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Influence of Stream Permanence, Predation, and Invasive Species on Crayfish in the Ozark Highlands with an Emphasis on Species of Greatest Conservation Need (*Orconectes marchandi*, *Orconectes eupunctus* and *Cambarus hubbsi*)

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Influence of Stream Permanence, Predation, and Invasive Species on Crayfish in the Ozark Highlands with an Emphasis on Species of Greatest Conservation Need (*Orconectes marchandi*, *Orconectes eupunctus* and *Cambarus hubbsi*)

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Biology

by

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University of Massachusetts Amherst
Bachelor of Science in Fisheries Ecology and Conservation, 2015

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This thesis is approved for recommendation to the Graduate Council.

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Abstract

Due to the ecological importance of crayfish and the increasing vulnerability of freshwater systems due to habitat loss, invasive species, and hydrologic alteration, understanding crayfish-environment relationships is crucial in the context of aquatic species conservation. I sought to examine the influence of hydrologic variation among intermittent and permanent streams on crayfish occupancy, abundance, predation risk, and potential vulnerability to invasive species effects. I conducted crayfish and environmental data sampling during two consecutive summers across 20 Ozark streams of differing permanence levels (10 intermittent, 10 permanent). In these same streams, I conducted fish and scat surveys over the course of four seasons. In addition, I performed population modeling based on previously collected data to understand the population dynamics of three crayfish species of greatest conservation need (*Orconectes eupunctus*, *Orconectes marchandi*, and *Cambarus hubbsi*) in the Spring River drainage of Arkansas and Missouri and assess their risk of invasive species and drought effects. I determined that occupancy of all crayfish species collected in my study was related to stream permanence. In most cases, crayfish abundance was largely related to stream permanence rather than local habitat. I documented that two species of conservation concern (i.e., *Orconectes williamsi*, *Orconectes meeki*) appear dependent on intermittent streams. Stream permanence, however, did not appear to influence crayfish predation by riparian mammals in my study. Crayfish predation pressure by mammals appeared to be determined by season and was strongest in spring and summer. The prevalence of fish prey in mammalian diets appeared to be strongly influenced by a season by stream permanence interaction, and this may be the first study to document such an observation. Lastly, my population modeling procedure indicated the potential of the Spring River to serve as a refuge for *O. eupunctus* and *C. hubbsi* when invasion and

drought effects are strong in the surrounding area. Conversely, the Spring River appears to serve as a barrier to dispersal for *O. marchandi* which could result in high extinction risk of the population under invasive species effects. This thesis adds to the knowledge of flow-ecology relationships and invasive species effects in the Ozark Highlands.

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Dedication

I dedicate this thesis to my parents who raised me to understand and appreciate nature, and above all, encouraged me to never compromise my happiness, individuality, and curiosity.

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List of Published Papers

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Introduction

Crayfish fulfill a crucial role within the ecosystems they occur and can be considered keystone species (Momot 1995). In addition to accounting for a significant proportion of macroinvertebrate biomass (> 50% in many aquatic systems) (Momot 1995), crayfish act as predators (Momot 1995, Stenroth and Nyström 2003), consumers of autotrophs (Momot 1995, Dorn and Wojdak 2004), detritivores (Stenroth and Nyström 2003), and prey for fish (Rabeni 1992) and terrestrial animals (Boyle et al. 2014). Additionally, crayfish function as bioprocessors of vegetation and carrion (Taylor et al. 2007) by converting coarse particulate organic matter to fine particulate organic matter that is a food resource for other organisms (Whitledge and Rabeni 1997).

Crayfish are among the most imperiled freshwater taxa largely due to their high levels of endemism (Taylor et al. 1996, Taylor et al. 2007) and taxa with restricted natural ranges are especially susceptible to decline (Taylor et al. 2007). Both anthropogenic and natural disturbance can be a cause of crayfish imperilment. Habitat loss and degradation has been identified as the greatest threat to global biodiversity (Ehrlich, 1988) and in freshwater systems, habitat alteration can arise in a number of ways. Dams, urbanization, channelization, and water withdrawal each contribute to the anthropogenic alteration of natural flows (Poff et al. 1997). In arid and semi-arid regions, temporary habitat loss via natural seasonal drought can lead to the prolonged duration of low flows or zero-flow days.

Recently, the natural flow regimes within the Ozark Highlands of Arkansas and Missouri were classified (Leasure et al. 2016). This region is characterized by seven natural flow regimes (Groundwater Stable, Groundwater, Groundwater Flashy, Perennial Runoff, Runoff Flashy, Intermittent Runoff, and Intermittent Flashy). The critical components of flow regimes (i.e., the

magnitude, frequency, duration, timing, and rate of change of hydrologic conditions (Richter et al. 1996)) are highly variable across the region. Some flow regimes (e.g., Intermittent Flashy streams) experience drying for 1 to 3 months per year, including complete drying in late summer. Whereas others (e.g., Groundwater Flashy streams) never dry completely (Leasure et al. 2016).

Stream drying is an important mechanism that influences predator-prey relationships and crayfish behavior. Summer drying can lead to intermittent or isolated habitats. During drought, biotic interactions (e.g., competition, predation) may intensify (Hodges and Magoulick 2011) due to high densities of organisms restricted to a limited area. In addition, water depth has shown to be positively related to crayfish survival in the presence of predators. Crayfish are known to occupy areas with deeper depths to avoid terrestrial predation (Gelwick 2000), and crayfish mortality via terrestrial predation may be lower in areas of deep water relative to shallow water (Englund and Krupa 2000). Drying not only increases predation risk, it also elicits differential behavioral responses such as reduced foraging, shifts in distribution, and burrow construction (Gelwick 2000). Some crayfish, such as primary or secondary burrowers, are able to construct deep, vertical burrows to the water table during stream drying (Crandall and Buhay 2008). While all crayfish are capable of burrowing to some degree (e.g., by constructing small, simple burrows under large substrate for moisture and cool temperatures (Jones and Bergey 2007)), not all crayfish succeed and may suffer reductions in population densities, shifts in reproductive timing, and reduced body size (Taylor 1988).

Invasive species are a major threat to ecosystem integrity and to date, there are no signs of a significant reduction of this pressure (Secretariat of the Convention of Biological Diversity 2010). There is a wide body of literature that has examined relationships between invasive and native crayfish (e.g., Westhoff et al. 2012, Larson et al. 2009, Larson and Magoulick 2009,

Magoulick and DiStefano 2007, Rabalais and Magoulick 2006 (a,b)). The mechanisms by which a native species of crayfish becomes displaced by an invasive species have been identified as competition, reproductive interference or hybridization, differential predation, and disease transmission (Lodge et al. 2000). The establishment of many invasive crayfish is often related to change that creates environments that are more favorable to introduced species and unfavorable to native species (Holdich et al. 1997) and potentially habitat loss due to seasonal stream drying.

In the Ozark Highlands of Arkansas and Missouri, the Spring River drainage houses three species of crayfish that are “Species of Greatest Conservation Need (SGCN)”: the Mammoth Spring crayfish (*Orconectes marchandi*), the Coldwater Crayfish (*Orconectes eupunctus*), and the Hubbs’ crayfish (*Cambarus hubbsi*). *Orconectes marchandi*, and *O. eupunctus*, are two of the most geographically restricted stream crayfish and are considered imperiled in Arkansas, Missouri and globally, are candidates for listing by the U.S. Fish and Wildlife Service under the U.S. Endangered Species Act. Populations of *O. marchandi* are limited to the Spring River drainage of southern Missouri and northern Arkansas, whereas populations of *O. eupunctus* are limited to the Eleven Point, Spring and Strawberry River drainages. In Arkansas, *O. marchandi* are mainly found in small streams in the eastern portion of the Spring River drainage, whereas *O. eupunctus* are mainly found in larger, spring-fed rivers (Flinders and Magoulick 2005).

Native to the White, Arkansas, and Kansas river drainages, *Orconectes neglectus* (Ringed crayfish [Pfliger 1996]) has invaded portions of the Spring River drainage in southern Missouri and northern Arkansas (Flinders and Magoulick 2005). Currently, *O. eupunctus*, which was once abundant in the Spring River drainage has been displaced by *O. neglectus*. *Orconectes neglectus* is now the dominant crayfish species in portions of the West Fork Spring River and the upper South Fork Spring River where *O. eupunctus* was formerly abundant (Magoulick and DiStefano

2007). *Orconectes eupunctus* still persists in the Spring River drainage, but its abundance has declined in the upstream areas that *O. neglectus* inhabits (Flinders and Magoulick 2005).

The mechanism of *O. eupunctus* displacement by *O. neglectus* remains unclear. It does not appear that *O. neglectus* displaces *O. eupunctus* by forcing them into different habitats (Rabalais and Magoulick 2006, a), and both juvenile (Larson and Magoulick 2009) and adult male (Rabalais and Magoulick 2006, b) competition did not appear to drive displacement. However, it is likely that the demonstrated high tolerance to drought of *O. neglectus* compared to *O. eupunctus* may inhibit recolonization of *O. eupunctus* after stream rewetting via priority effects (Larson et al. 2009). Nonetheless, *O. neglectus* has shown to be a successful invader in the Spring River drainage as demonstrated by the decline of *O. eupunctus* in its former range. In order to direct conservation measures of the remaining *O. eupunctus* in the Spring River drainage and the other imperiled crayfish species in the drainage (*C. hubbsi*, *O. marchandi*), it is essential to understand the population demography of these species in the context of a spreading invasive species and the probable intensified drought due to future climate change.

Given the imperiled status and high level of endemism seen in numerous Ozark crayfish species, it is vital to understand the combined effects of stream permanence, predation, and the impacts of invasive species. While the seasonal drying of intermittent streams in this region is a natural process, the pressures of human water use coupled with global climate change may induce additional stress on the region's sensitive aquatic biota in the future. Information gained from the establishment of flow-crayfish ecology relationships may provide insight into the importance of sustainable water use in the Ozark Highlands. Specifically in the Spring River drainage, where an invasive species is spreading and where two of the region's most geographically-restricted stream crayfish occur (*O. eupunctus* and *O. marchandi*), we intend to

understand the status and threats present so that we may inform future conservation decisions. Findings from this research will inform conservation and management of crayfish of greatest conservation need in the Ozark Highlands.

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**Stream permanence influences crayfish occupancy and abundance in the Ozark Highlands,
USA**

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Abstract

Despite the ecological importance and often imperiled status of crayfish in freshwater systems, few studies have examined crayfish use of intermittent streams. Crayfish use of intermittent streams is especially important to understand in the face of global climate change which may lead to additional stress exerted on aquatic biota in the future. We examined the influence of stream permanence and local habitat on crayfish occupancy and species densities in the Ozark Highlands, USA. Sampling took place from June to July in 2014 and 2015. We used a quantitative kickseine method to sample crayfish presence and abundance at 20 stream sites with 32 surveys per site in the Upper White River drainage, and associated local environmental variables were also measured in each year. Site occupancy and detection probabilities were modeled in Program PRESENCE. Multiple linear regression modeling was conducted to determine relationships between individual crayfish species densities and environmental variables. Occupancy of all crayfish species was related to stream permanence. *Orconectes meeki* was found exclusively in intermittent streams. *Orconectes neglectus* and *Orconectes luteus* had higher occupancy and detection probability in permanent streams than in intermittent streams and *Orconectes williamsi* was associated with intermittent streams. Estimates of detection probability ranged from 0.56 to 1 which is high relative to other studies that have examined crayfish detection probabilities. Multiple regression modeling indicated that species densities were largely related to stream permanence rather than local habitat, with the exception of *O. williamsi*. Individual species densities did not differ by year but total crayfish densities were significantly lower in 2015. Increased precipitation and discharge in 2015 likely led to lower crayfish densities seen during this year. Our study demonstrates that crayfish distribution and abundance is strongly influenced by stream permanence. Some species, including those of

conservation concern (i.e., *O. williamsi*, *O. meeki*), appear dependent on intermittent streams and managers should consider intermittent streams as an important component of freshwater biodiversity.

Introduction

Headwater streams are vital links in maintaining ecological integrity for most watersheds and are the most abundant streams, comprising $> 2/3$ of total stream length in a river network (Leopold et al. 1964, Meyer et al. 2003, Freeman et al. 2007). They are responsible for maintaining water quality, nutrient cycling, flood mitigation, as well as providing habitat and food for an array of organisms (Meyer et al. 2003). Headwater streams often have an abundance of refuge habitats, feeding and nursery grounds, and movement corridors, making them the most varied of lotic habitats (Meyer et al. 2007). Thus, headwater streams can harbor a unique complement of species and can be considered a major contributor to the overall biological diversity in riverine systems. Although headwater streams provide a wealth of ecosystem services and dominate the landscape, they are threatened due to the combination of legal uncertainty and human land and water use. Inconsistencies exist under the U.S. Clean Water Act that obfuscate whether headwater streams are afforded the same legal protection as larger, perennial streams (Nadeau and Rains 2007, USEPA and USACE 2007). Urbanization, agriculture, mining, and hydrologic alteration pose threats to the integrity of running water (Malmqvist and Rundle 2002) and headwater streams are especially susceptible due to their lack of legal protection and tendency to be intermittent, unnamed and unmapped.

Many headwater streams are intermittent and are dry for significant periods (Meyer et al. 2003) and are prevalent in both arid and semi-arid regions on every continent (Datry et al. 2014). Intermittent streams are vital in contributing to the biological integrity and diversity of river systems. The dynamics of intermittent streams regulate the persistence of, and provide spawning habitat for many aquatic species, including threatened species. The hydrologic variability inherent to intermittent streams fosters habitats that may limit use by some species, which may

create areas with limited predation or competitive pressure. For example, rainbow trout (*Oncorhynchus mykiss*) spawning was greater in an intermittent stream than in several permanently flowing tributaries in California, presumably due to a lack of brook trout in the intermittent reaches which may allow greater rainbow trout fry survival (Erman and Hawthorne 1976). Flashy flow regimes in intermittent streams help scour and maintain large pools which serve as refuges for fish during drying events, including the threatened Arkansas darter (*Etheostoma cragini*) in southeast Colorado (Labbe and Fausch 2000). Furthermore, hydrologic variability leading to low flows benefits native galaxiid fish at the expense of an exotic salmonid (Leprieur et al. 2006). It is known that intermittent streams are capable of supporting different benthic communities than permanent streams (e.g., Delucchi and Peckarsky 1989, Feminella 1996, Flinders and Magoulick 2003).

Crayfish fulfill a crucial role in many headwater streams, contributing to ecosystem functioning in many ways, and often acting as keystone species (Momot 1995). They are a major contributor to production, accounting for > 50% of macroinvertebrate biomass in many freshwater ecosystems (Momot 1995). Crayfish act as predators (Momot 1995, Stenroth and Nyström 2003), consumers of autotrophs (Momot 1995, Dorn and Wojdak 2004), detritivores (Stenroth and Nyström 2003), and prey for fish (e.g., smallmouth bass) (Rabeni 1992) and terrestrial animals (Boyle et al. 2014). They function as bioprocessors of vegetation and carrion (Taylor et al. 2007) by converting coarse particulate organic matter to fine particulate organic matter that is a food resource for other organisms (Whitledge and Rabeni 1997). Crayfish are among the most imperiled freshwater taxa (Taylor et al. 1996, Taylor et al. 2007) and show a high level of endemism. Thus they may be especially susceptible to the consequences of habitat degradation (Taylor et al. 2007).

Despite the importance of crayfish in streams and the vulnerability of intermittent streams, few studies have examined crayfish use of intermittent streams. DiStefano et al. (2009) found that two species of crayfish persist through stream drying by burrowing in the hyporheic zone and Flinders and Magoulick (2003) determined that 2 of 4 crayfish species studied occurred in greater densities in intermittent streams than in permanent streams. These studies indicate that crayfish are capable of withstanding drought by burrowing and intermittent streams may be more favorable to some crayfish species than permanent streams. In addition, recent distributional studies of Ozark crayfishes (e.g., *Orconectes meeki* and *O. williamsi*) suggested that these crayfish are likely to occur in many intermittent streams (Westhoff et al. 2006, DiStefano et al. 2008, Herleth-King 2015) and intermittent streams typically may not be sampled representatively in surveys for rare aquatic species (DiStefano et al. 2009). Intermittent streams are of great importance but are often neglected in research and monitoring.

Many crayfish are both of great conservation concern and are often cryptic and difficult to detect. Despite the importance of accounting for imperfect detection to effectively model species distribution and abundance (MacKenzie et al. 2006), very few studies have examined detectability when examining crayfish species distributions. Pearl et al. (2013) compared detection probabilities of the introduced *Orconectes neglectus* and *Procambarus clarkii* (detected at 0.11 and 0.44, respectively) and the native *Pacifastacus leniusculus* (detected at 0.24) in the Pacific Northwest. Frisch et al. (2016) detected *Cambarus* spp. at 0.36 in the Little Tennessee River basin of the Southern Appalachians and found detection negatively related to pebble size. Understanding patterns of detectability is especially important for crayfish due to their ecological importance and imperilment.

Although seasonal stream drying in the Ozark Highlands is a natural process in intermittent streams, global climate change and the pressures of human water use may induce additional stress on the region's crayfish in the future. The ecological importance of crayfish coupled with their imperiled status, indicate the need to characterize their use of intermittent streams to establish effective conservation and monitoring strategies. Our objectives were to 1) determine crayfish occupancy, detection probabilities, and species densities in intermittent versus permanent streams in the Ozark Highlands ecoregion and 2) examine the influence of stream permanence and landscape to local habitat variables on crayfish species densities.

