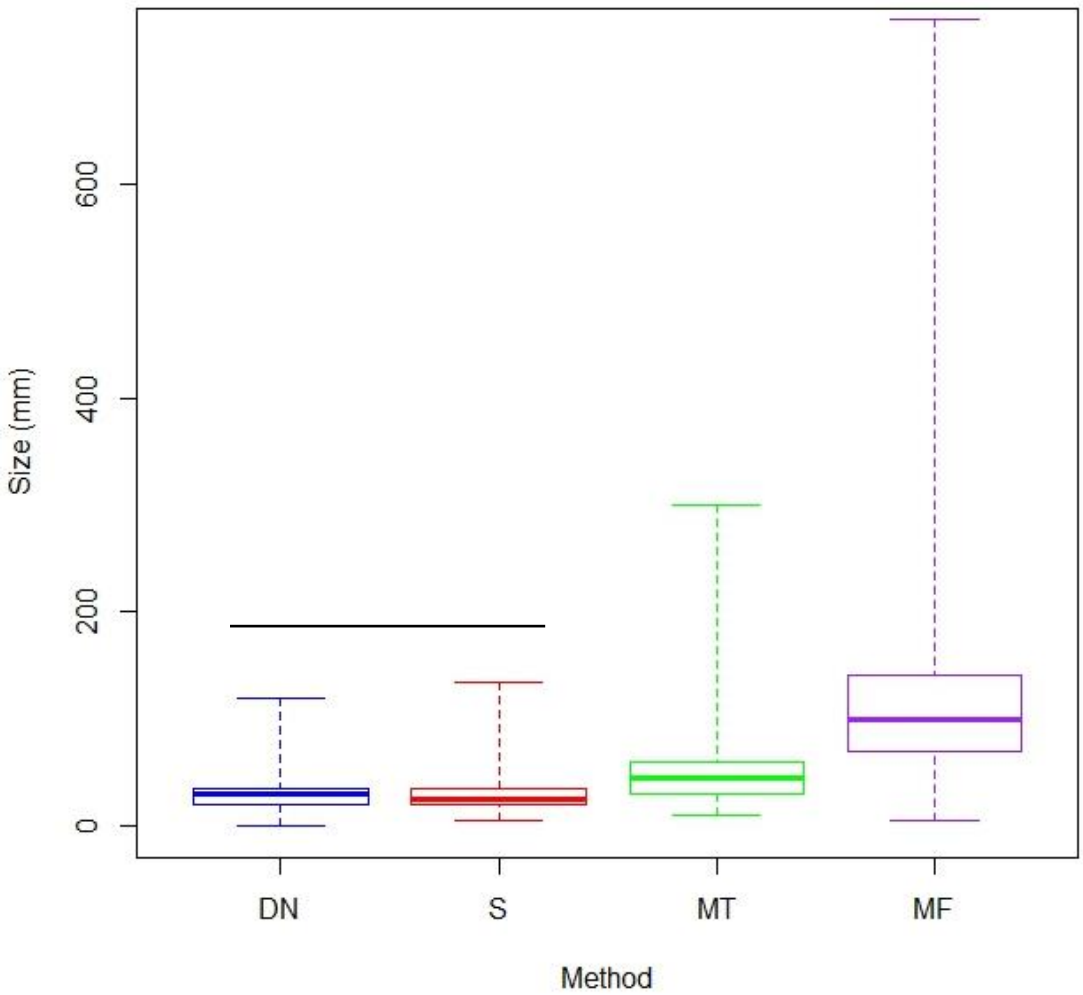

Fig. 2 Median fish total lengths (TL) caught by four sampling methods in a study of 29 Missouri wetlands 2015-2016. Box= 25th and 75th percentiles, whiskers= minimum and maximum values. Line over bars indicate no statistical difference. Wilcoxon rank test ($p<0.001$). (DN= dipnet, S= seine, MT= minnow trap, MF= mini-fyke net)

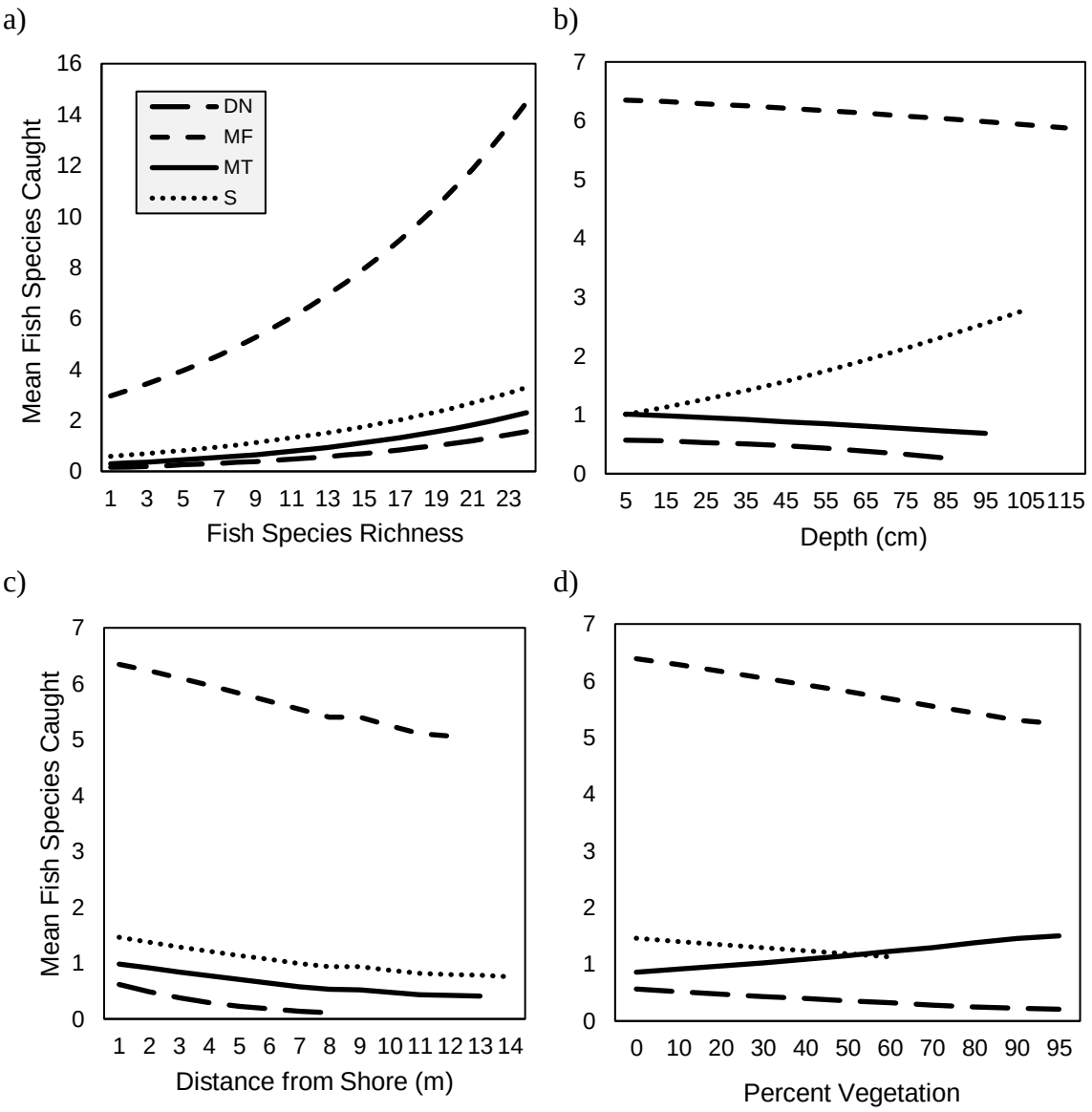

Fig. 3 Predicted number of fish species caught with four sampling methods across the observed ranges of fish species richness, depths, distance from shore, and vegetation, holding all other variables constant at the mean and averaging across regions, seasons and years (2015-2016) in a study of Missouri wetlands. (DN= dipnet, MF= mini-fyke net, MT= minnow trap, S= seine)

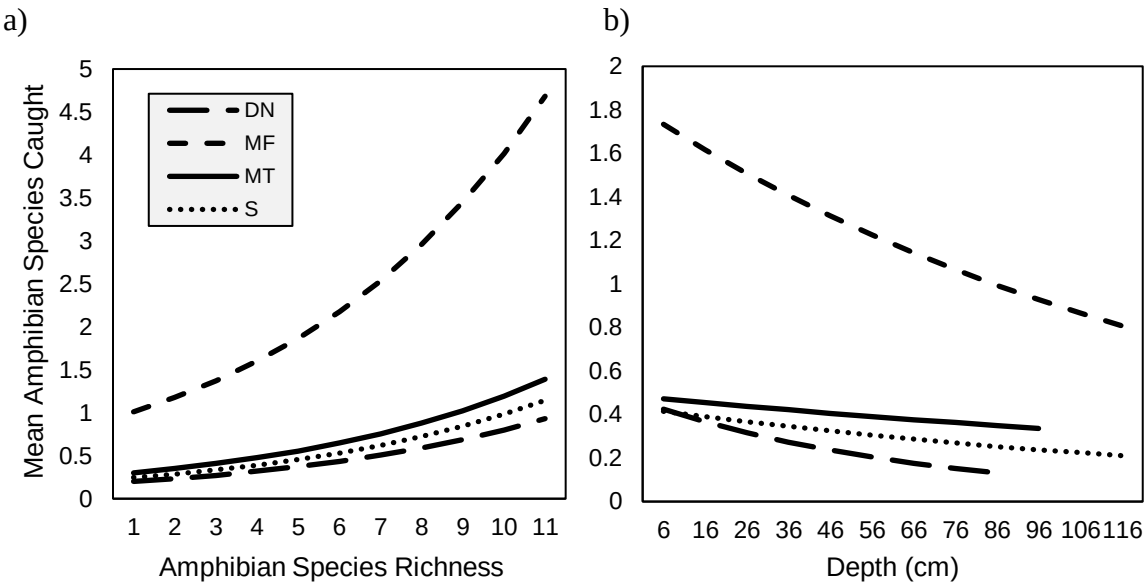

Fig. 4 Predicted number of amphibian species caught with four different sampling methods across the observed ranges of amphibian species richness and depths holding all other variables constant at the median and averaging across regions, seasons and years (2015-2016) in a study of Missouri wetlands. (DN= dipnet, MF= mini-fyke net, MT= minnow trap, S= seine)

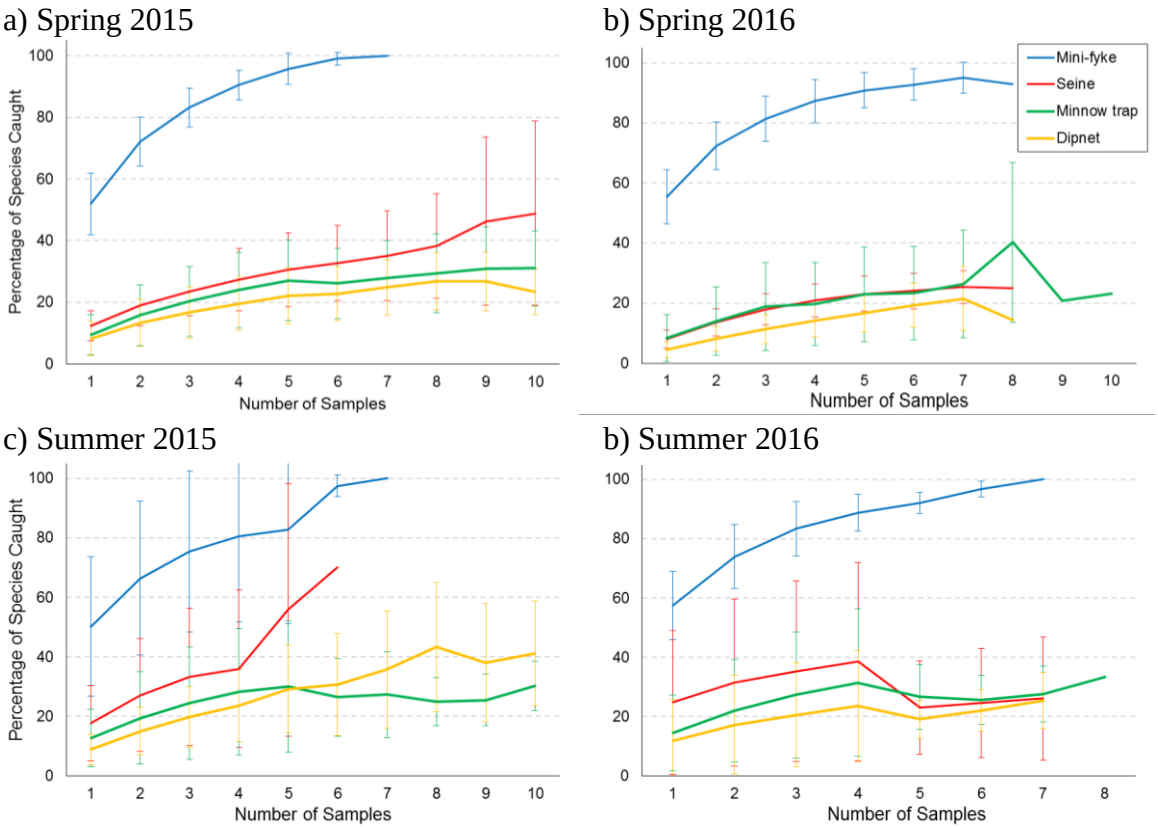

Fig. 5 Species accumulation curves for fish species for the four sampling methods. Each line is the mean percentage of species caught across wetlands from all study areas in the state of Missouri, 2015-2016 (Error bars show standard deviation)

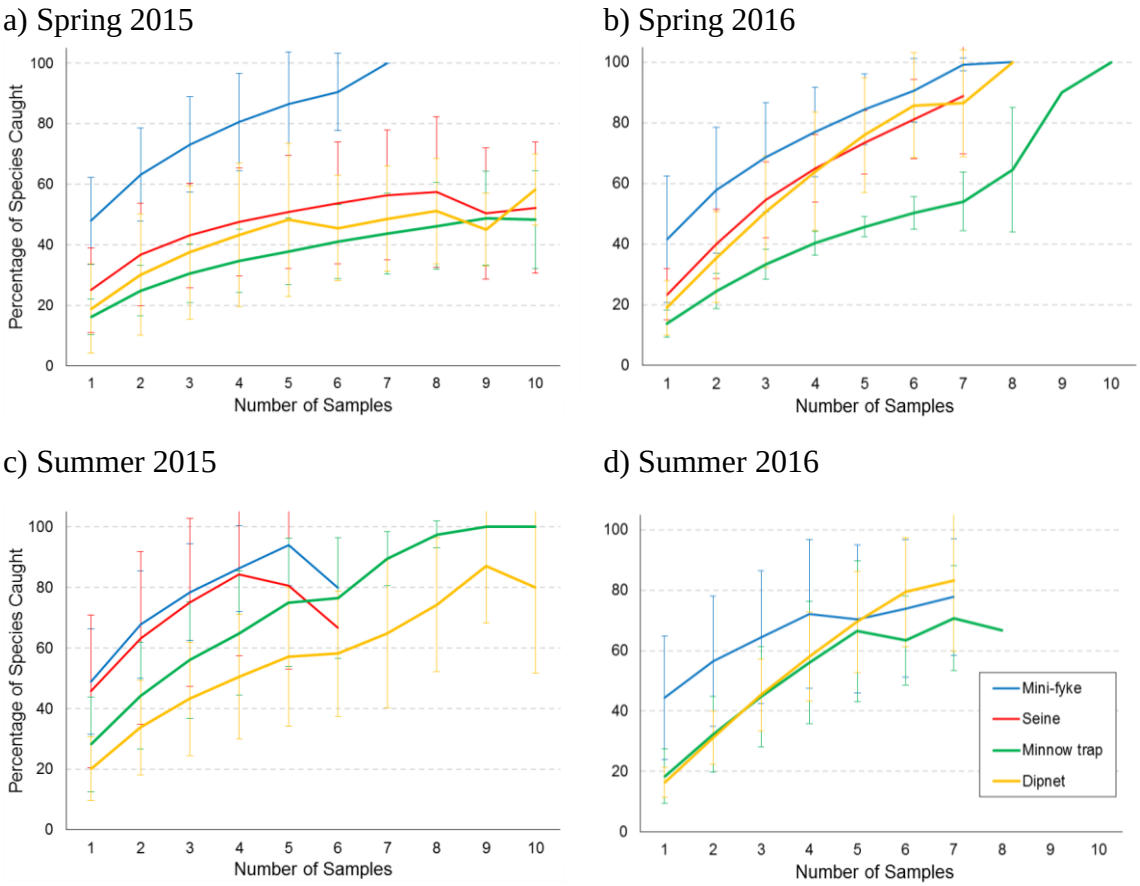

Fig. 6 Species accumulation curves for amphibian species for the four sampling methods. Each line is the mean percentage of species caught across wetlands from all study areas in the state of Missouri, 2015-2016. (Error bars represent standard deviation)

CHAPTER 2

INFLUENCE OF HYDROLOGY AND HABITAT CHARACTERISTICS ON FISH AND AMPHIBIAN SPECIES RICHNESS IN RESTORED FLOODPLAIN WETLANDS

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ABSTRACT

Freshwater wetland species, particularly fish and amphibians, are some of the most imperiled biota on our planet and many now rely on restored wetland areas for survival. Ensuring suitable fish and amphibian habitat occur periodically within restored wetland complexes will likely depend on developing a greater understanding of how these taxa utilize these highly altered wetland ecosystems and applying this knowledge to management decisions to meet the unique life-history needs of these species. We sampled 29 intensively managed wetlands across the state of Missouri during spring and summer of 2015 and 2016 to determine how fish and amphibian species richness is influenced by within wetland habitat characteristics, landscape scale features, and frequency and type of hydrologic connectivity. We detected a total 54 fish and 15 amphibian species and found wetland species richness fluctuated spatially and temporally. Modeling indicated that annual and seasonal amphibian species richness were positively influenced by the MRAV region and well water management and negatively impacted by the frequency of flooding events. Fish seasonal and annual richness was positively influenced by hydrologic connectivity but we saw no relationship with flood frequency. Seasonal and annual fish and amphibian richness was positively related to sample year 2015.

