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Notes and Discussion

Pond-Breeding Amphibian Community Composition in Missouri

Abstract.—We examined pond-breeding amphibian community composition at 210 ponds in Missouri between 2002 and 2012 using drift fence, dipnet, and funnel trap data. We encountered a total of 20 pond-breeding amphibian species in the combined surveys. We also examined whether the presence of American Bullfrogs, *Lithobates catesbeianus*, and fish influenced these patterns of diversity. Our results indicate the presence of American Bullfrogs, fish, and their interaction influenced the community composition of amphibians at these sites but in opposite patterns. American Bullfrogs often had a positive relationship with the total number of species, total caudate species, and total anuran species, whereas fish presence was negatively associated overall with species diversity, and the presence of both American Bullfrogs and fish was negatively associated with anuran species diversity. It is important to have baseline community species composition data from wide geographical ranges so spatiotemporal changes in community structure can be noted and assessed.

Introduction

Understanding the mechanisms that influence patterns of community structure is a fundamental goal in ecology. Knowledge of these mechanisms can be critical in evaluating the strength of species interactions, how food webs function, and how both local and regional biodiversity is maintained (Morin, 2011). Yet baseline information on community structure in natural systems is often lacking or poorly developed for comparisons of communities across large geographic areas (*e.g.* entire states), which is needed to understand if and how spatiotemporal changes occur in community composition.

Information on the mechanisms that influence amphibian community structure is increasingly necessary and important as many communities face threats such as invasive species, habitat alteration, or climate change (Parmesan, 2006; Semlitsch *et al.*, 2009) and are experiencing widespread declines (Stuart *et al.,* 2004). Management decisions hinge upon correct assessments of how these threats influence communities or ecosystems, resulting in a need for contemporary studies on community structure as a reference point for tracking potential changes.

Pond-breeding amphibians are model taxa for examining patterns of community structure. Populations are centered around breeding ponds, with larval stages occupying ponds for a few weeks to several months prior to completing metamorphosis (Wilbur,1980). Aquatic stages face a variety of threats including contamination and invasive species (Blaustein *et al.*, 2011), making population monitoring critical to determine both current patterns of community structure and potential factors already present that influence species composition. We examined pond amphibian community structure at breeding sites across the state of Missouri and examined whether the presence of American Bullfrogs, *Lithobates catesbeianus*, and fish influenced these patterns of diversity.

Methods

We sampled for amphibians in 210 ponds and wetlands between 2002 and 2012 in Missouri, U.S.A. (*see* electronic appendix 1 https://mospace.umsystem.edu/xmlui/handle/10355/40553). Sampling at these sites was conducted with drift fences around ponds or a combination of dipnetting and funnel trapping within ponds. Drift fences and pitfall buckets were installed and maintained around two ponds at the University of Missouri's Thomas S. Baskett Wildlife Research and Education Area (38.74847N, -92.20211)Boone County in 2002, and around five ponds at the Missouri Department of Conservation's (MDC) Daniel Boone Conservation Area (DBCA; within 1km of 38.771952N, -91.388054W), Warren County in 2005 (Hocking *et al.*, 2008; Semlitsch *et al.*, 2009).

Dipnet and funnel trap sampling was conducted at 49 constructed sites in 26 northern Missouri counties in 2006 (methods and sites in Shulse *et al.*, 2010). Sampling at these sites was conducted in three rounds, March/April, May/June, and July/August of 2006, with one set of dipnets and one night of trap sampling per site visit. We also sampled at 154 sites at Fort Leonard Wood (FLW; within 4km of 38.74847N, -92.20211W), Pulaski County, in 2012. Sampling the FLW sites was conducted in February/March and April/May of 2012, with three consecutive days of dipnetting and nights of trapping conducted concurrently in each round (Peterman *et al.*, 2014). Dipnet sweeps and number of funnel traps were scaled to the surface area of the ponds (one per 25 m^2 of pond surface area; Shulse *et al.*, 2010), and the location of dipnet sweeps and placement of traps within each pond were proportional to the aquatic habitat types present

Amphibians and fish were identified in the field to species when possible and released at site of capture; species of leopard and Pickerel Frog tadpoles were combined into one category as they are visually indiscernible. Fish species included Mosquitofish (*Gambusia* sp), sunfish (*Lepomis* sp), bass (*Micropterus* sp), catfish (Ictaluridae), and minnows (Cyprinidae).