Methods

Study area

Recently, the natural flow regimes in the Ozark-Ouachita Interior Highlands of Arkansas, Missouri, and Oklahoma were classified (Leasure et al. 2016). Sets of flow metrics were used that best quantified several ecologically-important components of these natural flow regimes. Leasure et al. (2016) found the Ozark Highlands ecoregion included permanent groundwater-influenced streams with relatively low hydrologic variability, as well as intermittent streams. Streams classified as Intermittent Flashy have small drainage areas (8 to 22 km²) and exhibit substantial flow variability (i.e., drying for one to three months per year), including complete drying in late summer. Groundwater Flashy streams were found within a range of drainage areas from 11 to 3,237 km². These streams had less daily flow variability than any runoff-dominated streams and never experienced complete drying (Leasure et al. 2016). We used Intermittent Flashy streams as intermittent streams and Groundwater Flashy streams as permanent streams due to the contrast in their hydrologic variability and tendency to experience complete (intermittent) or no seasonal drying (permanent). Both flow classes are considered “flashy”

which is a common stream type in the Ozark Highlands ecoregion (Leasure et al. 2016) and refers to the rapid rates of change from one magnitude to another. Ten intermittent streams and ten permanent streams were selected for study (Fig. 1). Streams were candidates for selection if they were within a drainage area smaller than 200 km², at least 10 km away from one another, and had a probability of ≥ 0.50 of being in the correct hydrologic regime, as per models in Leasure et al. (2016). Ten streams of each level of permanence were randomly selected from the group of streams fitting these criteria. Accessibility and the availability of riffle habitats was determined in candidate streams by visiting them prior to sampling. Land use within subwatersheds (HUC 12) of study streams was predominantly forest, pasture, hay, and livestock farming (Homer et al. 2015). Natural vegetation consisted of hickory (*Carya* spp.) and oak (*Quercus* spp.) forest. Streambeds consisted largely of boulder, cobble, pebble, and gravel substrates with occasional bedrock outcroppings. All study streams had a well-defined riffle-pool sequence typical of Ozark streams.

Field sampling

The 20 streams were each sampled in 2014 (10 June - 23 July) and 2015 (10 June - 17 July). Four distinct riffle habitats were selected in each stream and eight 1 m² areas were randomly chosen in each riffle for sampling. Crayfish were dislodged from a 1 m² area (determined by a quadrat made of PVC) by thoroughly kicking and disturbing the substrate directly upstream of a seine net (3 mm mesh, 1.8 m l X 1.1 m h). Crayfish dislodged from the substrate were washed into the seine net by the water current and by pulling the seine through the sample area. All crayfish collected were identified to species and were released to their collection sites.

We determined how environmental variables differed among streams of varying permanence and how these variables influenced crayfish abundance. Four variables (% forest cover, water depth, current velocity, and surface substrate size) were used in addition to stream permanence. Variables were selected based on previous studies that showed their importance for crayfish occupancy and abundance (e.g., Flinders and Magoulick 2005, Magoulick and DiStefano 2007, Larson and Olden 2013, Parvulescu et al. 2013, Nolen et al. 2014). Current velocity and depth were measured directly in front of each sampling location (32) in each stream using a Marsh McBirney® model 2000 flow meter (0.6-depth, ms^{-1}). Surface substrate composition was estimated within each 1m^2 quadrat. Percent sand (0-0.1 cm diameter), gravel (0.1-3 cm diameter), pebble (3-6 cm diameter), cobble (6-12 cm diameter), and boulder (>12 cm diameter) were visually estimated (Rabalais and Magoulick 2006). Percent forest cover was determined using ArcMap (ver. 10.3.1) and the National Land Cover Database 2011 (Homer et al. 2015). The percentage of forested land relative to all other land cover types was tabulated within each study subwatershed at the HUC 12 level.

Data Analysis

We used Principal Components Analyses (PCA) as a predictor variable reduction procedure. All predictor variables were $\log_{10}(x+1)$ transformed to meet the assumptions of normality and homogeneity of variance. PCA was performed separately for 2014 and 2015. The first two principal components were used as model predictor variables for both years. Using PCA for variable reduction simplified our modeling structure and helped avoid autocorrelation of predictor variables. Large differences in the contribution of predictor variables to each principal component facilitated making biological interpretations. The PCA for both years yielded one factor with an Eigenvalue > 1. The second factor was used in both years because in both analyses

it had Eigenvalues close to 1 (2014: 0.94, 2015: 0.97) and we wanted to include a factor correlated with a local habitat variable.

Program PRESENCE (Version 10.9, Proteus Research and Consulting Ltd., Dunedin, New Zealand) was used to estimate site occupancy rates (ψ) and detection probabilities (p) for the four most common crayfish species (*Orconectes luteus*, *O. meeki*, *O. neglectus* and *O. williamsi*) of the seven species collected. Site occupancy and detection probabilities of *O. williamsi* were not performed for permanent streams since this species only occupied one permanent stream. The remaining three species (*Orconectes longidigitus*, *O. punctimanus*, and *O. ozarkae*) were found at a small proportion of sites (< 20%), were not collected in both years, or models did not converge, and were therefore excluded. Models to determine the influence of stream permanence and environmental variables on crayfish occupancy and detection often exhibited poor fit and overdispersion due to small sample size and complete separation among response variables. Therefore, site occupancy and detection probabilities for each species were determined using the null (constant) model by year and stream permanence.

We used multiple linear regression to determine relationships between crayfish species densities and environmental variables to further understand the influence of stream permanence on crayfish. Two candidate models included each of the principal components separately and one candidate model included both principal components as the global model. The three models were run separately for each year for each of the four species. Species densities were $\log_{10}(x+1)$ transformed to meet the assumptions of normality and homogeneity of variance. Model selection was based on the small-sample size-corrected Akaike Information Criterion values (ΔAIC_c ; Akaike 1973, Burnham and Anderson 2002) and models were considered supported by the data if ΔAIC_c was less than 2.0 (Burnham and Anderson 2002). Model fit was assessed with R^2 . In

addition, effect of year and stream permanence on crayfish densities, diversity, and environmental variables was examined using two-way ANOVA. Simpson's Diversity was calculated based on all seven species collected. A false discovery rate correction was used to control Type I error rates (Benjamini and Hochberg 1995).

Results

A total of 3,830 crayfish was collected in 2014 and 1,887 were collected in 2015. There was no year by stream permanence interaction for any response variables examined (Table 1). Individual species densities differed significantly by stream permanence but did not differ by year (Fig. 2, Table 1). The pattern of crayfish species occurrence by stream permanence was the same in both years but with lower densities in 2015 (Fig. 2). *Orconectes luteus* and *O. neglectus* densities were significantly greater in permanent streams than in intermittent streams whereas *O. meeki* and *O. williamsi* densities were significantly greater in intermittent streams than in permanent streams (Fig. 2, Table 1). Total crayfish densities were significantly greater in 2014 than in 2015 but did not differ by stream permanence (Fig. 2, Table 1). Simpson's diversity did not differ by stream permanence or year (Table 1).

Summer 2014 had slightly less than average precipitation (June: 2.24 cm., July: 2.62 cm.) whereas summer 2015 was substantially wetter than average (June: 4.55 cm., July: 11.66 cm.) (NOAA 2016). Current velocities were significantly greater in 2015 than in 2014 and were significantly greater in permanent streams than in intermittent streams (Table 1). Depths were significantly greater in 2015 and in permanent streams (Table 1). Substrate size did not differ by year but was significantly greater in intermittent streams (Table 1).

Detection probability and site occupancy

Detection probabilities were moderate to high for all species in both stream types during both years (Fig. 3). In intermittent streams, *O. williamsi* had the highest detection probabilities in both years ($p \geq 0.94$) whereas *O. meeki* had the lowest detection probabilities in 2014 ($p = 0.70$) and *O. neglectus* had the lowest detection in 2015 ($p = 0.58$) (Fig. 3). *Orconectes neglectus* and *O. luteus* both had high detection probabilities in permanent streams in both years ($p \geq 0.87$) (Fig. 3). *Orconectes meeki* was not detected in permanent streams and detection probabilities of *O. williamsi* could not be estimated for this stream type due to model failure based on only one detection of this species in both years. There was a moderate positive correlation between crayfish density and detection probability ($r = 0.54$).

Orconectes neglectus, the most commonly captured species, had the highest site occupancy rates in both years in permanent streams ($\text{psi} = 1$) and *O. luteus* had moderately high occupancy in this stream type ($\text{psi} \geq 0.60$) (Fig. 3). *Orconectes meeki* did not occupy permanent streams and *O. williamsi* occupied only one permanent stream. All species occupied intermittent streams in both years but some to a greater extent. *Orconectes williamsi* had the highest occupancy in intermittent streams in 2014 ($\text{psi} = 0.80$) and *O. meeki* had the highest occupancy in 2015 within this stream type ($\text{psi} = 0.90$) (Fig. 3). *Orconectes luteus* had the lowest occupancy in intermittent streams in both years ($\text{psi} = 0.20$) (Fig. 3).

Density relationships

The first two principal components for 2014 explained 78.3% of the variation in the covariate data. In 2014, intermittent streams were strongly positively correlated (0.83) with PC1 and contributed 49.11%, and substrate size was strongly positively correlated (0.94) with PC2

and contributed 56.38%. In 2015, the first two factors explained 68.3% of the variance in the covariates. Intermittent streams were positively correlated (0.77) with PC1 and contributed 53.92% and current velocity was strongly positively correlated (0.97) with PC2 in 2015 and contributed 66.44%. (Table 2).

Multiple regression modeling indicated that species densities were largely determined by PC1 rather than PC2 (Table 3). In both years, *O. luteus* densities were related to PC1 (Table 3). Densities of *O. luteus* were significantly negatively related to PC1 in 2014 (coef = -0.23; SE = 0.07; $p < 0.01$) and negatively related to PC1 in 2015 (coef = -0.12; SE = 0.06; $p = 0.07$).

Orconectes meeki densities were related to PC1 in 2014 (Table 3). Both the global model and the PC1 model were equally supported in 2015 (Table 3). Densities of *O. meeki* were significantly positively related to PC1 in 2014 (coef = 0.13; SE = 0.06; $p = 0.05$) and in 2015 (coef = 0.07; SE = 0.02; $p = 0.01$). Based on the global model, densities were also positively related to PC2 in 2015 (coef = 0.04; SE = 0.02; $p = 0.06$).

The global model and the PC1 model were equally supported in 2014 for *O. neglectus* (Table 3). Densities of *O. neglectus* were significantly negatively related to PC1 in 2014 (coef = -0.13; SE = 0.05; $p = 0.01$) and 2015 (coef = -0.09; SE = 0.03; $p < 0.01$). Based on the global model, *O. neglectus* densities were positively related to PC2 in 2014, though this relationship was not significant (coef = 0.08; SE = 0.04; $p = 0.10$).

Orconectes williamsi densities were related to PC1 in 2014 (Table 3) and densities were significantly positively related to PC1 (coef = 0.22; SE = 0.09; $p = 0.02$). Unlike all other study species, the PC2 model was equally supported as the global model for the top ranked model in 2015 (Table 3). Densities of *O. williamsi* were significantly negatively related to PC2 in 2015

(coef = -0.11; SE = 0.04; p = 0.02) but were positively related to PC1, however, this relationship was not significant (coef = 0.06; SE = 0.04; p = 0.13).

Discussion

Site occupancy of all crayfish species differed by stream permanence in both years. Stream permanence is known to have a significant effect on biotic communities. Most studies focused on determining the influence of stream permanence on aquatic invertebrates have studied benthic insects. Stream permanence influences insect emergence (Delucchi and Peckarsky 1989) and riffle permanence can affect the structure of benthic insect assemblages (Feminella 1996). Flinders and Magoulick (2003) demonstrated the importance of stream permanence for lotic crayfish and found that crayfish density and crayfish-environment relationships differed between intermittent and permanent streams. Unlike Flinders and Magoulick (2003), which detected all crayfish species within their study in both stream types throughout the Spring River drainage of Arkansas and Missouri, we found one species (*O. meeki*) to occupy intermittent streams exclusively and another species (*O. williamsi*) nearly did so. Two species in the Flinders and Magoulick (2003) study (*O. marchandi* and *O. punctimanus*) were found in significantly greater densities in intermittent streams than in permanent streams whereas densities of two species (*Cambarus hubbsi* and *O. ozarkae*) did not differ by stream type. Our study found all four species of crayfish to differ significantly by stream permanence (Table 1). Our findings agree with Flinders and Magoulick (2003) that stream permanence appears to be of great importance for crayfish occupancy and abundance.

Individual species densities were determined by both stream permanence and local habitat variables. Linkages between crayfish density and local habitat variables have been illustrated in a number of studies (Flinders and Magoulick 2005, Westhoff et al. 2006,

Magoulick and DiStefano 2007, DiStefano et al. 2008, Nolen et al. 2014, Herleth-King et al. 2015). Nolen et al. (2014) determined that landscape variables have a stronger influence on crayfish distributions but local habitat variables were more important in explaining crayfish abundances. The present study reinforces the idea that crayfish presence may be determined by landscape-scale variables (e.g., stream permanence) whereas crayfish abundance is more apt to be determined by both landscape-scale and local habitat variables.

Interannual variability in precipitation likely contributed to higher total crayfish densities in 2014 and differences in local habitat variables. More precipitation in 2015 elicited the faster current velocities and deeper water depths recorded during this year. Average substrate size did not differ between years which was expected. However, average substrate size was larger in intermittent streams which may be related to high annual maximum flow and high flood frequency seen in streams of this flow class (Leasure et al. 2016). Smaller substrate particles may get washed out during high flow events in intermittent streams which may explain the larger average substrate size in these streams. More precipitation and the increased frequency of high flow events in 2015 presumably led to the lower crayfish densities seen during the second year of the study.

Unlike previous studies, *O. williamsi* densities were significantly negatively associated with current velocities in 2015. Westhoff et al. (2006) found that *O. williamsi* densities were positively associated with higher current velocities and Herleth-King et al. (2015) found this species to occur in riffles rather than pools, where current velocities are faster. While these previous studies suggest that *O. williamsi* is associated with faster current velocities this was not the case during the second year of our study. We did not discern a relationship between *O. williamsi* and water depth in our study, but this species is known to be positively associated with

shallow depths (Westhoff et al. 2006). It is possible that intensified local habitat conditions in 2015 brought on by precipitation were too extreme and led to reduced total crayfish densities during that year, despite previous documentation of *O. williamsi* being associated with fast current velocities. Relationships between current velocity and depth were not revealed in our models for the remaining species. However, previous studies have indicated *O. meeki* as more of a habitat generalist than some of its species associates (e.g., *O. williamsi*; DiStefano et al. 2008) and *O. neglectus* is often observed in slower velocity pools (Westhoff et al. 2006). One possible explanation for the different species-environment relationships observed in our study versus previous studies may be because our study only focused on riffle habitats. This may explain why water depth was not seen as an important habitat variable in our study and the unanticipated negative association among *O. williamsi* and current velocity. Differences in interannual precipitation over the course of the study may have also played a role in our observations and these differences can alter what we term “intermittent” and “permanent.”

Surprisingly, total crayfish densities did not differ by stream permanence. According to Resh et al. (1988), it is expected that standing crop biomass would be lower in streams that undergo frequent disturbance such as seasonal drying. Since streams with permanent flow tend to have a more stable range of physical and chemical conditions than intermittent streams (Boulton and Lake 1990), we anticipated that this consistency in habitat may foster greater crayfish densities in permanent streams however, this was not the case. Our results also do not coincide with Flinders and Magoulick (2003) who found greater crayfish densities in intermittent streams than permanent streams in the Spring River drainage of Arkansas and Missouri. It is possible that a difference in crayfish densities was undetected between the two stream types because the dry period in the intermittent streams was not intense enough to elicit negative population effects

during the period of study. While 2014 had slightly less precipitation in the summer months than average, neither year had substantial seasonal drought as is typical for this region (Magoulick 2000, Larson et al. 2009) which supports that intermittent and permanent streams are dynamic by nature and are closely linked to interannual variability in precipitation. There was no difference detected in species diversity between the two stream types indicating intermittent streams may be as capable of supporting diverse and abundant crayfish populations as permanent streams.

Unlike most studies of crayfish distribution, ours is one of the few to model crayfish detection probabilities and account for imperfect detection. Detection probabilities are important to consider when examining species distribution and without them, biased conclusions may be drawn (MacKenzie et al. 2006) and this may be especially important for rare and imperiled species. Crayfish detection probabilities were moderate to high for all species in both years (2014: 0.70-0.98, 2015: 0.56-1.00). These detection probabilities are high compared to the few previous studies that examined crayfish detection probability. In the Pacific Northwest, Pearl et al. (2013) detected the native *Pacifastacus leniusculus* at 0.24 and the invasive *Orconectes neglectus* and *Procambarus clarkii* at 0.11 and 0.44, respectively. In the Little Tennessee River basin of the Southern Appalachians, Frisch et al. (2016) detected *Cambarus* spp. at 0.36. Our crayfish detection probabilities may have been higher due to differences in stream size, species behavior, local habitat conditions, and sampling techniques.

Local density affects the detection probability of most species and when the density of a species increases, the probability of detection often increases (Royle and Nichols 2003). We saw a moderate positive relationship between crayfish densities and detection probability ($r = 0.54$). Since this relationship was not strong, it is likely that our sampling method was sufficient at detecting crayfish even at low densities. Furthermore, detection probabilities remained high

during the wet summer of 2015 when depths were deeper and velocities were faster. Our approach appears to be effective at sampling riffle-dwelling crayfish in small streams. It is important to recognize that our sampling regime focused only on riffle habitats. It is known that some of the species we collected also occur in other habitats such as pools (*O. neglectus* [Westhoff et al. 2006], *O. meeki* [DiStefano et al. 2008]) which we did not sample. Therefore, this may confound interspecies comparisons.

Spatial segregation by stream permanence was present across our study sites. Intermittent streams were clustered in the southwest portion of the study area whereas permanent streams were clustered in the central part of the study area (Fig. 1). The clustering of our sites occurred at least in part because stream permanence is determined by geographic variation in vegetative cover, climate, geology, topography, and river size (Poff et al. 1997). Due to this, regional patterns are inherent to flow classification. We intended to use additional landscape level variables in our models. However, all variables examined (e.g., geology, soils, elevation) were correlated with stream permanence. For example, intermittent streams were dominated by sandstone geology and were at higher elevations than permanent streams. Permanent streams were predominantly comprised of limestone and dolomite geology, agricultural land cover, and were at lower elevations. Therefore, it may be appropriate to view stream permanence as a surrogate for a suite of landscape variables due to the interconnected nature of hydrology and landscape-scale factors.

We documented that two imperiled species of crayfish (*O. meeki* and *O. williamsi*) occupy intermittent streams throughout the Upper White River drainage in Arkansas and Missouri. This study adds to our knowledge base of crayfish use of intermittent streams and the importance of stream permanence for biotic assemblages. Our research confirms that small,

headwater streams support a diversity of organisms including those of conservation concern. Intermittent streams may be neglected in research and monitoring which may bias knowledge of aquatic species distributions and potentially affect conservation actions. Since intermittent streams are important for these and other imperiled crayfish such as *Orconectes marchandi* (Flinders and Magoulick 2003), they should be considered in surveys of imperiled biota. Our study is applicable to the current management of intermittent streams due to the ongoing controversy related to the U.S. Clean Water Act (Nadeau and Rains 2007, USEPA and USACE 2007) and the types and sizes of streams afforded protection under this policy. In addition, predicted changes in climatic patterns will likely elicit significant low-flow periods that may lead to local or total extinctions of imperiled species (Humphries and Baldwin 2003) which may include *O. meeki* and *O. williamsi*. The effects of climate change and subsequent alterations to hydrologic regimes will likely have broad impacts on imperiled aquatic biota on a global scale. Crayfish use of intermittent streams suggests that these streams are crucial for the conservation of these species and the integrity of freshwater systems in the face of global climate change.