INTRODUCTION

Freshwater ecosystems are among the most threatened systems in the world (Ricciardi and Rasmussen 1999; Dudgeon et al. 2006), with freshwater wetlands considered among the most endangered. The United States has experienced a 53% reduction in wetland habitats since the 1780's, and Missouri exceeds the national average with >85% of wetlands destroyed (Dahl 2006). Major causes of wetland loss include draining and filling for agriculture and river modification projects involving damming, channelization, and levee construction that alter flow regimes and disrupt exchanges between the main channel and floodplain wetlands (Dahl 1990; Epperson 1992; Galat et al. 1998; Kingsford 2000, Kingsford and Thomas 2004). Concerns over declining wildlife populations, waterfowl in particular, spawned early conservation efforts aimed at preserving existing wetlands and replacing those already lost (Galat et al. 1998; Dahl 2006). In Missouri, much of the restoration focused on establishing the state's extensive system of wetland complexes, mostly concentrated along river and stream floodplains. To reestablish any kind of ecosystem function, these restored wetland areas are now intensively managed through a combination of controlled water level manipulations and repeated soil disturbance. Although wetland management has historically focused on providing suitable habitat for migratory waterbirds, perspectives are changing and agencies are beginning to encourage actions that meet the habitat needs of a wider range of wetland dependent species (Galat et al. 1998), as illustrated by recent wetland enhancement projects that work to restore the natural topography. Ensuring suitable fish and amphibian habitat occur periodically within restored wetland complexes will likely depend on developing a greater understanding of how these taxa rely on the existing

actively managed wetland habitats and applying this knowledge to management decisions to meet the unique life-history needs of these species.

Ninety eight percent of U.S. rivers are affected by some type of flow regulation (Vitousek et al. 1997), and these hydrologic modifications have extensive impacts on riverine floodplain ecosystems (Vitousek 1997; Kingsford 2000) including connected wetlands, chiefly through disruption of flood pulses that allow for lateral transfer of biota and nutrients between river channels and the floodplain (Junk et al. 1989). Flood pulses are a major force driving the productivity of these ecosystems, and many species are specifically adapted to the predictable, periodic flooding and drying cycles (Junk et al. 1998). In many floodplain ecosystems of North America, like those in Missouri, the regulation of large rivers has eliminated regular flooding or severely altered the timing and intensity of flood events, disrupting the lateral transfer of nutrients, organic matter, and biota (Ward and Stanford 1995; Galat et al. 1998; Tockner et al. 2010; Covino 2017). With reduced or altered hydrologic connectivity, floodplain wetlands and their biota, especially sensitive species like fish and amphibians, can be severely impacted (Ward et al. 1999; Kingsford and Thomas 2004). In many restored or constructed floodplain wetlands, intensive management regimes seek to mimic the historic flood pulses through controlled flooding and regular soil disturbance (Galat et al. 1998), but we don’t know how this intensive management is impacting wetland dependent species like fish and amphibians.

Conserving and restoring wetland function is essential as wetlands provide valuable resources to fish and amphibians at various stages throughout their life cycles. Many fish and amphibian species depend on wetlands to breed and develop (Holland

1986; Turner et al. 1994; Kilgore and Baker 1996; Semlitsch and Bodie 1998; Semlitsch 2000; Gorman et al. 2014). Some species spend the majority of their lifetime in wetland habitats while others periodically utilize them for foraging or nursery areas (Lambou 1963; Copp and Peňáz 1988; Junk et al. 1989; Batzer and Wissinger 1996; Kilgore and Baker 1996; O'Connell 2003; Miranda 2005; Petranka et al. 2006; Poi de Neiff et al. 2009). Floodplain wetlands can also serve as important corridors for fish movement, both for migration and movement between main river channels and spawning grounds (Burgess et al. 2013), and refugia for both riverine and resident wetland fish, as they provide protection from harsh river conditions and predators (Guillory 1979; Copp and Peňáz 1988; Turner et al. 1994). Due to their biphasic lifecycle, many amphibians use both aquatic and terrestrial environments during their life as they breed and lay eggs in wetlands and then metamorphose from an aquatic larval stage to a terrestrial adult stage (Wilbur 1980; Semlitsch 2000). Consequently, semiaquatic ecotones and terrestrial habitats adjacent to inundated wetlands are equally important as wetlands in meeting species life-history requirements (Semlitsch 2000; Houlahan and Findlay 2003). Therefore, understanding how management and restorations shape fish and amphibian species distributions in managed wetlands is essential to ensure wetland areas function properly to mitigate for historic wetland losses.

Aspects of hydrologic connectivity are commonly identified as the most important factor influencing fish and amphibian species distributions in systems where the natural hydrology has been highly altered. Beesley et al. (2014) reported wetland water source, method of water delivery, and timing best explained fish recruitment in enhanced floodplain wetlands. A study by Baber et al. (2001) found that connectivity between

wetlands and permanent water bodies was the primary source influencing fish assemblage and Dembowksi and Miranda (2011) reported higher fish species richness in a section of an oxbow that was connected to a river. In wetlands that experience natural and managed flooding, natural flooding can cause larger changes in fish community structure (Stoffels et al. 2014). Similarly, isolated wetlands and wetlands that experience more frequent drying were less likely to contain fish (Snodgrass et al. 1996). Amphibian species richness and abundance can be negatively impacted by disturbance caused by flood events (Morand and Joly 1995). Richter and Azous (1995) hypothesized that increases in the duration and frequency of water level fluctuations within wetlands contributed to lower species richness of amphibian species. Therefore, hydrologic connectivity of restored wetlands is one factor that may affect fish and amphibians. While research focusing on the response of fish populations to altered flow regimes are numerous, literature examining these trends in amphibian population are much more limited, and more research is needed (Poff and Zimmerman 2010).

Landscape scale habitat also affects fish and amphibian species. Amphibian species are sensitive to landuse surrounding wetland habitats as many species emigrate from a wetland following breeding or metamorphosis into the adjacent upland to forage and find refuge from desiccation, predation, and freezing during the remainder of the year (Madison 1997; Richter et al. 2001). Amphibian species abundance and richness typically respond positively to increased proportion of wetlands (Jacob and Houlahan (1997; Knutson et al. 1999; Houlahan and Findlay 2003; Burne and Griffin 2005) and forest cover (deMaynadier and Hunter 1995; Mitchell et al. 1997; Kolozsvary and Swihart 1999) in the surrounding landscape. Extent of forest and wetlands in the surrounding

landscape have been linked to increased measures of fish biotic integrity and help shape fish species assemblages (Roth et al. 1996). Roads and other forms of urban development have been associated with reduced species richness and abundance of both fish and amphibian taxa (Lehtinen et al. 1999; Mensing et al. 1999; Wang et al. 2001; Houlahan and Findlay 2003; Jacobs and Houlahan 2011). Esselman et al. (2011) found urbanization, pollution, pasture land and dams densities all decreased percent intolerant fish at a site. While stream fish studies provide useful insight, research investigating the influence of landscape characteristics on wetland fish species typically focus on the effects of hydrology with less attention to potential influence of surrounding landscape features.

Fish and amphibian species richness may also be affected by habitat conditions within individual wetland units, but similar to landscape factors, studies finding relationships between fish species and within wetland habitat factors are limited (Semlitsch 2000; Houlahan and Findlay 2003; Burne and Griffin 2005; Shulse et al. 2010). Burne and Griffin (2005) found increased amphibian species richness with increased wetland hydroperiod and increased density of emergent vegetation. Increased wetland area has been linked to increased species richness for both taxa (Houlahan and Findlay 2003; Burne and Griffin 2005; Lubinski et al. 2008). However, the presence of fish, specifically predatory fish, is commonly associated with lower amphibian species richness (Hecnar and M'Closkey 1997), and can cause changes in amphibian species composition (Babbitt and Tanner 2000). Fish species assemblages can be shaped by individual tolerance to water quality metrics like turbidity (Brazner and Beals 1997; Lubinski et al. 2008) and dissolved oxygen (Chapman et al. 1996). While the effects of

local and landscape scale variables on fish and amphibian species richness and distribution are well documented, it is unclear if these trends persist in actively managed wetland habitats where habitat conditions are explicitly controlled by management activities, particularly by manipulation of water levels.

The objective of this study was to investigate the influence of wetland hydrology on the distribution of fish and amphibian species in actively managed wetlands. Specifically we quantified the fish and amphibian species richness of wetlands to evaluate how species richness is affected by wetland hydrologic conditions including connectivity during unplanned flood events and controlled inundation from various managed water sources. We also wanted to account for the influence of local and landscape scale factors including within-wetland habitat characteristics, upland land use, temporal and spatial variation. Investigating the species utilizing Missouri public wetlands and understanding their relationships with wetland conditions will provide managers and researchers with more information about the conditions present in their wetlands to help evaluate current management regimes and tailor future decisions to best suit the natural communities. Understanding these relationships will help guide future wetland restoration projects and aid in the development of effective management strategies that create suitable fish and amphibian habitat in highly altered wetlands.

METHODS

Study areas

We sampled 29 intensively managed wetland units, across six study areas that were equally distributed among the Central Dissected Till Plains, Osage Plains, and Mississippi River Alluvial Basin ecoregions (Nigh and Schroeder 2002) of Missouri (Fig.

1). Twenty three wetlands were managed by the Missouri Department of Conservation and six were managed by the U.S. Fish and Wildlife Service. Specific study sites include: Duck Creek Conservation Area (CA), Otter Slough CA, Schell Osage CA, Four Rivers CA, Fountain Grove CA, and Swan Lake National Wildlife Refuge. These study areas were predominantly managed for moist soil plants and were equipped with levees and water control structures that allowed for precise water level manipulations. Managed water sources (water intentionally added in order to flood wetlands and influence soil moisture) varied for each wetland unit and included ground water delivered from wells (n= 9), river water delivered by pump or ditches (n=12), and water from reservoirs (n=8) that was either pumped or gravity fed. In addition to managed inundation, some wetland units received water from adjacent creeks and rivers during uncontrolled, overbank flood events. Many wetland units contained deep borrow ditches alongside levees that had a high likelihood of holding water for most or all of the year.

Wetland characteristics, including area, water depth, and habitat complexity varied by season, weather events, and management goals. Water level manipulation is an adaptive process, so each wetland was managed differently according to annual site-specific goals. Wetlands were usually drawn down in late spring to facilitate seed germination and promote vegetation growth, and flooded in the fall or early winter to provide habitat for waterbird species. Sampling locations for fish and amphibians were located at wetland depths ranging from 2 to 288 cm, while inundated area of sampled wetlands ranged from 0.3 to 111.1 hectares. Vegetation composition within seasonally flooded wetlands varied extensively among season and years, due to factors such as the timing of flood and drawdown events and natural weather conditions. Naturally occurring

vegetation was distributed along hydrologic gradients within wetland units. Common wetland flora included smartweeds (*Polygonom spp.*), beggarticks (*Bidens spp*.), barnyardgrasses (*Echinochloa spp.*), spike rushes (*Eleocharis spp.*), crabgrasses, and panic grasses. Small areas of agricultural row crops, primarily corn (*Zea mays*), were also commonly found in managed wetlands. While most wetlands were manipulated under similar water management regimes, they differed in the level and degree of disturbance. Disturbance can be applied in several ways including extended inundation, prescribed burning, and mechanical manipulations like mowing tilling, and disking.

Fish and amphibian sampling

In order to capture pre-drawdown and pre-flooding conditions and maximize the probability of detecting the full suite of fish and amphibian species present, we sampled wetland units during spring (March-May) and summer (June-August) of 2015 and 2016. The same wetland units were sampled during both seasons each year and across both years at each study area, if possible. Specific sampling locations within a wetland were randomly selected and were stratified between nearshore (within 20 m of shore) and offshore (all other inundated area within the wetland). The offshore area was divided into 3 sections and samples were randomly assigned to a section.

To begin sampling, we visualized the boundary of each wetland unit as a compass and randomly selected a degree between 1 and 360. The primary sample site in a wetland was placed within 20 m from shore at the randomly selected degree, with a paired sample site offshore. A randomly generated list was used to select the specific sampling method used at each site (see below for a description of the sampling methods). Proceeding along

the edge of the wetland, successive pairs of samples were situated at random distances from the previous nearshore sample according to the estimated boundary of the inundated portion of the wetland. The goal was to collect the same number of samples with each method in each unit (~40 samples/wetland), but the actual number of samples within a wetland varied depending on the extent of inundated area and the accessibility and suitability of that area (i.e. water depths >1.5m and mats of dense vegetation prevented sampling in some areas) at the time of sampling, with greater numbers of samples in larger, more accessible wetlands. Sampling locations were at least 50 m apart in an effort to reduce bias of sample autocorrelation, and exceeded distances recorded in previous research (Heyer et al. 1994; Buech and Egeland 2002).

Surveys included four sampling methods: mini-fyke nets, minnow traps, dip netting, and seining (see Chapter1). Mini-fyke nets (60 by 120 cm frame, 7.6 m lead, and 0.32-cm mesh) were deployed from shore or structure within the wetland that could act as a barrier (i.e. hunting blind) using rebar stakes on the end of the lead line and the bound end of the hoop section. Minnow traps (with 0.32-cm mesh and 2.54-cm openings) were attached to a stake secured in the substrate. Mini-fyke nets and minnow traps were set in mid-afternoon/evening and left overnight to include both diurnal and nocturnal species (Bonar et al. 2009; Heyer et al. 1994). Soak times for mini-fyke nets and minnow traps averaged 17.2 hours and did not exceed 24 hours. The contents of each net were immediately sorted or placed into aerated live wells and sorted soon after.

Seine hauls (1.2 m x 4.5 m and 0.32-cm mesh) were ~ 6 m long with two people holding each side and walking the seine forward while pressed to the bottom of the wetland (Bonar et al. 2009). Contents of each seining effort were placed into an aerated

live well and sorted according to species. Individual dipnet (40 x 23 x 30.5 cm frame and 0.32-cm mesh) samples consisted of 10-15 sweeps distributed across all available habitat at the randomly assigned sampling site (Heyer et al. 1994; Mensing et al. 1998; Hamer and Parris 2011). Sweeps were ~ 1 m long with the net pressed to the substrate, pulling the net towards the observer (Heyer et al. 1994). Species detected in each sample were sorted immediately.

All fish and amphibians captured were identified to species, counted, measured, and returned to the water. If identification was impossible in the field, voucher specimens were collected and identified in the lab at the University of Missouri. If an individual was suspected to be rare, it was photographed to verify identification and released. Voucher specimens were euthanized and preserved using a 10% formalin solution or 70% ethanol (Heyer et al. 1994; Kelsch and Shields 1996). All observers were trained in fish and amphibian identification prior to field sampling. Sampling was carried out under University of Missouri Animal Care and Use Committee permit #8211 and MDC permit #16717.

Within wetland habitat characteristics

In addition to species-specific data, wetland specific habitat characteristics were measured and recorded on a local scale for each wetland unit. Wetland area (ha) was defined as the inundated area of the wetland at the time of sampling and determined by visually estimating the boundary of inundated area on-site and quantified using ArcMap 10.3 GIS software (2014 Environmental Systems Research Institute Inc., Redlands, California). Maximum and mean water depth (cm) were calculated based on depth measurements recorded at each sample site. Maximum and mean water temperature (°C)

and minimum and mean dissolved oxygen (mg/L) were calculated from measurements taken at each sample site using a handheld meter (YSI Pro2030, YSI Incorporated, Yellow Springs, Ohio). We visually assessed the habitat complexity of each unit by estimating the percentage of inundated area comprised of vegetation or open water. We further described vegetation by estimating the percentage of vegetation falling into each of six categories: submergent vegetation (i.e. coontail and elodea), emergent vegetation (i.e. cattails, smartweed, and sedges), small woody vegetation (i.e. button bush and rose mallow), agricultural crops (i.e. corn), trees (i.e. willows) or other (i.e. floating vegetation like duckweed). Vegetation categories were similar to that used by Burne and Griffin (2005), but were adjusted to incorporate the agricultural crops. Visual estimations fell into one of six ranges (0-5, 5-25, 25-50, 50-75, 75-95, and 95-100%) and the median of each category was used in statistical analysis (Burne and Griffin 2005).