Statistical Analyses: We characterized amphibian communities in Missouri using data collected by all survey methods but used data from sites surveyed with dipnets and funnel traps for statistical analysis because they represented a greater range of sites in Missouri. The reduced dataset was further truncated to include only those taxa that were identified to the level of species, and life stages were merged into a single binary response variable equivalent to 'detected' or 'not detected' for each locality. The binary dummy variable was used to calculate diversity at three taxonomic levels: (1) 'Total', for all amphibian taxa excluding American Bullfrogs; (2) 'Salamanders', for all seven caudate taxa; (3) 'Frogs', for 12 anuran taxa excluding American Bullfrogs. We calculated diversity as the number of species detected within the taxonomic group at a site.

To determine whether American Bullfrogs, fish, or their interaction predicted total amphibian species diversity, salamander species diversity, or frog species diversity in Missouri, we fitted generalized linear mixed-effect regression (GLMER) models with a binomial error term in the package 'lme4' (Bates *et al.*, 2014). In these models American Bullfrog presence, fish presence, and their interaction were fixed effects; pond was nested in ecoregion as random effects to capture regional variation. We included ecoregion as a random effect to account for the potential for species assemblages to differ based on ecoregion-specific attributes. The study sites were located in either the Ozark Plateau (n=174) in the southern portion of the state or the Central Dissected Till Plains (n=36)in the North (Etheridge, 2009). Model fit was evaluated based on likelihood ratio tests and Akaike's Information Criterion (AIC) following maximum likelihood estimation and the Laplace approximation.

RESULTS

We detected 20 of the possible 27 pond-breeding amphibian species in the study areas (Daniel and Edmond, 2013; Fig. 1). The most common species encountered were anurans: Green Frogs (*Lithobates clamitans*, 56% of sites), American Bullfrogs (*L. catesbeianus*, 55%), and members of the leopard frog complex (50%). Frequently encountered caudates were Central Newts (*Notophthalmus viridescens louisianensis*; at 49% of sites), Ringed Salamanders (*Ambystoma annulatum*, 42%), and Spotted Salamanders (*A. maculatum*, 40%). Pond-breeding species that were within our sampling range but were not encountered included the Northern Crawfish Frog (*L. areolatus*), Plains Spadefoot Toad (*Spea bombifrons*), Eastern Narrow-mouthed Toad (*Gastrophryne carolinensis*), and Four-toed Salamander (*Hemidactylium scutatum*). Tiger Salamanders (*A. tigrinum*) and Western Narrow-mouthed Toads (*G. olivacea*) were encountered at one site each. Fish were encountered at 22% (n=46) of the ponds sampled.

Overall, American Bullfrogs were positively associated with total amphibian diversity while fish were negatively associated with diversity (Fig. 2). Both single factor GLMER models (using fish or American Bullfrogs as fixed effects) performed better than the null model containing only random effects (Table 1**A**). The model with both fixed effects performed better than either single factor model (X^2=35.65, df=1, P=2E^{-9}), and adding the interaction term (fish*American Bullfrogs) provided no benefit (Table 1A). For the two parameter model, random effect variances were small (Pond=0.08, Ecoregion=0.03), and the change in the slope parameter estimated for the fixed effects were both significant (American Bullfrogs: Z=6.454, P=1E^{-10}; Fish: Z=-5.988, P=2E^{-9}), indicating American Bullfrogs are positively associated with increased diversity and fish are negatively associated (Table 2**A**).

Salamander diversity was slightly higher in the presence of American Bullfrogs, but there was a strong negative association between fish presence and salamander diversity (Fig. 2). The single factor model including American Bullfrogs as a fixed effect showed no improvement over the null model, but the model containing fish presence as a fixed effect was informative (X^2=45.17, df=1, P=2E^{-11}; Table 1**B**).