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Tables and Figures

Table 1. Effect of year and stream permanence (two-way ANOVA p-values) on crayfish species densities, total crayfish densities, Simpson's Diversity, and local habitat variables. False discovery rate correction was used to control Type I error rates.

Variable	Year	Stream Permanence	Year * Stream Permanence
<i>Orconectes luteus</i>	0.25	< 0.01	0.26
<i>Orconectes meeki</i>	0.24	0.01	0.25
<i>Orconectes neglectus</i>	0.18	< 0.01	0.17
<i>Orconectes williamsi</i>	0.18	0.02	0.17
Total crayfish density	0.02	0.81	0.75
Simpson's Diversity	0.97	0.81	0.75
Water depth (m)	0.01	0.02	1.00
Current velocity (m/s)	< 0.01	0.02	1.00
Substrate size	0.66	0.02	1.00

Table 2. Principal Components Analyses (PCA) of five environmental covariates describing 20 streams in 2014 and 2015 in the Upper White River drainage of Arkansas and Missouri, USA. Correlations between covariates and the first two PCA factors (PC1 and PC2) and the relative contribution of covariates to these factors are indicated.

Site Covariate	PC1		PC2	
	Contribution (%)	Correlation	Contribution (%)	Correlation
2014				
Intermittent streams	49.11	0.83	16.65	-0.28
Water depth	12.43	-0.21	16.10	0.27
Current velocity	9.47	0.16	8.09	-0.14
Average substrate size	11.83	-0.20	56.38	0.94
% Forested land	17.16	0.30	2.81	-0.05
2015				
Site Covariate	PC1		PC2	
	Contribution (%)	Correlation	Contribution (%)	Correlation
Intermittent streams	53.92	0.77	9.84	0.14
Water depth	12.92	-0.18	14.29	-0.21
Current velocity	6.16	0.09	66.44	0.97
Average substrate size	11.49	0.16	3.10	0.05
% Forested land	15.52	0.22	6.35	0.09

Table 3. Multiple linear regression model output for four crayfish species in 2014 and 2015 in the Upper White River drainage of Arkansas and Missouri, USA. PC1 and PC2 relationships are shown in Table 2. P-values correspond to statistical significance of models. P-values of coefficients shown in text.

Species	Model	AIC_c	ΔAIC_c	r²	p
2014					
<i>O. luteus</i>	PC1	13.32	0.00	0.40	< 0.01
	PC2	23.32	10.01	0.00	0.79
	Global	16.43	3.11	0.40	0.01
<i>O. meeki</i>	PC1	8.24	0.00	0.20	0.05
	PC2	12.58	4.34	0.01	0.66
	Global	11.04	2.79	0.22	0.12
<i>O. neglectus</i>	PC1	-0.58	0.00	0.31	0.01
	PC2	4.91	5.49	0.09	0.21
	Global	-0.54	0.04	0.41	0.01
<i>O. williamsi</i>	PC1	23.31	0.00	0.26	0.02
	PC2	29.12	5.81	0.01	0.65
	Global	26.03	2.73	0.28	0.06

Table 3. Cont.

Species	Model	AIC_c	ΔAIC_c	r²	p
2015					
<i>O. luteus</i>	PC1	9.20	0.00	0.17	0.07
	PC2	12.69	3.49	0.01	0.61
	Global	12.02	2.81	0.19	0.17
<i>O. meeki</i>	PC1	-29.58	1.26	0.31	0.01
	PC2	-24.97	5.87	0.14	0.11
	Global	-30.83	0.00	0.45	0.01
<i>O. neglectus</i>	PC1	-25.55	0.00	0.42	<0.01
	PC2	-15.20	10.35	0.03	0.47
	Global	-23.41	2.14	0.45	0.01
<i>O. williamsi</i>	PC1	-1.59	4.37	0.09	0.19
	PC2	-5.95	0.00	0.27	0.02
	Global	-5.55	0.40	0.37	0.02

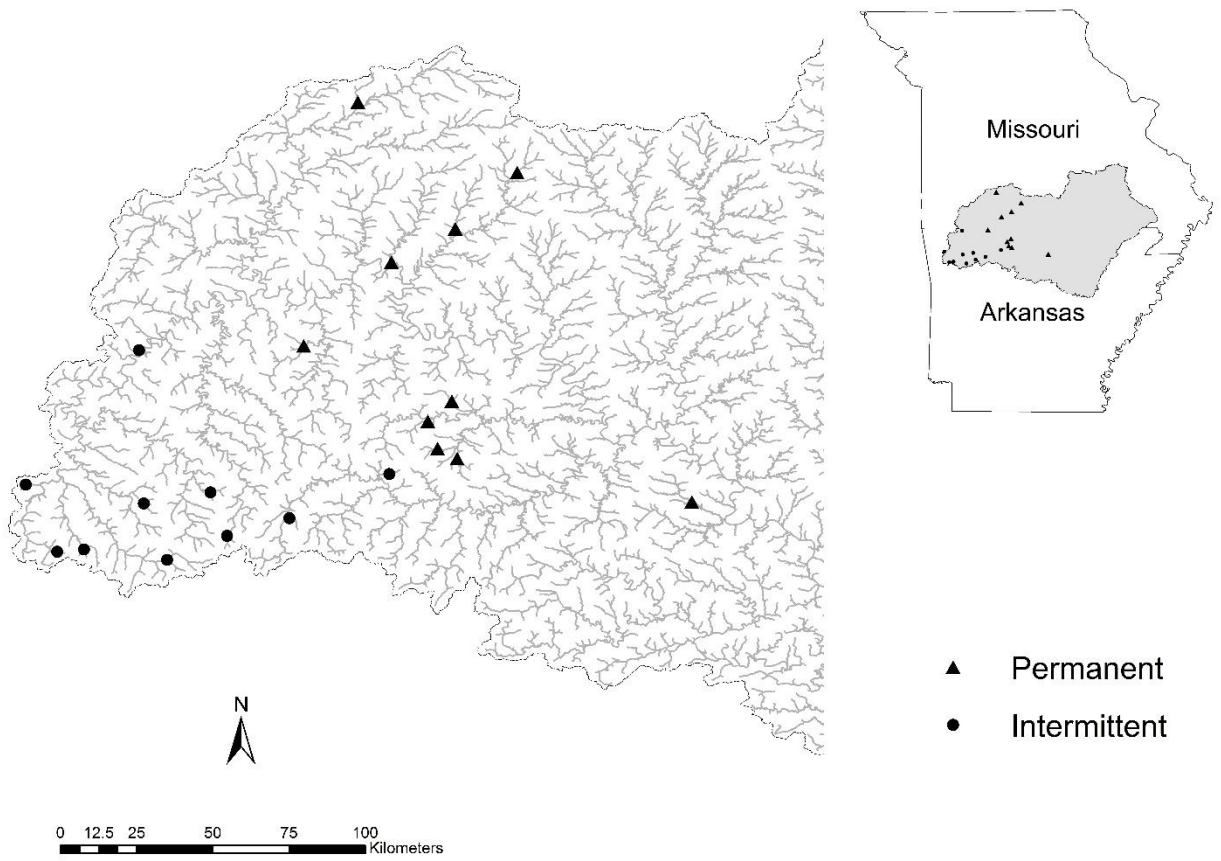


Fig. 1. Study area and sample locations within the Upper White River drainage of Arkansas and Missouri, USA.

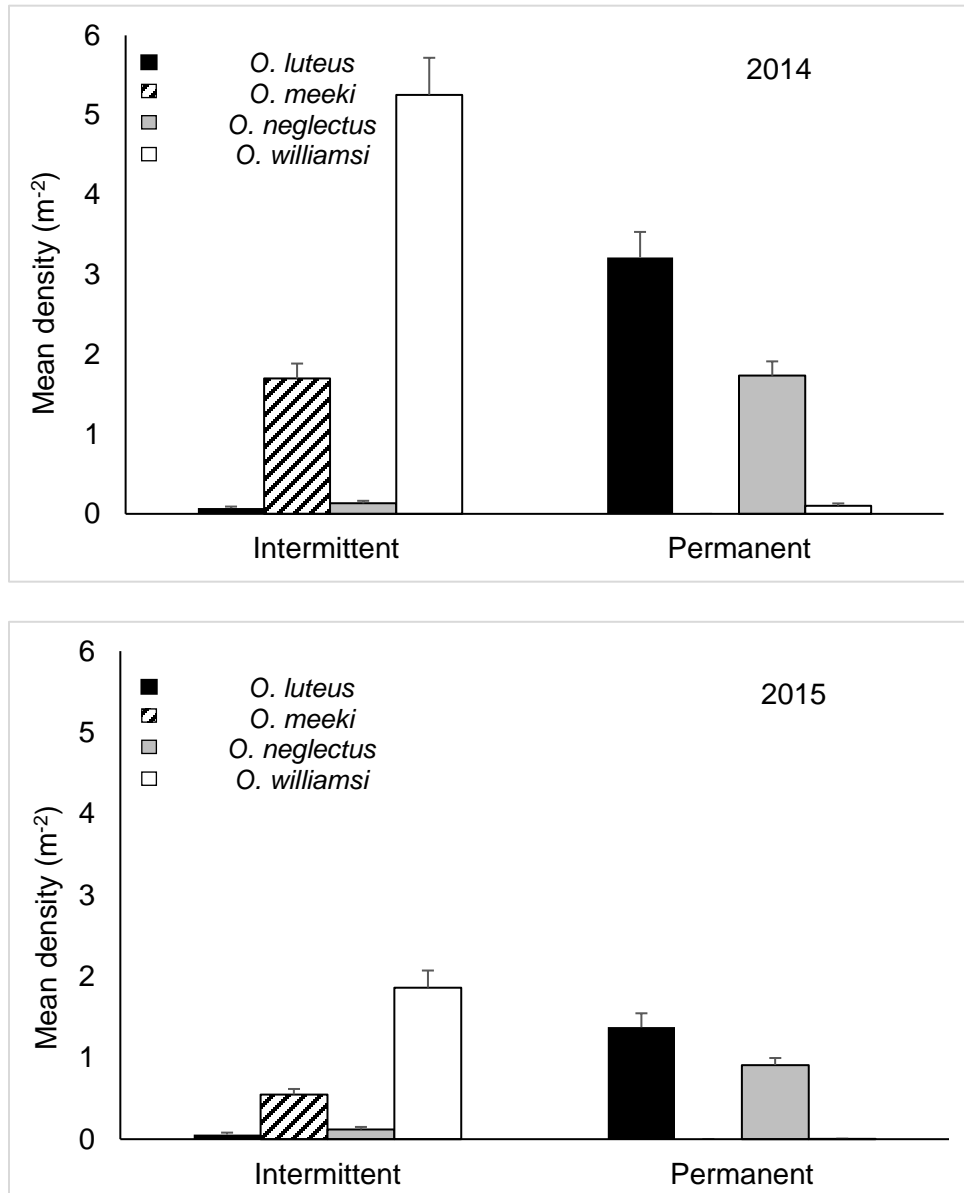


Fig. 2. Mean densities (\pm SE) by stream permanence of four most common crayfish species captured in 2014 and 2015 in 20 streams in the Upper White River drainage of Arkansas and Missouri, USA.

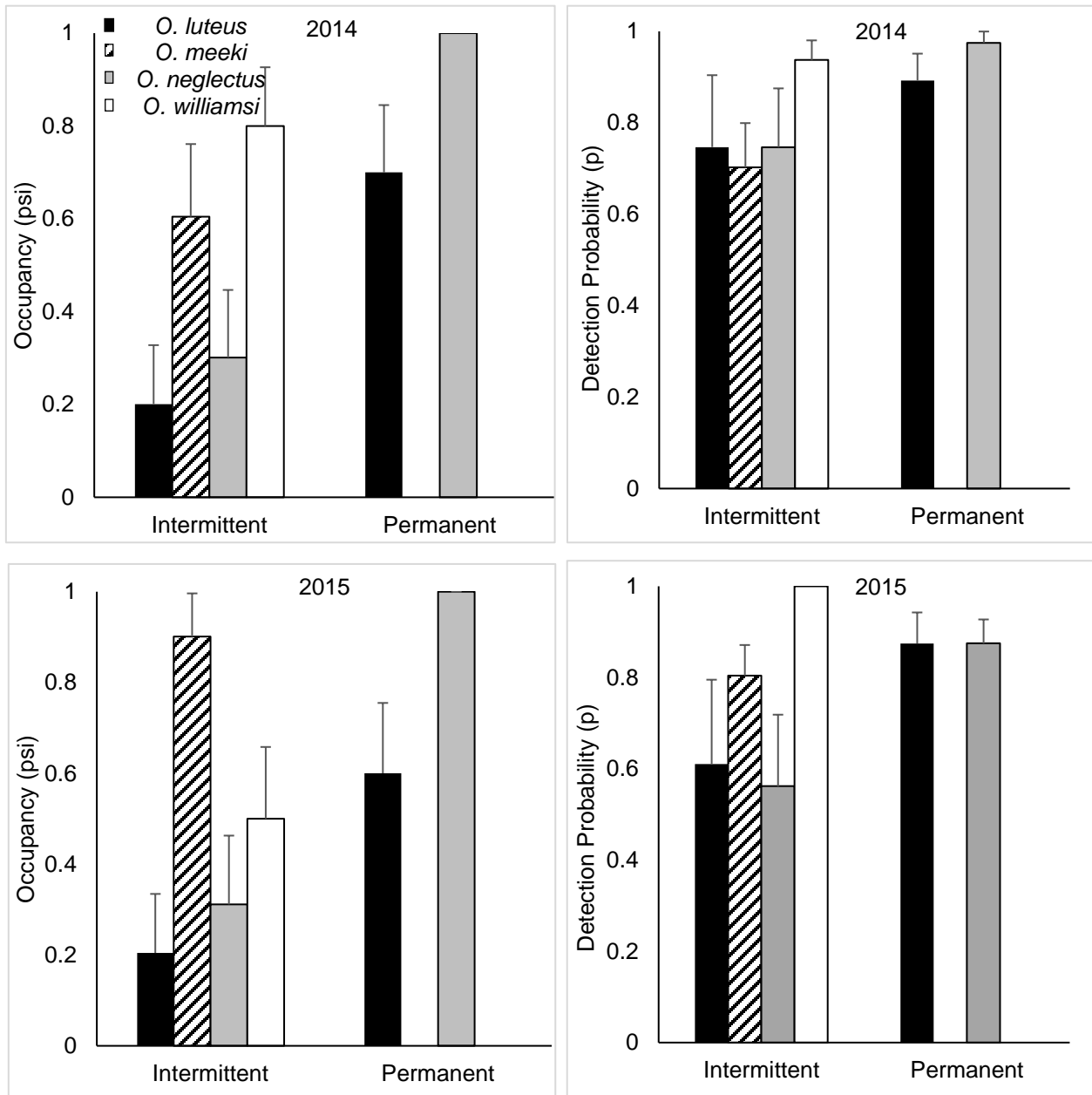


Fig. 3. Site occupancy (left panels) and detection probability (right panels) for all species in 2014 (top panels) and 2015 (bottom panels) by stream permanence across 20 streams in the Upper White River drainage of Arkansas and Missouri, USA. Missing bars indicate species absence in stream type or model failure due to only one detection event (i.e., *O. williamsi* in permanent streams).

Effect of stream permanence on predation risk of lotic crayfish by riparian and aquatic predators

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Abstract

Given the importance of crayfish in stream ecosystems, gaining insight into the role of stream permanence in maintaining predator-prey interactions is critical. Our objectives were to 1) determine the influence of stream permanence and season on crayfish predation and 2) assess the role of stream permanence and crayfish density on the presence of predators, while accounting for imperfect detection. We conducted surveys of crayfish density, fish presence, mammalian scat, and environmental variables within 10 intermittent and 10 permanent streams in the Ozark Highlands. We used occupancy modeling and logistic regression to assess the relationship between predator presence, crayfish density, and environmental variables. Stream permanence did not play a role in determining the relative frequency of occurrence or volume percentage of occurrence of crayfish prey in mammalian diets. However, season influenced the percent volume of crayfish found in mammal scats, and the volume percentage of crayfish prey was significantly greater in spring and summer compared to fall. The relative frequency and volume percentage of fish prey was strongly influenced by a season and stream permanence interaction which to our knowledge, is the first instance of this observation. Raccoons had the highest detection probability whereas American mink and river otter had low detection probabilities. River otter occupancy was positively associated with intermittent streams whereas centrarchid, raccoon, and American mink occupancy was negatively associated with intermittent streams. While our study did not indicate a relationship between stream permanence and predation risk, future hydrologic variability associated with climate change may alter crayfish predation risk and could have widespread implications for aquatic biota and predator-prey interactions.

Introduction

Crayfish fulfill a crucial role in lotic systems and can be considered both keystone species (Nyström et al. 1996) and ecosystem engineers (Creed and Reed 2004). They are a major contributor to production (Momot 1995) and act as predators (Momot 1995, Stenroth and Nyström 2003), consumers of autotrophs (Momot 1995, Dorn and Wojdak 2004), and detritivores (Stenroth and Nyström 2003). Due to their high contribution to macroinvertebrate biomass in many freshwater ecosystems (often >50% [Momot 1995]), crayfish serve as an important prey source for aquatic (Nyström et al. 2006), riparian (e.g., raccoon [*Procyon lotor*; Dorney 1954]; river otter [*Lontra canadensis*; Dekar et al. 2010]; American mink [*Neovison vison*; Wolff et al. 2015]), and avian predators (e.g., great blue heron [*Ardea herodias*; Hunt et al. 1995]). Despite the multifunctional role of crayfish in stream ecosystems and their importance in lotic food webs, crayfish are among the most imperiled freshwater taxa and are often highly endemic (Taylor et al. 1996, Taylor et al. 2007).