Landscape characteristics

We estimated the percent upland within 200 m of the wetland comprised of five vegetation categories using ArcMap 10.3 GIS software (2014 Environmental Systems Research Institute Inc., Redlands, California) and 2014 National Agricultural Imagery Program (NAIP) digital aerial imagery (2 m resolution), coupled with on-site verification. Wetland boundaries were drawn to create polygons of each unit and generate 200 m surrounding buffer polygons. Area within the buffer was identified as one of six habitat types including: Forest, Wetland, Grassland, Developed, and Agriculture. Forest was defined as an area that was predominantly covered by trees. Wetland included surrounding managed wetland units. Any area predominantly covered by grasses was categorized as Grassland. Developed areas included human created structures like roads,

buildings, and utility structures. Crops and crop fields in the surrounding upland were included in the Agriculture category. Agricultural crops planted within adjacent wetland units were not separated from the Wetland measure.

Hydrologic characteristics

We estimated quantitative and qualitative metrics of hydrologic characteristics for each wetland unit using data provided by area managers, U.S. Geological Survey (USGS) river gage data, and on-site observations. Connectivity was defined as the ability of a wetland to flood from an adjacent creek, river, or stream outside of planned management actions (i.e. overbank flooding) and was categorized as a binary variable (yes or no). The average number of floods per year (FPY) was calculated separately for each wetland unit using data retrieved from the nearest, most accurate river gage (U.S. Geological Survey 2016) and records kept by area managers. A unit was considered to flood based on gage data when the recorded river height exceeded the known height of the wetland levee. Averages were based off data from 2011-2015, and were considered representative of a range of annual weather conditions. Water Source was defined as the source of the water introduced into wetland units for management actions and fell into three categories: well, river, or reservoir.

Regional species pool

The regional species pool can affect local species richness (Cornell and Lawton 1992), so we accounted for this by calculating Fish and Amphibian Species Pool metrics reflecting the number of species that could have occurred at each study area (Appendix B). To establish a list of species potentially present, we used the Missouri Department of Conservation Fish Community Database and Missouri Herpetological Atlas (Daniel and

Edmund 2017). The Fish Community Database is a compilation of stream and river fish records from 1923 to the present collected by MDC staff, and researchers from academic or governmental agencies under the permission of a Wildlife Collectors Permit. Our study areas had not been previously sampled extensively, thus we derived a regional fish species list by including records from within the corresponding eight digit Hydrologic Unit Codes (HUC 8) and a regional amphibian species pool with records from within natural division units and sections described by Thom and Wilson (1983). The hydrologic units are part of a hierarchical, nested mapping system of all watersheds within the United States, and HUC 8 units represent the subbasin level, or medium-sized river basins. The HUC units were aggregated into a standard layer in the Watershed Boundary Dataset by the United States Department of Agriculture-Natural Resources Conservation Service (USDA-NRCS), the Environmental Protection Agency (EPA), and the United States Geological Survey (USGS). The natural divisions, defined by Thom and Wilson (1983), incorporate factors such as geology, physiography, drainage and geology to divide the state into 6 relatively unique divisions with 19 subsections. All study areas were contained within individual HUC 8 units or natural division divisions/sections with the exception of Four Rivers CA, which was located within four HUC 8 watersheds. To increase accuracy and eliminate unlikely species (i.e. extirpated species), we only included species that have been detected since 1980. We further refined the amphibian list by only including wetland breeding species to eliminate species that reside exclusively in terrestrial environments or in aquatic systems not present in the study areas (i.e. Ozark streams). Cope's gray treefrogs (*Hyla chrysocelis*) and Eastern gray treefrogs (*Hyla versicolor*) were grouped into a "gray treefrog complex" due to the extreme

similarity of external characteristics and challenges in differentiating between species. Any species detected in a wetland that was not included in the regional species pool list was excluded from the species richness count in analysis to prevent statistical dependence between variables (Cam et al. 2000).

DATA ANALYSIS

We pooled species caught by all methods for analysis. The number of species detected in each wetland during each season was considered the representative seasonal species richness and total species caught in a wetland during spring and summer of each year was considered the representative annual species richness. We evaluated species richness because it is a commonly used, easily calculated metric that allowed for basic comparison between wetland units (Gotelli and Colwell 2001) and, because of its widespread use in scientific studies, it allowed for comparison of our results to other studies (Fleishman et al. 2006). From an ecological perspective, species richness can be an indicator of the overall ecosystem health and is often used to identify areas of conservation importance (Meir et al. 2004).

To test the effects of wetland hydrologic connectivity and managed water source on fish and amphibian species richness, while accounting for within-wetland habitat conditions, upland landuse, temporal variables, and sampling, we developed a set of *a priori* candidate models (Table 3). Candidate models included biologically relevant biotic and abiotic variables representing specific hypothesis for what might influence fish and amphibian species richness. Prior to running models we evaluated multicollinearity of independent covariates using Pearson correlation coefficient to avoid including highly correlated variables ($r \geq 0.70$) in the same model (Burnham and Anderson 2002).

Amphibian Species Pool was correlated with Water Source (r=0.79) and moderately correlated with Region (r=0.66). Fish Species Pool was highly correlated with Water Source (r=0.85). Effects of the managed water source on species richness was one of our main research questions and thus, we eliminated fish and amphibian Species Pool metrics from the modeling process. We hypothesized that different ecological mechanisms influenced fish and amphibian species richness on a seasonal and annual basis and thus we ran separate sets of generalized linear mixed models for each taxa and richness metric (annual or seasonal). More stable, landscape scale variables were included in the annual richness models while both landscape and ephemeral, local scale variables were included in the seasonal richness models (Table 3). Based on our small sample size, we limited the number of parameters in each model to a maximize model parsimony and avoid over-fitting the models (Burnham and Anderson 2002). To account for spatial dependence we included Study Area as a random effect in all models while Season, and Year were included as a fixed effect in seasonal richness models and Year was included as a fixed effect in annual richness models. Our response variables, fish or amphibian richness, were count data and therefore non-normally distributed. In addition to being non-normal, fish data were zero-inflated, so these models were fit with the function “glmmadmb” and run using the “glmmADMB” package (Fournier et al. 2012), which incorporated the excess zeros in the data. Annual fish richness models were run using a Poisson distribution and log link, while seasonal models were better fit with a negative binomial distribution and log link. Amphibian richness models were run using the “lme4” package (Bates et al. 2015) and fit with the “glmer” function using a Poisson distribution and log link. All modeling was done in program R (R Core Team 2015).

Models were ranked using Akaike's information criteria corrected for sample size (AICc) and the model with the lowest AICc was considered to have the best fit, relative to its complexity, and be the best supported model (Burnham and Anderson 2002). Remaining models in the set were ranked and evaluated according to differences in AICc (ΔAICc) from the best supported model. Models within 0-2 ΔAICc values of the best fit model are considered to have substantial empirical support, models within 4-7 ΔAICc have considerably less support, while models >10 ΔAICc have essentially no support (Burnham and Anderson 2002). Akaike weights (*wt*) were used to further assess the likelihood of remaining models (Burnham and Anderson 2002). The importance of parameters and their effects were determined by examining 95% confidence intervals (CI). Significant parameters were those whose CI values did not overlap zero (Mazerolle 2006). We used the fitted models to plot the means and standard errors of covariates with significant parameter estimates. We were also interested in evaluating the influence of flooding frequency (i.e. FPY) on fish and amphibian richness, however river gage data used to compile flood frequency information were unavailable for the Duck Creek study site. Instead of reducing the sample size for all modeling by excluding records from this site, we ran a separate FPY model with a subset of data for each taxa and richness metric (annual and seasonal) and evaluated parameter estimates .

RESULTS

Over the two year study we sampled 29 wetlands and collected 2,176 samples. We captured 213,592 total individuals comprised of 64,178 individual amphibians and 149,414 individual fish. In 2015 we collected 1,178 samples and in 2016 we collected 998 samples. In 2015 we collected 692 samples in the spring and 486 in the summer. In

2016 we collected 633 samples in the spring and 365 in the summer. We detected a total of 15 amphibian and 54 fish species (both adult and juveniles) across all study areas. Five of the 54 fish species were considered Missouri Species of Conservation Concern (SOCC) including bantam sunfish (*Lepomis symmetricus*), brown bullhead (*Ameiurus nebulosus*), flier (*Centrarchus macropterus*), lake chubsucker (*Erimyzon sucetta*), and starhead topminnow (*Fundulus dispar*). No amphibian SOCC were detected.

Fish were captured at 97% of the wetland units and amphibians were detected at 100% of the wetland units (Table 4). The most widely detected species of fish, Green sunfish (*Lepomis cyanellus*), was detected in 28 of 29 (97%) wetlands and was also the most abundant fish species caught (Table 4). Other commonly encountered and abundant fish were Western mosquito fish (86%, n=21,035) and black bullhead (82%, n=18066). The most widely detected species of amphibian, American bullfrog (*Lithobates catesbeianus*), was detected in 29 of 29 (100%) wetlands and was also the most abundant amphibian species caught (Table 4). Southern leopard frogs (89%, n=27,206) were the second most widely distributed and abundant amphibian species. All other amphibian species were found in less than half of the wetlands (≤14 wetland units) and were much less abundant (n<1400).

Wetland species richness varied across sites and taxa. Fish species richness ranged between 0 to 24 species among individual wetlands, and average species richness within an individual wetland fluctuated by as much as 16 species between seasons (Fig. 2). At one study area, Otter Slough, we consistently detected fewer fish species (<5) than other sample areas. Amphibian species richness ranged between 0 to 11 among individual wetlands and the greatest fluctuation in average wetland species richness was

approximately 4 species between seasons (Fig. 3). We detected different total numbers of fish species between seasons each year, but detected the same total number of amphibian species in both seasons within each year.

Seasonal fish richness was best described by the Connectivity model and no other model was within 2 ΔAICc or had substantial model weight (*wt*≤0.03) (Table 5). Seasonal fish richness increased with wetland hydrologic connectivity and was lowest in 2016 (Table 6; Figure 4a and b). Annual fish species richness also was best predicted by the Connectivity model (*wt*= 0.99), and no other model ranked within 2 ΔAICc (Table 5). Annual fish species richness was higher in wetlands that were hydrologically connected and lower in the sample year 2016 (Fig. 5a and b). When we ran the separate FPY model using a subset of the data (excluding the study area with no flood records), FPY did not have a significant parameter estimate in the seasonal (PE=0.14, SE=0.08, p>0.05) or annual (PE=0.21, SE=0.11, p>0.05) fish species richness models.

Seasonal amphibian richness was best predicted by the Region model and the Water Source model had moderately high empirical support (Table 7; ΔAICc=2.48) but low model weight (*wt*=0.19). Annual amphibian species richness was best described by the Region model, but the Water Source model also had high empirical support (ΔAICc=0.72) and comparable weight (*wt*=0.31) (Table 7). Region, Water Source, and Year had significant parameter estimates and showed the same directional relationships in both the seasonal and annual richness model set (Table 6). Annual and seasonal amphibian species richness was greatest in the Mississippi River Alluvial Valley and lowest in the Osage Plains (Fig 6a and Fig 7a). Wetlands inundated with well water contained greater seasonal and annual amphibian species richness than wetlands

inundated with reservoir or river water (Fig 6b and fig7b). We found greater seasonal and annual amphibian richness in 2015 than 2016 (Fig 6c and 7c). When we ran the separate FPY model to see if richness was impacted by flooding frequency, we found that annual and seasonal amphibian species richness decreased with the number of flooding events per year (Fig. 8a and b).

DISCUSSION

We detected a diverse set of fish and amphibian species using wetlands across the state (Sexton 1986; Galat et al. 1998; Lubinski et al. 2008; Montgomery 2009; Shulse 2010; Beesley et al. 2012), suggesting that these reconstructed wetland areas are an important resource for wetland taxa in areas that have experienced historic wetland declines. Our study found that hydrologic connectivity was a positive predictor of fish species richness while managed water source influenced amphibian species richness, and these relationships varied among regions and years. Collectively, results indicate that wetland hydrology is a major factor generating patterns of species richness for both taxa, emphasizing the importance of tailoring water management regimes and restoration activities to the characteristics present and suitability of the wetlands for the chosen activities.

We detected 54 fish species across 97% of surveyed wetland units and 15 amphibian species across 100%. Not surprisingly, the most common and abundant fish and amphibian species detected in our study are widely distributed in the state and numerous throughout their range (Pflieger 1975; Johnson 2000). Green sunfish are exceptionally tolerant of poor environmental conditions, often detected in sites with degraded water quality and habitat structure, allowing them to thrive in almost all

managed wetlands (Karr 1981). Because of their introduction for mosquito control, mosquito fish (including Western mosquito fish caught in our study) are among the most widespread freshwater fish in the world (Pyke 2005). American bullfrogs often colonize newly constructed wetlands and can thrive in those that provide permanent water for overwintering tadpoles (Boone and Semlitsch 2004). Southern leopard frogs are also able to utilize a wide range of wetland habitats and are found across the majority of the state (Johnson 2000). Presence of these species in almost all of the wetlands sampled would indicate that wetland units are holding water throughout the year, despite their location on the landscape. Due to the presence of permanent water, these managed wetlands may not represent the full range of hydrologic conditions that historically existed in floodplain ecosystems and may discourage species that prefer ephemeral wetlands from breeding and utilizing these areas.

More interestingly, we detected a number of uncommon species including 5 Missouri SOCC (bantam sunfish, flier, brown bullhead, lake chubsucker, and Starhead topminnow). While detection of SOCC species is encouraging, 4 of the 5 species were detected exclusively at the Duck Creek study area, and only few individuals of the other species were detected at other areas. Presence of sensitive species can indicate wetland health, but our research does not indicate trends in these populations and further research is needed to determine the persistence and health of SOCC populations in wetlands. We also caught several nonnative fish species including common carp, bighead carp, goldfish, silver carp and grass carp. Common carp were detected in 21 out of 29 wetlands sampled and were the 10th most abundant species, while the other nonnative species were detected in fewer numbers in less than half of wetlands sampled. While these species do

not seem to be the dominant species inhabiting Missouri wetlands it is important to monitor these population to ensure these areas do not contribute to their proliferation or spread. Our results illustrate the diverse assemblage of fish and amphibian species relying on Missouri's restored wetland habitats, and emphasize the need to identify specific wetland features structuring their distribution and use.

We found increased seasonal and annual fish species richness in wetlands that were hydrologically connected during flood events, however species richness was substantially lower in 2016. Connected wetland units, that received floodwater from adjacent creeks and rivers, may have exhibited greater fish species richness because flooding facilitated fish movement and exposure to a new source of colonists that may have been more diverse than those found in managed water sources. We observed no indication that flooding frequency had further impact on fish species richness. Our findings are supported by similar studies that found wetland fish species richness and abundance were positively influenced by hydrologic connectivity to outside water bodies (Galat et al. 1998; Snodgrass et al. 1996; Baber et al. 2002; Beesley et al. 2014). Significant effects of landscape-scale processes, including hydrologic connectivity, suggest colonization dynamics are likely governing patterns of fish species richness (Snodgrass et al. 1996; Baber et al. 2002; Beesley et al. 2014) more than small-scale habitat preferences. Uncontrolled flood events may allow greater numbers of fish species to colonize wetland units compared to controlled inundation from managed water sources, and thus play a larger role in determining overall fish species richness. Managed water was often pumped from a river or reservoir and delivered to wetland units via ditches and culvert pipes, and our study suggests this system may reduce fish species

richness. Water delivery via pipes and ditches can reduce colonization by restricting fish movement into wetland units (MacDonald and Davies 2007; Katano et al. 2013; Beesley et al. 2014), and pumping can injure or kill fish (Baumgartner et al. 2009; Thompson et al. 2011). Other managed water was pumped from ground wells and, presumably, did not introduce any fish species to wetlands. Greater numbers of fish species may occur in connected compared to unconnected wetlands (Snodgrass et al. 1996 and Galat et al. 1998), and "natural" flooding may contribute to greater changes in fish community structure than controlled flooding (Stoffels et al. 2014). The frequent, seasonal drying of actively managed wetlands means that it is possible fish species may be periodically locally extirpated and colonization of new species only occurs in wetlands following inundation from natural flooding or as part of management actions.

Our results found that amphibian species richness was mainly influenced by region and a hydrologic variable, managed water source, which is similar to other studies (Sexton and Phillips 1986; Richter and Azous 1995; Tockner et al. 1999). Annual and seasonal amphibian richness was greatest in the Mississippi River Alluvial Valley ecoregion and in wetlands inundated with well water compared to river or reservoir fed wetlands. While controlled inundation using water from rivers and reservoirs is more likely to introduce fish and other biota that can predate or compete with amphibian species compared to water pumped from ground wells, we observed no indication that amphibian species richness was inversely related to fish species richness. This contradicts other amphibian studies that found lower amphibian richness when fish were present (Sexton and Phillips 1986; Hecnar and M'Closkey 1997; Morand and Joly 1995; Snodgrass 2000; Knutson et al. 2004), but most of the studies examining this relationship

were conducted in more upland sites where amphibian species are less likely to co-exist with fish. Further investigation of the relationship between floodplain fish and amphibians is needed. We found that Amphibian Species Pool was strongly correlated with Water Source (r=0.79) and moderately correlated with Region (r=0.66), and support for these variables could indicate that wetland species richness may be influenced by the existing species pool. It has been proposed that the number of species present in the regional species pool can affect local species richness (Cornell and Lawton 1992), suggesting that species richness may be greater in some areas solely because more species are present in that region. Our results found that more species were collected in the MRAV region which also had the greatest species pool compared to other ecoregions. However, our results may be confounded by water source as the majority of wetlands in the MRAV region also had well water as the managed water source. In natural floodplain ecosystems, the highest amphibian richness would have likely been further from the main channel in more isolated, ephemeral wetlands, so wetlands managed with well water may be replicating these habitats better than river or reservoir filled wetlands.

