



Does habitat matter? Assessing food resource and trophic niche differences of primary burrowing crayfishes in multiple habitats

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Abstract Understanding the functional roles of species is invaluable to conservation planning and effective ecosystem management. We used stable isotope analyses to examine the functional role of two primary burrowing crayfishes from the Piedmont region of South Carolina, USA, across both roadside and field habitats. *Distocambarus youngineri* served as a secondary consumer in roadside and field habitats, with similar trophic positions between habitats, sex, and size. *Distocambarus youngineri* relied heavily on primary and secondary consumers, with invertebrates contributing up to 82% of their somatic tissue growth. Conversely, *D. crockeri* served as secondary

consumers in roadside habitats and tertiary consumers in field habitats. In field habitats, *D. crockeri* relied heavily on primary and secondary consumers, with invertebrates contributing up to 84% of their somatic tissue group. In comparison, *D. crockeri* in roadside habitats relied principally on primary consumers (up to 42% of tissue composition). In fields, *D. crockeri* had higher carbon and nitrogen isotope ratios, as well as a larger trophic niche than those in roadside habitats. Our models suggest *D. crockeri* in roadside habitats consume more vegetation than populations in fields. Our analysis indicated considerable variation in trophic position, niche space, and dietary contribution between primary burrowing crayfish species, highlighting the ecological distinctness of primary burrowing crayfishes, occupying similar habitats but serving different roles within their ecosystems. These findings also highlight the important role primary burrowing crayfish play in these ecosystems, by regulating herbivore numbers, which helps ensure ecosystem stability, as well as facilitates energy flow to higher trophic levels.

Keywords Burrowing crayfish · Semiterrestrial habitats · Functional role · Resource use · Stable isotopes

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Introduction

Understanding species resource use and functional roles are invaluable in conservation planning and effective ecosystem management. Trophic interactions can greatly influence population sizes, community structure, and ecosystem processes (Estes et al., 2011; Frisch et al., 2014); thus, understanding these interactions is key to effective ecosystem management. Resource use and functional roles of species are useful in understanding trophic interactions because they provide information such as diet and trophic level, which gives insight on energy transfer from producer to consumer. This information is imperative in understanding species' roles within ecosystems (Ginzberg & Arditi, 2012), as well as the plasticity of those roles in different habitats. Despite the importance of understanding trophic dynamics, it remains poorly understood for many groups, including highly imperiled crayfishes.

Crayfish are an important component of freshwater and terrestrial ecosystems, often acting as keystone species and ecosystem engineers as well as playing a major role in food web dynamics (Creed Jr. & Reed 2004; Reynolds et al., 2013; Richman et al., 2015). Crayfish have been described as detritivores, omnivores, and carnivores, fulfilling several functional roles (e.g., primary consumers, scavengers, and predators; Thoma & Armitage, 2008; Usio & Townsend, 2008; Taylor & Soucek, 2010; Stites et al., 2017). Nonetheless, our understanding of crayfishes functional roles is primarily based off of crayfishes within lotic habitats, with only two studies assessing burrowing crayfish living in semiterrestrial habitats (Bloomer et al., 2022; Graham et al., 2022). Comparisons between aquatic and terrestrial ecosystems have demonstrated clear differences in food web structure, with top-down control greater in water than on land (Shurin et al., 2002). Additionally, less herbivory, more decomposers, and more detrital accumulation often occur in terrestrial than aquatic ecosystems (Shurin et al., 2006). Thus, our current understanding of stream crayfish trophic roles cannot be extended to burrowing crayfishes.

Burrowing crayfish diets have been explored through foraging behavior, gut content, and stable isotope analyses. Foraging studies have found reduced vegetation around burrow entrances (Loughman et al., 2015, 2019), plant matter in resting

chambers (Foltz II et al., 2018; Loughman et al., 2019), and remains of small vertebrates in burrows (Thoma & Armitage, 2008). Studies also report burrowing crayfish exhibiting sit-and-wait predation at the entrance of their burrows (Thoma & Armitage, 2008; Diehl et al., 2022; Graham et al., 2022), with prey varying from small worms and ants (Diehl et al., 2022; Graham et al., 2022) to larger vertebrates (e.g., salamanders and frogs; Thoma & Armitage, 2008; McCormack & Coughran, 2011). Similarly, studies assessing gut content and stable isotope analyses highlight the importance of invertebrates in crayfish diets (Bloomer et al., 2022; Graham et al., 2022), with crayfish trophic positions similar to other invertebrate primary consumers (Bloomer et al., 2022). Despite these recent gains in understanding burrowing crayfish diets, conducting comprehensive diet studies of burrowing crayfishes presents several challenges, including assessing all prey items, due to the small size, heterogeneity, and cryptic nature of some prey, and having to take into account the influence of seasonal changes, habitat variation, and environmental stressors. For example, techniques such as gut content analyses are only able to assess what is currently in crayfish guts, and crayfish gastric mills crush food items making them unidentifiable or biased toward food items that take longer to break down (Vannote & Ball, 1972; Lorman & Magnuson, 1978; Momot, 1995). Stable isotope approaches can help remedy some of these issues with comprehensive diet studies determining the functional trophic role of a given crayfish in an ecological system. Specifically, stable isotope approaches integrate food sources over time and reveal what the animal has assimilated, allowing for a more inclusive diet estimation than other techniques. Additionally, most studies assess a limited number of sites and habitats; thus, species variation within habitats and trophic plasticity based on species use of various habitat types has not been assessed.

Past studies have connected the trophic position of crayfishes with aspects of trophic ecology theory, such as trophic position typically increasing when crayfish body size increases (Roth et al., 2006; Larson et al., 2010, 2017; Kreps et al., 2016; Stites et al., 2017). In addition, trophic ecology theory predicts that a species trophic position will differ when habitat and resources change (Carscallen et al., 2012; Dixon et al., 2012; Sánchez-Hernández & Amundsen, 2018). However, no studies have assessed variation in

the trophic position of burrowing crayfishes among habitats. Burrowing crayfishes occur across many habitat types from wetlands that are frequently inundated (e.g., roadside ditches) to semiterrestrial habitats that experience surface water inundation more rarely (e.g., prairies), with single species populations occurring over a heterogeneous mix of habitats. Many crayfish are also omnivores, and most omnivore studies highlight their flexibility in feeding (Fagan, 1997; Lancaster et al., 2005; Anderson & Cabana, 2007; Olsson et al., 2008). Understanding the differences in food resources and trophic positions of species utilizing numerous ecosystem types is a basis for understanding food web dynamics and ecosystem function.

We used stable isotope approaches to examine the flexibility of nutritional resources, energy pathways, and functional role (i.e., trophic position) of two burrowing crayfish across two distinct habitat types. The Newberry Burrowing Crayfish, *Distocambarus youngineri* Hobbs & Carlson, 1985 and the Piedmont Prairie Burrowing Crayfish, *D. crockeri* Hobbs & Carlson, 1983 are both endemic to the Piedmont region of South Carolina, USA. Both species are often found in historical prairie habitats, poorly drained areas where the ground is saturated during wet parts of the year (Eversole & Welch, 2010). However, they have distinct ranges, with *D. youngineri* found only in Newberry and Laurens counties, South Carolina, within the Southern Outer Piedmont ecoregion and *D. crockeri* found within 5 counties throughout the Carolina Slate Belt ecoregion. Burrowing crayfish are a critically imperiled group, accounting for 15% of the US crayfish species but representing 32% of critically imperiled crayfish (Welch & Eversole, 2006; Bloomer et al., 2021). Additionally, *Distocambarus youngineri* is ranked as critically imperiled globally and subnationally (G1/S1), and at the highest level for South Carolina species of greatest conservation needs (SGCN). *Distocambarus crockeri* is ranked as vulnerable globally and subnationally (G3/S3), and at a high level for South Carolina SGCN. Despite their conservation status, their nutritional and energetic ecology are poorly understood. Understanding the trophic position of imperiled species is essential for effective conservation. These species may occupy unique roles in food webs, and changes in their trophic interactions could signal ecosystem instability or other threats. By identifying their trophic position, managers can predict cascading effects of their decline and

design strategies that maintain ecological balance and resilience. Furthermore, there is an urgent need to improve management of these species and gain a better understanding of their ecological functions to guide management actions and preserve or enhance semiterrestrial ecosystems.