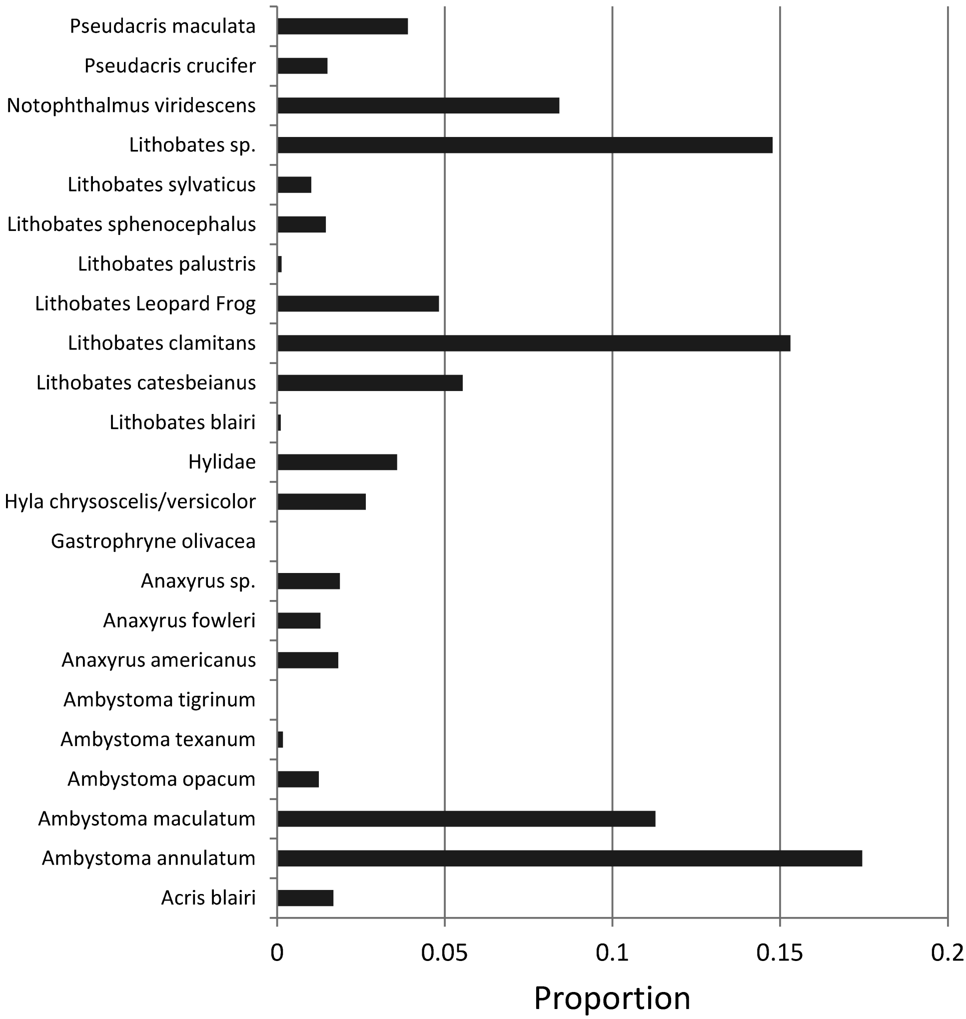

FIG. 1.—Proportion of amphibian species detected in ponds of Missouri, U.S.A., during drift fence (2002, 2005) and dipnet/funnel trap (2006, 2012) sampling

The model with both fixed effects performed better than the fish-only single factor model (X^2=6.94, df=1, P=0.008), but adding the interaction term (fish*American Bullfrogs) provided no benefit (Table 1**B**). For the two parameter model, random effect variances were again small (Pond=0.00, Ecoregion=0.85), and the change in the slope parameter estimated for the fixed effects were both significant (American Bullfrogs: Z=2.626, P=0.009; Fish: Z=-5.856, P=$5E^{-9}$), meaning American Bullfrogs are slightly positively associated with increased salamander diversity and fish are negatively associated with salamander diversity (Fig. 2**B**).