Crayfish predation risk is largely determined by habitat characteristics. Risk from aquatic or terrestrial predators is often related to water depth. Crayfish predation risk from aquatic predators (e.g., fish) is typically greater in deep water (Mather and Stein 1993, Flinders and Magoulick 2007, Clark et al. 2013). Deeper water may be limiting to some terrestrial predators, while others such as river otters are able to forage in both deep and shallow habitats due to their ability to dive for extended periods of time (Ben-David et al. 2000). Due to this, aquatic predators may show greater crayfish consumption in wet or non-drying conditions, whereas terrestrial predators may show greater crayfish consumption in shallow water and during periods of drought (Englund and Krupa 2000, Englund 1999). Predation risk is also closely linked to the availability of substrate and substrate particle size as these features can offer hiding cover. In a

crayfish tethering experiment in northern Wisconsin lakes, crayfish predation was higher in sand habitats compared to habitats containing cobble or macrophytes (Kershner and Lodge 1995). Larger substrates such as large rocks or boulders have more interstitial space and therefore provide crayfish with more protection from predators (Stein and Magnuson 1976) and these substrates may be especially important as refugia for small-bodied crayfish where predation is high (Clark et al. 2013). When water levels are low, protection afforded by large substrate sizes or submerged vegetation may be important for avoidance of predators (Wolff et al. 2016).

Due to the importance of instream habitat for crayfish predation risk, it is likely that crayfish predation risk differs among streams of varying hydrologic patterns. The natural flow regime of streams is closely linked to physical habitat. The movement of sediment, woody debris, and other transportable materials by flowing water is responsible for maintaining a variety of features such as substrate size, channel morphology, and habitat heterogeneity (Poff et al. 1997). Due to the importance of habitat in determining crayfish predation risk, assessing the role of stream permanence and seasonality as related to crayfish predation is important in understanding their habitat requirements and distribution patterns.

Given the crucial role of crayfish in stream ecosystems coupled with their imperiled status, gaining insight into the role of stream permanence in maintaining predator-prey interactions is critical, especially in the context of global climate change. Alterations to precipitation regimes will likely result in more extreme hydrologic events (e.g., flooding, drought) (Wuebbles and Hayhoe 2004) which may alter biotic relationships. Our objectives were to 1) determine the influence of stream permanence and season on crayfish predation and 2) assess the role of stream permanence and crayfish density on the presence of aquatic and mammalian predators, while accounting for imperfect detection.

Methods

Study Area

The natural flow regimes in the Ozark-Ouachita Interior Highlands of Arkansas, Missouri, and Oklahoma were recently classified (Leasure et al. 2016). The Ozark Highlands ecoregion included both intermittent streams with highly variable hydrology and permanent groundwater-influenced streams with relatively low hydrologic variability (Leasure et al. 2016). Streams classified as Intermittent Flashy streams have small drainage areas (8 to 22 km²) and exhibit a great deal of flow variability (e.g., drying for one to three months per year), including typical complete drying in late summer. Groundwater Flashy streams showed a wide range of drainage areas (11 to 3237 km²), had less daily flow variability than runoff-dominated streams and never experienced complete drying (Leasure et al. 2016). We examined Intermittent Flashy streams as intermittent streams and Groundwater Flashy streams as permanent streams, due to the contrast in their hydrologic variability and tendency to experience complete seasonal drying (intermittent) or no seasonal drying (permanent). Both flow classes are considered “flashy” which is a common stream type in the Ozark Highlands ecoregion (Leasure et al. 2016) and refers to the rapid rates of change from one magnitude to another. Ten intermittent streams and ten permanent streams were selected for study (Fig. 1).

Land use within subwatersheds (HUC 12) of study streams was predominantly forested, pasture, hay, and livestock farming (Homer et al. 2015). Natural vegetation consisted of hickory (*Carya* spp.) and oak (*Quercus* spp.) forest. Streambeds consisted largely of boulder, cobble, pebble, and gravel substrates with occasional bedrock outcroppings. All study streams had a well-defined riffle-pool sequence that is typical of Ozark streams.

Site Selection

Streams were candidates for selection if they were within a drainage area smaller than 200 km², at least 10 km away from one another, and had a probability of ≥ 0.50 of being in the correct hydrologic regime, as given by models in Leasure et al. (2016). We randomly selected 10 streams of each level of permanence from the group of streams fitting these criteria.

Accessibility and the availability of riffle habitats were determined in candidate streams by visiting them prior to sampling.

Crayfish surveys

To determine the influence of crayfish density in riffle habitats on the presence of predators, crayfish were sampled in the 20 streams during 2015 (10 June - 17 July). Four distinct riffle habitats were selected in each stream and eight 1 m² areas were randomly chosen in each riffle. In each sampling area, crayfish were dislodged from a 1 m² area (determined by a quadrat made of PVC) by thoroughly kicking and disturbing the substrate directly upstream of a seine net (6 mm mesh, 1.8 m l X 1.1 m h). Crayfish dislodged from the substrate were washed into the seine net by the water current and by pulling the seine through the sample area. All crayfish collected were identified to species and were released to their collection sites.

Abiotic variable data collection

At each crayfish sampling location (32) in each stream, current velocity and depth were measured directly in front of the sampling location using a Marsh McBirney® model 2000 flow meter (0.6-depth, ms⁻¹). Substrate composition was estimated at each sampling location within the 1 m² quadrat. Percent sand (0-0.1 cm diameter), gravel (0.1-3 cm diameter), pebble (3-6 cm diameter), cobble (6-12 cm diameter), and boulder (>12 cm diameter) was visually estimated

(Rabalais and Magoulick 2006). Percent forest cover was determined using ArcMap (ver. 10.3.1) and the National Land Cover Database 2011 (Homer et al. 2015). The percentage of forested land relative to all other land cover types was tabulated within each study subwatershed at the HUC 12 level. Drainage area of HUC 12 subwatersheds was determined using the National Hydrography Dataset Plus (USEPA and USGS 2005).

Scat surveys

Mammal scats were collected during summer 2015 (3 Aug – 29 Aug), fall 2015 (16 Oct – 20 Oct), winter 2016 (29 Jan – 1 Feb), and spring 2016 (14 May – 18 May) at each site. Trained observers walked along both stream banks and surveyed up to 10 m from the stream edge. Surveys encompassed the four riffles (and pools in between and on edges) sampled for crayfish in summer 2015. Mink scat was identified by its twisted appearance with tapered ends, raccoon scat was distinguished by its tubular and smooth shape with blunt ends, and otter scat was identified by its irregular shape with tapered or blunt ends which is sometimes found in amorphous piles (Levine and Mitchell 2008). Individual scats were held in plastic bags and frozen until processing.

Laboratory processing

Each scat sample was soaked in water until prey remains were separated. Samples were washed through a sieve (500 μ mesh) and air dried. Prey remains were sorted into eight prey classes (mammal, crayfish, fish, bird, insect, reptile, plant, and unknown) using a dissecting microscope (10 x). The unknown prey class was comprised of unidentifiable bones that may have belonged to birds, mammals, or reptiles. These broad prey classes were sufficient to

understand the importance of crayfish prey in the diets of riparian mammals. Exoskeleton fragments, chelae, scales, bones, teeth, fur, and feathers were used to identify prey.

Diet metrics

Two diet metrics were used to assess the seasonal importance of crayfish in the diets of riparian mammals among permanent and intermittent streams. Relative frequency of occurrence of each prey class was calculated as the number of occurrences of each prey class divided by the total number of occurrences of identified prey (Ciucci et al. 1996). The volume percentage of each prey class was visually estimated to the nearest 5% as the percentage of each prey class in each scat (McDonald and Fuller 2005). Both metrics were used in analyses as each metric has pros and cons, and it has been suggested that frequency of occurrence should not be the sole method to assess carnivore diet (Klare et al. 2011). The frequency of occurrence metric may overestimate the contribution of small amounts of prey and may also overestimate less digestible prey and underestimate more digestible prey (Klare et al. 2011). The volume percentage metric has the potential to underestimate the contribution of highly digestible prey and does not account for variation in scat sizes (Klare et al. 2011). To minimize the biases of using a single metric, we used both in our analyses (Zabala and Zuberogoitia 2003). The effect of stream permanence and season on volume percentage and relative frequency of occurrence of each prey class was examined using two-way ANOVA. If ANOVA indicated a difference among groups, Tukey's HSD test was used to determine which groups differed. Since multiple ANOVA's were run, we used false discovery rate correction to control Type I error rates (Benjamini and Hochberg 1995). All analyses related to diet metrics were performed using the data from the three mammal species detected.

Aquatic predator surveys

Snorkel surveys were conducted from 10 June - 17 July 2015 and coincided with crayfish sampling. Pool habitats between and on the edges of riffle habitats sampled for crayfish were sampled. Trained observers documented the presence of three common species of stream-dwelling centrarchids (i.e., smallmouth bass [*Micropterus dolomieu*], largemouth bass, [*Micropterus salmoides*], and Ozark bass [*Ambloplites constellatus*]). Fish smaller than 100mm in length were not recorded. Since fish are gape-limited predators, small or young of year fish rarely consume crayfish (Roell and Orth 1993, Rabeni, 1992, Dorn and Mittelbach 1999) and were therefore excluded from analyses.

Variable Reduction

To simplify our modeling procedure and avoid correlation among predictor variables, Principal Components Analysis (PCA) was used as a predictor variable reduction procedure. All predictor variables were $\log_{10}(x+1)$ transformed to meet the assumptions of normality and homogeneity of variance. The first two principal components were used as model predictor variables for both years. Large differences in the contribution of predictor variables to each principal component facilitated making biological interpretations. The first and second factors of the PCA had Eigenvalues > 1 .

Mammal occupancy

Program PRESENCE (Version 10.9, Proteus Research and Consulting Ltd., Dunedin, New Zealand) was used to estimate occupancy rates (the proportion of area occupied in each site; ψ) and detection probabilities (p) for the three riparian mammals detected throughout the study. Four models were run for each species of mammal and for all mammals combined. Two

models included each principal component separately as the occupancy parameter, one model included both principal components as the occupancy parameters (global model), and one model had a constant occupancy parameter (null model). Model selection was based on the small-sample size-corrected Akaike Information Criterion values (ΔAIC_c ; Akaike 1973, Burnham and Anderson 2002) and models were considered supported by the data if ΔAIC_c was less than 2.0 (Burnham and Anderson 2002). We anticipated that precipitation may play a role in our ability to detect mammal scats, therefore three detection covariates (i.e., Julian date, amount of precipitation within the last seven days, and the number of days since last precipitation event ≥ 2 inches) were examined. None of the detection covariates performed better than the null (constant) model for detection. Therefore, detection was modeled as a constant throughout the modeling procedure. Two models for American mink and one model for river otter did not converge presumably due to our limited detection of these species and complete separation among response variables. For these reasons, we could not obtain a reliable estimate for site occupancy of these species. Site occupancy estimates for raccoon and all mammals combined and detection probabilities for all species were based on the most supported model.

Fish presence

Logistic regression modeling was used to determine the relationship between the presence of fish predators and site-level variables. Two candidate models included each of the principal components separately and one candidate model included both principal components as the global model. The three models were once for all fish predators and sizes ($>100\text{mm}$) combined. The Chi-Square Goodness for Fit Test and McFadden's R^2 were calculated to assess model fit of the global model.

Results

Mammal diet

There was no stream permanence by season interaction for relative frequency of occurrence metrics for any prey type except fish (Table 1). Individually, stream permanence and season did not play a role in influencing the relative frequency of any prey type (Table 1, Fig. 2).

For the volume percentage metric, fish were the only prey item influenced by the stream permanence by season interaction (Table 1). Percent volume of fish prey was 100% (fish were the only prey type found) in winter in permanent streams but contributed very little during other seasons (<2%) (Fig. 3). During all seasons except winter, percent volume of crayfish prey was highest, ranging from 46-78% in permanent streams (Fig. 3). All remaining prey classes contributed little volume with the exception of mammal prey in fall (35%) in permanent streams (Fig. 3). In intermittent streams, percent volume of fish prey was <2% during winter and was low during all other seasons (Fig. 3). Crayfish prey had the highest percent volume in all seasons (40-71%) except fall which was dominated by mammal (31%), fish (23%), and plants (21%). (Fig. 3). Stream permanence did not play a role in influencing percent volume of all prey types other than fish (Table 1, Fig. 2).

The percent volume of crayfish prey was influenced by season (Table 1, Figs. 2 & 3). The volume percentage of crayfish prey was significantly greater in spring ($p=0.02$) and summer ($p < 0.01$) compared to fall but no significant differences were detected between winter and other seasons (Fig. 3). Relative frequency of occurrence of crayfish prey exhibited a similar pattern and was greater in spring and summer compared to fall (Fig. 2), though this relationship was not

significant (Table 1). With the exception of fish, the consumption of all prey types were not influenced by season for either metric (Table 1).

Occupancy models

The first two principal components explained 62% of the variation in the covariate data. Intermittent streams were strongly positively correlated (0.81) with PC1 and contributed 35.53%. Percent forested land was also strongly positively correlated (0.96) with PC1 and contributed 42.11%. Crayfish density was strongly positively correlated (0.99) with PC2 and contributed 70.21% (Table 2).

For all mammals combined, the null, global, and PC1 models were equally supported (Table 3). Occupancy of all mammals was positively related to PC1 (coef=2.05; SE=4.74; 95% CI=-7.24–11.34) and negatively related to PC2 (coef=-0.31; SE=0.88; 95% CI=-2.03–1.41), but the relationships were not significant. The global model fit the data well (Chi-square p=0.47) with no overdispersion (c-hat=0.66).

The null, global, and PC2 models were equally supported for raccoon (Table 3). Raccoon occupancy was negatively related to PC1 (coef=-2.07; SE=1.41; 95% CI=-0.69–4.83) and PC2 (coef=-2.34; SE=1.50; 95% CI=-5.28–0.60), but neither of these relationships were significant. The global model fit the data well (Chi-square p=0.41) and there was no overdispersion (c-hat=0.81).

Only two of the four models converged for American mink. The PC2 model was supported more than the global model (Table 3). American mink occupancy was negatively related to PC2 (coef=-1.71; SE=0.33; 95% CI=-2.36–1.06) and this relationship was significant.

The global model fit the data well (Chi-square $p=0.30$) and there was minimal overdispersion ($\hat{c}=1.16$).

Three of the four models converged for river otter. The null, PC1, and PC2 models were equally supported (Table 3). River otter occupancy was positively related to PC1 (coef=1.69; SE=1.52; 95% CI=-1.30–4.67) and PC2 (coef=1.51; SE=4.83; 95% CI=-7.96–10.98), but these relationships were not significant. Since the global model did not converge, model fit was assessed based on the PC1 and PC2 models. These models fit the data well (PC1 Chi-square $p=0.37$; PC2 Chi-square $p=0.43$) and there was minimal overdispersion (PC1 $\hat{c}=1.33$; PC2 $\hat{c}=1.29$).

Occupancy and detection

Raccoons were the most commonly detected mammal species and had the highest naïve and site occupancy rates (naïve occupancy=0.55; $\psi=0.64$; SE=0.14; CI=0.35–0.85). While occupancy rates could not be modeled reliably for American mink and river otter, their naïve occupancy rates were 0.50, and 0.10, respectively. For all mammals species combined, naïve occupancy was 0.75 and modeled site occupancy was 0.84 (SE=0.12; CI=0.48–0.97). Raccoon also had the highest detection probability ($p=0.39$; SE=0.09; CI=0.24–0.57). American mink and river otter had low detection probability (mink: $p=0.15$; SE=0.04; CI=0.09–0.24; otter: $p=0.03$; SE=0.02; CI=0.01–0.10). The detection probability of all mammals was 0.43 (SE=0.08; CI=0.30–0.58).

Fish presence

Presence of fish of the *Micropterus* and *Ambloplites* genera (i.e., smallmouth bass [*Micropterus dolomieu*], largemouth bass [*Micropterus salmoides*], and Ozark bass [*Ambloplites*

constellatus) greater than 100mm in length were negatively related to PC1 (coef=-0.91; SE=0.53; CI=-0.91-0.13) and positively related to PC2 (coef=-0.19; SE=0.47; CI=-0.19-0.73), but these relationships were not significant. The model fit of the global model was acceptable ($X^2=0.14$) but little variance was explained by the global model (13%).

Discussion

The volume percentage and relative frequency of crayfish prey in mammalian diets did not differ in permanent streams compared to intermittent streams. Other studies that have indicated that crayfish predation in deeper depths is dominated by aquatic predators rather than terrestrial predators (Mather and Stein 1993, Flinders and Magoulick 2007, Clark et al. 2013). In our study, riffle depth was deeper in permanent streams than in intermittent streams, therefore, we expected crayfish consumption by mammals to be lower in permanent streams than in intermittent streams due to the probable difficulty for mammals to access crayfish in deep water. Some terrestrial predators such as river otter are known to dive for extended periods of time and can therefore access prey in both shallow and deep habitats (Ben-David et al. 2000). However, due to the small number of scats collected from river otter throughout the study, this explanation may not be the reason we saw more intense crayfish predation in permanent streams.

Both the relative frequency and volume percentage of fish prey found in mammal diets was strongly influenced by the interaction of stream permanence and season. In permanent streams, fish were the dominant prey item by volume during winter but comprised very little prey volume in other seasons in permanent streams. Percent volume of fish in intermittent streams was not the dominant prey type in any season. Since some stream-dwelling crayfish are known to seek refuge among rocks and mud during winter to escape the threat of freezing (Aiken 1968), and several other prey classes are likely less active (i.e., reptiles, mammals, and insects)

or not in bloom (plants) during winter, it is reasonable that fish were the dominant prey type by percent volume in winter. However, this effect was only seen in permanent streams. One possible explanation for this result is that fish may be less available as prey in intermittent streams due to low winter flows. While we did not measure environmental variables (e.g., water depth, current velocity) during winter, it is known that Intermittent Flashy streams tend to have a higher frequency of no-flow days and low-flow spells and a lower baseflow index than Groundwater Flashy streams (Leasure et al. 2016). Since there was no precipitation over the winter sampling period for mammal scats (NOAA 2017), and some fish have been documented to be intolerant of low-flow conditions (e.g., low maximum water depth and slow average velocity) (Birnbaum et al. 2007), it is possible that low winter flows led to lower fish densities in intermittent streams than in permanent streams, making them a more accessible prey item in permanent streams during winter. To our knowledge, ours is the first study to examine the influence of a season by stream permanence interaction on aquatic prey and future studies should examine this interaction over a broader spatial scale to determine whether this relationship is generalizable.