We found that increased number of floods per year were associated with lower amphibian species richness, which was similar to Richter and Azous (1996). Frequent flooding in connected wetlands may create highly disturbed habitat for wetland breeding amphibian species that typically prefer lentic wetlands (Semlitsch 2000). Frequently flooded sites often experience increased substrate deposition and subsequent declines in riparian cover, creating greater temperature fluctuations all of which decrease the potential to support a wide range of amphibian species (Morand and Joly 1995). Wetlands subject to repeated flooding events may exhibit lower amphibian species

richness because a single flood event could eliminate amphibian species from a pool by flushing them out or introducing predators that decimate the population, requiring species to begin the process of colonization over again (Ward and Blaustein 1994).

Our results indicated substantial annual variation in both fish and amphibian species richness, with 2016 generally having lower richness. We suspect the difference in species richness between years, specifically fewer species in 2016, may be attributed to management of the wetland units. During the 2016 field seasons, the Missouri Department of Conservation conducted flights to obtain Lidar imagery of many state wetland areas so wetlands were drawn down and kept water levels low to accommodate the acquisition of topographic data. Uncontrolled flood events periodically inundated many study units, but because water control structures were left open, the wetlands quickly drained. Sampling sometimes followed these flood events, taking advantage of temporary increased water levels, but we also encountered extremely low water levels in some units throughout 2016. In general, drier conditions limited our ability to sample and likely decreased the number of species present in wetland units. Significant differences in species richness between years, likely associated with management actions, highlights the influence of anthropogenic manipulation on species use and distribution in actively managed wetlands.

Fish and amphibian species richness were not strongly predicted by within-wetland and upland habitat. Similar to other studies, hydrology was the major force driving patterns in fish and amphibian species distribution and richness (Richter and Azous 1995; Babbitt and Tanner 2000; Baber et al. 2002; Bouvier et al. 2009; Beesley et al. 2014), and connectivity, region, and water source may overshadow any effects of

within-wetland or upland habitat variables at the spatial scale of our study. In these actively managed wetlands, distribution and composition of vegetation within wetlands, as well as the extent and depth of wetland flooding, are often deliberately, and precisely manipulated by wetland managers (Fredrickson and Leigh 1998). Habitat manipulations within wetlands are an adaptive process, based on the habitat requirements of wetland species, previous wetland conditions, spatial relationships between to wetland and connected waterways, and management objectives (Galat et al. 1998), and may be too unpredictable and inconsistent for patterns in fish and amphibian richness to reflect trends in habitat preference observed in unmanaged wetlands. The nested location of individual wetland units within larger, constructed wetland complexes, all of which were in rural areas, may cause homogenous surrounding landscape features (i.e. almost all wetlands are surrounded by gravel roads and other wetland units), decreasing the explanatory power of these variables and resulting in lack of support for upland habitat models. Further, characterization of upland habitat only included areas within 200 m of the wetland and may not have described landscape features on a sufficient scale to capture effects of landscape scale variables on fish or amphibian species, as some species respond more to catchment level disturbance than local landuse (Findlay and Houlahan 1997; Mensing et al. 1998; Lammert and Allan 1999; Esselman et al. 2011). The use of species richness as a response variable may also diminish effects of habitat features as individual species exhibit difference habitat preferences. Another downside of using species richness is that it doesn’t provide any information about the type of species present (e.g. distinguishing between native and nonnative species) (Fleishman et al. 2006). Further examination of individual species and species’ traits would provide greater insight into

the quality of these wetland areas. Additional research is needed to illuminate habitat preferences of specific species.

Contrary to our results, studies conducted in unmanaged wetlands reported that patterns of fish or amphibian species richness and distribution were mainly determined by taxa and species-specific preferences of within-wetland characteristics like amount and composition of vegetation, wetland size, water depth, water quality, and presence of predators (Hecnar and M'Closkey 1997; Babbitt and Tanner 2000; Winemiller et al. 2000; Knutson et al. 2004; Burne and Griffin 2005; Werner et al. 2007; Shulse et al. 2010). Associations between fish and amphibian species richness and upland characteristics like forest cover, development, and the proximity/extent of wetlands in surrounding areas is also well documented in the literature (Findlay and Houlahan 1997; Knutson et al. 1999; Houlahan and Findlay 2003; Gray et al. 2004; Shulse et al. 2010). Many studies investigating the influence of wetland hydrology on fish and amphibians in wetlands focus on the effects of wetland hydroperiod or wetland size and depth in relation to wetland permanence and do not investigate aspects of hydrologic connectivity. While this may be appropriate for isolated, unmanaged wetlands, our results suggest that variables describing hydrologic connectivity and water source should be incorporated into studies of restored wetlands.

CONCLUSIONS

Our research indicates that in highly modified, actively managed wetland systems, hydrologic connectivity and managed water source are major forces impacting the distribution and richness of fish and amphibian wetland taxa. The variety of species detected in our study indicates that restored wetlands can provide suitable habitat for a

range of species and taxa, including sensitive species, but this will likely require thoughtful design and construction. Future wetland restoration should continue to consider hydrologic factors and determine appropriate hydrologic regimes based on project objectives and the species likely to use the area. If the goal is to accommodate the full gradient of wetland dependent species within a wetland complex, outfitting some wetlands with a direct connection to an outside water source, like a river, will promote the greatest utilization by fish species by providing the best access, while inundating other impoundments using well water might support the greatest range of amphibian species by allowing water delivery during key times of the year with less disturbance. .

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TABLES

Table 1. Description of hydrologic, within-wetland, upland landuse, spatial, and temporal covariates included in regression analysis predicting fish and amphibian species richness in 29 managed wetlands in Missouri, 2015-2016.

Variable type	Variable name	Description
Hydrologic	Connectivity	Unmanaged hydrologic wetland connectivity (wetland is connected=Y, wetland is not connected=N)
	WaterSource	Source of the water introduced to wetland unit for management purposes (River, Reservoir, or Well)
	FPY	Average annual flooding frequency (river gage data from 2011-2015)
Within wetland habitat (Local)	PerVeg	Total percentage of all vegetation (Submergent, emergent, woody, agricultural a trees) within the wetland unit
	Sub	% submergent vegetation of total wetland vegetation
	Eme	% emergent vegetation of total wetland vegetation
	Wood	% woody vegetation of total wetland vegetation

	Ag	% agricultural crops of total wetland vegetation
	Tree	% trees of total wetland vegetation
	T	Average temperature (^{0}C) across sample sites
	DO	Average dissolved oxygen (mg/L) across sample sites
	Depth	Average water depth (cm) across sample sites
	Area	Area (ha) of wetland at time of sampling
	Sample	Number of samples collected in wetland during seasonal sampling period
Upland landuse (Landscape)	For	% forest in immediate upland landscape (within 200 m)
	Wet	% wetland in immediate upland landscape (within 200 m)
	Grass	% grassland in immediate upland landscape (within 200 m)
	Dev	% developed areas in immediate upland landscape (within 200 m)
	Ag	% cropland in immediate upland landscape (within 200 m)
	MaxSize	Maximum size (ha) of wetland unit when flooded to full pool
	Sample	Sum of samples collected during spring and summer sampling periods each year
Spatial/temporal variables	Region	Ecoregion location of study area (Central Dissected Till Plains, Osage Plains, or Mississippi River Alluvial Valley)
	Study Area	Study area location of wetland unit (Fountain Grove CA, Swan Lake NWR, Four Rivers CA, Schell-Osage CA, Duck Creek CA, Otter Slough CA)
	Season	Seasonal sampling period (Spring or Summer)
	Year	Year of sampling (2015 or 2016)
	FishSpRich	Number of fish species in the regional species pool
	AmphSpRich	Number of amphibian species in the regional species pool

Table 2. Mean and standard deviation (in parentheses) of covariates across categories of hydrologic connectivity and managed water source for study of relationships of fish and amphibian species richness in Missouri wetlands, 2015-2016.

	Connectivity		Water Source		
Covariate	Yes	No	Well	River	Reservoir
FishSpPool	71.8 (14.2)	90.7 (12.3)	95.0 (0)	68.5 (8.7)	60.9 (7.6)
AmphSpPool	18.2 (3.6)	22.0 (2.9)	23.0 (0.0)	17.8 (2.9)	15.3 (2.5)
PerVeg (%)	41.7 (29.0)	38.3 (22.1)	52.6 (26.2)	25.5 (18.3)	51.8 (30.4)
Sub (%)	12.6 (14.5)	12.2 (19.3)	18.7 (17.6)	4.7 (4.4)	17.4 (18.8)
Eme (%)	18.9 (19.2)	16.9 (19.7)	24.3 (21.7)	11.6 (13.0)	22.3 (20.7)
Wood (%)	8.4 (8.7)	4.4 (2.9)	5.1 (4.2)	7.4 (5.7)	11.4 (12.5)
Ag (%)	1.4 (4.6)	3.8 (7.5)	2.0 (5.5)	1.9 (1.8)	1.6 (5.0)
Tree (%)	1.5 (2.1)	3.8 (3.7)	2.5 (3.7)	1.8 (2.1)	1.3 (1.6)
T (^{o}c)	26.2 (5.8)	29.1 (6.1)	29.2 (5.4)	26.0 (5.3)	24.7 (6.7)
DO (mg/L)	8.2 (2.5)	9.4 (2.2)	8.0 (2.7)	9.3 (2.0)	7.8 (2.6)
Depth (cm)	32.8 (11.0)	29.4 (9.9)	27.1 (6.7)	34.0 (9.9)	35.7 (14.0)
Area (ha)	42.8 (58.3)	24.9 (16.5)	17.3 (14.5)	59.2 (64.1)	35.4 (53.9)

For (%)	22.4 (15.3)	16.7 (15.2)	18.4 (20.0)	22.4 (12.4)	23.3 (11.9)
Wet (%)	40.2 (22.8)	33.2 (12.7)	32.5 (15.6)	39.8 (24.7)	45.9 (19.5)
Grass (%)	11.1 (12.8)	4.9 (2.4)	17.5 (17.0)	8.1 (4.6)	2.8 (3.0)
Dev (%)	8.7 (3.5)	8.1 (3.6)	7.1 (2.8)	9.7 (3.7)	8.5 (3.3)
Ag (%)	10.7 (11.6)	35.6 (16.4)	23.1 (19.2)	11.7 (12.5)	11.8 (12.6)
MaxSize (ha)	121.9 (90.4)	77.7 (50.6)	48.4 (20.5)	181.7 (68.8)	84.9 (83.0)
FPY	3.1 (1.1)	0.0 (0.0)	0.0 (0.0)*	3.5 (1.1)	2.1 (0.9)
Sample	23.7 (6.9)	22.0 (7.0)	23.0 (7.7)	23.7 (6.2)	23.5 (7.1)

*FPY data was unavailable for wetland units within the Duck Creek study area

Table. 3 Models and covariates for study of relationships of fish and amphibian species richness in Missouri wetlands, 2015-2016. All models include StudySite as a random factor.

Model type	Model Name	Variables in Model
Seasonal species richness	Temporal	Year, Season
	Region	Year, Season, Region
	Water Source	Year, Season, WaterSource
	Connectivity	Year, Season, Connectivity
	Sampling Intensity	Year, Season, NumberOfSample
	Area	Year, Season, Area
	Total Vegetation	Year, Season, PerVeg
	Vegetation Types	Year, Season, Sub, Eme, Wood, Ag, Tree
	Mean Water Quality	Year, Season, Tmean, DOmean, Dmean
	Extreme Water Quality	Year, Season, Tmax, DOmin, Dmax
	Fish (amphibian analysis)	Year, Season, FishSeasonalRich
Annual species richness	Temporal	Year
	Region	Year, Region
	WaterSource	Year, WaterSource
	Connectivity	Year, Connectivity

Sampling Intensity	Year, NumberOfSample
Maximum Wetland Size	Year, MaxWetlandSize
Sampling Intensity	Year, NumberOfSample
Upland Landuse	Year, For, Wet, Grass, Dev, Ag
Fish (only for amphibians)	Year, FishAnnualRich

Table 4. The 15 most widely distributed (as frequency out of 29 wetlands) and most abundant (out of total individuals caught) fish and amphibian species caught during a study of Missouri wetlands between 2015 and 2016.

Taxa	Species	# of wetlands	Species	Individuals
Fish	Green sunfish	28	Green sunfish	35859
	W. mosquito fish	25	Gizzard shad	21288
	Black bullhead	24	W. mosquito fish	21035
	Bluegill	24	Black bullhead	18066
	Black crappie	22	White crappie	8499
	White crappie	22	Emerald shiner	6477
	Common carp	21	Black crappie	3866
	Emerald shiner	20	Bantam sunfish	3079
	Yellow bullhead	20	Bluegill	3067
	Gizzard shad	17	Common carp	3024
	Golden shiner	17	Orangespotted sunfish	1653
	Orangespotted sunfish	16	Golden shiner	1043
	Shortnose gar	16	Lake chubsucker	948
	Goldfish	13	Grass pickerel	845
	Largemouth bass	13	Cypress darter	635
Amphibians	American bullfrog	29	American bullfrog	32998

S. leopard frog	26	S. leopard frog	27206
N. cricket frog	14	Green treefrog	1366
Green frog	10	American toad	387
Gray treefrog	9	Green frog	342
Green treefrog	8	Central newt	263
American toad	7	N. cricket frog	257
Central newt	7	Fowler's toad	186
Western lesser siren	6	N. spring peeper	172
Plains leopard frog	4	W. lesser siren	118
Fowler's toad	3	E. narrowmouth toad	64
E. narrowmouth toad	2	Gray treefrog	63
W. chorus frog	2	Woodhouse's toad	18
N. spring peeper	1	Plains leopard frog	6
Woodhouse's toad	1	W. chorus frog	5

Table 5. Number of parameters (k), delta AICc (ΔAICc), Akaike's weight (wt), and log likelihood for generalized linear mixed models predicting annual (n= 45) and seasonal (n=89) fish species richness of wetlands across the state of Missouri, 2015-2016.

Model type	Model name	k	ΔAICc	wt	logLik
Seasonal richness	Connectivity	6	0.00	0.90	-250.20
	Sampling Intensity	6	6.85	0.03	-253.62
	Total Vegetation	6	7.29	0.02	-253.84
	Temporal	5	7.94	0.01	-255.32
	Water Source	7	8.85	0.00	-253.44
	Mean Water Quality	8	9.90	0.00	-252.76
	Area	6	10.14	0.00	-255.27
	Region	7	10.35	0.00	-254.19
	Vegetation Type	10	10.42	0.00	-250.51
	Extreme Water Quality	8	12.03	0.00	-253.83
Annual richness	Connectivity	4	0.00	0.99	-121.23
	Temporal	3	11.78	0.00	-128.32
	Sampling Intensity	4	11.83	0.00	-127.14
	Water Source	5	13.69	0.00	-126.80
	Max Wetland Size	4	13.78	0.00	-128.12
	Region	5	14.35	0.00	-127.13
	Upland Landuse	8	19.52	0.00	-125.49

Table 6. Top ranked model parameter estimates (Estimate), standard errors (SE), P values (P), and 95% confidence intervals upper (UCL and LCL) for fixed effects variables from generalized linear mixed effects models predicting fish and amphibian species richness in Missouri wetlands, 2015-2016.

Model	Parameter	Estimate	S.E.	*p*-value	LCL	UCL
Fish						
Seasonal richness	(Intercept)	0.71	0.23	0.002	0.26	1.16
	Connectivity:Y	1.92	0.24	0.000	1.45	2.38
	Year:2016	-0.23	0.10	0.018	-0.32	-0.05
Annual richness	(Intercept)	0.93	0.22	0.000	0.49	1.37
	Connectivity:Y	1.93	0.23	0.000	1.48	2.38
	Year:2016	-0.19	0.08	0.021	-0.35	-0.03
Amphibian						
Seasonal richness	(Intercept)	1.67	0.15	0.000	1.37	1.97
	Region:CDTP	-0.63	0.20	0.002	-1.02	-0.23
	Region:OP	-1.09	0.23	0.000	-1.53	-0.64
	Year:2016	-0.48	0.13	0.000	-0.74	-0.22
	WaterSource:Reservoir	-0.70	0.25	0.004	-1.19	-0.22
	WaterSource:River	-0.90	0.23	0.000	-1.35	-0.46
Annual richness	(Intercept)	1.81	0.30	0.000	1.23	2.40
	Region:CDTP	-0.66	0.23	0.006	-1.11	-0.20
	Region:OP	-0.98	0.25	0.000	-1.48	-0.48
	Year:2016	-0.39	0.16	0.022	-0.71	-0.06

WaterSource:Reservoir	-0.67	0.26	0.012	-1.18	-0.17
WaterSource:River	-0.88	0.23	0.000	-1.34	-0.42

Table 7. Number of parameters (k), delta AICc (ΔAICc), Akaike's weight (wt), and log likelihood for generalized linear mixed models predicting annual (n= 45) and seasonal (n=89) amphibian species richness of wetlands across the state of Missouri, 2015-2016.