For frog diversity the single factor model with fish as a fixed effect showed no improvement over the null model, but the model containing American Bullfrog presence demonstrated a significant improvement (X^2=37.44, df=1, P=$9E^{-10}$; Table 1C). The model with both fixed effects performed better than the American Bullfrog single factor model (X^2=8.50, df=1, P=0.004), and frog species diversity was best predicted by the model including the interaction of American Bullfrog and fish

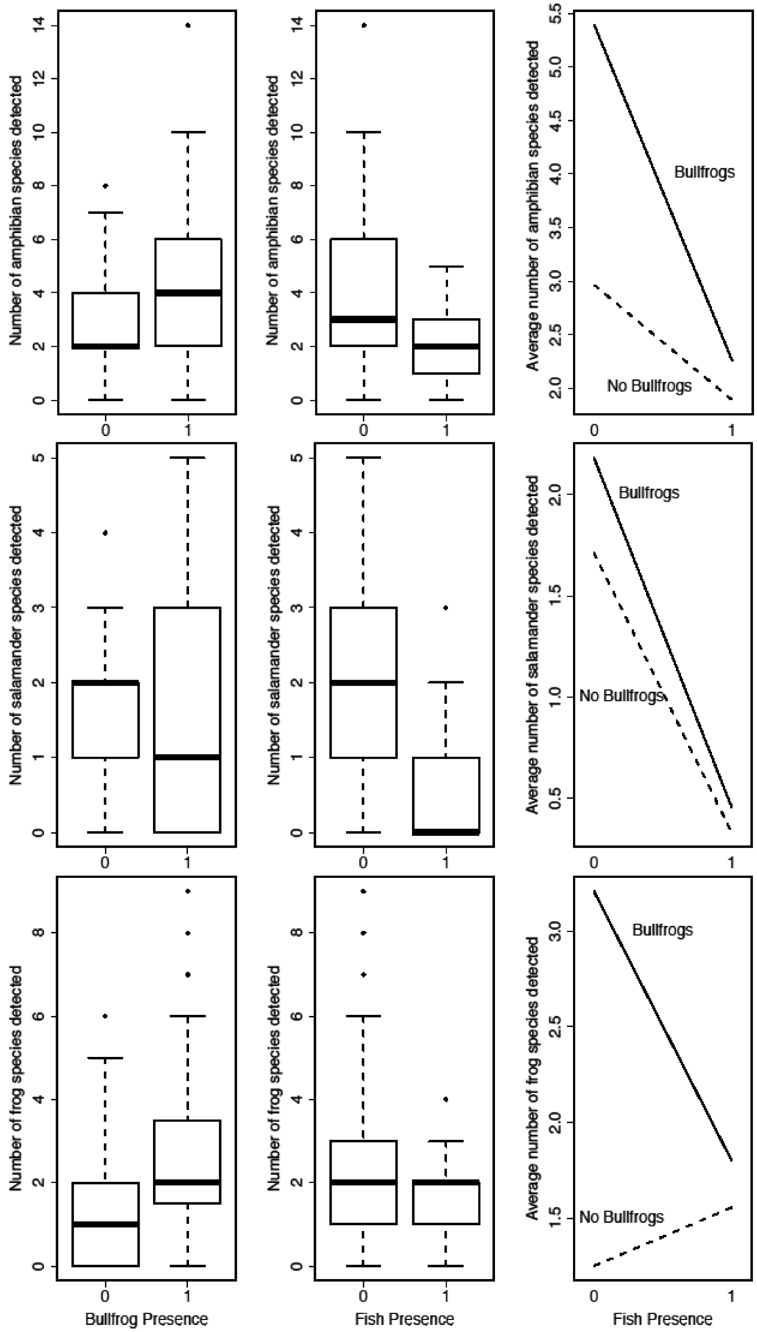

FIG. 2.—The effects of American Bullfrogs, *Lithobates catesbeianus*, and fish on frog (bottom), salamander (middle) and overall (top) species diversity at 211 pond sites sampled in Missouri, USA, between 2002 and 2012

TABLE 1.—Comparison of generalized linear mixed models for the effect of American Bullfrogs and fish on species diversity. All models include the random effect variables 'Pond' nested within 'Ecoregion'. The models with the lowest AIC values are in bold. Based on the deltaAIC values there is some support for the interaction models even though the ''best'' models seem to be the additive models for 'Total' and 'Salamanders'