Season influenced the volume percentage of crayfish prey in mammal scats. Crayfish predation was highest in spring and summer relative to winter and fall which is in alignment with findings from previous studies. Dekar et al. (2010) found that the frequency of occurrence of crayfish in river otter scats was highest in summer and lowest in winter. Crayfish have also been documented as the dominant component of American mink diet in summer (Wolff et al. 2015). Seasonal variation in the prevalence of crayfish as a prey source for terrestrial and aquatic predators may be especially pronounced in the Ozark Highlands where many streams are intermittent and experience prolonged drought during the summer months. It is known that

aquatic organisms (e.g., crayfish) can become exposed to harsh biotic and abiotic conditions including increased vulnerability to predation (Magoulick and Kobza 2003). Therefore, we anticipated greater crayfish predation by mammals during summer when water depths were likely lowest and crayfish were at their most vulnerable. However, this does not explain why we saw high crayfish predation during spring when flows are typically highest and depths are deepest. In addition to water depth, seasonal variation in water temperature may play a role in the accessibility of crayfish prey. Aiken (1968) documented that adult *Orconectes virilis* move to deeper water in colder months and while they did not appear to burrow, they were found to seek protection between mud and rocks to escape the threat of freezing. During colder seasons, it is possible that the tendency for crayfish to seek refuge among mud and rocks may make them less apt to be captured by terrestrial predators which may in part explain the higher prevalence of crayfish in the diets of mammals during the spring and summer we observed.

Occupancy of mammalian predators was related to stream permanence, forested land, and crayfish density. The occurrence of semi-aquatic predators is often linked to seasonal prey availability (e.g., fish, crayfish) which can be tied to landscape characteristics. The abundance of fishes that may serve as prey for mammals is often higher in areas of woody debris (Angermeier and Karr 1984) which is closely linked to riparian woodland cover (Jeffress et al. 2011). River otter occupancy was positively associated with intermittent streams and forested land.

Occupancy of river otter has been linked to forested land in previous studies. In Kansas, river otter occupancy was shown to increase with the proportion of woodland cover (Shardlow 2005). The occupancy of American mink and raccoon was negatively associated with intermittent streams and forested land. It is unsurprising that raccoons were negatively associated with forested land due to their ability to thrive in human-dominated landscapes (Prange et al. 2004)

Site occupancy of American mink has been shown to be negatively associated with urbanization (Wolff et al. 2015), and human disturbance (Racey and Euler 1983, Brzeziński et al. 2012) however, none of our study streams were set in urban areas. In our study, the watersheds with a low proportion of forested land were mainly comprised of farmland or pasture with relatively little human influence. Since the intermittent streams and forested land covariates were highly correlated (Table 2), we are unable to discern to whether mammal occupancy is driven by stream permanence or land cover.

American mink and raccoon occupancy was negatively associated with summer crayfish densities in riffles. It is likely that American mink select sites based on high prey concentrations (Wolff et al. 2015) and this may be possible for other predators such as raccoon, so this result was surprising. One potential reason for this observation could be that crayfish densities were reduced by the presence of American mink and raccoon and subsequent predation pressure. It is also important to recognize that our crayfish sampling regime focused only on riffle habitats. Future studies related to crayfish consumption should include crayfish sampling from a variety of habitats.

Accounting for imperfect detection is important when modeling species distribution and abundance (MacKenzie et al. 2006). In our study, detection probabilities of raccoon were moderate whereas American mink and river otter had low detectability. Jeffress et al. (2011b) detected river otter by scat and tracks and detection probabilities varied by substrate type but ranged from 0.18 (snow) to 0.60 (mud) which is higher than our detection probability for river otter. While none of the detection covariates we examined were better at describing mammal occupancy than the null models, Wolff et al. (2015) found that the observer and the amount of rainfall for seven days prior to surveying was most effective at modeling the detection

probability of American mink and Kowalski et al. (2015) found that raccoon detectability was greatest in areas of high road and trail densities. It is important to acknowledge that our detection probabilities for American mink and river otter were low compared to other studies and in order to more effectively determine the presence of semi-aquatic mammals, it would likely be advantageous to utilize multiple tracking methods (e.g., tracks, scats, observation) rather than scat samples alone.

Fish presence was not significantly associated with stream permanence, forested land, or crayfish density in riffle habitats. Larger centrarchids are known to use deep habitats with slow velocities (Lobb and Orth 1991), and while smaller centrarchids utilize shallow habitats (Lobb and Orth 1991), they were not the focus of our study due to their inability to consume larger crayfish and were not documented. Since permanent streams had significantly deeper depths than intermittent streams in our study, we expected fish presence to be highest in permanent streams, but this was not the case. We also anticipated a possible relationship between fish presence and crayfish density. Since larger fish (which were the focus of our study) are not restricted by gape size and are capable of consuming both juvenile and adult crayfish, we expected a negative association between fish presence and crayfish density since crayfish densities could become depleted by fish predation. To gain a better understanding of these relationships, future studies should implement more frequent fish surveys and crayfish sampling from pool habitats.

Our occupancy modeling often displayed little separation among models and most relationships between predator occupancy and environmental covariates were insignificant. While general trends between predator occupancy, stream permanence, forested land, and crayfish density were discerned, it is possible that these factors are not the most important in driving predator occupancy. It may be advantageous to incorporate a more comprehensive suite

of landscape-level variables that may better explain predator occupancy, since our study only included six (three landscape, three local) variables. We intended to use additional landscape level variables in our models. However, all variables examined (e.g., geology, soils, elevation) were correlated with stream permanence. This is somewhat unsurprising since hydrology is determined by landscape scale variation such as vegetative cover, climate, geology, and topography (Poff et al. 1997). It may be appropriate to view stream permanence as a surrogate for a suite of landscape variables due to the interconnected nature of hydrology and landscape-scale factors. In addition, surveys for mammalian predators were only conducted once per season and fish surveys were only conducted once in summer, more frequent surveys may reinforce our findings.

It is also important to recognize the caveats associated with the diet metrics used. Since the frequency of occurrence metric may overestimate less digestible prey (Klare et al. 2011), it is possible that this metric's caveats may skew our ability to truly identify the role of stream permanence in determining predation of crayfish. Since both the relative frequency of occurrence and volume percentage metrics of crayfish prey were not significantly different by stream permanence, we must interpret these results with caution.

Our study demonstrates the importance of stream permanence in facilitating predator-prey interactions. In the face of global change, models have indicated that much of North American will likely experience an increased frequency of summer drought and more intense precipitation events which will lead to increased flooding (Wuebbles and Hayhoe 2004). Such precipitation changes are likely to lead to greater temporal fluctuations in flow regimes of streams and may lead to variation in water depths (Wuebbles and Hayhoe 2004) outside of the range that native organisms are adapted to. In addition, the projected intense low-flow periods

may lead to local or total extinctions of aquatic biota (Humphries and Baldwin 2003). Such hydrologic variability may alter crayfish predation risk and could have widespread implications for aquatic biota and predator-prey interactions.

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Tables and Figures

Table 1. Effect of stream permanence and season (two-way ANOVA p-values) on volume percentage and relative frequency of occurrence of eight prey classes. False discovery rate correction was used to control Type I error rates.

Variable	Stream Permanence	Season	Stream Permanence * Season
Relative Frequency			
Crayfish	0.21	0.14	0.85
Mammal	0.90	0.28	0.85
Fish	0.46	0.64	0.04
Insect	0.90	0.76	0.85
Bird	0.90	0.64	0.28
Reptile	0.84	0.64	0.85
Plant	0.84	0.64	0.85
Volume Percentage			
Crayfish	0.42	0.02	0.80
Mammal	0.99	0.28	0.89
Fish	0.74	0.18	< 0.01
Insect	0.99	0.69	0.80
Bird	0.99	0.69	0.74
Reptile	0.86	0.69	0.89
Plant	0.86	0.69	0.80

Table 2. Principal Components Analyses (PCA) of six environmental covariates describing 20 streams in the Upper White River drainage of Arkansas and Missouri, USA. Correlations between covariates and the first two PCA factors (PC1 and PC2) and the relative contribution of covariates to these factors is indicated. Eigenvalue for PC1 = 1.56, PC2 = 1.12.

Site Covariate	PC1		PC2	
	Contribution (%)	Correlation	Contribution (%)	Correlation
Intermittent streams	35.53	0.81	12.77	-0.18
Crayfish Density	3.95	-0.09	70.21	0.99
Water Depth	8.33	-0.19	8.51	-0.12
Current Velocity	6.14	-0.14	4.96	0.07
Drainage Area (km ²)	3.95	-0.09	2.84	-0.04
% Forested Land	42.11	0.96	0.71	0.01

Table 3. Models of site occupancy (ψ) and detection probability (p) for three species of mammals individually and combined. Parameters were fixed (.) or allowed to vary with PCA factors (PC1 and PC2) derived from six site covariates. Models that converged out of the total set of four are included.

Model by species	$\Delta AICc$	wi
<i>All mammals</i>		
$\psi(.), p(.)$	0.00	0.37
$\psi(PC1, PC2), p(.)$	0.08	0.35
$\psi(PC1), p(.)$	1.34	0.19
$\psi(PC2), p(.)$	2.66	0.10
<i>Raccoon - Procyon lotor</i>		
$\psi(.), p(.)$	0.00	0.41
$\psi(PC2), p(.)$	0.54	0.31
$\psi(PC1, PC2), p(.)$	1.70	0.17
$\psi(PC1), p(.)$	2.62	0.11
<i>American mink - Neovison vison</i>		
$\psi(PC2), p(.)$	0.29	0.35
$\psi(PC1, PC2), p(.)$	3.46	0.07
<i>River otter - Lontra canadensis</i>		
$\psi(.), p(.)$	0	0.49
$\psi(PC1), p(.)$	0.8	0.33
$\psi(PC2), p(.)$	1.95	0.18

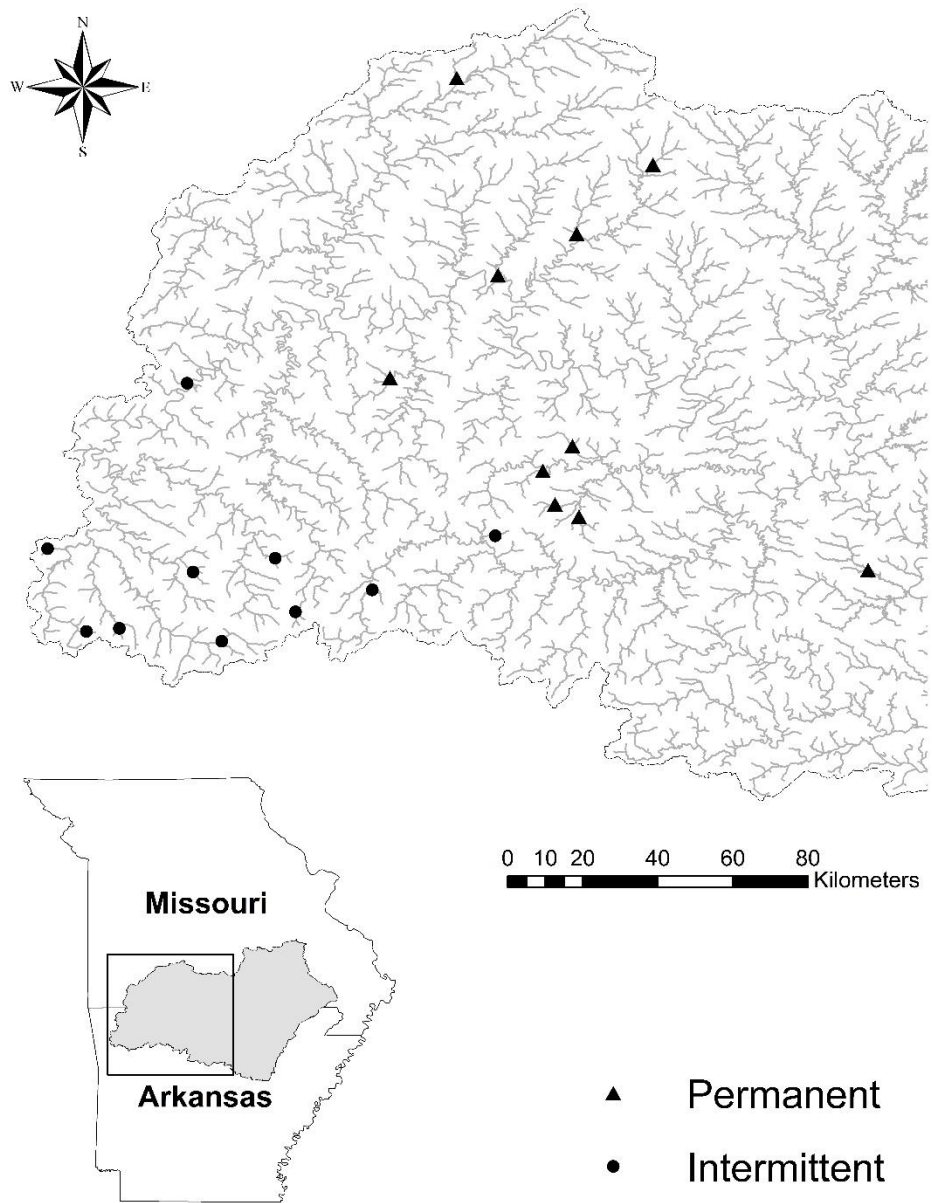


Fig. 1. Study area and sample locations within the Upper White River drainage of Arkansas and Missouri, USA.

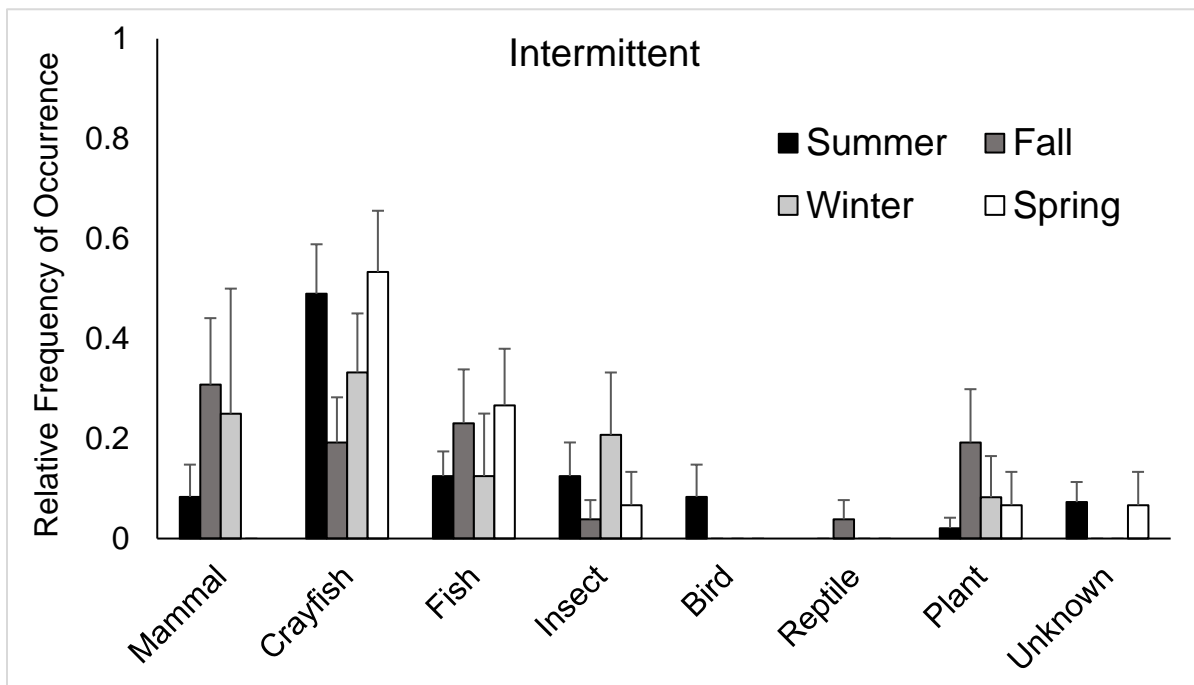
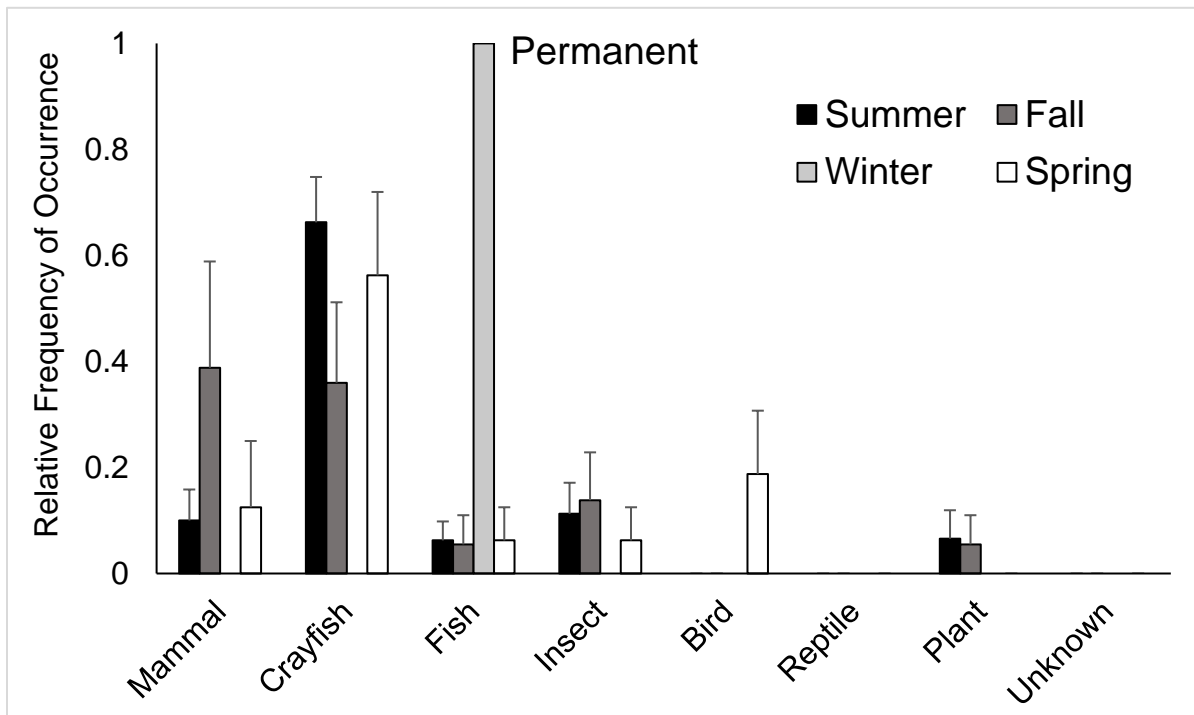


Fig. 2. Relative frequency of occurrence of eight prey classes in the diet of riparian mammals (raccoon, American mink, otter) in permanent (top) and intermittent (bottom) streams. Error bars represent standard errors.