Materials and methods

Study area/site selection

To select habitat types and sites, we performed surveys of *Distocambarus* species throughout areas of the southern outer piedmont and slate belt ecoregions of South Carolina in February–March 2020 and 2022. Sites were sampled in the late winter/early spring due to the water table being highest during this time of year (Welch & Eversole, 2006), increasing the chances of collecting primary burrowing crayfish without causing great destruction to their habitats. Our surveys identified *D. youngineri* and *D. crockeri* within numerous habitat types including fields, vernal pools, roadside habitats, and forests. For both species, several field and roadside populations were found. Thus, sites were selected for isotopic analysis in both field and roadside habitats. Field sites included areas maintained for powerline rights-of-ways, managed as wildlife openings (i.e., agriculture-like lands that are plowed and managed to grow crops and/or to attract wildlife for hunting), or farmlands. Roadside habitats refer to habitats that appear to have been created by the construction of roadways (e.g., roadside ditches).

Eight *D. youngineri* sites (4 field and 4 roadside habitats) were selected within Newberry County, SC based on accessibility and abundance of target species (Fig. 1, Online Resource 1). Two field sites were farmlands actively managed for agriculture (*D. youngineri* Field 2 and 3), and two field sites were managed powerline right-of-way. Surface water was not observed at the roadside habitats, all of which were actively maintained by mowing. Ten *D. crockeri* sites (5 field and 5 roadside habitats) were selected within Edgefield, McCormick, Greenwood, and Saluda counties, SC based on accessibility and abundance of the target species (Fig. 1, Online Resource 1). All field sites were managed as wildlife openings and all roadside habitats were managed as roadside

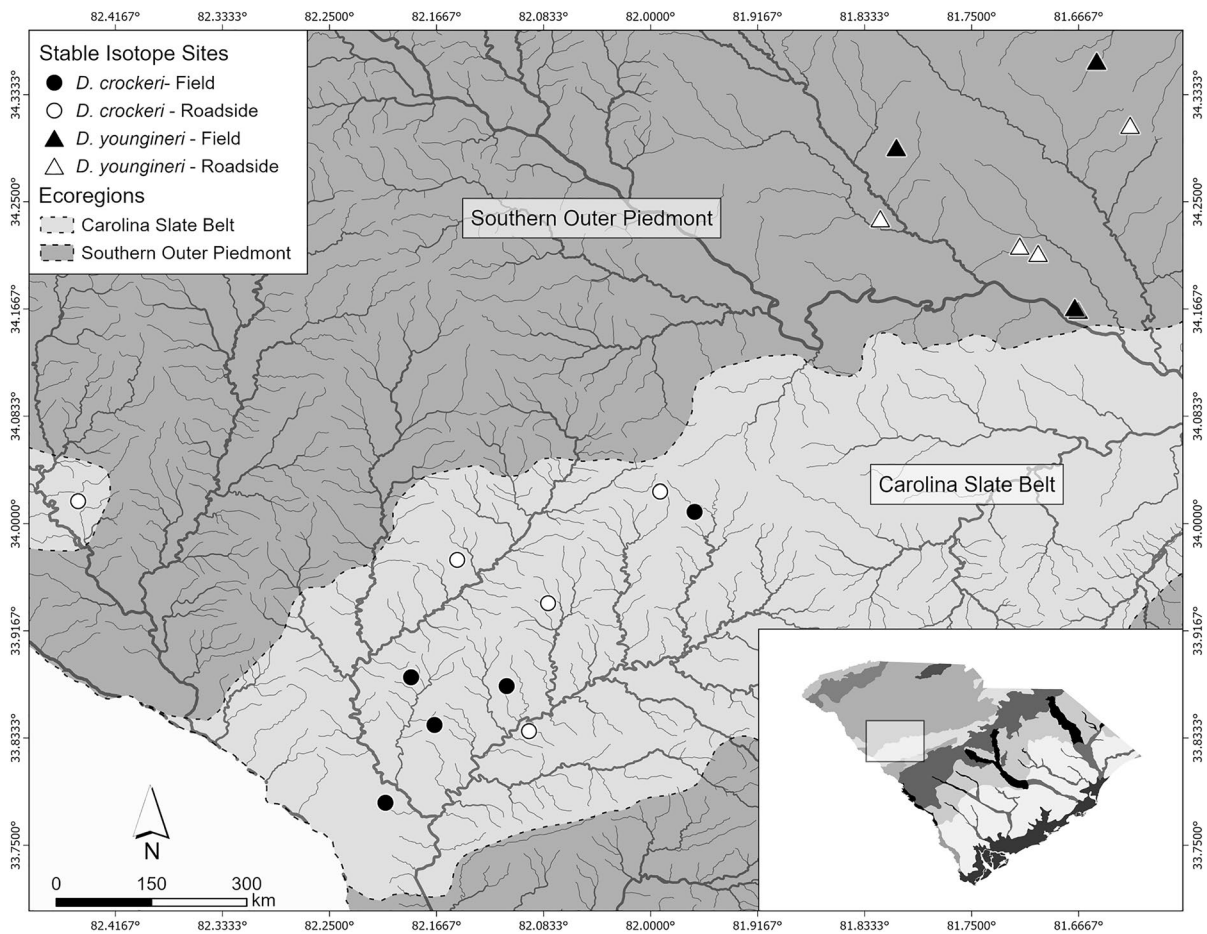


Fig. 1 Map of *Distocambarus youngineri* and *D. crockeri* field and roadside habitat sampling sites

ditches, retaining water during the winter and spring months and during heavy rains events.

Stable isotope sampling

Distocambarus crockeri and *D. youngineri* sites were sampled for stable isotope collections March 2021 and March 2022, respectively. Mean temperature and rainfall data were checked from the National Oceanic and Atmospheric Administration, National Weather Service (<https://www.weather.gov/wrh/Climate>), and were similar in both sampling years, indicating that data from the two years should be comparable with regard to interannual variations in weather. Crayfish collection occurred via burrow excavation at all *D. youngineri* sites and all *D. crockeri* field sites. Crayfish were collected with dipnets at *D. crockeri*

roadside habitat sites because all *D. crockeri* roadside sites were ditches filled with water. Target species were the only or most abundant crayfish species collected at each site. At each site, 6–12 adult (total carapace length [CL] ≥ 15 mm) crayfish of the target species were captured, with a minimum of 3 individuals of each sex (Online Resource 1, 2). Carapace length was measured for most collected *D. youngineri* and *D. crockeri*. Samples of potential food sources such as the dominant live vegetation (grasses, *Plantago major* L., *Solidago* spp., *Taraxacum officinale* F.H. Wigg), detrital leaves (*Quercus* spp., *Carya* spp., *Nyssa* spp., etc.), and terrestrial invertebrates (Carabidae, Hymenoptera, Isopoda, Lycosidae, Oligochaeta, etc.) were also collected from the same sites. Samples of all potential food sources were made by hand at all *D. youngineri* sites and all *D. crockeri* field sites. At

D. crockeri roadside habitat sites, vegetation was collected by hand and all other food sources were collected with dipnets. We classified all invertebrate into primary and secondary consumer groups. All herbivore invertebrates were classified as primary consumers, and all carnivore or omnivore invertebrates were classified as secondary consumers. All crayfish and food sources were kept on ice in the field and frozen for later analysis.