Model type	Model name	k	ΔAICc	wt	logLik
Seasonal richness	Region	6	0.00	0.65	-149.48
	Water Source	6	2.48	0.19	-150.72
	Sampling Intensity	5	5.84	0.04	-153.55
	Area	5	5.88	0.03	-153.57
	Temporal	4	5.94	0.03	-154.73
	Fish	5	7.01	0.02	-154.14
	Connectivity	5	7.03	0.02	-154.15
	Total Vegetation	5	8.06	0.01	-154.66
	Extreme Water Quality	7	11.45	0.00	-154.03
	Mean Water Quality	7	11.56	0.00	-154.08
	Vegetation Types	9	12.72	0.00	-152.22
Annual richness	Region	5	0.00	0.44	-79.53
	Water Source	5	0.72	0.31	-79.89
	Sampling Intensity	4	3.06	0.10	-82.32
	Temporal	3	3.51	0.08	-83.76
	Connectivity	4	5.27	0.03	-83.43
	Maximum Wetland Size	4	5.37	0.03	-83.48
	Fish	4	5.90	0.02	-83.75
	Upland Landuse	8	12.06	0.00	-81.33

FIGURES

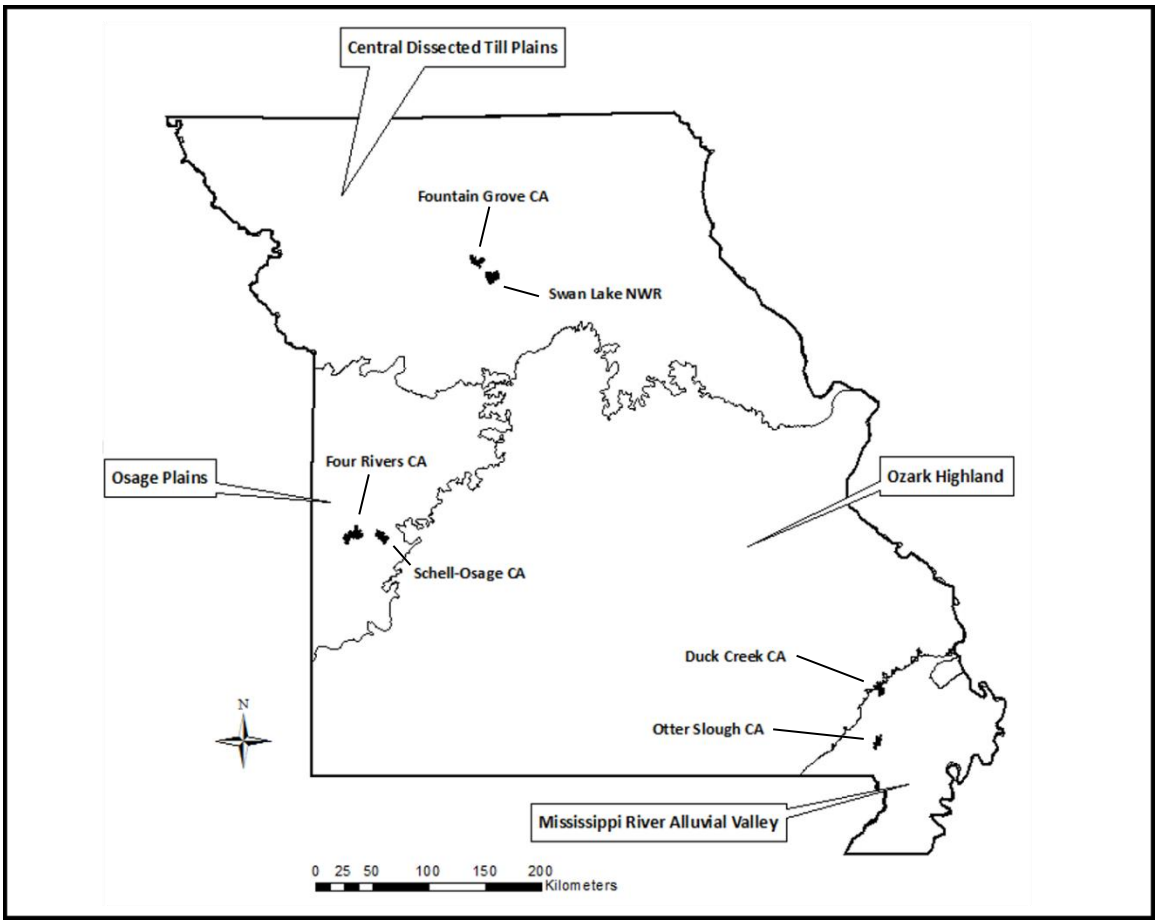

Fig. 1 Map of 6 study areas and ecoregions used for a study investigating patterns of fish and amphibian species richness in wetlands across the state of Missouri, 2015-2016.

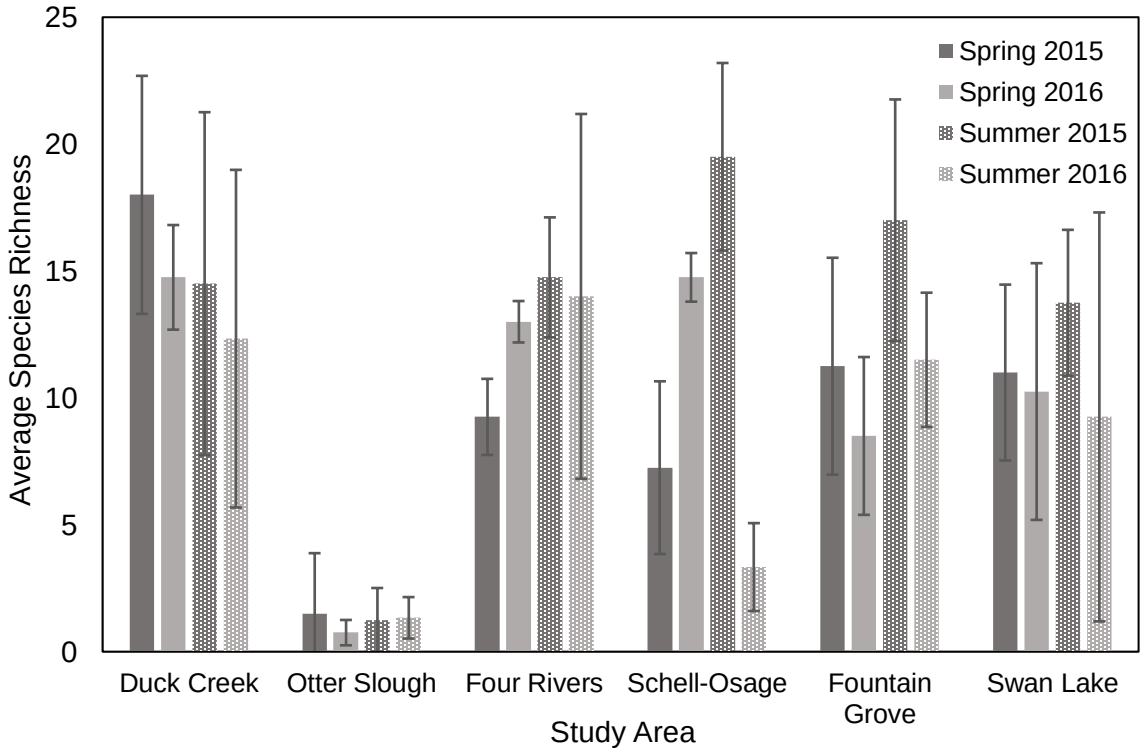

Fig. 2 Average fish species richness of wetlands across all six study areas in the state of Missouri during spring and summer 2015-2016. Error bars represent standard deviation.

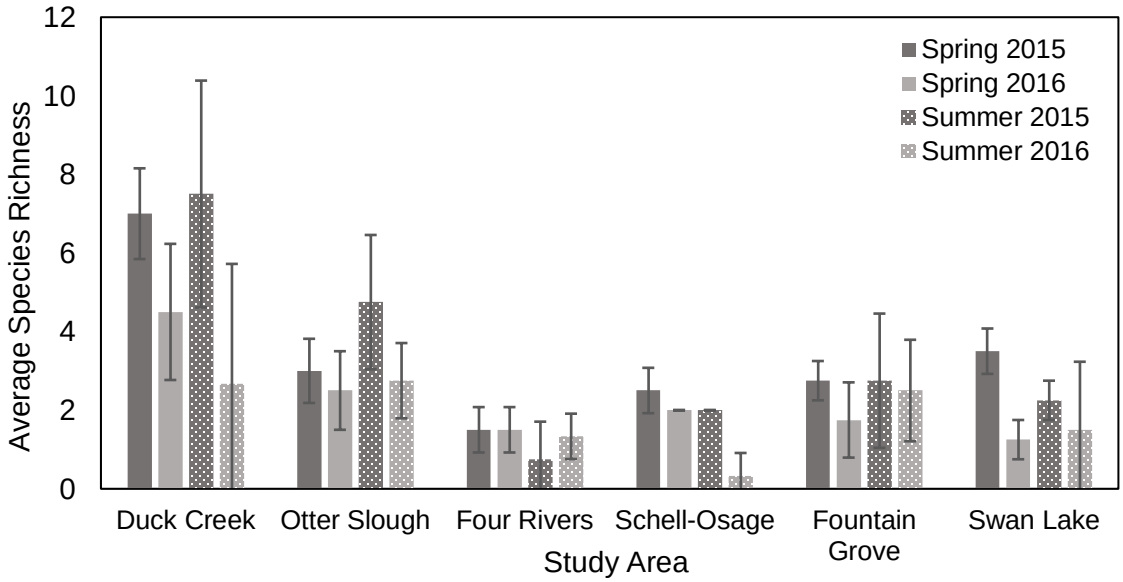

Fig. 3 Average amphibian species richness of wetlands across all six study areas in the state of Missouri during spring and summer 2015-2016. Error bars represent standard deviation.

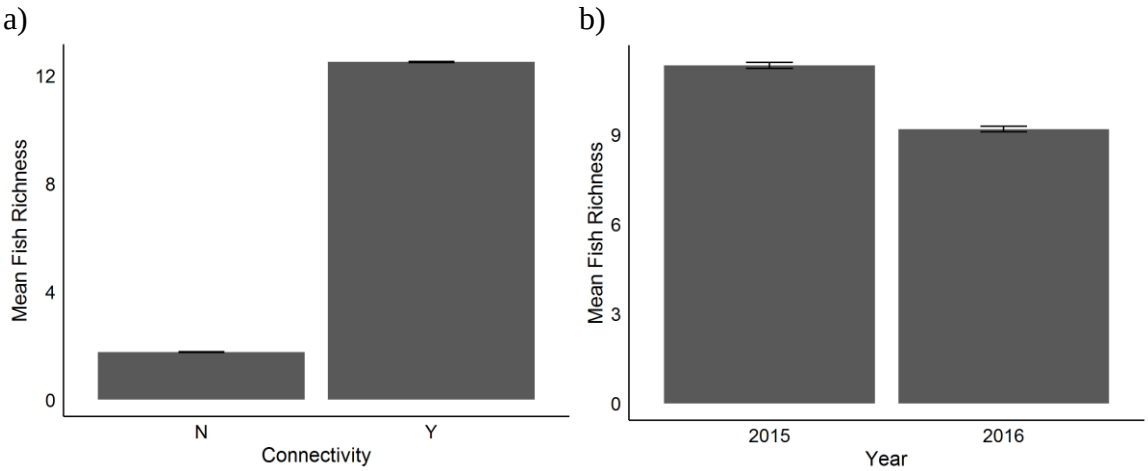

Fig. 4 Mean values of seasonal fish species richness fitted using generalized linear mixed models across levels of Connectivity (a) and Year (b) in a study of fish and amphibian species richness patterns in wetlands in Missouri, 2015-2016. Errors bars show standard error. (N=No connectivity, Y= connectivity).

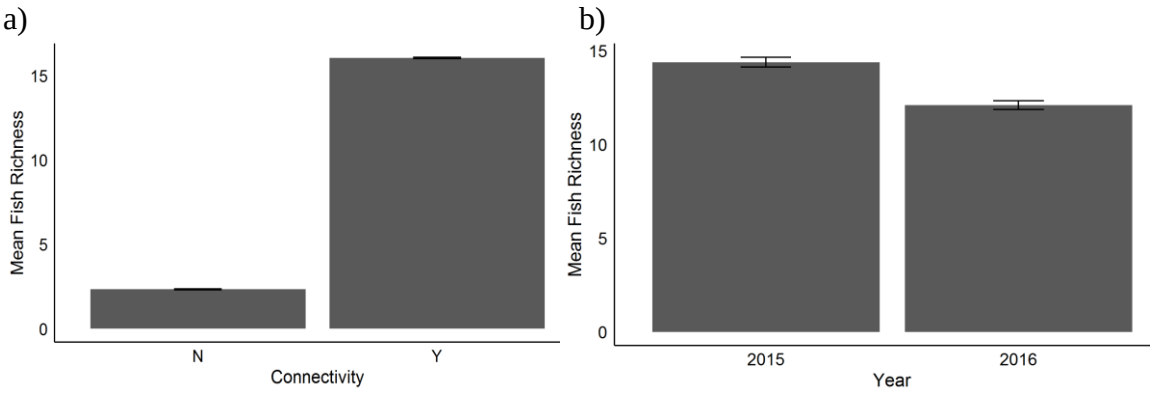

Fig. 5 Mean values of annual fish species richness fitted using generalized linear mixed models across levels of Connectivity (a) and Year (b) in a study of fish and amphibian species richness patterns in wetlands in Missouri, 2015-2016. Error bars show standard error. (N= No connectivity, Y= connectivity).

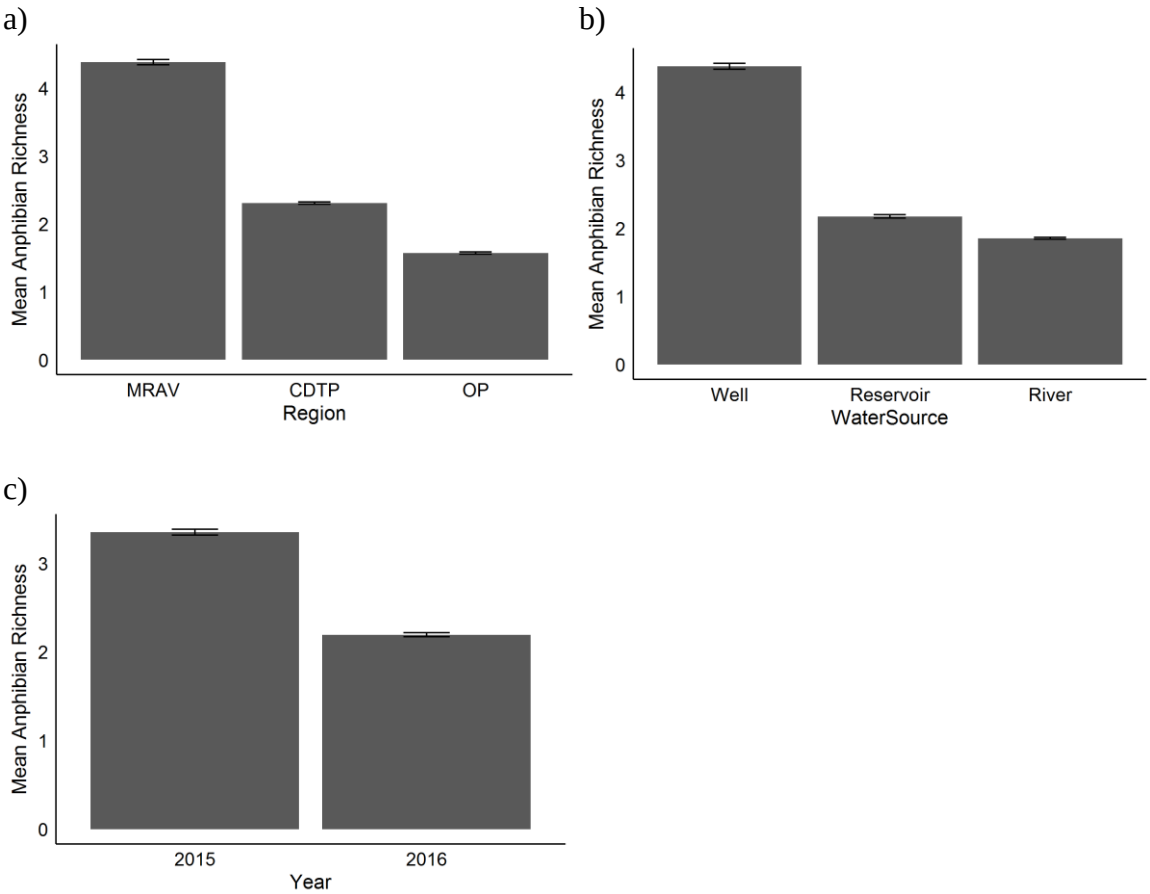

Fig. 6 Mean values of seasonal amphibian species richness fitted using generalized linear mixed models across levels of Region (a), Water Source (b), and Year (c) in a study of fish and amphibian species richness patterns in wetlands in Missouri, 2015-2016. Error bars show standard error. (MRAV= Mississippi River Alluvial Valley, CDTP= Central Dissected Till Plains).