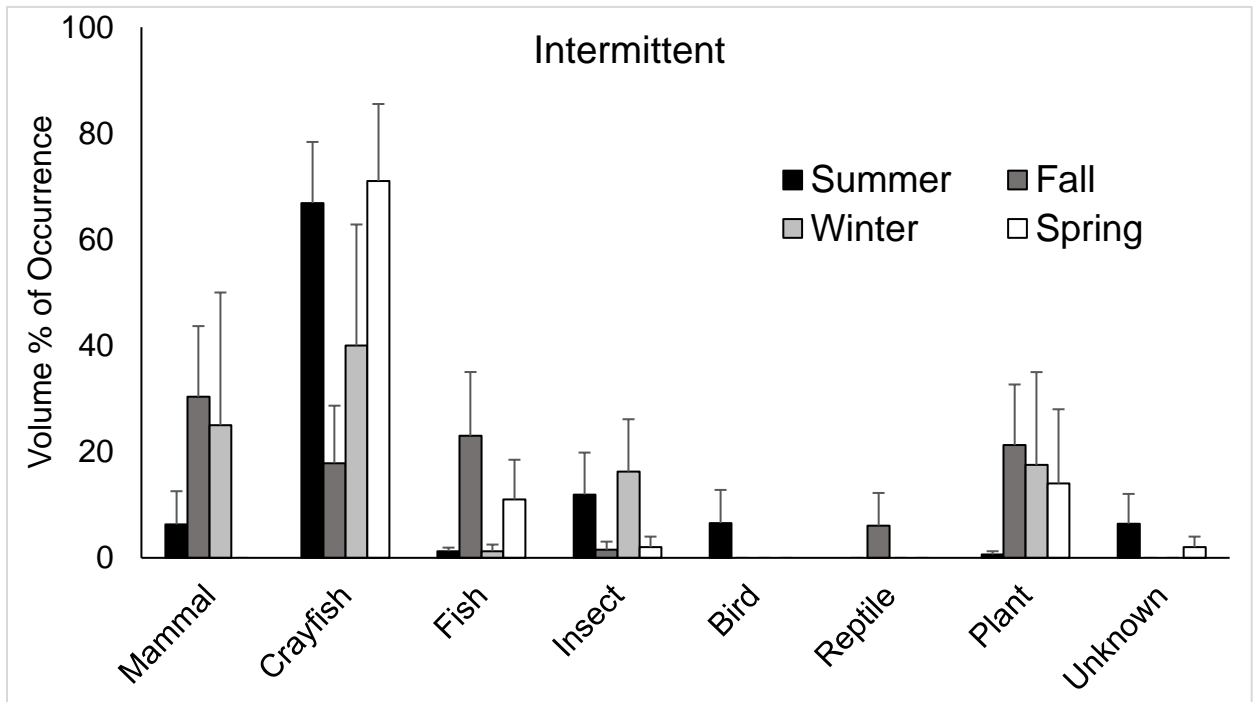
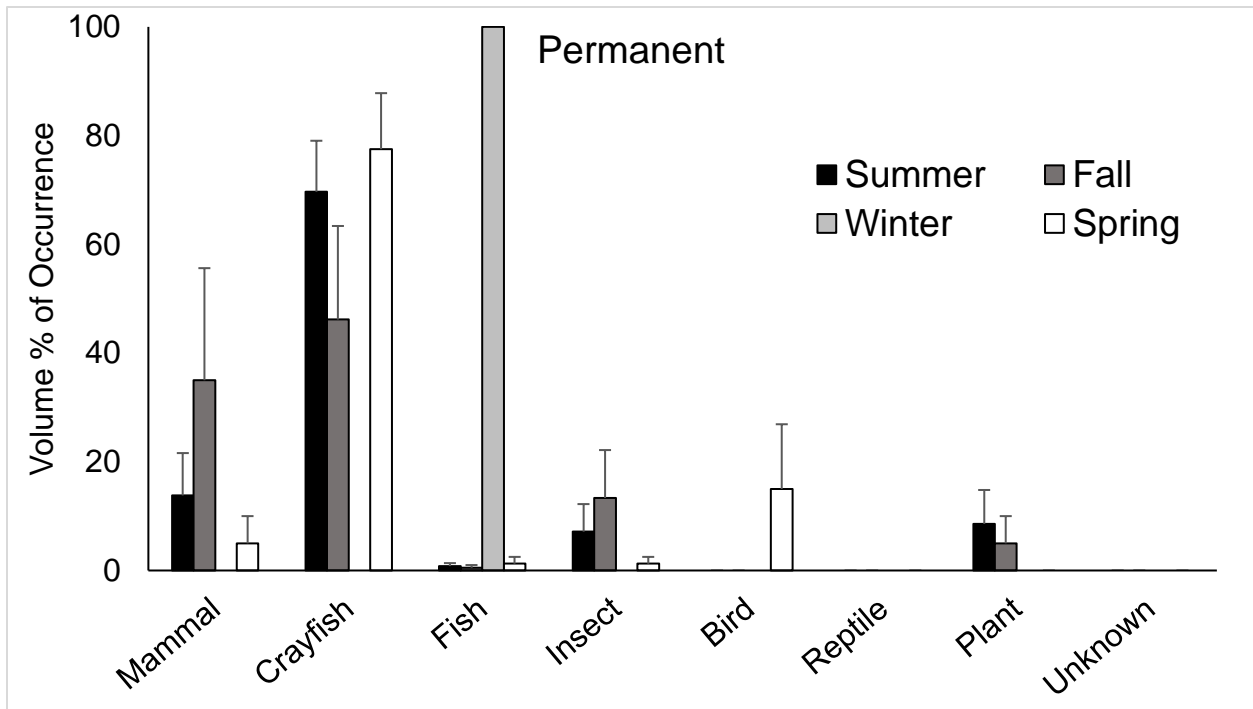


Fig. 3. Volume percentage of occurrence of eight prey classes in the diet of riparian mammals (raccoon, American mink, otter) in permanent (top) and intermittent (bottom) streams. Error bars represent standard errors.

Modeling effects of crayfish invasion and drought on crayfish population dynamics

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Abstract

In addition to being narrow-ranged endemics, three crayfish species of conservation concern (*Orconectes eupunctus*, *Orconectes marchandi*, *Cambarus hubbsi*) in the Spring River drainage of Arkansas and Missouri are facing invasive species effects by the recently introduced *Orconectes neglectus*. Our objectives were to model the population dynamics of these imperiled species, assess how these populations may be affected under increased invasion effects and intensified drought, and determine the potential of the Spring River to serve as a refuge from invasion and drought effects. RAMAS-Metapop was used to construct stage-based demographic models. Field data was used to determine locations of populations and obtain estimates of initial abundances and carrying capacities. Terminal extinction risk, median time to quasi-extinction, and metapopulation occupancy were used to assess population viability under different scenarios. Models indicated that the Spring River has potential to act as a refuge for *O. eupunctus* and *C. hubbsi* and allowed the populations to persist even when invasion and drought effects were extreme in the adjacent South Fork of the Spring River. Conversely, the Spring River acting as a barrier to dispersal for *O. marchandi* led to reduced quasi-extinction times and the greatest extinction risk under most invasion scenarios. *Cambarus hubbsi* appears to be highly susceptible to decline if vital rates are reduced due to simulated drought since they have low reproductive potential and mature slowly. Better estimates of stage specific survival and fecundity coupled with an increased understanding of the mechanisms of displacement by *O. neglectus* could reinforce our findings. Limiting the spread of invasive species, maintaining natural habitat and hydrologic regimes, and gaining insight into life histories and demographic parameters will increase our ability to conserve endemic crayfish in the Ozark Highlands.

Introduction

Crayfish fulfill a crucial role in aquatic ecosystems and often act as keystone species (Momot 1995). They are a major contributor to production and account for > 50% of macroinvertebrate biomass in many freshwater ecosystems (Momot 1995). Crayfish act as predators (Momot 1995, Stenroth and Nyström 2003), consumers of autotrophs (Momot 1995, Dorn and Wojdak 2004), detritivores (Stenroth and Nyström 2003), and prey for fish (e.g., smallmouth bass) (Rabeni 1992) and terrestrial animals (Boyle et al. 2014). They function as bioprocessors of vegetation and carrion (Taylor et al. 2007) by converting coarse particulate organic matter to fine particulate organic matter that is a food resource for other organisms (Whitledge and Rabeni 1997).

Despite the importance of crayfish in aquatic systems, they are among the most imperiled freshwater taxa (Taylor et al. 1996, Taylor et al. 2007) and show high levels of endemism. At least 382 species of crayfish inhabit North America (Crandall and Buhay 2008) and nearly half (48%) of these species are possibly extinct, endangered, threatened, or vulnerable (Taylor et al. 2007). Due to this degree of imperilment, crayfish are expected to experience high extinction rates (Ricciardi and Rasmussen 1999). In addition, a deficit in basic information related to crayfish life history, range, and species associations exists for most species (Taylor et al. 1996, Lodge et al. 2000).

Within the Spring River drainage of north-central Arkansas and south-central Missouri, there are three species of crayfish that are of conservation concern: *Orconectes eupunctus* (coldwater crayfish), *Orconectes marchandi* (Mammoth Spring crayfish), and *Cambarus hubbsi* (Hubbs' crayfish). *Orconectes eupunctus* is considered critically imperiled in the state of Missouri and globally imperiled (S1, G2; Missouri Natural Heritage Database 2017) and it is

estimated that fewer than 10,000 individuals of *O. eupunctus* exist (DiStefano et al. 2010). *O. marchandi* is considered critically imperiled/imperiled in Missouri and imperiled globally (S1/S2, G2; Missouri Natural Heritage Database 2017). Both *O. eupunctus* and *O. marchandi* are being considered for listing under the U.S. Endangered Species Act.

While considered stable and not in need of conservation attention by Taylor et al. (2007), it has been suggested that the conservation of *C. hubbsi* should be prioritized due to its limited range within the Ozark Highlands and contribution to regional taxonomic diversity (Pflieger 1996, Crandall 1998). In addition, this species may be categorized as a K life-history strategist based on its documented slower growth and lower reproductive potential compared to *Orconectes* species (Larson and Magoulick 2011). These factors may increase the susceptibility of *C. hubbsi* to environmental impacts and invasive species (Larson and Magoulick 2011).

In addition to these native species being imperiled and having narrow ranges, an invasive species, *Orconectes neglectus* (ringed crayfish), now occurs in high numbers in portions of the Spring River drainage (Flinders and Magoulick 2005). Native to the White, Arkansas, and Kansas river drainages of the central United States, it is known that *O. neglectus* was introduced into the Spring River drainage between 1984 and 1998 (Magoulick and DiStefano 2007). Previous research indicates that *O. neglectus* has become established throughout the West Fork of the Spring River and into portions of the South Fork Spring River (Magoulick and DiStefano 2007) and it appears to have replaced some native species of crayfish that were formerly abundant in this area. Although *O. eupunctus* and *C. hubbsi* made up a substantial proportion of the crayfish community in the West Fork of the Spring River during 1984 (Pflieger, unpubl. data), studies found no *O. eupunctus* and *C. hubbsi* in the West Fork of the Spring River during collections in 1998 and 1999 (Flinders and Magoulick 2005, Magoulick and DiStefano 2007),

suggesting that one or both species were extirpated by *O. neglectus*, which now dominates this area. Since *O. neglectus* has the potential to expand its distribution through the entirety of the Spring River drainage, further displacement of native, endemic crayfish may occur in the future.

Understanding the potential consequences of an advancing invasive species is critical in the context of native crayfish conservation in the Spring River drainage. Since additional stressors including habitat loss and fragmentation and intensified drought due to the impacts of climate change may exacerbate invasive species effects, it is important to understand the population dynamics of native crayfish and their vulnerability to future environmental change and invasion. Our objectives were to 1) model the population dynamics of imperiled crayfish of the Spring River drainage, 2) assess how these populations may be affected under different scenarios including invasive species impacts and intensified drought, and 3) determine the potential for the Spring River to serve as a refuge from invasion and drought effects.

Methods

Study Area

The Spring River drainage in north-central Arkansas and south central Missouri (36°N 91°W) is located in the Salem Plateau physiographic region of the Ozark Plateau (Fig. 1). The underlying bedrock consists predominantly of permeable dolomites, cherts, and limestone, leading to a Karst topography (Adamski et al. 1995). No major urban areas are within the drainage and land use is predominantly pasture for livestock and forest comprised mainly of oak (*Quercus* spp.) and hickory (*Carya* spp.). Streambeds consist largely of boulder, cobble, pebble, and gravel substrates with occasional bedrock outcroppings and study streams generally contain well-defined riffle-pool sequences typical of Ozark streams.

Field Surveys

Multiple sampling events occurred to determine the distribution of several crayfish species throughout the Spring River drainage. Sites along the South Fork, West Fork, Spring River, and several tributaries within the drainage were surveyed both in 1998-1999 and 2010-2011 (Flinders and Magoulick 2005, Nolen et al. 2014). Crayfish were collected using a quantitative kick-net method in which crayfish were dislodged from a 1m² area by kicking and disturbing the substrate directly upstream of a 1.5 x 1.0 m seine net (mesh size = 3mm) (Mather and Stein 1993). In 1998 and 1999, three to five replicate 1m² kick-net samples were collected in each of riffle, run, pool, backwater, and vegetation habitats at each site. In 2010 and 2011, three replicate 1m² kick-net samples were collected in riffle and run habitats. In each sample, sex, and carapace length (to the nearest 0.1mm) were recorded for all individuals and densities of all crayfish species were documented. These data allowed us to determine stage class structure, locations of extant populations of the species of interest, and estimate initial abundances of each species among populations for our modeling procedure.

Model Overview

RAMAS-Metapop (Applied Biomathematics, Setauket, New York, USA) was used to construct a demographic model of the three imperiled crayfish species in the Spring River drainage. RAMAS-Metapop is a valid and sufficiently accurate tool for assessing and managing imperiled species (Brook et al. 2000). For each of the three species, models consisted of three demographic stages: juveniles, young adults, and old adults. Environmental stochasticity was incorporated into the models by randomly sampling mean survival and fecundity from the stage matrix and standard deviations from the standard deviation matrix within the program (Akçakaya 1991). Ceiling type density dependence was incorporated into the model by estimating the

carrying capacity of each subpopulation. Since not directly measured, estimates of carrying capacity were based on the area of a habitat unit or “subpopulation” (i.e., riffle or run) and the average densities of each species found per 1m² during sampling. Since only females were modeled, we defined carrying capacity as the approximate number of females that would occur in a habitat unit. A spatial component of the models was incorporated by inputting the relative position of each subpopulation as X and Y coordinates. Dispersal was based on a distance-function matrix in which dispersal between subpopulations decreased with increasing distance. In order to determine the median time to quasi-extinction, we based our extinction threshold on effective population size estimates from Gouin et al. (2011) for *Austropotamobius pallipes*, an endangered European freshwater crayfish, since we were unable to find published data related to extinction thresholds of *Orconectes* species. For *A. pallipes*, estimates of effective population size were variable but ≤ 150 in most populations examined (Gouin et al 2011). Therefore, the effective population size used in our models was 150. All models were run for 1,000 iterations over 100 years. We used terminal extinction risk, median time to quasi-extinction (in years), and metapopulation occupancy to assess population viability.

Model Parameterization

Fecundity estimates for each crayfish species were based on published literature (Flinders and Magoulick 2005, Larson and Magoulick 2008, Larson and Magoulick 2011) (Table 1). Since the published fecundity data did not explicitly incorporate stage or age and since larger crayfish are expected to be more fecund (Kirkpatrick 1984) we assumed young adult crayfish to be 50% less fecund than old adult crayfish. We used age-based survival estimates from Hein et al. (2006) that were based on *Orconectes rusticus* (Table 1). Since *Cambarus hubbsi* is characterized as having a different life history than *Orconectes* species (Larson and Magoulick 2011), we chose

to use a slightly higher juvenile survival rate for *Cambarus hubbsi* (Table 1). Without modifying the juvenile survival rate of *C. hubbsi*, the models showed the metapopulation to go extinct quickly under baseline conditions which we felt was unrealistic. This was likely due to the low reproductive potential of *C. hubbsi* (Larson and Magoulick 2011).

Baseline Scenario

Our modeling procedure included different scenarios. The baseline scenario represented no further spread of *O. neglectus* throughout the Spring River drainage. The parameters used for fecundity and survival were the values found in the literature (Table 1) and carrying capacity for each subpopulation was that of a natural carrying capacity without any invasion effects. Baseline carrying capacities were based on abundance estimates for each species within subpopulations (*O. eupunctus* and *C. hubbsi*: K=55, *O. marchandi*: K=110). This baseline scenario was used to compare relative changes in population viability with invasion and drought.

Invasion and Drought Scenarios

To simulate the potential impacts of increased spread of *O. neglectus*, carrying capacities for the focal species were reduced due to the presence of *O. neglectus*. Since the extent to which *O. neglectus* invasion will reduce carrying capacities in each subpopulation is unknown and the mechanisms of displacement by *O. neglectus* have not been documented with certainty, invasion effects on each metapopulation were modeled over a range of carrying capacities at the subpopulation level at 20%, 40%, 60%, and 80% reductions to baseline carrying capacities. We simulated potential effects of drought on crayfish population dynamics by reducing vital rates (fecundity and survival) of the focal species. We chose to reduce vital rates because drought has been linked to reductions in mean body size which is likely to decrease reproductive output and

increase predation risk (Taylor 1988), thereby reducing fecundity and increasing mortality. This scenario was modeled over a range of reductions in baseline vital rates (5%, 10%, and 15% reductions). Since drought has been suggested to play a role in exacerbating invasive species effects (Larson et al. 2009), we included a scenario in which invasion and drought were additive. This scenario was run over a range of reduced carrying capacities and vital rates: 20% reduction in K and 5% reduction in vital rates, 40% reduction in K and 10% reduction in vital rates, and 60% reduction in K and 15% reduction in vital rates

The Spring River as a refuge

We also modeled the potential for the Spring River to serve as a refuge for *O. eupunctus* and *C. hubbsi*. The Spring River is a large, spring-fed river and invasive species effects by *O. neglectus* remain undetected. Based on previous research (Larson et al. 2009), we hypothesized that the Spring River may act as a refuge for *O. eupunctus* and *C. hubbsi* and invasive species and drought effects may be relaxed in this area. To model the Spring River as a potential refuge, we included a scenario in which carrying capacities and vital rates were reduced in the South Fork subpopulation (60% reduction in K, 15% reduction in vital rates) but were left at baseline levels in the Spring River subpopulation. Since *O. marchandi* prefers lower order streams and does not occur in the South Fork or Spring River but in their tributaries (Flinders and Magoulick 2005), *O. marchandi* was excluded from this scenario.