Laboratory analysis

Samples were prepared for stable isotope analysis at the US Forest Service, Southern Research Station, Center for Bottomland Hardwoods Research, Aquatic Conservation and Ecology Team lab (Oxford, MS). At least 10 mg of each sample was dried at 60 °C for at least 48 h. Abdomen tissue samples from tail muscle were used from individual crayfish. Vegetation and detritus were rinsed to remove debris before drying, while guts of large invertebrates (e.g., crickets and grasshoppers) were removed to avoid gut contamination. Whole bodies of small invertebrates (e.g., isopods) were used in analyses. Numerous invertebrates were grouped in one sample to meet the 10 mg required weight. After drying, samples were shipped to Washington State University Stable Isotope Core Laboratory (Pullman, WA) for stable isotope measurements of carbon and nitrogen isotopic ratios. Samples were ground to homogenous consistency, and approximately 2 and 3 mg of animal and plant tissue, respectively, were used in analyses.

For carbon 13:12 and nitrogen 15:14 isotopic analyses, samples were converted to N₂ and CO₂ with an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA). These gases were separated with a 3 m gas chromatography column and analyzed with a continuous flow isotope ratio mass spectrometer (IRMS) (Delta PlusXP, Thermofinnigan, Bremen) (Brenna et al., 1997; Qi et al., 2003). Isotopic reference materials were interspersed with samples for calibration. Contribution of ¹⁷O was corrected by the IRMS software using the Santrock correction (Santrock et al., 1985). Quality control was performed using reference materials with similar composition to samples and interspersed with samples in all analytical sequences. Reference materials of NIST1547, a peach leaf, and NIST1577b, a bovine liver, were used to assure both repeatability and reproducibility of isotope results

and element compositions. Analytical precision for samples was less than 0.3 parts per mil.

Data analysis

Isotope signatures were used to calculate crayfish trophic positions (TP) using the equation:

$$TP_{\text{crayfish}} = TP_{\text{base}} + (\delta^{15}N_{\text{crayfish}} - \delta^{15}N_{\text{base}}) / \Delta N$$

where base refers to invertebrate primary consumers (Post, 2002), $TP_{\text{base}} = 2$, and $\delta^{15}N_{\text{base}}$ refers to the average $\delta^{15}N$ of primary consumers at the site. The ΔN was set at 3.40 based on an established average fractionation rate (Vander Zanden & Rasmussen, 2001; Post, 2002). Taxa groups were combined into categories: detritus, live vegetation, invertebrate primary consumers (e.g., Hymenoptera, Isopoda, Euryuridae, Lumbricidae, Gastropoda, and Oligochaeta), and invertebrate secondary consumers (e.g., Carabidae, Lycosidae, Geophilidae, and Pisauridae), as well as male and female crayfishes shown separately.

To assess if *D. youngineri* and *D. crockeri* within field and roadside habitats share a similar trophic niche, we assessed the percentage of stable isotope niche overlap among groups. We calculated standard ellipse areas corrected for small sample sizes (SEAc) as a measure of the stable isotope niche size for each species within each habitat type using the Stable Isotope Bayesian Ellipses (SIBER) R package (Jackson et al., 2011). Standard ellipses contain ~40% of the individuals in the stable isotope space (Jackson et al., 2011). The degree of stable isotope niche overlap (percentage of standard ellipse overlapped) between field and roadside habitats for *D. youngineri* and *D. crockeri*, separately, was calculated as the proportion of the non-overlapping area between the two standard ellipses.

To assess if crayfish resource use differed between habitat types or sex, we used linear mixed-effect (lme) models for *D. youngineri* and *D. crockeri* separately. In each model, trophic position or $\delta^{13}C$ was the response variable, habitat and sex were fixed effects, and site was the random effect. In addition, we used lme models to assess if there were shifts in crayfish resource use with size. In each model, trophic position or $\delta^{13}C$ was response variables, crayfish size was the predictor variable, and site was the repeated measure. Because we did not measure the CL of all

crayfish collected, models assessed the subset of the specimens measured. Crayfish trophic positions and sizes were log-transformed so that data were normally distributed. All tests were conducted using the *lmer* package in R (Kuznetsova et al., 2015). Tukey's post hoc analysis was used to assess which habitat type or sex differed for any significant ($P < 0.05$) results.

To determine relative contribution of resources (detritus, vegetation, and other invertebrate) to crayfish diets, we used Bayesian mixing models in R package *simmr* (Parnell & Inger, 2016) for *D. youngineri* and *D. crockeri* separately. Models were run with unconverted $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of individual crayfish in mixing models. We used corrected values of 2.4‰ for $\delta^{15}\text{N}$ and 0.40‰ for $\delta^{13}\text{C}$ for vegetation (McCutchan Jr. et al., 2003) and 3.23‰ for $\delta^{15}\text{N}$ and 0.47‰ for $\delta^{13}\text{C}$ for invertebrates (Vander Zanden & Rasmussen, 2001). Values of food sources must be corrected before being used in mixing models to account for differences in isotope ratios between a consumer and its food source due to metabolic processes (Ben-David et al., 1997; Phillips 2012). Using corrected values ensures more accurate interpretation of the data.

Results

In total, 92 and 75 *D. youngineri* and *D. crockeri* were collected, respectively (Table 1). For *D. youngineri*, 40 males and 52 females were collected, with 47 and 45 specimens collected from fields and roadside habitats, respectively. For *D. crockeri*, 37 males and 38 females were collected, with 34 and 41 specimens collected from fields and roadside habitats, respectively. We collected detritus, live vegetations, primary consumers, and secondary consumers from each of our study sites (Online Resource 2 and 4). Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for *Distocambarus* spp. varied among sites (Figs. 2 and 3; Table 1; Online Resource 3 and 5), indicative of consumption of a range of carbon and nitrogen sources.

Trophic positions

Distocambarus youngineri had a broad trophic position (Table 1; Fig. 2), ranging from 1.89 to 4.25. *Distocambarus youngineri* trophic niche was similar within fields and roadside habitats for both the carbon

and nitrogen stable isotope axes (Fig. 4). There was little variation in niche size between habitat types (SEA_c : field=24.47; roadside habitat=27.82), with 38% overlap observed between *D. youngineri* trophic niche in each habitat (Fig. 4). There was no variation in *D. youngineri* trophic position between sex (Fig. 2; lme model: $F_{1,89}=0.06$, $P=0.80$), habitat type (lme model: $F_{1,89}=0.67$, $P=0.44$), or size ($F_{1,90}=1.29$, $P=0.26$). Additionally, there was no variation in *D. youngineri* $\delta^{13}\text{C}$ levels between sex (Fig. 2; lme model: $F_{1,89}=0.18$, $P=0.68$), habitat type ($F_{1,89}=0.24$, $P=0.64$), or size (lme model: $F_{1,90}=0.00$, $P=0.99$). We observed similar structure among sites, with male and female *D. youngineri* $\delta^{15}\text{N}$ levels similar to secondary consumers in both field and roadside habitat sites (Fig. 2; Online Resource 2, 3).