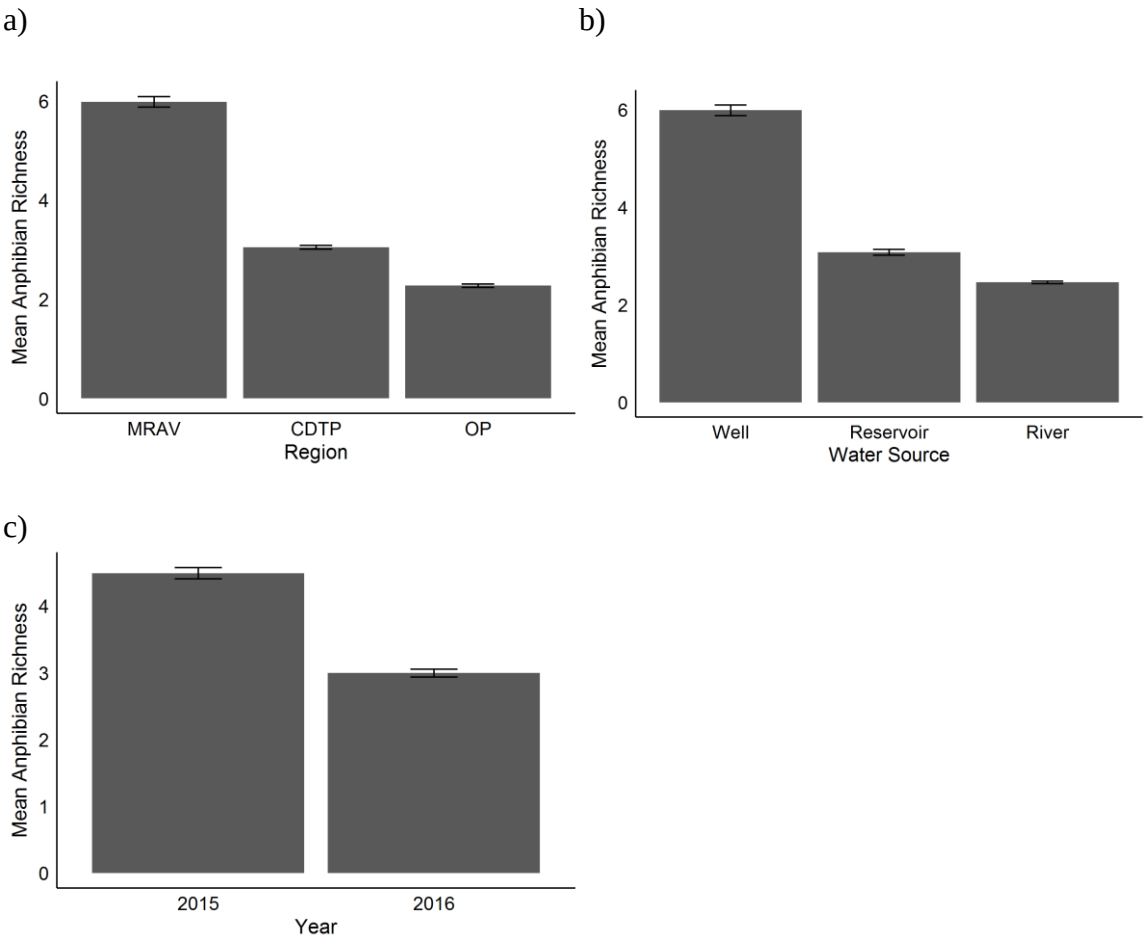

Fig. 7 Mean values of annual amphibian species richness fitted using generalized linear mixed models across levels of Region (a), Water Source (b), and Year (c) in a study of fish and amphibian species richness patterns in wetlands in Missouri, 2015-2016. Error bars show standard error. (MRAV= Mississippi River Alluvial Valley, CDTP= Central Dissected Till Plains).

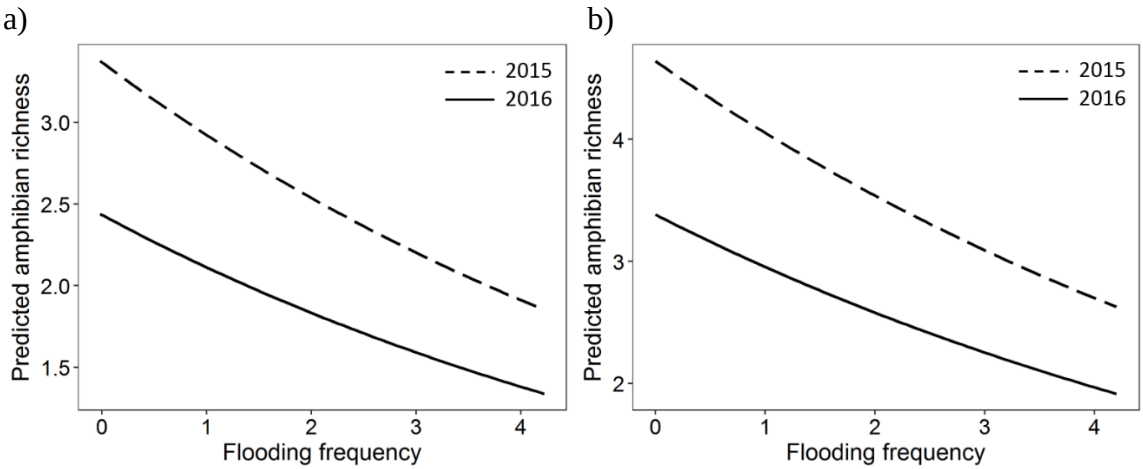

Fig. 8 Mean values of seasonal (a) and annual (b) amphibian species richness across a range of flooding frequency and years, fitted from generalized linear mixed models in a study of fish and amphibian species richness patterns in wetlands in Missouri, 2015-2016.

CHAPTER 3

CONSIDERATIONS FOR SURVEYING FISH AND AMPHIBIANS IN MISSOURI WETLANDS

Julia G. Kamps, Craig. P. Paukert, Elisabeth B. Webb

SYNOPSIS

This summary provides information to help managers select the most appropriate sampling method based on monitoring objectives, habitat and species characteristics, and available resources to determine fish and amphibian species richness in Missouri wetlands. While not an exhaustive review, all methods included (mini-fyke net, minnow trap, dipnet, and seine) have been tested in Missouri managed wetland complexes.

INTRODUCTION

Missouri wetlands are home to variety of fish and amphibian species. With a limited amount of wetland habitat present on the landscape, managing to meet the needs of a diverse assemblage of wetland dependent species is essential for the health of animal populations and the greater wetland ecosystem. In order to provide adequate habitat and make informed management decisions, it is important for managers to be able to identify species using the wetlands and monitor how they respond to wetland management actions. With limited time and resources, it is imperative to use efficient and effective sampling methods.

TYPES OF SURVEY METHODS

Survey methods can be divided into two classes of equipment: active and passive. Determining which class of equipment to use is the first step in choosing specific survey methods.

Table 1. Attributes of active and passive sampling methods.

Active	Passive
Rely more on the skill of the observer to capture fish and amphibians species	Rely on the ability of the net to capture and retain fish and amphibian species
Requires constant operation	Typically set and left overnight
Requires 1 or 2 people Especially effective for sessile species	Can be done by 1 person, easier with 2 Especially effective for mobile species
Can be done in a single day	Multi-day sampling

SURVEY METHOD DESCRIPTIONS AND SPECIFICATIONS

Passive

- Mini-fyke net: a mesh net consisting of two rectangular frame sections followed by a series of circular funnels, with a vertical lead of mesh attached to the front rectangular frame
    - Dimensions: 60 x 120 cm frame, 7.6 m lead, and 0.32-cm mesh
    - Price: $350.00
    - Pros: effective for fish and amphibian detection, detects individuals across widest size range, can be used by one person
    - Cons: bulky/heavy, expensive, requires overnight deployment
- Minnow trap: a two piece metal, mesh trap circular in shape with inward facing funnels at each slightly tapered end
    - Dimensions: 2.54-cm openings and 0.32-cm mesh
    - Price: $32.00
    - Pros: portable, easy to set in thick vegetation, effective for amphibian detection, can be set by one person

    - Cons: catch restricted to smaller individuals, requires overnight deployment, not very effective for fish detection

Active

- Dipnet: a mesh bag suspended from a metal, D shaped frame attached to the end of a long wooden handle
    - Dimensions: 40 x 23 x 30.5 cm frame, and 0.32-cm mesh
    - Price: $45.00
    - Pros: portable, done by one person in one day, effective for amphibian detection
    - Cons: catch restricted to smaller individuals, not very effective for fish detection, catch restricted to smaller individuals
- Seine: a rectangular section of mesh suspended between two wooden pole handles with weights strung from the bottom and buoys attached across the top
    - Dimensions: 1.2 x 4.5 m and 0.32-cm mesh
    - Price: $81.00
    - Pros: relatively affordable, portable, can be done in one day
    - Cons: need 2 people to operate, not effective in thick vegetation or floating algae, not very effective for detecting fish or amphibian species, catch restricted to smaller individuals

Sampling equipment can be purchased at a range of prices, and all equipment should last across multiple sampling seasons. It is also important to consider the tradeoffs between price of the equipment and costs associated with manpower needed to use it.

SELECTION OF SURVEY SITES

Water depth, vegetation density, and distance from shore influenced method effectiveness and should be considered when placing individual samples. If water is limited, it may be beneficial to take samples in deeper water near water control structures or in borrow pits.

Distance from shore

- Less fish were detected in samples further from shore
- Amphibian species detection was not influenced by distance
- Nearshore sampling will save time and effort while maximizing species detection

Depth

- Trends indicate fish and amphibian species are detected in greater numbers in shallower depths with most methods
- All sampling is generally restricted to wadeable water depths but passive methods can be deployed in deeper depths if using a boat
- Passive methods require water depths great enough to cover the openings and allow fish and amphibians to enter the net
- Seining is more effective detecting fish species in the deeper range of wadeable depths

Vegetation

- Minnow traps caught greater numbers of fish species with increasing vegetation density while trends suggest dipnets, mini-fyke nets, and seines caught greater numbers of fish species in less dense vegetation
- Amphibian species detection was not influenced by vegetation

- Passive equipment is easier to use in dense vegetation than active methods (active methods can snag, allowing individuals to escape)
- Avoid suspended debris and algae when using active methods to avoid clogging mesh

SAMPLING EFFORT

In general, an average of 6-7 samples with a mini-fyke net was able to detect the majority (>90%) of fish and amphibian species in a wetland unit across seasons. While minnow traps and dipnets can detect 100% of amphibian species in a wetland, these methods are inconsistent and performed very differently across years. This is demonstrated by the wide range of percentages seen at the bottom of the decision tree (Fig. 2). Mini-fyke nets were much more consistent. If monitoring is taking place in dry conditions (i.e. during the summer), and the goal is to detect all amphibian species present, it would be beneficial to take additional samples with a minnow trap or dipnet. See decision trees for additional information about sampling effort and expected results (Fig. 1 and 2).

SAMPLE TIMING

Timing of sampling is highly dependent on the objectives of monitoring efforts. Sampling might be done to gain information for upcoming management decisions, investigate species use based on environmental conditions (i.e floods or drought), or ideally on a regular basis to observe trends over time. Regular monitoring throughout the year and across years would likely provide the most useful information regarding changes in species composition and abundances as species assemblage changes on a seasonal and annual basis.

- Greater numbers of amphibian species were detected during spring

- Greater numbers of fish species were detected during summer following flood events

SPECIES IDENTIFICATION

Being able to properly identifying species caught during surveys is an important part of monitoring efforts. Utilizing interdisciplinary teams among organizations, institutions, or within Divisions can be a beneficial experience for researchers, species experts, and managers to interact, examine shared questions, and learn. The majority of fish and amphibians found in Missouri wetlands can be easily identified with the help of a comprehensive field guide. If identification is not possible by the observer, species can be documented in photographs or preserved for identification by an expert at a later time. Below are recommended field guides:

Pflieger, W.L. 1997. The fishes of Missouri, Revised edition. Missouri Department of Conservation,

Jefferson City, Missouri.

Johnson, T.R. 2000. The amphibians and reptiles of Missouri. Missouri Department of Conservation, Jefferson City, Missouri.

DECISION TREES

The decision trees below are provided to help managers choose the ideal sampling method to achieve their monitoring objectives. We included the number of sample sites required with each method necessary to detect the maximum percentage of species present in a wetland based on species accumulation curves (Chapter 1). In addition to survey timing, it is important to consider the limitations of each method regarding wetland habitat characteristics (i.e. water depth and vegetation).

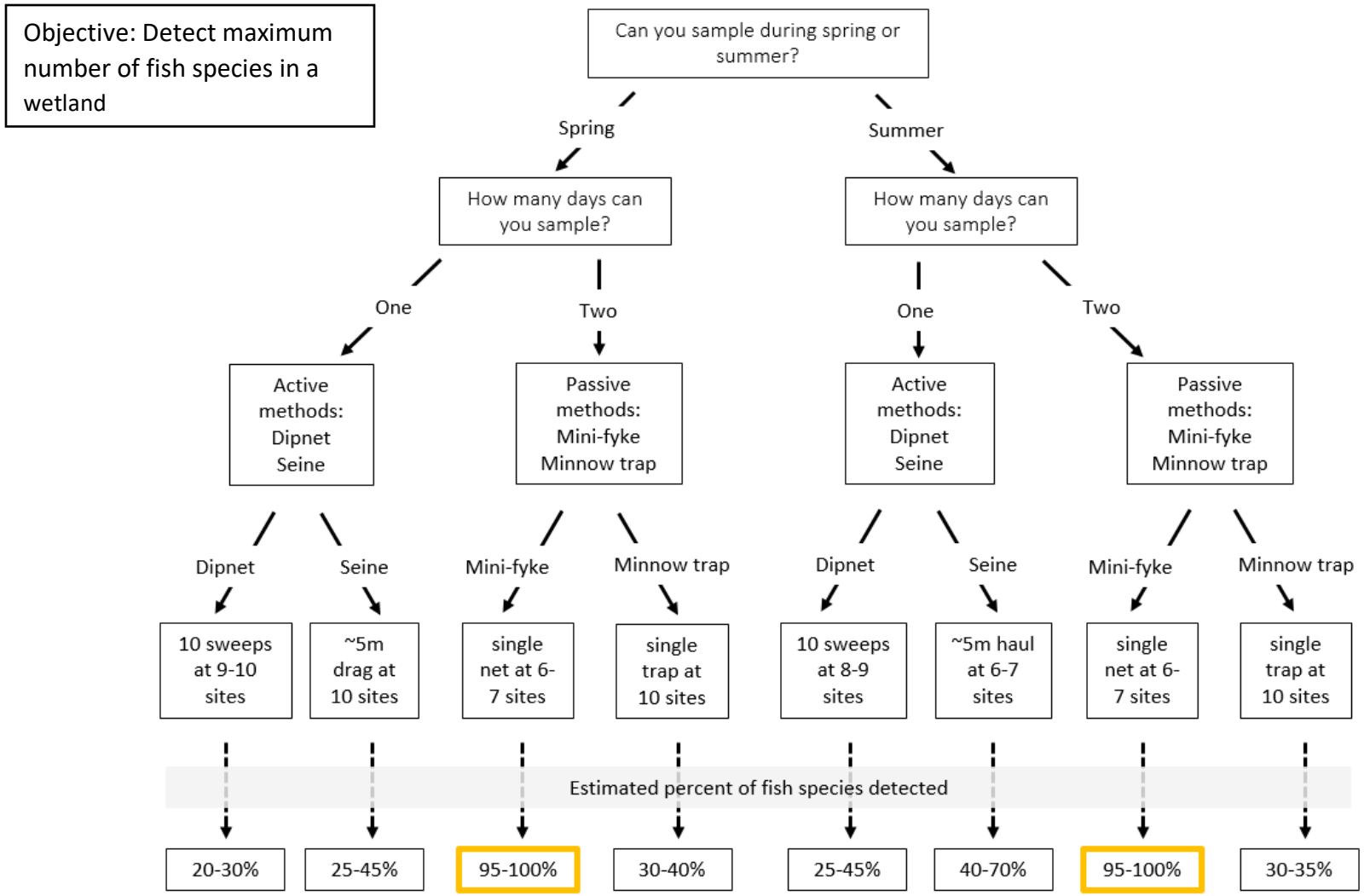

Fig. 1 Decision tree to help detect maximum number of fish species in a wetland. Highlighted boxes indicate highest percentage of total fish species detected per wetland.