Response	Model	Df	AIC	Δ AIC	logLik
A. Total Diversity	Null (intercept)	3	905.63	53.67	-449.82
	Bullfrog	4	885.60	33.64	-438.80
	Fish	4	887.49	35.53	-439.74
	Bullfrog+Fish	5	851.96	0.00	-420.98
	Bullfrog*Fish	6	852.07	0.11	-420.03
B. Salamanders	Null (intercept)	3	615.94	48.11	-304.97
	Bullfrog	4	616.56	48.73	-304.28
	Fish	4	572.77	4.94	-282.38
	Bullfrog+Fish	5	567.83	0.00	-278.92
	Bullfrog*Fish	6	569.83	2.00	-278.92
C. Frogs	Null (intercept)	3	760.26	44.49	-377.13
	Bullfrog	4	724.82	9.05	-358.41
	Fish	4	760.73	44.96	-376.37
	Bullfrog+Fish	5	718.32	2.55	-354.16
	Bullfrog*Fish	6	715.77	0.00	-351.88

presence ($X^2=4.56$, df=1, $P=0.033$; Table 2**C**). For the interaction model, random effect variances remained small (Pond=0.13, Ecoregion=0.00) and significant estimates were found for American Bullfrogs ($Z=7.108$, $P=1E^{-12}$), and the interaction term ($Z=-2.24$, $P=0.025$), but not for fish (Table 2**C**). This means American Bullfrog presence was positively associated with increased diversity of other frogs, but when fish and American Bullfrogs were both present, frog diversity was slightly reduced (Fig. 1**C**).

DISCUSSION

Habitat generalists such as *Lithobates catesbeianus, L. clamitans, L. sphenocephalus, Anaxyrus americanus, Notophthalmus viridescens louisianensis, Hyla chrysoscelis/versicolor, Pseudacris crucifer* and *P. maculata* were encountered across our sites, as expected from distribution records (Daniel and Edmond, 2013). Two species that had historically wide distributions but were rare in our surveys were *L. palustris* and *Ambystoma tigrinum*. Low detection of *L. palustris* may be explained by true absence in the areas or that they were present in the larval stage where they would have been classified in the ''Leopard Frog complex'' or ''*Lithobates sp*.'' categories, given the difficulty in differentiating their tadpoles from those of other leopard Frog species. Historically, *A. tigrinum* had a wide distribution across the state but there are few current site records (Daniel and Edmond, 2013), suggesting our low encounter frequency may be a result of low site occupancy by *A. tigrinum* or could be an indication of population decline. Lack of detection of *Gastrophryne* may be due to the timing of our sampling combined with their rapid larval development. Other species, such as *L. areolata*, have a more restricted range outside of the areas we sampled. More targeted surveys and data are needed for these species of concern, which will help create a clearer understanding of rare species and overall community structure.

The results of our analyses indicate the presence of American Bullfrogs, fish, and their interaction influenced the community composition of amphibians but in opposite patterns. American Bullfrogs generally had a positive relationship with all groupings of species, whereas fish presence was negatively associated with species diversity. Although negative impacts of American Bullfrogs on all life stages of several other anuran species, particularly those where American Bullfrogs have been introduced, have been documented (Keisecker and Blaustein, 1998; Lawler *et al.*, 1998; Boone *et al.*, 2004; Pittman *et al.*,

TABLE 2.—Regression coefficients for fixed and random factors of the best supported models (See Table 1). American Bullfrog effects are positive and larger for frogs than salamanders, fish effects are negative and larger for salamanders than frogs. Random effect variances are small, and for salamanders all random variation is explained by Ecoregion, with the opposite being true for frogs. The frog interaction term shows the amount that the increase in diversity associated with American Bullfrog presence is reduced when fish are also present