Distocambarus crockeri had a broad trophic position (Table 1; Fig. 3), ranging from 1.47 to 4.80. *Distocambarus crockeri* trophic niche differed between field and roadside habitats along both the carbon and nitrogen stable isotopes axis (Fig. 4), with *D. crockeri* in fields having more enriched carbon and nitrogen levels. *Distocambarus crockeri* trophic niche size was also larger in fields than roadside habitats (SEA_c : field=17.36; roadside habitat=6.85). Similarly, *D. crockeri* trophic position and $\delta^{13}\text{C}$ levels in fields were higher than those from roadside habitats (lme model of TP: $F_{1,69}=9.36$, $P=0.02$; lme model of $\delta^{13}\text{C}$: $F_{1,69}=27.50$, $P<0.001$) (Fig. 4). *Distocambarus crockeri* within fields had $\delta^{15}\text{N}$ levels above our sampled secondary consumers in 40% of field sites, indicating that they can serve as tertiary consumers in field habitats (Fig. 3; Online Resource 4, 5). *Distocambarus crockeri* within roadside habitats had $\delta^{15}\text{N}$ levels with similar values as secondary consumers (Fig. 3; Online Resource 4, 5). There was no variation between male and female *D. crockeri* trophic positions (lme model: $F_{1,69}=0.09$, $P=0.76$) or $\delta^{13}\text{C}$ levels (lme model: $F_{1,69}=0.99$, $P=0.32$) (Fig. 3; Online Resource 4, 5). There was also no relationship between *D. crockeri* size and trophic position (lme model: $F_{1,69}=1.19$, $P=0.28$).

Food resources

Our Bayesian mixing models suggested that invertebrates contributed up to 82% of the tissue composition of *D. youngineri*, ranging from 77 to 82% among

Table 1 *Distocambarus* species mean ($\pm$ SD) trophic position (TP), carbon and nitrogen stable isotope values, and carapace length (CL), as well as number of sampled individuals between sexes per site

	Males					Females				
	N	TP	$\delta^{15}\text{C}$	$\delta^{15}\text{N}$	CL	N	TP	$\delta^{15}\text{C}$	$\delta^{15}\text{N}$	CL
<i>D. youngineri</i>										
Field 1	5	2.98 (0.10)	-26.45 (0.33)	6.00 (0.33)	20.7 (3.49)	7	2.80 (0.11)	-26.23 (1.14)	5.41 (0.36)	27.2 (3.28)
Field 2	5	3.24 (0.08)	-26.08 (0.58)	10.57 (0.29)	24.6 (2.45)	6	3.23 (0.13)	-26.01 (0.41)	10.52 (0.44)	26.6 (2.26)
Field 3	4	3.47 (0.35)	-18.52 (2.80)	7.65 (1.19)	26.4 (3.79)	8	3.46 (0.42)	-20.45 (3.48)	7.60 (1.42)	29.3 (4.00)
Field 4	6	3.00 (0.16)	-27.06 (1.24)	5.53 (0.55)	24.6 (3.40)	6	3.14 (0.32)	-24.80 (4.66)	6.01 (1.09)	31.9 (2.31)
Total	20					27				
Roadside 1	5	3.59 (0.49)	-25.02 (0.71)	9.26 (1.68)	24.8 (4.16)	7	3.83 (0.21)	-23.34 (1.85)	10.09 (0.72)	25.4 (2.41)
Roadside 2	6	2.38 (0.15)	-25.62 (1.52)	3.85 (0.51)	22.9 (4.24)	6	2.19 (0.29)	-25.89 (2.41)	3.18 (0.97)	25.3 (4.47)
Roadside 3	4	2.65 (0.17)	-17.72 (1.14)	4.70 (0.57)	26.1 (0.06)	5	2.89 (0.15)	-18.24 (1.27)	5.50 (0.50)	24.3 (7.34)
Roadside 4	5	2.80 (0.24)	-25.37 (0.88)	4.23 (0.80)	21.0 (3.61)	7	2.81 (0.22)	-25.97 (0.65)	4.26 (0.76)	27.8 (2.96)
Total	20					25				
<i>D. crockeri</i>										
Field 1	3	3.69 (0.11)	-16.33 (1.34)	7.20 (0.39)		3	3.62 (0.08)	-16.46 (0.99)	6.95 (0.28)	
Field 2	4	3.98 (0.31)	-16.04 (0.42)	7.79 (1.04)		3	4.17 (0.59)	-17.74 (1.00)	8.43 (2.01)	
Field 3	3	2.26 (0.19)	-21.92 (0.53)	3.97 (0.63)		3	2.42 (0.16)	-22.57 (0.59)	4.52 (0.56)	
Field 4	3	3.34 (0.14)	-19.69 (3.56)	5.92 (0.49)		3	3.26 (0.21)	-20.81 (3.61)	5.67 (0.73)	
Field 5	4	3.19 (0.18)	-14.30 (0.57)	8.32 (0.61)		5	3.20 (0.06)	-14.81 (1.44)	8.33 (0.20)	
Total	17					17				
Roadside 1	4	2.14 (0.17)	-24.58 (0.11)	3.77 (0.57)	20.0 (1.98)	5	1.96 (0.16)	-24.79 (0.44)	3.17 (0.54)	19.8 (4.07)
Roadside 2	4	2.11 (0.46)	-24.92 (2.45)	2.79 (1.58)	23.1 (3.89)	4	2.01 (0.52)	-24.67 (2.93)	2.46 (1.75)	17.3 (0.64)
Roadside 3	3	2.60 (0.03)	-20.62 (0.23)	3.41 (0.09)	22.3 (1.92)	4	2.69 (0.11)	-21.39 (1.34)	3.70 (0.39)	28.5(0.80)
Roadside 4	4	2.22 (0.09)	-26.72 (0.29)	1.70 (0.32)	22.5 (3.46)	3	2.28 (0.12)	-26.68 (0.33)	1.91 (0.40)	19.4 (3.18)
Roadside 5	5	2.15 (0.31)	-24.95 (1.39)	2.85 (1.07)	23.3(3.32)	5	2.11 (0.22)	-24.61 (0.95)	2.72 (0.74)	19.8(1.18)
Total	20					21				

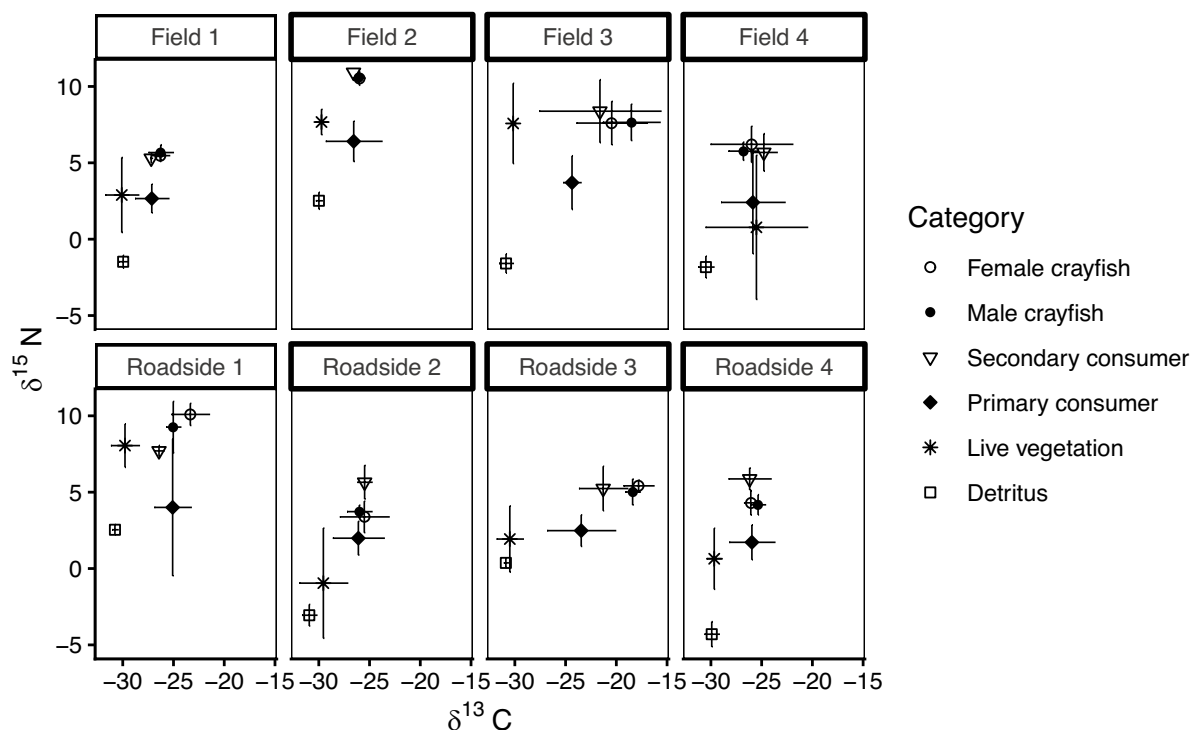


Fig. 2 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic signatures for *Distocambarus youngineri* male and females captured in all field and roadside sites. Error bars show the standard deviation of isotope signatures of all individuals and vegetative samples