Parameter Validation

To assess the accuracy of our parameters and modeling process, we conducted a preliminary analysis for a subpopulation of *O. eupunctus* in an upper reach of the West Fork. This subpopulation had high densities of *O. eupunctus* (3.48 individuals m⁻²) in 1999 (Flinders

and Magoulick 2005) but upon revisiting this site in 2015, *O. eupunctus* had been extirpated and *O. neglectus* occurred in high densities (Magoulick et al., unpubl. data). Therefore, when using the abundance of *O. eupunctus* in 1999, we estimated this subpopulation to go extinct in our model within approximately 15 years. This subpopulation was modeled to have a median quasi-extinction time of 10.3 years when carrying capacities were reduced by 90% in the subpopulation due to the hypothetical presence of *O. neglectus*. Since in our model, this subpopulation went extinct within the timeframe we observed the true extirpation of *O. eupunctus* from this area, we proceeded to use these baseline parameter estimates throughout the remainder of our modeling procedure.

Sensitivity Analysis

To determine the sensitivity of model parameters, each parameter was varied by $\pm 25\%$ of the baseline estimate while holding all other parameters constant (Akçakaya 2000). Terminal extinction risk was used to measure the effect of each parameter and absolute values of low estimates were subtracted from absolute values of high estimates. Sensitivity analyses were conducted for each of the three focal species.

Results

O. eupunctus

The baseline scenario for *O. eupunctus* resulted in a terminal extinction risk of nearly zero (0.002). After 100 years, the cumulative probability of quasi-extinction was less than 0.01. All 12 subpopulations were expected to remain occupied over 100 years.

Reductions in carrying capacity due to the presence of *O. neglectus* led to an increased terminal extinction risk at all levels of reduction (Fig. 2) and a reduction in median time to quasi-

extinction (Fig. 3) after a 40% reduction in carrying capacity. 3 of the 12 subpopulations would remain occupied after 100 years when carrying capacities are reduced by 80%.

For *O. eupunctus*, reduced vital rates due to drought led to an increased terminal extinction risk (Fig. 2) and a reduction in median time to quasi-extinction (Fig. 3) once vital rates were reduced by 10%. It would take 65 years for less than one subpopulation to remain occupied.

If the additive effects of invasion and drought led to reduced carrying capacities and vital rates, terminal extinction risk would increase (Fig. 2) and median time to quasi-extinction would decrease (Fig. 3) for *O. eupunctus*. Less than one subpopulation would remain occupied under the most severe reductions in carrying capacities and vital rates after 42 years.

With an extreme invasion and drought scenario occurring in the South Fork subpopulation (60% reduction in K, 15% reduction in vital rates) but not in the Spring River, the chance of terminal extinction was low (extinction risk=0.06) (Fig. 4) and over 100 years, the cumulative probability of quasi-extinction was 0.16. Of the 12 subpopulations, 7 were expected to remain occupied over 100 years and all 7 remaining subpopulations were those occurring in the Spring River.

O. marchandi

The baseline scenario for *O. marchandi* resulted in a terminal extinction risk of zero. After 100 years, the cumulative probability of quasi-extinction was zero. Of the 16 subpopulations, 8 were expected to remain occupied over 100 years.

A reduction in carrying capacity due to the presence of *O. neglectus* led to an increased terminal extinction risk (Fig. 2) and a reduction in median time to quasi-extinction (Fig. 3). Times ranged from 47-83 years for less than one subpopulation expected to remain occupied.

Reductions in vital rates due to the influence of drought led to an increased terminal extinction risk (Fig. 2) and a reduction in median time to quasi-extinction (Fig. 3) once vital rates were reduced by 10%. Times ranged from 48-77 years for less than one subpopulation to remain occupied.

For *O. marchandi*, the additive effects of invasion and drought led to an increased terminal extinction risk (Fig. 2) and a reduction in median time to quasi-extinction (Fig. 3). It would take 31-60 years for less than one subpopulation to remain occupied under the additive effects scenario.

C. hubbsi

For *C. hubbsi*, the baseline scenario resulted in a terminal extinction risk of 0.003. After 100 years, the cumulative probability of quasi-extinction was 0.03. 13 of the 14 subpopulations were expected to remain occupied over 100 years.

Reductions in the carrying capacity of *C. hubbsi* due to *O. neglectus* invasion led to an increased terminal extinction risk (Fig. 2) and a reduction in median time to quasi-extinction (Fig. 3). Under the most severe reduction in carrying capacity (80%), one subpopulation was expected to remain occupied after 100 years.

Reduced vital rates due to drought led to an increased terminal extinction risk (Fig. 2) and a reduction in median time to quasi-extinction (Fig. 3) for *C. hubbsi*. It would take 46-75 years for less than one subpopulation to remain occupied.

The additive effects of invasion and drought led to an increased terminal extinction risk (Fig. 2) and a reduction in median time to quasi-extinction (Fig. 3). It would take 37-90 years for less than one subpopulation to remain occupied under the additive effects scenario.

An extreme invasion and drought scenario (60% reduction in K, 15% reduction in vital rates) occurring in the South Fork and baseline vital rates and carrying capacities in the Spring River would lead to a terminal extinction risk of 0.30 (Fig. 4) and a median time to quasi-extinction of 93 years. Of the 14 subpopulations, 6 were expected to remain occupied over 100 years and the 6 remaining subpopulations only occurred in the Spring River.

Sensitivity Analyses

Sensitivity analyses indicated that juvenile survivorship was the most sensitive parameter in the models for all species (Fig. 5). No other parameters were sensitive for *O. eupunctus* and *O. marchandi* but adult 1 survivorship, adult 2 fecundity, and adult 1 fecundity were close to being sensitive in the *C. hubbsi* models (Fig. 5).

Discussion

Our models indicated that the Spring River has the potential to serve as a refuge for *O. eupunctus* and *C. hubbsi* even when invasion and drought effects are strong in the South Fork. Our model predicted that none of the South Fork subpopulations of *O. eupunctus* and *C. hubbsi* would remain occupied over 100 years but several of the Spring River subpopulations would remain extant. Despite the modeled loss of *O. eupunctus* and *C. hubbsi* in the South Fork, the population as a whole was expected to persist with a low risk of extinction. It is probable that *O. neglectus* will reach the Spring River eventually, but whether they become established in a large river is uncertain. *O. neglectus* has been shown to be negatively associated with water depth in large rivers (Flinders and Magoulick 2005). Since both *O. eupunctus* and *C. hubbsi* have been demonstrated to have positive associations with current velocity and large *O. eupunctus* are known to be positively associated with water depth (Flinders and Magoulick 2005), it is possible

that the habitat present in the Spring River is more favorable to *O. eupunctus* and *C. hubbsi* than *O. neglectus*.

While the Spring River has the potential to aid in the persistence of *O. eupunctus* and *C. hubbsi*, it appears to serve as a barrier to dispersal for *O. marchandi* (Flinders and Magoulick 2005), which, as a result, appeared to be highly susceptible to invasion impacts. *O. marchandi* was expected to experience quasi-extinction within about 70 years under a 20% reduction in carrying capacity due to invasion. Of the three species, *O. marchandi* has the highest reproductive potential but its subpopulations are spatially isolated so that movement between subpopulations is unlikely. For our modeling procedure, we hypothesized that dispersal of *O. marchandi* between subpopulations was negligible based on the distance between subpopulations and the documented limited dispersal capabilities of Ozark crayfish species (e.g., *Orconectes hylas* can advance upstream at a rate of 128 m/year in its invasive range [DiStefano and Westhoff 2011]). Currently, the extent of gene flow between subpopulations of *O. marchandi* in the Spring River drainage is being examined (Magoulick et al. unpubl. data) and this information will be incorporated into future models of *O. marchandi* population dynamics.

Based on our models, if *O. neglectus* reaches the smaller order streams where *O. marchandi* occurs, the effects could be drastic due to the spatial isolation of these subpopulations. Under the invasion scenarios, *O. marchandi* had the highest terminal extinction risk of the three species examined with the exception of *C. hubbsi* under the 20% reduction in carrying capacity scenario. However, reductions in vital rates due to drought had relatively minor effects on *O. marchandi* until reductions were substantial (>10%). The additive effects scenarios greatly affected the extinction risk of *O. marchandi* even under the most mild invasion and drought scenario. Our models indicate that potential reduced carrying capacities of *O. marchandi*

due to *O. neglectus* invasion could severely limit the persistence of *O. marchandi*, but that reductions in vital rates due to drought would be less severe unless combined with invasion effects, presumably due to the high reproductive potential of *O. marchandi*.

Of the three species examined, *C. hubbsi* appeared to be the most susceptible to decline when vital rates were reduced due to simulated drought. Baseline fecundities were low for *C. hubbsi* and drastically limited the persistence of this species when they were reduced. Terminal extinction risk was highest for *C. hubbsi* and time to quasi-extinction was shortest even at mild reductions in vital rates. The additive effects of invasion and drought had a severe effect on *C. hubbsi* and this species had the most rapid time to quasi extinction under all three of the additive effects scenarios. Reducing carrying capacities due to invasion had an intermediate yet pronounced effect on the extinction risk of *C. hubbsi* compared to *O. eupunctus* and *O. marchandi* but invasion had the strongest effect on the time to extinction for *C. hubbsi*.

Under invasion impacts, *O. eupunctus* had the lowest terminal extinction risk and longest time to quasi-extinction compared to *C. hubbsi* and *O. marchandi*. *Orconectes eupunctus* was not expected to experience quasi-extinction within 100 years until carrying capacities were reduced by 60%. With reductions in vital rates, *O. eupunctus* was intermediately affected and the additive effects scenarios led to a rapid time to quasi-extinction and high extinction risk when severe. *Orconectes eupunctus* has an intermediate baseline fecundity of the three species examined and dispersal between the South Fork and Spring River subpopulations of *O. eupunctus* is more likely than dispersal of *O. marchandi* due to distance, which may explain why invasion and drought impacts were not as severe for this species.

Sensitivity analyses indicated that juvenile survival is the most sensitive parameter in the models of the three species. This suggests that uncertainty in our predictions can be reduced if

better estimates of this parameter can be obtained for the species of interest. One weakness of our modeling procedure is that we did not have stage or age based survival estimates for our focal species and had to base our estimates on different, yet related, species. In addition, the estimates of fecundity we acquired from the literature were not age based which caused us to estimate the difference in fecundity between age one adults and age two adults. Our study was limited in the sense that the data collected for these species was not collected with the intention of conducting stage-structured population modeling. Future efforts to monitor the population status of these species should incorporate age specific survival and fecundity.

While our models suggest that the spread of *O. neglectus* to the tributaries of the Spring River could be detrimental to the persistence of *O. marchandi*, it is unknown whether *O. neglectus* would negatively affect or displace *O. marchandi*. However, since *O. neglectus* has shown no distribution or density relationship with stream size and has been found within streams of varying orders (i.e., intermittent headwaters of the West Fork, the main channel of the West Fork, and the South Fork [Flinders and Magoulick 2005]), this suggests that *O. neglectus* could persist in the lower order streams *O. marchandi* inhabits. Furthermore, *O. marchandi* has been associated with slower moving, shallow water with gravel, pebble, and cobble substrates and since both small and large *O. neglectus* have been documented to be negatively associated with water depth (Flinders and Magoulick 2005), this also suggests that *O. neglectus* may be capable of persisting in the same habitat and streams occupied by *O. marchandi*. *Orconectes marchandi* has been documented to occur in higher densities in intermittent streams than in permanent streams in the Spring River drainage (Flinders and Magoulick 2003) and is likely able to endure low-flow or drought conditions, but to date, the drought tolerance of *O. marchandi* has not been directly examined. If *O. marchandi* is as capable of withstanding drought as *O. neglectus*

(Larson et al. 2009), it is possible that *O. marchandi* displacement by *O. neglectus* may be less probable or less severe. In order to more adequately predict the likelihood of invasion effects on *O. marchandi*, potential mechanisms of displacement by *O. neglectus* and drought tolerance should be examined.

Determining the true mechanism of displacement of native crayfish by *O. neglectus* would increase the accuracy of our predictions. Invasive crayfish have been demonstrated to negatively impact native crayfish in several ways such as through disease transmission (Alderman et al. 1990), reproductive interference and hybridization (Butler and Stein 1985, Perry et al. 2001), interspecific competition (Hill and Lodge 1994, Gherardi and Cioni 2004), and habitat displacement with subsequent predation by fish (DiDonato and Lodge 1993, Garvey et al. 1994). Despite numerous research efforts, the mechanism of *O. eupunctus* displacement by *O. neglectus* remains unclear. It does not appear that *O. neglectus* displaces *O. eupunctus* by forcing them into different habitats (Rabalais and Magoulick 2006, a), and both juvenile (Larson and Magoulick 2009) and adult male (Rabalais and Magoulick 2006, b) competition did not appear to drive displacement. It has been demonstrated that *O. neglectus* is far more tolerant to desiccation than *O. eupunctus* and that this resistance to drying could inhibit recolonization by *O. eupunctus* when flow resumes through priority effects (Larson et al. 2009). This research suggests that drought and invasion may act cooperatively in the spread of *O. neglectus*, and it has been suggested that other potential mechanisms (e.g., differential predation, reproductive interference) should be examined (Larson and Magoulick 2009). To date, the capability to withstand drought has not been studied for *C. hubbsi* or *O. marchandi*, but due to the limited reproductive potential and slow growth of *C. hubbsi* and the isolation of *O. marchandi* subpopulations, understanding drying effects and potential mechanisms of displacement of these species is crucial.

While our modeling procedure did not allow for the direct modeling of invasive-native species interactions, water level and drought has been shown to change these interactions in other population models. In their stage-structured models of invasive crayfish (*Orconectes rusticus*) and native sunfishes (*Lepomis* spp.) in northern Wisconsin lakes, Hansen et al. (2013) demonstrated that drought reduced crayfish recruitment that allowed the native sunfish to dominate. In our study, the opposite result was seen in that additive effects of drought and invasion decreased the chance of persistence of native crayfish. This difference may have been related to the Hansen et al. (2013) study occurring in lakes and with both fish and crayfish whereas our models were based in streams and with crayfish only. Future modeling efforts of the Spring River drainage crayfish could include multi-species models to better investigate invasive-native crayfish interactions.

The effects of drought on crayfish is understudied in the context of population modeling and more is known about amphibians. Cayuela et al. (2016) used a stochastic matrix population model to simulate the effects of drought on an endangered toad (*Bombina variegata*) in southern France. Models indicated that severe drought had a negative impact on fecundity and survival at various ontogenetic stages and that drought frequency negatively influenced the population growth rate. Other studies have indicated adult survival to be positively correlated with rainfall (*Rana sylvatica*: wood frog [Berven 1990]) and that survival in breeding ponds of *Ambystoma tigrinum tigrinum* (eastern tiger salamander) tends to be higher in years of high or average precipitation (Church et al. 2007). Despite the lack of studies related to how drought may facilitate changes in crayfish demographic parameters, it is possible that drought may elicit similar responses as in other semi aquatic species (e.g., amphibians).

Our modeling procedure demonstrates the potential importance of the Spring River as a refuge for *O. eupunctus* and *C. hubbsi* from invasion and drought effects. The spread of *O. neglectus* to the lower order tributaries of the Spring River where *O. marchandi* occurs could result in a rapid decline of *O. marchandi* due to the isolation of these subpopulations. While it is possible that *O. neglectus* will reach the Spring River and its tributaries over time, efforts should be made to prevent the further spread of *O. neglectus* by restricting human-mediated movement of this species for bait, which has been implicated as a potential vector of alien crayfish establishment (Lodge et al. 2000, DiStefano et al. 2009). Since abiotic disturbance, such as drought, is potentially important in native species displacement and may be capable of reducing crayfish vital rates, the maintenance and restoration of natural habitat and disturbance regimes may be crucial to conserve native species. Obtaining reliable estimates of demographic parameters, gaining insight into mechanisms of displacement, and limiting the further spread of invasive species will increase our ability to conserve endemic crayfish.

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Tables and Figures

Table 1. Baseline model parameter estimates for each species and stage.