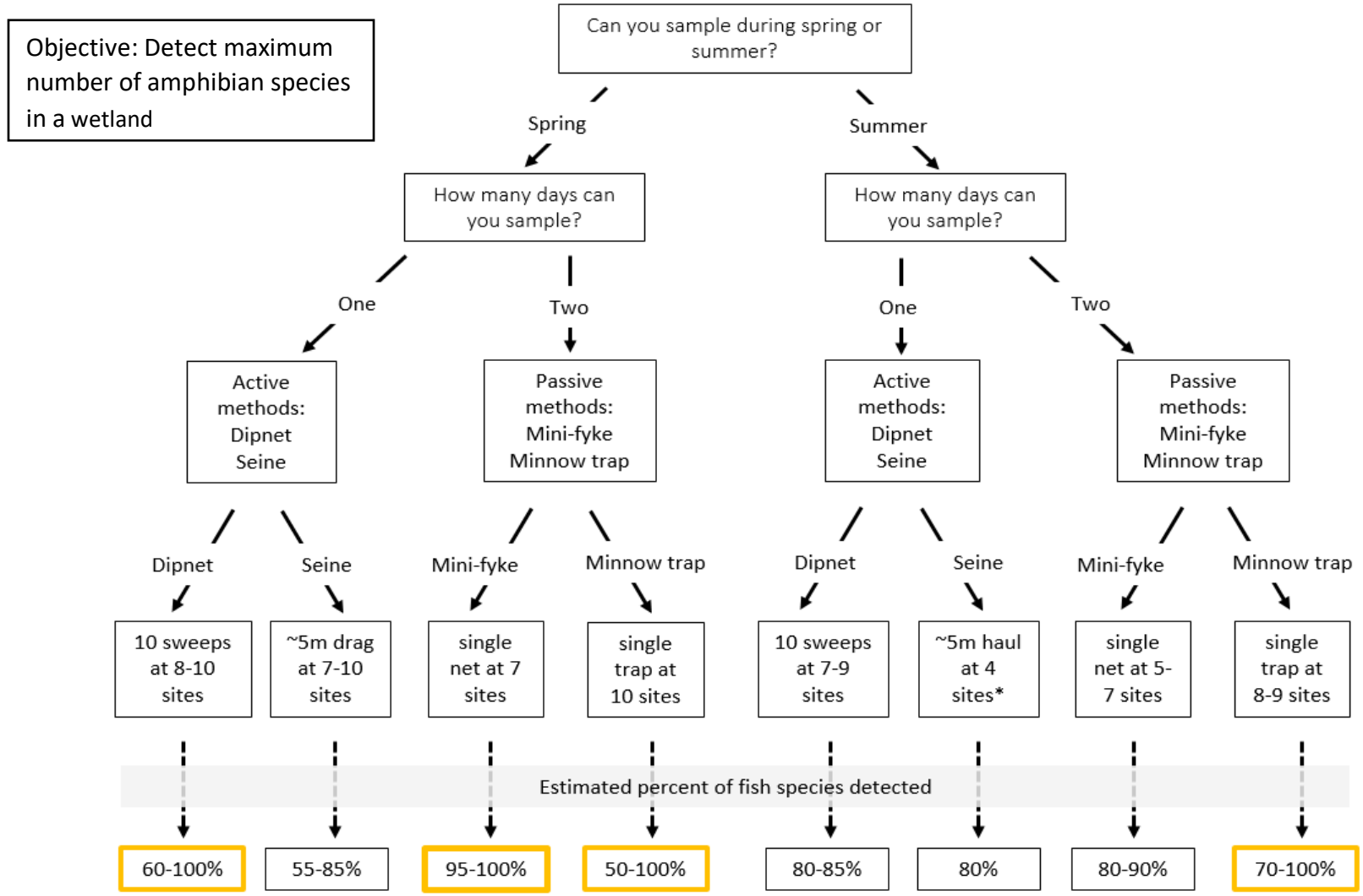

Fig. 2 Decision tree to help detect maximum number of amphibian species in a wetland. Highlighted boxes indicate highest percentage of total amphibian species richness per wetland

SPECIES CO-OCCURRENCE

By looking at species co-occurrence patterns, observers can gain insight into the greater fish or amphibian community with limited species detection. We used the cooccur function in package Cooccur in R to create heatmaps representing pairwise species combinations and their co-occurrence signs (positive or negative). We built data matrices using presence-absence data from seasonal wetland sampling efforts to exclude species detected during different seasons at the same wetland unit. Species pairs that were expected to occur together at less than one site were excluded from analysis. We calculated the number of positive, negative, and random species associations, as well as number of pairs found (Table 2). Heatmaps show species with significant positive and negative associations moving from those with the strongest negative associations on the left to those with the strongest positive associations on the right.

Table 2. Number of species pairs found, pairs analyzed, positive pair associations, negative pair associations, and random pair associations in an analysis of fish and amphibian species co-occurrence using seasonal presence-absence data from Missouri wetlands, 2015-2016.

Species	# pairs found	# pairs analyzed	Positive associations	Negative associations	Random associations
Fish	1431	663	202	53	408
Amphibians	105	47	12	0	35
Both	2346	1044	291	69	684

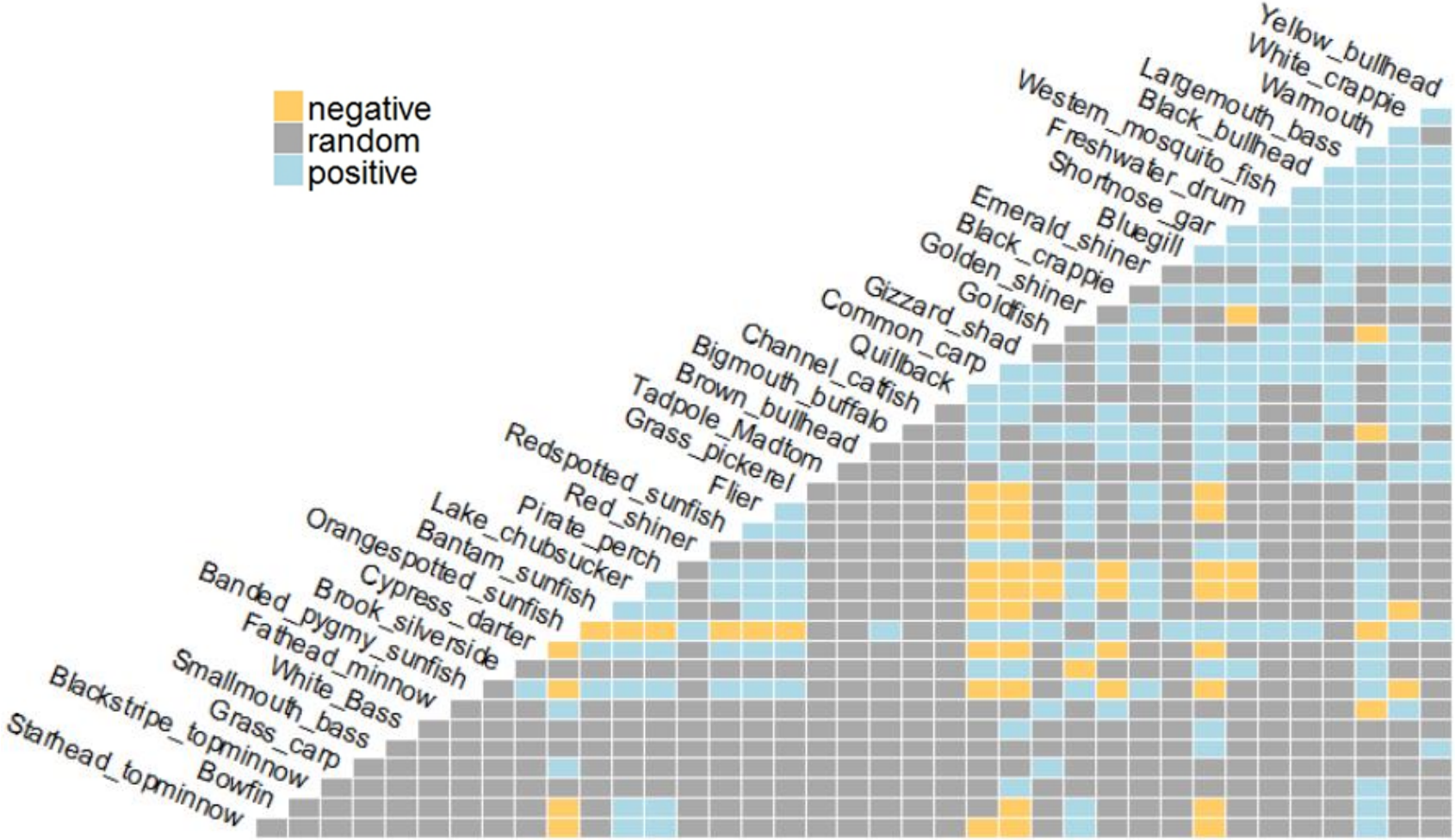

Fig. 3 Fish species association heat map using collective species detection data from a study of wetlands in Missouri, 2015-2016

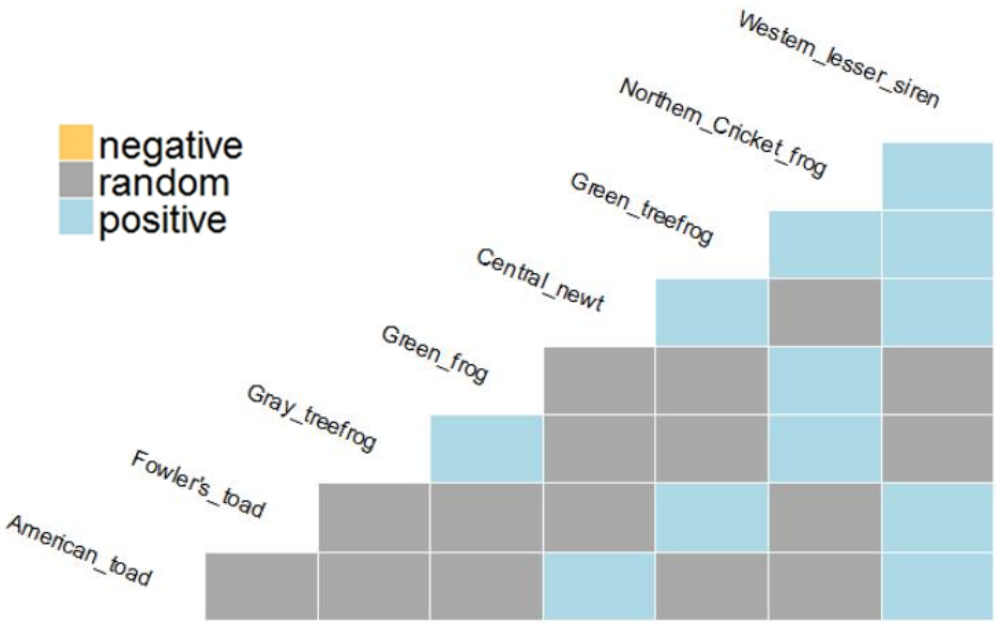

Fig. 4 Amphibian species association heat map using collective species detection data from a study of wetlands in Missouri, 2015-2016

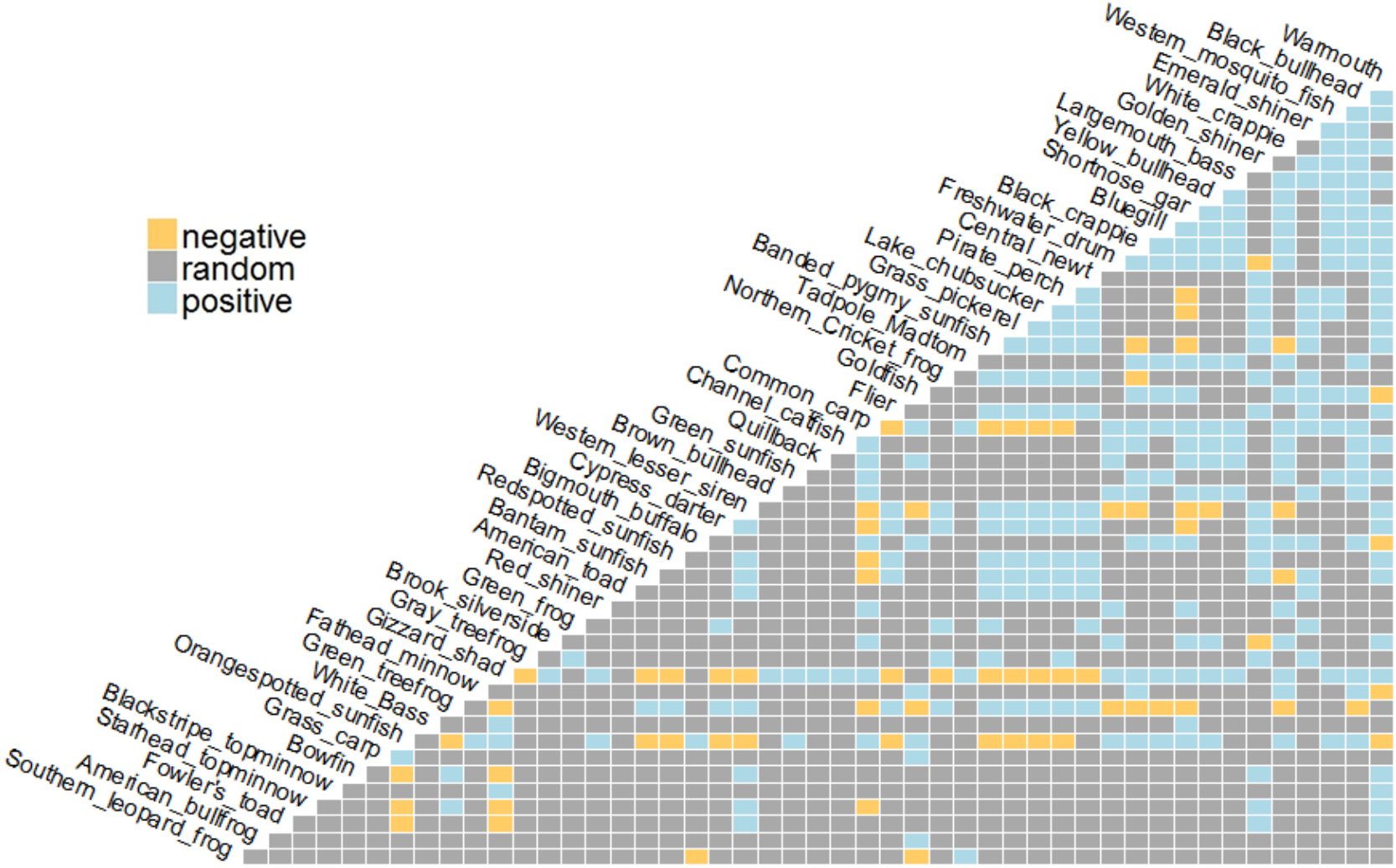

Fig. 5 Fish and amphibian species association heat map using collective species detection data from a study of wetlands in Missouri, 2015-2016

FUTURE RESEARCH

Information gained from this study will reduce uncertainty associated with fish and amphibian monitoring efforts and increase sampling efficiency to aid managers and researchers in answering other wetland-related research questions. While our study detected a number of amphibian species, it would be interesting to compare amphibian detection using mini-fyke nets, dipnets, and minnow traps to aural amphibian sampling which is likely better at detecting adult amphibians (Heyer 1994). While these results give us a good snapshot of the fish and amphibian species using Missouri wetlands, continued monitoring of species richness, abundance, and population trends over time will provide the most information regarding the effects of wetland management and the ability of managed wetland habitats to provide essential habitats to these taxa. Protocols outlining the most effective and efficient sampling techniques should make monitoring more feasible for wetland managers and researchers. It would be especially informative to track population trends of SOCC and invasive species. Species detections from this study could be used to further evaluate relationships between individual species, their patterns of co-occurrence, and how these patterns influence overall wetland species assemblages. Information regarding the impacts of flooding and managed water sources on wetland species is important to consider when designing new wetland complexes, and sampling protocols evaluated here could be used to monitor species before, during, and after wetland enhancement projects to help determine how changes to wetland hydrology impact species access to and use of wetlands.