males and females in fields and roadside habitats (Table 2). Results indicated that *D. youngineri* relied heavily on primary (e.g., Euryuridae, Lumbricidae, Gastropoda) and secondary consumers (e.g., Carabidae, Geophilidae, Pisauridae), attributing up to 43% and 38% of their tissue composition, respectively. Mixing models also indicated that detritus and vegetation contributed up to 13 and 17% of crayfish tissue, respectively.

Our Bayesian mixing models suggested that invertebrates contributed up to 84% and 59% of *D. crockeri* tissue composition within fields and roadside habitats, respectively (Table 2). Results indicated that *D. crockeri* within field habitats relied heavily on secondary and primary consumers, attributing up to 50% and 34% of their tissue composition, respectively. Conversely, *D. crockeri* within roadside habitats relied primarily on primary consumers (up to 42% of tissue composition), with all other groups (e.g., secondary consumers, vegetation, detritus) contributing evenly (~20%) to its tissue composition. Mixing models also indicated that detritus and vegetation are

a more important part of the tissue composition of *D. crockeri* in roadside habitats (up to 42%) than those in fields (up to 21%).

Discussion

Understanding the trophic position of imperiled species is essential for effective conservation and management to maintain ecological balance and resilience within ecosystems. Thus, our goal was to understand the trophic and nutritional ecology of two imperiled crayfishes, *D. youngineri* and *D. crockeri*, within two habitat types. Results of our stable isotope analyses show a high degree of flexibility in nutritional resources, energy pathways, and trophic position for two burrowing crayfishes. As one of the first isotopic analyses performed on primary burrowing crayfishes, our results indicate that burrowing crayfish may operate at higher trophic levels than expected, with previous studies indicating that while crayfish are omnivores they prefer

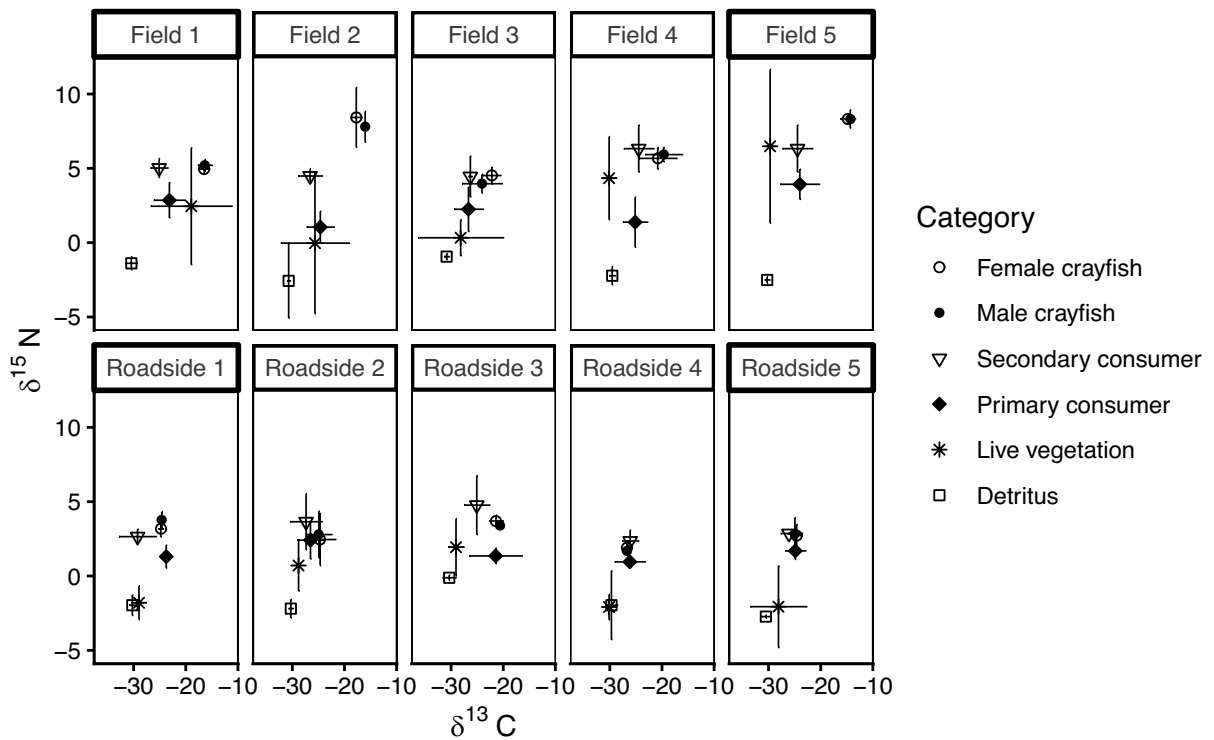


Fig. 3 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic signatures for *Distocambarus crockeri* male and females captured in all field and roadside sites. Error bars show the standard deviation of isotope signatures of all individuals and vegetative samples

Fig. 4 Stable isotope values of *Distocambarus youngineri* and *D. crockeri* with associated isotopic niche (correct standard ellipses (SEAc)) for field and roadside habitat populations

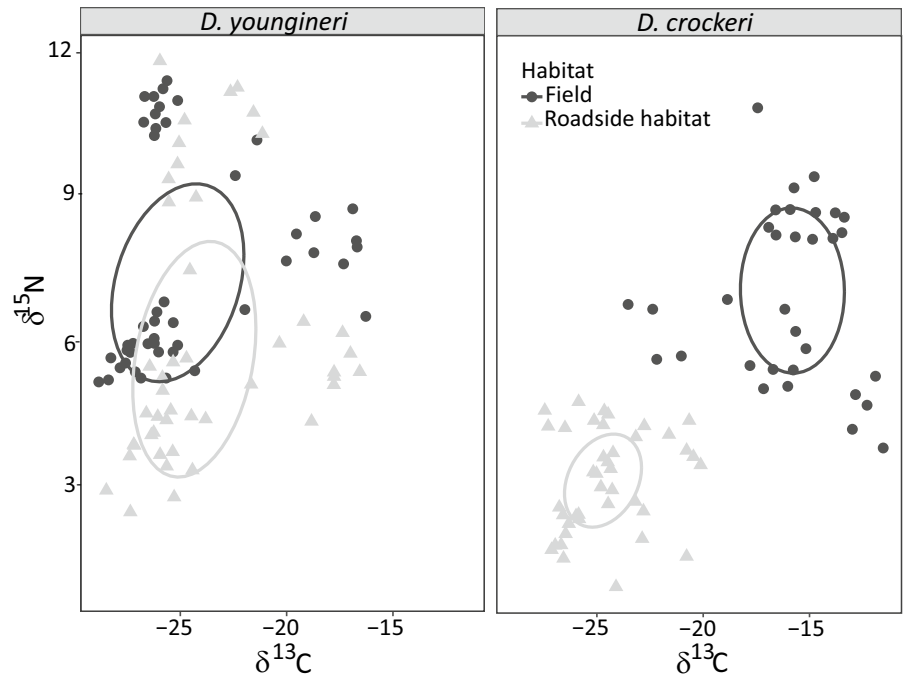


Table 2 *Distocambarus* species proportional contribution of food sources ($\pm$ SD) for males and females at field and roadside habitats