Response	Model	Fixed Effect	Est.	SE	z	Pr(>|z|)	Random Effect	Var	SD
A. Total Species	Bullfrog+Fish	(Intercept)	0.940	0.152	6.166	7.01E-10	Pond	0.080	0.283
		Bullfrog	0.574	0.089	6.454	1.09E-10	Ecoregion	0.031	0.176
		Fish	-0.736	0.123	-5.988	2.12E-09			
B. Salamanders	Bullfrog+Fish	(Intercept)	-0.253	0.674	-0.376	7.07E-01	Pond	0.000	0.000
		Bullfrog	0.297	0.113	2.626	8.64E-03	Ecoregion	0.849	0.921
		Fish	-1.398	0.239	-5.856	4.73E-09			
C. Frogs	Bullfrog*Fish	(Intercept)	0.153	0.113	1.357	1.75E-01	Pond	0.133	0.365
		Bullfrog	0.945	0.133	7.108	1.18E-12	Ecoregion	0.000	0.000
		Fish	0.223	0.315	0.709	4.78E-01			
		Interaction	-0.791	0.353	-2.240	2.51E-02			

2013), we found an overall positive association of American Bullfrogs with amphibian species diversity across Missouri. Our results should not be interpreted as American Bullfrogs causing an increase in amphibian species diversity. Rather, we feel that other factors that were not included in our study, such as diversity of terrestrial landscape around the pond, diversity of aquatic microhabitats within the pond, and hydroperiod contribute to the increased amphibian species diversity at the sites where amphibian diversity was high, and these were sites that were also associated with American Bullfrogs. The fish-free American Bullfrog ponds in our study were typically large, permanent ponds set in a diverse terrestrial landscape that included field, forest and edge habitats that would be inhabited by a wide variety of pond-breeding amphibians that would use the pond. These permanent ponds provided deep waters for species with larvae that overwinter (*L. catesbeianus and L. clamitans*) and peripheral shallow waters for species with larvae that develop more rapidly such as toads (*Anaxyrus* sp,), as well as accommodating species with intermediate larval development times such as *H. chrysoscelis/versicolor*. These sites also had diverse aquatic microhabitats including open water, leaf litter, submerged, floating and emergent vegetation, offering an assortment of substrate options for oviposition and larval habitat. Fish were encountered in fewer of our study sites and had a negative overall association with all species groupings. The negative impacts of fish, native and introduced, on both salamander and frog species has been widely documented (Hecnar and M'Closkey, 1997; Smith *et al.*, 1999; Shulse *et al.*, 2010; Drake *et al.*, 2014). Semlitsch *et al.* (2015) reported amphibian abundance, species richness, and diversity decreased with an increase in pond size due primarily to the presence of fish.

Our summary and analysis of pond-breeding amphibian community structure on a state-wide scale provides a baseline of information and a beginning assessment of potential factors that impact community structure on a large scale. It also affords an opportunity to subsequently examine how and if spatiotemporal changes occur in amphibian community composition.

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DANA L. DRAKE AND BRITTANY H. OUSTERHOUT, Division of Biological Sciences, University of Missouri, Columbia, 65211; JARRETT R. JOHNSON, Department of Biology, Western Kentucky University, 1906 College Heights Blvd., #11080, Bowling Green, 42101; THOMAS L. ANDERSON AND WILLIAM E. PETERMAN, Division of Biological Sciences, University of Missouri, Columbia, 65211; CHRISTOPHER D. SHULSE, Missouri Department of Transportation, P.O. Box 270, Jefferson City, 65102; DANIEL J. HOCKING, Department of Natural Resources and the Environment, 114 James Hall, University of New Hampshire, Durham, 03824; KENTON L. LOHRAFF, US Army IMCOM & FLW, DPW Natural Resources Branch, 1334 First St., Bldg 2222, Fort Leonard Wood, Missouri 65473; ELIZABETH B. HARPER, Division of Natural Resource Management and Ecology, Paul Smith's College, Paul Smiths, New York 12970; TRACY A. G. RITTENHOUSE, Department of Natural Resources and the Environment, University of Connecticut, 1376 Storrs Road, Unit 4087, Storrs, 06269; BETSIE B. ROTHERMEL, Archbold Biological Station, 123 Main Dr., Venus, Florida 33960; and LORI S. EGGERT AND RAYMOND D. SEMLITSCH, Division of Biological Sciences, University of Missouri, Columbia, 65211. *Submitted 16 January 2014; Accepted 28 February 2015.*

[1] Corresponding author: e-mail: Drake.Dana.L@gmail.com