APPENDIX A

Table A1. List of all fish species detected in 29 wetlands at six study areas across the state of Missouri, 2015-2016. (DC=Duck Creek, FG= Fountain Grove, FR= Four Rivers, OS= Otter Slough, SO= Schell-Osage, and SL=Swan Lake)

	2015						2016					
Species	DC	FG	FR	OS	SO	SL	DC	FG	FR	OS	SO	SL
Banded pygmy sunfish	x						x					
Bantam sunfish	x						x					
Bighead carp		x										
Bigmouth buffalo		x				x		x				x
Black bullhead	x	x	x		x	x	x	x	x		x	x
Black crappie	x	x	x		x	x	x	x	x		x	x
Blackstripe topminnow			x								x	
Bluegill	x	x	x		x	x	x	x	x		x	x
Bluntnose minnow		x										
Bowfin	x						x					
Brook silverside			x		x				x		x	
Brown bullhead			x		x							
Channel catfish		x	x		x							x
Common carp	x	x	x	x	x	x		x	x		x	x
Cypress darter	x						x					
Emerald shiner	x	x	x	x	x	x	x	x	x			x
Fathead minnow		x				x		x				
Flathead catfish					x							
Flier	x						x					
Freshwater drum		x	x		x	x			x		x	x
Gizzard shad		x	x	x	x	x		x	x		x	x
Golden shiner	x	x		x	x	x	x	x				x
Goldfish	x	x				x		x	x			x
Grass carp		x			x	x	x					x
Grass pickerel							x					
Green sunfish		x	x	x	x	x	x	x	x	x	x	x
Lake chubsucker	x						x					
Largemouth bass	x	x	x		x	x			x		x	x
Logperch			x		x							
Longear sunfish	x											
Longnose gar	x						x					
Orangespotted sunfish		x	x		x	x		x	x		x	x
Pirate perch	x						x					
Quillback		x	x		x				x			
Red shiner			x					x	x		x	x
Redspotted sunfish	x						x					

River carpsucker			x									
	2015						2016					
Species	DC	FG	FR	OS	SO	SL	DC	FG	FR	OS	SO	SL
Shortnose gar		x	x		x	x	x	x	x		x	x
Silver Carp		x										
Slender madtom											x	
Slough darter							x					
Smallmouth bass			x		x							
Smallmouth buffalo									x			x
Spotted bass	x		x									
Spotted gar	x											
Starhead topminnow	x						x					
Striped bass					x							
Tadpole madtom					x			x				x
Walleye					x							
Warmouth	x		x		x		x		x		x	
Western mosquito fish	x	x	x		x	x	x	x	x	x	x	x
White bass									x			x
White crappie	x	x	x		x	x	x	x	x		x	x
Yellow bullhead	x	x	x		x	x	x	x	x		x	x
Total species	25	24	25	5	27	19	24	18	21	2	18	23

Table A2. List of all amphibian species detected in 29 wetlands at six study areas across the state of Missouri, 2015-2016. (DC=Duck Creek, FG= Fountain Grove, FR= Four Rivers, OS= Otter Slough, SO= Schell-Osage, and SL=Swan Lake)

	2015						2016					
Species	DC	FG	FR	OS	SO	SL	DC	FG	FR	OS	SO	SL
American bullfrog	x	x	x	x	x	x	x	x	x	x	x	x
American toad	x			x					x	x		x
Central newt	x				x	x	x					
Eastern narrowmouth toad	x						x					
Fowler's toad	x						x					
Gray treefrog	x			x		x		x				
Green frog	x	x	x	x	x	x		x				
Green treefrog	x			x			x			x		
Northern cricket frog	x	x		x	x	x	x	x	x	x		x
Northern spring peeper	x											
Plains leopard frog		x			x							
Southern leopard frog	x	x	x	x	x	x	x	x	x	x	x	x
Western chorus frog						x						
Western lesser siren	x			x			x					
Woodhouse’s toad		x										
Total species	12	6	3	8	6	7	8	5	4	5	2	4

APPENDIX B

Table B1. List of fish species in regional species pool potentially present at each study area according to Missouri Fish Community Database records for study of 29 wetlands across the state of Missouri, 2015-2016. (DC=Duck Creek, FG= Fountain Grove, FR= Four Rivers, OS= Otter Slough, SO= Schell-Osage, and SL=Swan Lake)

Species	DC	FG	FR	OS	SO	SL
Banded darter	x		x	x	x	
Banded pigmy sunfish	x			x		
Banded sculpin			x		x	
Bantam sunfish	x			x		
Bigmouth buffalo	x	x	x	x	x	x
Bigmouth shiner		x				x
Black buffalo	x			x		
Black bullhead	x	x	x	x	x	x
Black crappie	x	x	x	x	x	x
Black redhorse		x	x		x	x
Blacknose shiner			x		x	
Blackside darter	x			x		
Blackspotted topminnow	x		x	x	x	
Blackstripe topminnow	x		x	x	x	
Blacktail shiner	x		x	x	x	
Bleeding shiner			x		x	
Blue catfish		x				x
Blue sucker	x			x		
Bluegill	x	x	x	x	x	x
Bluntnose darter	x			x		
Bluntnose minnow	x	x	x	x	x	x
Bowfin	x			x		
Brassy minnow		x				x
Brindled madtom	x			x		
Brook silverside	x		x	x	x	
Brown bullhead	x			x		
Bullhead minnow	x	x		x		x
Central stoneroller	x	x	x	x	x	x
Chain pickerel	x			x		
Channel catfish	x	x	x	x	x	x
Common carp	x	x	x	x	x	x
Common shiner		x	x		x	x
Creek chub	x	x	x	x	x	x
Creek chubsucker	x			x		

Species	DC	FG	FR	OS	SO	SL
Cypress darter	x			x		
Cypress minnow	x			x		
Dollar sunfish	x			x		
Dusky darter	x			x		
Eastern redfin shiner	x			x		
Emerald shiner	x	x	x	x	x	x
Fantail darter			x		x	
Fathead minnow	x	x	x	x	x	x
Flathead catfish	x	x	x	x	x	x
Flier	x			x		
Freckled madtom	x		x	x	x	
Freshwater drum	x	x	x	x	x	x
Ghost shiner			x		x	
Gizzard shad	x	x	x	x	x	x
Golden redhorse		x	x		x	x
Golden shiner	x	x	x	x	x	x
Goldeye		x				x
Goldfish		x				x
Goldstripe darter	x			x		
Grass carp		x				x
Grass pickerel	x			x		
Green sunfish	x	x	x	x	x	x
Greenside darter			x		x	
Harlequin darter	x			x		
Highfin carpsucker	x			x		
Hornyhead chub			x		x	
Ironcolor shiner	x			x		
Johnny darter	x	x	x	x	x	x
Lake chubsucker	x			x		
Largemouth bass	x	x	x	x	x	x
Largescale stoneroller			x		x	
Least darter			x		x	
Logperch	x	x	x	x	x	x
Longear sunfish	x	x	x	x	x	x
Longnose gar	x	x	x	x	x	x
Mimic shiner	x			x		
Mississippi silvery minnow	x			x		
Mud darter	x			x		
Northern hog sucker	x		x	x	x	
Northern orangethroat darter	x		x	x	x	

Species	DC	FG	FR	OS	SO	SL
Northern studfish	x		x	x	x	
Orangespotted sunfish	x	x	x	x	x	x
Orangethroat darter		x	x		x	x
Ozark minnow			x		x	
Pirate perch	x			x		
Plains minnow		x				x
Pugnose minnow	x			x		
Quillback	x	x	x	x	x	x
Rainbow darter			x		x	
Red shiner	x	x	x	x	x	x
Redear sunfish	x		x	x	x	
Redfin shiner	x	x	x	x	x	x
Redspotted sunfish	x			x		
Ribbon shiner	x			x		
River carpsucker	x	x	x	x	x	x
River darter	x			x		
Sand shiner		x	x		x	x
Scaly sand darter	x			x		
Shadow bass	x			x		
Shoal chub	x	x		x		x
Shorthead redhorse	x	x		x		x
Shortnose gar	x	x	x	x	x	x
Silver chub	x	x		x		x
Silver redhorse			x		x	
Slender madtom		x	x		x	x
Slenderhead darter	x		x	x	x	
Slough darter	x		x	x	x	
Smallmouth bass			x		x	
Smallmouth buffalo	x	x	x	x	x	x
Southern redbelly dace			x		x	
Speckled darter	x			x		
Spotted bass	x	x	x	x	x	x
Spotted gar	x			x		
Spotted sucker	x		x	x	x	
Starhead topminnow	x			x		
Steelcolor shiner	x			x		
Stippled darter			x		x	
Stonecat		x	x		x	x
Striped fantail darter	x		x	x	x	
Striped shiner	x		x	x	x	

Species	DC	FG	FR	OS	SO	SL
Suckermouth minnow	x	x	x	x	x	x
Tadpole madtom	x	x	x	x	x	x
Taillight shiner	x			x		
Trout-perch		x				x
Warmouth	x		x	x	x	
Weed shiner	x			x		
Western mosquitofish	x	x	x	x	x	x
Western redfin shiner		x	x		x	x
Western sand darter	x			x		
Western silvery minnow		x				x
White bass	x	x	x	x	x	x
White crappie	x	x	x	x	x	x
White sucker		x	x		x	x
Yellow bullhead	x	x	x	x	x	x
Total	95	57	75	95	75	57

Table B2. List of amphibian species in regional species pool potentially present at each study area according to Missouri Herpetological Atlas records for study of 29 wetlands across the state of Missouri, 2015-2016. (DC=Duck Creek, FG= Fountain Grove, FR= Four Rivers, OS= Otter Slough, SO= Schell-Osage, and SL=Swan Lake)

Species	DC	FG	FR	OS	SO	SL
American Bullfrog	x	x	x	x	x	x
American Toad	x	x	x	x	x	x
Blanchard's Cricket Frog	x	x	x	x	x	x
Boreal Chorus Frog		x	x		x	x
Cajun Chorus Frog	x			x		
Central Newt	x		x	x	x	
Eastern Narrow-mouthed Toad	x			x		
Eastern Spadefoot	x			x		
Eastern Tiger Salamander		x				x
Fowler's Toad	x	x		x		x
Gray Treefrog complex	x	x	x	x	x	x
Great Plains Toad		x				x
Green Frog	x	x	x	x	x	x
Green Treefrog	x			x		
Illinois Chorus Frog	x			x		
Marbled Salamander	x			x		
Mole Salamander	x			x		
Northern Crawfish Frog		x	x		x	x
Northern Leopard Frog		x				x
Plains Leopard Frog		x	x		x	x
Plains Spadefoot		x				x
Small-mouthed Salamander	x	x	x	x	x	x
Southern Leopard Frog	x	x	x	x	x	x
Spotted Salamander	x		x	x	x	
Spring Peeper	x	x	x	x	x	x
Three-toed Amphiuma	x			x		
Upland Chorus Frog	x			x		
Western Lesser Siren	x			x		
Wood Frog		x				x
Woodhouse's Toad		x				x
Total	21	17	13	21	13	17

APPENDIX C

Hydrographs for seasonal sampling periods in relation to 2015 (Fig. C1) and 2016 (Fig. C2) annual flood patterns recorded by USGS river gages near the Swan Lake NWR (a), Fountain Grove CA (b), Four Rivers CA (c), and Schell-Osage CA (d) study areas. Otter Slough CA did not flood and Duck Creek CA experienced smaller scale floods from local creeks and ditches where gage data is unavailable. These graphs help illustrate the dynamic nature of flooding at our study areas and the timing of sampling in relation to flood events. River gages are not located directly adjacent to study areas and hydrographs do not represent exact flooding regimes.

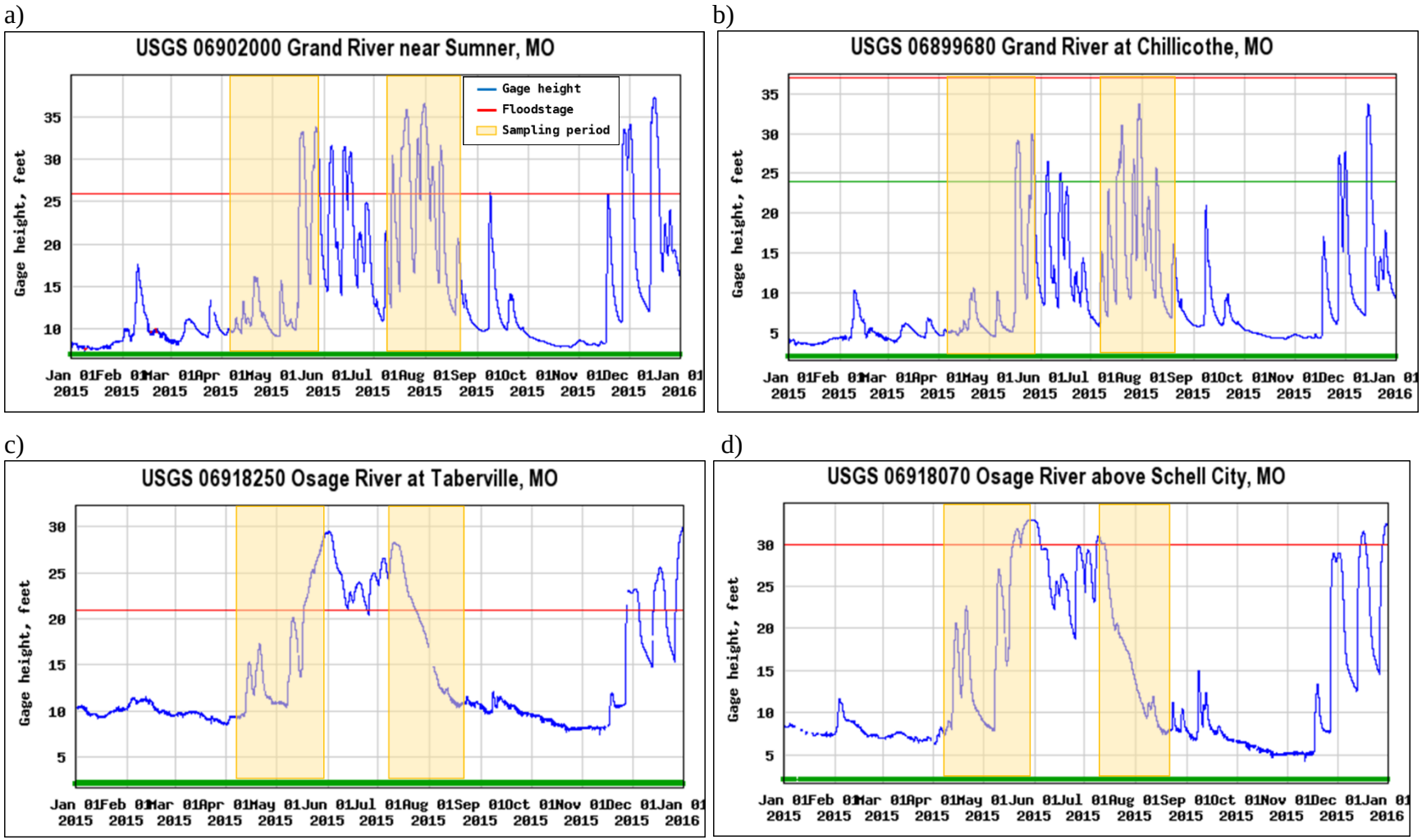

Fig. C1 Hydrographs showing annual flood patterns and seasonal sampling periods near a) Swan Lake NWR, b) Fountain Grove CA, c) Four Rivers CA, and d) Schell-Osage CA study areas during 2015 in study of 29 Missouri wetlands

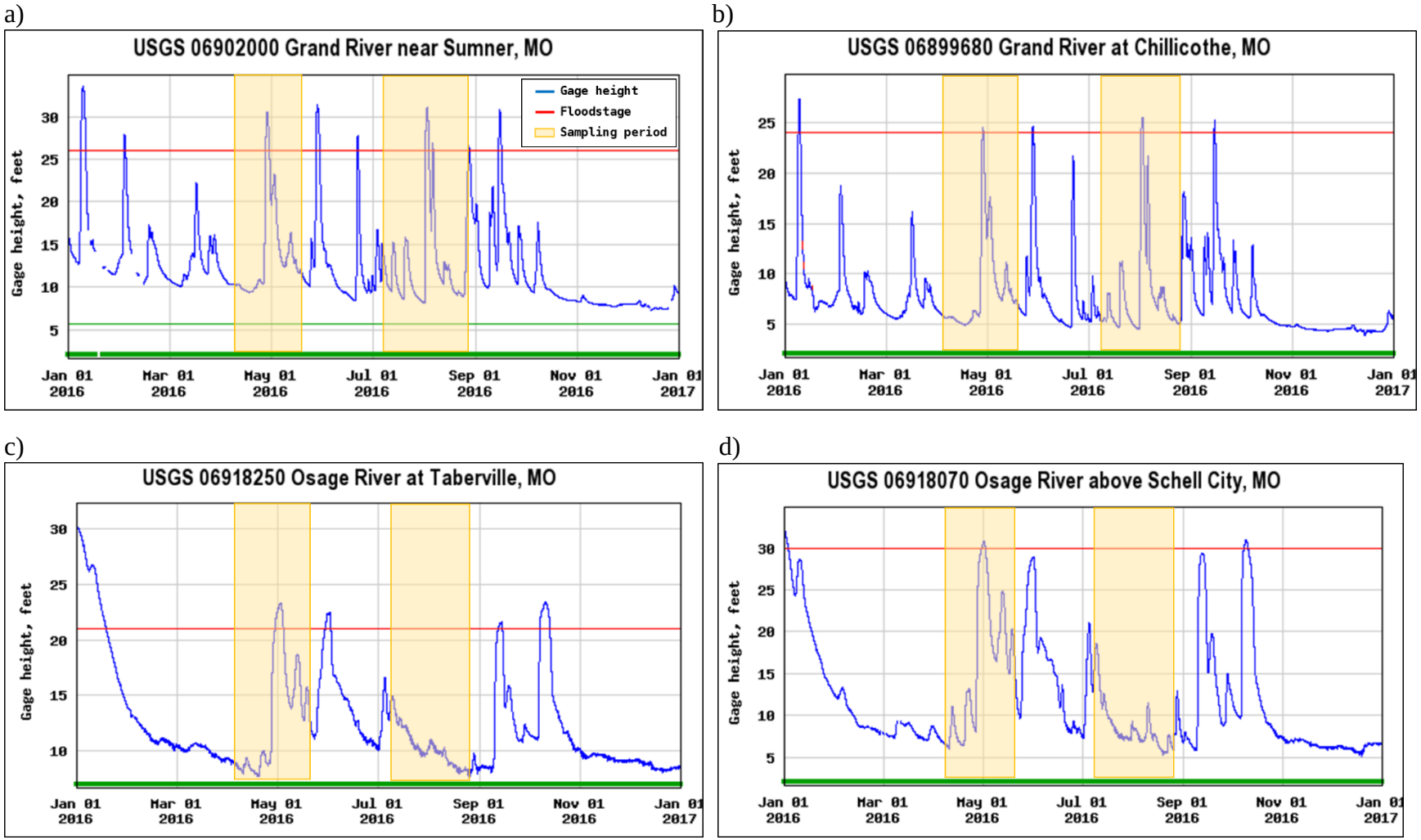

Fig. C2 Hydrographs showing annual flood patterns and seasonal sampling periods near a) Swan Lake NWR, b) Fountain Grove CA, c) Four Rivers CA, and d) Schell-Osage CA during 2016 in study of 29 Missouri wetlands