	Field		Roadside	
	Females	Males	females	Males
<i>D. youngineri</i>				
Secondary consumer	36.9 (14.5)	37.0 (15.4)	37.7 (20.5)	31.6 (17.8)
Primary consumer	39.8 (19.5)	35.0 (20.1)	43.8 (24.0)	43.4 (22.1)
Vegetation	13.6 (9.0)	16.5 (11.0)	9.0 (6.6)	12.0 (8.2)
Detritus	9.7 (6.0)	11.5 (6.9)	9.5 (6.2)	13.1 (7.7)
<i>D. crockeri</i>				
Secondary consumer	50.0 (14.7)	47.4 (17.7)	18.5 (7.6)	17.9 (7.9)
Primary consumer	34.1 (17.3)	31.4 (19.6)	40.3 (13.7)	41.7 (13.1)
Vegetation	9.9 (6.8)	12.9 (8.9)	19.8 (10.5)	20.3 (11.3)
Detritus	5.9 (3.7)	8.3 (5.2)	21.5 (10.9)	20.1 (10.6)

eating vegetation and serve similar roles are primary consumers or levels between primary and secondary consumers (Gherardi et al., 2004; Johnston et al., 2011). While *D. youngineri* and *D. crockeri* have omnivorous diets, invertebrates comprise a large portion of their energetic and nutritional needs and both species appear to serve as secondary or tertiary consumers within their ecosystems. Omnivory is advantageous because it provides flexibility to feed at both lower and higher trophic levels, allowing individuals to survive periods when high-quality food is not available, but consuming higher-quality food when it is available (Diehl, 2003; Kratina et al., 2010; France, 2012; Wootton, 2017). In the current study, up to 84% of *Distocambarus* spp. tissue originated from invertebrate prey and up to 40% of *Distocambarus* spp. diets included vegetation and detritus. Burrowing crayfish are known to exhibit predatory behaviors (Loughman, 2010; Graham et al., 2022) and, combined with our results, suggest that *D. youngineri* and *D. crockeri* actively target primary and secondary consumers when foraging for food. Variability in feeding activity likely has implications for crayfish roles in controlling invertebrate populations (Ushio & Townsend, 2004, 2008) and detrital processing (Momot, 1995; Usio, 2000). Historically, crayfishes were thought to have an opportunistic omnivore lifestyle, sustaining themselves on plant material and, if the opportunity arises, consuming animals (Momot, 1995; Bloomer et al., 2022). Our results highlight that energy and nutrition for burrowing crayfish are derived mainly from invertebrate food sources, although plant

matter and detritus are still an important component of their diets.

In the current study, differences were observed between habitats for both isotopes, with higher isotopic values and larger trophic niche breadth detected for *D. crockeri* in fields (wildlife openings) than roadside habitats (roadside ditches). Differences in *D. crockeri* $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ levels are likely due to a wider variety of plants (i.e., C_3 and C_4 plants) and invertebrates available in fields. The average $\delta^{13}\text{C}$ levels of *D. crockeri* in fields (mean $\delta^{13}\text{C}$ levels = -16‰) were higher than most C_3 vegetation $\delta^{13}\text{C}$ levels (mean $\delta^{13}\text{C}$ levels = -27‰). C_4 plants (i.e., millet, and corn) generally have higher $\delta^{13}\text{C}$ values (i.e., -13‰ ; Fry, 2006) and were planted in field sites during spring and summer months. Due to our sampling timing, these vegetation sources were not present and thus not collected and used in data analyses. Nonetheless, high $\delta^{13}\text{C}$ levels indicate C_4 vegetation may be an essential diet attribute of *D. crockeri* in fields. Similarly, differences in $\delta^{15}\text{N}$ levels are likely due to differences in invertebrate diversity present within each habitat type, with at least 7 and 4 invertebrate families present within field and roadside habitats, respectively (Online Resource 3). While invertebrate abundance was not formally accessed, there appeared to be more secondary consumers present at field than roadside sites, which may impact crayfish feeding strategies.

Trophic position and niche differences were detected between field and roadside habitats for *D. crockeri*, but not for *D. youngineri*. Differences in trophic position and niche of the same species may

result from differences in types of food consumed, food chain length, or environmental and ecological conditions (Parkyn et al. 2001; Beatty, 2006; Olsson et al., 2008; Sánchez-Hernández & Amundsen, 2018). Field and roadside ditch management can directly impact vegetation and invertebrate composition, changing the ecosystems' trophic structure (Wilson et al., 2005; Dollinger et al., 2017; Jakobsson et al., 2018). For example, herbicide use can reduce the diversity and abundance of plants and invertebrates within habitats (Haughton et al., 1999; Sullivan & Sullivan, 2003; Wilson et al., 2005), while planting crops may increase food type availability if both naturally occurring and planted vegetation are present within the system. Food availability also shifts throughout the year. Food may be limited in habitats where food is clumped or patchily distributed (Fero et al., 2007), as well as in managed fields where herbicide use and agricultural practices may remove large portions of vegetation and invertebrates during periods of the year (House, 1989; Brust, 1990; Haughton et al., 1999; Sullivan & Sullivan, 2003). Unlike *D. crockeri* roadside habitats, which were often ditches inundated with water during portions of the year, *D. youngineri* roadside habitats were more like fields (i.e., grassy open areas without a trench). Thus, differences between species may be due to environmental and community composition differences among sampling sites, and not species-specific traits. Because species ranges do not overlap, we could not assess sites with both species to understand if differences are due to species-specific traits or site composition.

Competition can also cause shifts in diets and trophic positions, with a reduction in niche size often occurring (Harrington et al., 2009; Jackson & Britton 2014; Baudry et al., 2024). *Cambarus latimanus* (LeConte, 1856) co-occurred with *D. crockeri* at all roadside sites, but *D. crockeri* was the only crayfish collected from all field sites. Additionally, *D. crockeri* had a smaller trophic niche in roadside than field sites. Conversely, *D. youngineri* co-occurred with *C. latimanus* at roadside sites, yet, trophic position and stable isotope analysis support that *D. youngineri* could target invertebrates as its main food sources and operate as secondary consumers in both habitat types. Because we did not collect large numbers of *C. latimanus* from each site, we did not include them in our analyses. Nonetheless, the shift in *D. crockeri*'s

diet from field to roadside sites may be due to niche partitioning (Stites et al., 2017). Similarly, Stites et al. (2017) showed that *Barbicambarus cornutus* (Faxon, 1884) and *B. simmonsii* Taylor & Schuster, 2010 occupied a higher trophic level than co-occurring crayfish species in the same habitat. *Barbicambarus* spp. were secondary consumers or predators, while co-occurring crayfish species were primary consumers. Furthermore, competition for food sources could be occurring at sites where *D. crockeri* and *C. latimanus* are both present, with *D. crockeri* shifting to a lower trophic level and *C. latimanus* potentially occupying the higher tertiary consumer level. Further analyses of these sites are required to determine the effect of *C. latimanus* on *D. crockeri* diet and trophic position.