Species	Parameter	Estimate	Source
<i>Orconectes eupunctus</i>	<i>Survival</i>		
	Juvenile	0.02	Estimates based on Hein et al. 2006
	Adult 1	0.58	
	Adult 2	0.11	
	<i>Fecundity</i>		
	Adult 1	30	Estimates based on Larson and Magoulick 2008
Adult 2	54		
<i>Orconectes marchandi</i>	<i>Survival</i>		
	Juvenile	0.02	Estimates based on Hein et al. 2006
	Adult 1	0.58	
	Adult 2	0.11	
	<i>Fecundity</i>		
	Adult 1	30	Estimates based on Flinders and Magoulick 2005
Adult 2	60		
<i>Cambarus hubbsi</i>	<i>Survival</i>		
	Juvenile	0.03	Estimates based on Hein et al. 2006
	Adult 1	0.58	
	Adult 2	0.11	
	<i>Fecundity</i>		
	Adult 1	17	Estimates based on Larson and Magoulick 2011
Adult 2	33		

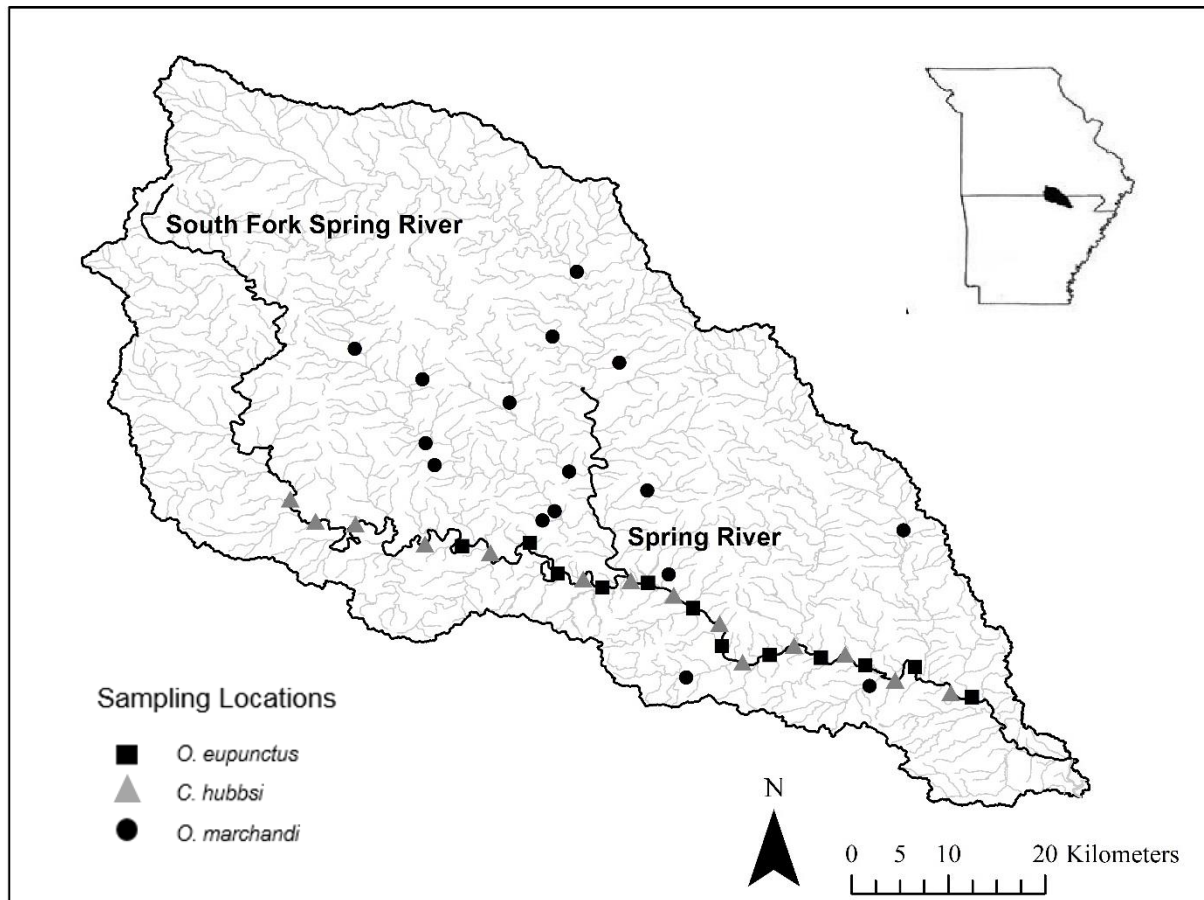


Figure 1. Study area in the Spring River drainage of Arkansas and Missouri with sampling locations of *O. eupunctus*, *C. hubbsi*, and *O. marchandi*. Sampling locations were used as subpopulations for the modeling procedure in RAMAS-Metapop.

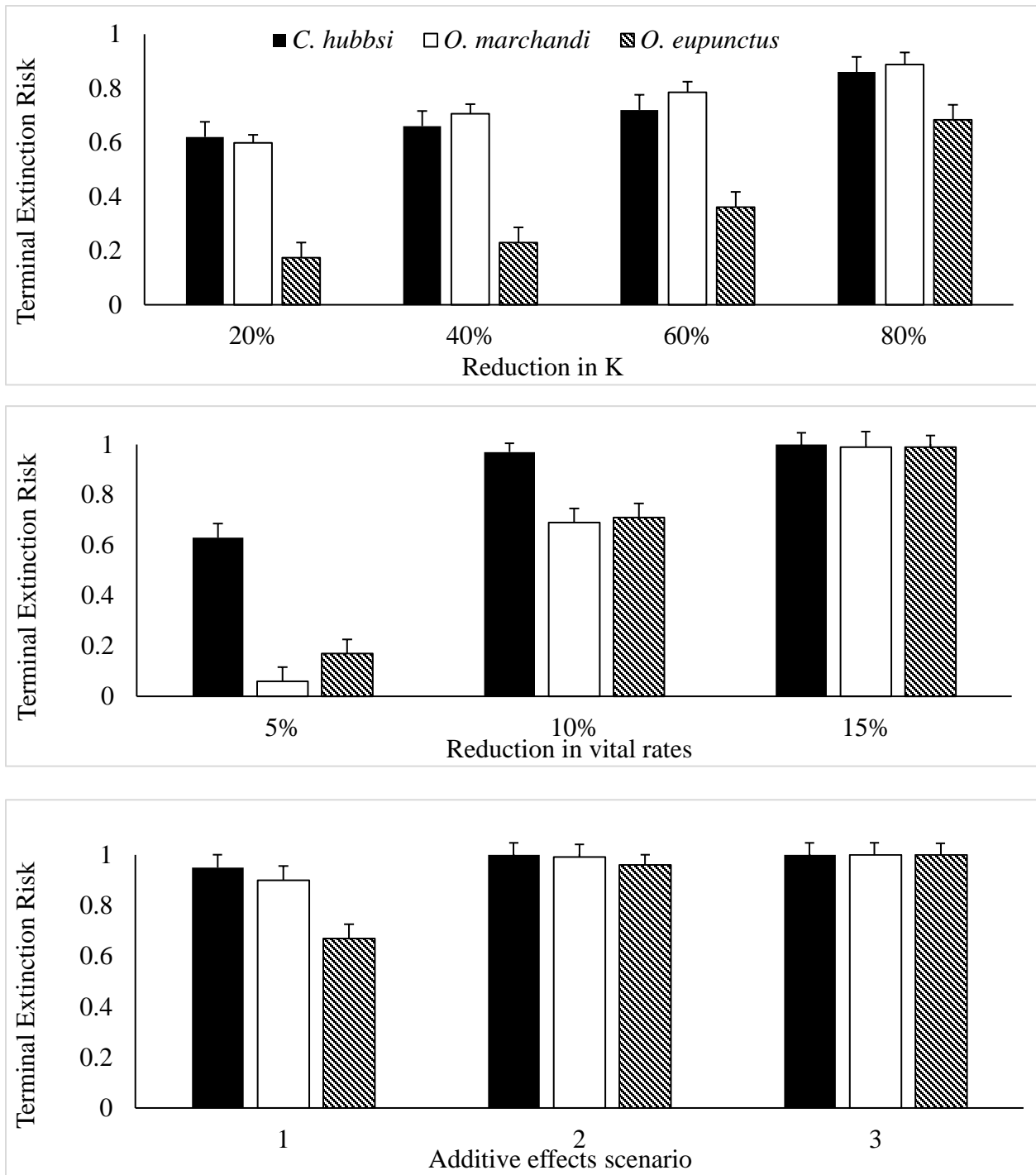


Figure 2. Terminal extinction risks for all species under reduced carrying capacities due to invasion (top), reduced vital rates due to drought (middle) and the additive effect of drought and invasion (bottom). Additive effects scenario 1: 20% reduction in K, 5% reduction in vital rates, scenario 2: 40% reduction in K, 10% reduction in vital rates, scenario 3: 60% reduction in K, 15% reduction in vital rates. Error bars indicate 95% confidence intervals.

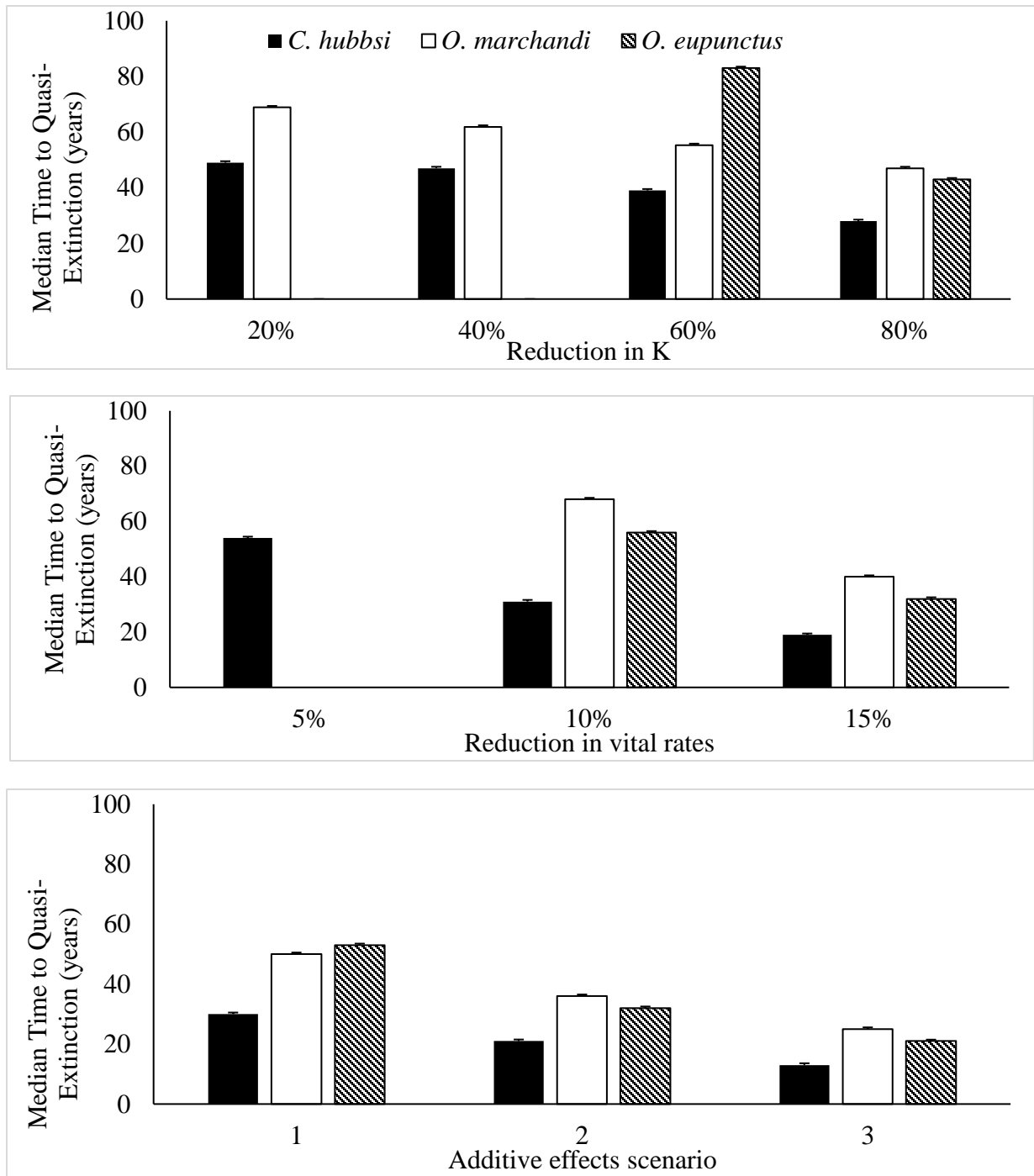


Figure 3. Median time to quasi-extinction for all species under reduced carrying capacities due to invasion (top), reduced vital rates due to drought (middle) and the additive effect of drought and invasion (bottom). Additive effects scenario 1: 20% reduction in K, 5% reduction in vital rates, scenario 2: 40% reduction in K, 10% reduction in vital rates, scenario 3: 60% reduction in K, 15% reduction in vital rates. No estimate for *O. eupunctus* under 20% and 40% reduction in carrying capacity and *O. marchandi* and *O. eupunctus* under 5% reduction in vital rates since quasi-extinction not predicted within 100 years. Error bars indicate 95% confidence intervals.

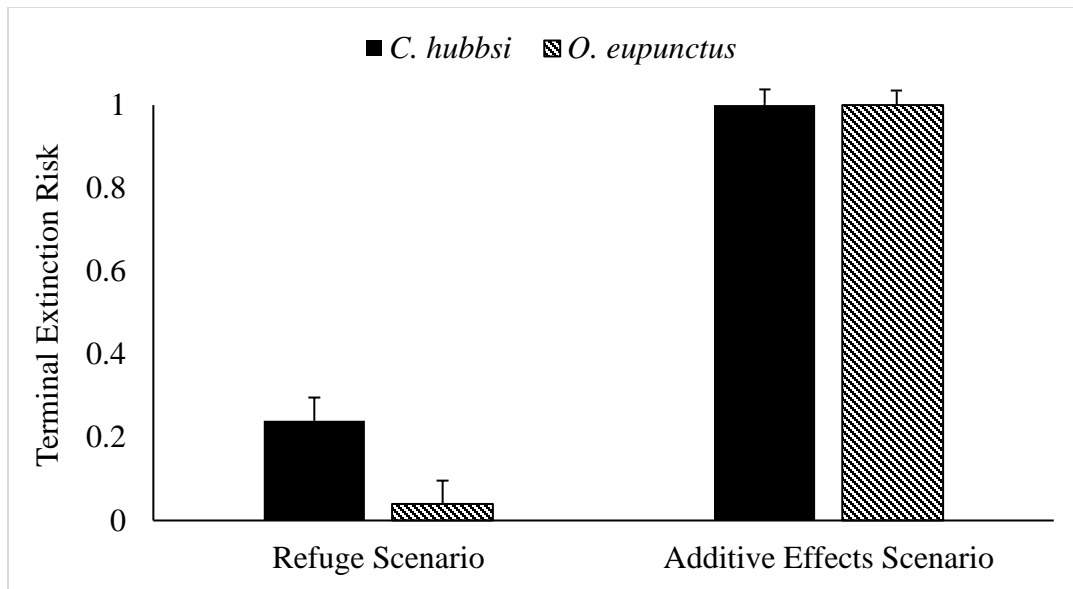


Figure 4. Terminal extinction risk for *C. hubbsi* and *O. eupunctus* with extreme invasion and drought effects under Spring River refuge scenario and additive effects scenario (no refuge). Error bars indicate 95% confidence intervals.

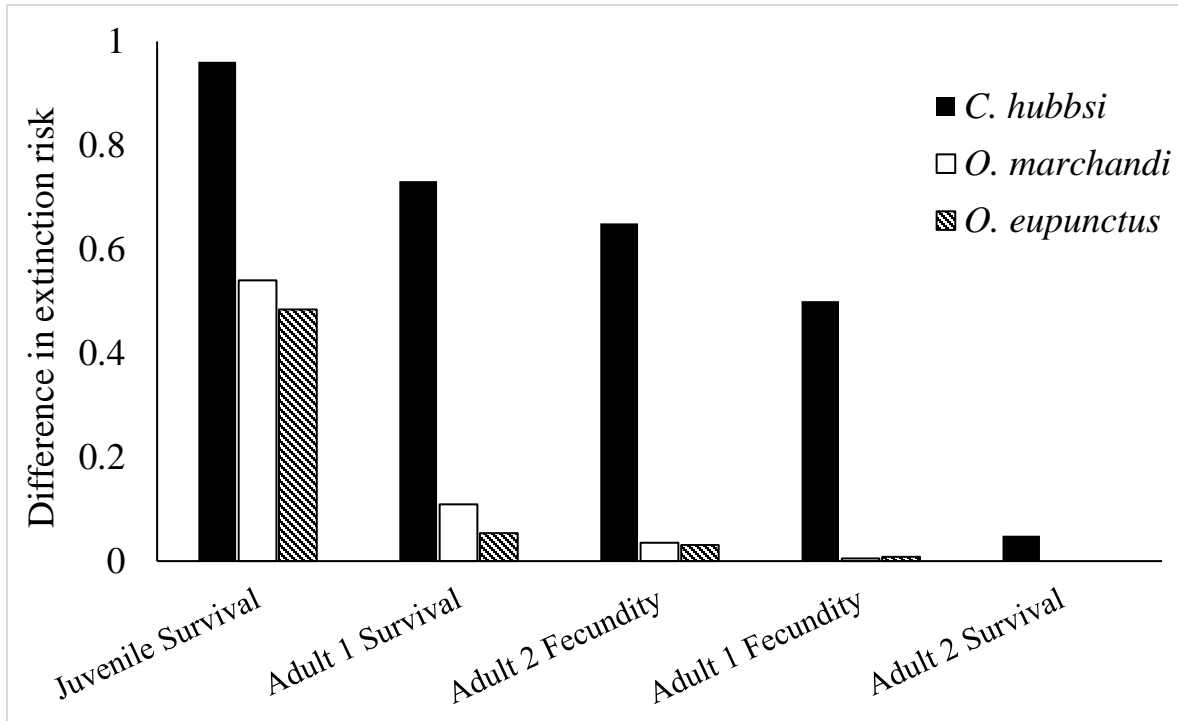


Figure 5. Sensitivity analysis derived from differences in terminal extinction risk between high and low parameter values for each species.

Conclusion

Our research reinforces the importance of stream permanence in determining species distributions, abundances, and biotic interactions. Due to our documentation of the importance of intermittent streams for two imperiled crayfish (*O. meeki* and *O. williamsi*), our research confirms that even small streams are capable of supporting a diverse array of organisms, including those of conservation concern. In addition, our research is pertinent to the ongoing legal uncertainty related to the U.S. Clean Water Act and the types and sizes of streams protected under this policy. Especially in the context of global climate change which is expected to alter hydrologic regimes, we encourage an awareness of intermittent streams in the development of future conservation strategies.

While we did not discern a relationship between crayfish predation risk and stream permanence, it is possible that predation-hydrology relationships may become clearer under increased hydrologic variability due to climate change. Such temporal fluctuations in flow regimes may create disturbance regimes and habitat characteristics that are outside of the range that native organisms are adapted to, and as a result, biotic interactions may change. Therefore, we recommend continued monitoring of predator-prey relationships in the context of hydrology.

Our modeling procedure demonstrated the potential increased effects of invasive species and drought in the Spring River drainage. This modeling procedure indicated the potential rapid decline of *C. hubbsi* due to drought on account of their low reproductive potential. The potential vulnerability of *O. marchandi* to invasion effects was also displayed and was likely amplified by the limited connectivity of subpopulations. Our study also indicated the potential of the Spring River to serve as a refuge from invasion and drought effects for *O. eupunctus* and *C. hubbsi*. This work also suggested the importance of obtaining accurate estimates of demographic parameters

of these species and future efforts should be made to ensure the collection of age specific survival and fecundity estimates when planning for future conservation.