Even though secondary consumers were present in both field and roadside habitats, *D. crockeri* populations in roadside habitats relied more on detrital and vegetation sources than secondary consumers. This could be due to crayfish foraging style (Bloomer & Taylor, 2022; Diehl et al., 2022; Graham et al., 2022). *Distocambarus crockeri* within fields may exhibit predatory behavior, similar to the Little Brown Mudbug (*Lacunicambarus thomai* Jezerinac, 1993; Graham et al., 2022), while those in roadside habitats may exhibit an opportunistic foraging behavior, similar to the Slenderwrist Burrowing Crayfish (*Fallicambarus petilicarpus* Hobbs & Robison, 1989; Bloomer et al., 2022). Foraging behavioral differences may be impacted by the presence of predators (Pecor & Hazlett, 2003; Diehl et al., 2022; Graham et al., 2022). Crayfish in fields without surface water may have higher predation risks, exhibiting more anti-predator behavior and less foraging, eating mostly plants and animals near their burrow, while crayfish in roadside habitats with surface water may have less predation risks and thus, spend more time foraging. These factors may also explain why field crayfishes had a larger trophic niche.

Although differences were not detected between *D. youngineri* trophic positions or niches in field and roadside habitats, management of sites did impact them. *Distocambarus youngineri* in farmed fields (Field 2 and 3) had higher nitrogen levels than non-farmed fields, likely due to the fertilization of these fields. Both isotopic niche and dietary contributions of *Procambarus clarkii* (Girard, 1852) were affected by the introduction of fertilizers in rice-crayfish coculture systems (Sun et al., 2023). Because

vegetation was nitrogen-enriched due to fertilization, crayfish isotopic signatures showed high levels of nitrogen similar to levels that would be present if high amounts of invertebrates were eaten. Nonetheless, dietary contribution analysis indicated that crayfish in fertilized fields did not consume more animal material, but rather they consumed more particulate organic matter (POM). Furthermore, increased $\delta^{15}\text{N}$ values in *D. youngineri* habitats may explain why analyses showed similar $\delta^{15}\text{N}$ values between all *D. youngineri* and field *D. crockeri* but consumptions of more vegetative food sources by *D. youngineri*.

Contrasting results of the relationship between body size and trophic position have been documented for crayfishes, with most studies assessing differences between juveniles and adults (Roth et al., 2006; Taylor & Soucek, 2010; Larson et al., 2017; Stites et al., 2017), and no studies assessing burrowing species. We did not see any correlations between size (range, *D. crockeri* = 16–29 mm CL; *D. youngineri* 16–35 mm CL) and trophic position. Similar to Stenroth et al. (2006), larger crayfishes were just as reliant on animal tissue as smaller crayfishes, with crayfish potentially obtaining their carbon and nitrogen from different sources (i.e., consuming other invertebrate for nitrogen and detritus for carbon). We considered all *Distocambarus* species used in our study adults (CL $\geq$ 16 mm), based on the smallest form 1 *Distocambarus* species (*D. carlsoni* Hobbs, 1983) collected having a 16 mm CL (unpublished data). Thus, we were not able to assess if diets changed between juvenile and adult *Distocambarus* spp. Including smaller individuals in future isotopic analyses could provide a better insight into diet changes, as juveniles were more commonly found occupying surface water of both roadside ditches and fields.

While isotopic analyses can integrate food sources over several months to years, dietary contribution and trophic positions are dependent on samples from our single collection. Thus, our dietary contribution and trophic positions analyses are limited to only food available during our March collections. For example, our isotopic analyses identify that C_4 plants were a key vegetation source for crayfish diets, but they were not represented at most sites. In addition, other key resources (i.e., primary and secondary consumers) that were present outside of our sampling window may not be represented in our dataset. Nonetheless, *D. youngineri* and *D. crockeri* are often in their

burrows (average depth of *D. crockeri* burrows 1.5 m; Welch & Eversole, 2006) during summer and fall months when rainfall is reduced and the water table is low (Eversole & Welch, 2010, 2013). Thus, our sampling likely represents most of the resources these species would encounter when foraging. Additionally, yearly differences were not observed for crayfishes or food sources available within sites, and precipitation and temperatures were similar between years, reducing the likelihood of interannual differences between species collected in 2021 and 2022. Trophic position can also be impacted by the baseline $\delta^{15}\text{N}$ values used in trophic analyses. Because we did not collect primary consumers from the same family at every sampling site, $\delta^{15}\text{N}$ values of all primary consumers collected from a site were averaged to assess trophic positions. Primary consumers collected were grazers that may present a certain degree of omnivory, and thus, may have a TP higher than 2 (Vander Zanden & Fetzer 2007). This discrepancy could lead to higher than normal TP values for our target species. Nonetheless, Letourneur et al. (2024) points out that a weakness of TP estimates is the use of a single baseline value, while we still used a single baseline value averaging multiple types of primary consumers allows us to incorporate different sources of nitrogen within a system. Additionally, average C:N biplots show that primary consumer $\delta^{15}\text{N}$ values fell between detritus and secondary consumers at all sites.

Despite the gain in understanding of the trophic interactions of burrowing crayfish, comprehensive food resource studies of burrowing crayfishes remain limited. Results of our analysis indicate there may be considerable variation in trophic position, niche space, and dietary contribution among primary burrowing crayfish species. Further, our analysis indicate the ecological distinctness of primary burrowing crayfishes, occupying similar habitats but serving different roles within their ecosystems. Studies have shown that primary burrowing crayfish often act as omnivores feeding on invertebrates, vegetation, and detritus, but the extent of species' reliance on these food sources and how habitat, competition, or predators impact food selection is poorly understood. Our findings highlight the important role primary burrowing crayfishes play in these ecosystems, by regulating herbivore numbers, which helps ensure ecosystem stability, as well as facilitates energy flow to higher trophic levels. Further, understanding a species' food

source and factors impacting food sources will allow conservationists to identify threats to the species' survival, protect and restore necessary habitats and food sources, and ensure ecosystem stability.

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Authors' contributions ZCB formulated the idea. ZCB, MRK, and ZJL designed the study. ZCB and HDW conducted the fieldwork. ZCB conducted the laboratory analyses. ZCB and HDW performed statistical analyses. ZCB and HDW wrote the manuscript. All authors provided editorial advice.

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Declarations

Conflict of interest There is no conflict of interest declared in this article.

References

- Anderson, C. & G. Cabana, 2007. Estimating the trophic position of aquatic consumers in river food webs using stable nitrogen isotopes. *The North American Benthological Society* 26: 273–285. [https://doi.org/10.1899/0887-3593\(2007\)26\[273:ETTPOA\]2.0.CO;2](https://doi.org/10.1899/0887-3593(2007)26[273:ETTPOA]2.0.CO;2).
- Baudry, T., J. Smith-Ravin, A. Arqu  , J. P. Go  t, J. Cucherousset, J. M. Paillisson & F. Grandjean, 2024. Trophic niche of the invasive *Cherax quadricarinatus* and extent of competition with native shrimps in insular freshwater food webs. *Biological Invasions* 26: 3227–3241. <https://doi.org/10.1007/s10530-024-03373-8>.
- Beatty, S. J., 2006. The diet and trophic positions of translocated, sympatric populations of *Cherax destructor* and *Cherax cainii* in the Hutt River, Western Australia: evidence of resource overlap. *Marine and Freshwater Research* 57: 825–835. <https://doi.org/10.1071/MF05221>.
- Ben-David, M., T. A. Hanley, D. R. Klein & D. M. Schell, 1997. Seasonal changes in diets of coastal and riverine mink: the role of spawning Pacific salmon. *Canadian Journal of Zoology* 75: 803–811. <https://doi.org/10.1139/z97-102>.
- Bloomer, C. C. & C. A. Taylor, 2022. Habitat suitability modelling of primary burrowing crayfishes, with a new state record for *Procambarus liberorum* Fitzpatrick, 1978 (Decapoda: Astacidea: Cambaridae). *Journal of Crustacean Biology* 42: ruac008. <https://doi.org/10.1093/jcbiol/ruac008>.
- Bloomer, C. C., C. A. Taylor & B. K. Wagner, 2022. Resource use by the slenderwrist burrowing crayfish, *Fallicambarus petilicarpus*. *Freshwater Crayfish* 27: 1–8. <https://doi.org/10.5869/fc.2022.v27-1.1>.
- Bloomer, C. C., R. J. DiStefano & C. A. Taylor, 2021. A global review of life history studies on burrowing crayfish. *Crustaceana* 94: 357–379. <https://doi.org/10.1163/15685403-bja10098>.
- Brenna, J. T., T. N. Corso, H. J. Tobias & R. J. Caimi, 1997. High-precision continuous-flow isotope ratio mass spectrometry. *Mass Spectrometry Reviews* 16: 227–258. [https://doi.org/10.1002/\(SICI\)1098-2787\(1997\)16:53.0.CO;2-J](https://doi.org/10.1002/(SICI)1098-2787(1997)16:53.0.CO;2-J).
- Brust, G. E., 1990. Direct and indirect effects of four herbicides on the activity of carabid beetles (Coleoptera: Carabidae). *Pesticide Science* 30: 309–320. <https://doi.org/10.1002/ps.2780300308>.
- Carscallen, W. M. A., K. Vandenberg, J. M. Lawson, N. D. Martinez & T. N. Romanuk, 2012. Estimating trophic position in marine and estuarine food webs. *Ecosphere* 3: 1–20. <https://doi.org/10.1890/ES11-00224.1>.
- Creed, R. P., Jr. & J. M. Reed, 2004. Ecosystem engineering by crayfish in a headwater stream community. *Journal of the North American Benthological Society* 23: 224–236. [https://doi.org/10.1899/0887-3593\(2004\)0232.0.CO;2](https://doi.org/10.1899/0887-3593(2004)0232.0.CO;2).
- Diehl, K. M., N. M. Storer, H. D. Wells, D. A. Davis, Z. J. Loughman & Z. A. Graham, 2022. On the surface or down below: field observations reveal a high degree of surface activity in a burrowing crayfish, the Little Brown Mudbug (*Lacunicambarus thomai*). *PLOS One* 17: e0273540. <https://doi.org/10.1371/journal.pone.0273540>.
- Diehl, S., 2003. The evolution and maintenance of omnivory: dynamic constraints and the role of food quality. *Ecology* 84: 2557–2567. <https://doi.org/10.1890/02-0399>.
- Dixon, H. J., M. Power, J. B. Dempson, T. F. Sheehan & G. Chaput, 2012. Characterizing the trophic position and shift in Atlantic salmon (*Salmo salar*) from freshwater to marine life-cycle phases using stable isotopes. *ICES Journal of Marine Science* 69: 1646–1655. <https://doi.org/10.1093/icesjms/fss122>.
- Dollinger, J., F. Vinatier, M. Voltz, C. Dag  s & J. S. Bailly, 2017. Impact of maintenance operations on the seasonal evolution of ditch properties and functions. *Agricultural Water Management* 193: 191–204. <https://doi.org/10.1016/j.agwat.2017.08.013>.
- Estes, J. A., J. Terborgh, J. S. Brashares, M. E. Power, J. Berger, W. Bond, S. R. Carpenter, T. E. Essington, R. D. Holt, J. B. C. Jackson, R. J. Marquis, L. Oksanen, T. Oksanen, R. T. Paine, E. K. Pikitch, W. J. Ripple, S. A. Sandin, M. Scheffer, T. W. Schoener, J. B. Shurin, A. R.

- E. Sinclair, M. E. Soule, R. Virtanen & D. A. Wardle, 2011. Trophic downgrading of planet Earth. *Science* 333: 301–306. <https://doi.org/10.1126/science.1205106>.
- Eversole, A. G. & S. M. Welch, 2010. Conservation of imperiled crayfish—*Distocambarus (Fitzcambarus) youngineri* Hobbs and Carlson 1985 (Decapoda: Cambaridae). *Journal of Crustacean Biology* 30: 151–155. <https://doi.org/10.1651/09-3154.1>.
- Eversole, A. G. & S. M. Welch, 2013. Ecology of the primary burrowing crayfish *Distocambarus crockeri*. *Journal of Crustacean Biology* 33: 660–666. <https://doi.org/10.1163/1937240X-00002176>.
- Fagan, W. F., 1997. Omnivory as a stabilizing feature of natural communities. *The American Naturalist* 150: 554–567. <https://doi.org/10.1086/286081>.
- Fero, K., J. L. Simon, V. Jourdie & P. A. Moore, 2007. Consequences of social dominance on crayfish resource use. *Behaviour* 144: 61–82.
- Foltz, D. A., II., N. M. Sadecky, G. A. Myers, J. W. Fetzner, S. A. Welsh, G. W. Stocker, M. G. Glon & R. F. Thoma, 2018. *Cambarus loughmani*, a new species of crayfish (Decapoda: Cambaridae) endemic to the pre-glacial Teays River Valley in West Virginia, USA. *Journal of Natural History* 52: 2875–2897. <https://doi.org/10.1080/00222933.2018.1557271>.
- France, R. L., 2012. Omnivory, vertical food-web structure and dystem productivity: stable isotope analysis of freshwater planktonic food webs. *Freshwater Biology* 57: 787–794. <https://doi.org/10.1111/j.13652427.2012.02744.x>.
- Frisch, A. J., M. Ireland & R. Baker, 2014. Trophic ecology of large predatory reef fishes: energy pathways, trophic level, and implications for fisheries in a changing climate. *Marine Biology* 161: 61–73. <https://doi.org/10.1007/s00227-013-2315-4>.
- Fry, B., 2006. Stable isotope ecology. Springer, New York, 318. <https://doi.org/10.1007/0-387-33745-8>.
- Gherardi, F., P. Acquistapace & G. Santini, 2004. Food selection in freshwater omnivores: a case study of crayfish *Austropotamobius pallipes*. *Archiv fuer Hydrobiologie* 159: 357–376. <https://doi.org/10.1127/0003-9136/2004/0159-0357>.
- Ginzberg, L. R. & R. Arditi, 2012. How Species Interact: Altering the Standard View on Trophic Ecology, Oxford University Press, Oxford: <https://doi.org/10.1093/acprof:osobl/9780199913831.001.0001>.
- Graham, Z. A., K. M. Diehl, D. Davis & Z. J. Loughman, 2022. Death from below: sit-and-wait predatory behavior in a burrowing crayfish (*Lacunicambarus thomai*). *Food Webs* 31: e00225. <https://doi.org/10.1016/j.fooweb.2022.e00225>.
- Harrington, L. A., A. L. Harrington, N. Yamaguchi, M. D. Thom, P. Ferreras, T. R. Windham & D. W. Macdonald, 2009. The impact of native competitors on an alien invasive: temporal niche shifts to avoid interspecific aggression? *Ecology* 90: 1207–1216. <https://doi.org/10.1890/08-0302.1>.
- Haughton, A. J., J. R. Bell, N. D. Boatman & A. Wilcox, 1999. The effects of different rates of the herbicide glyphosate on spiders in arable field margins. *The Journal of Arachnology* 27: 249–254.
- Hobbs, H. H., Jr., 1981. The Crayfishes of Georgia, Smithsonian Institution Press, Washington:
- Hobbs, H. H., Jr. and P. H. Carlson. 1983. *Distocambarus* (Decapoda: Cambaridae) elevated to generic rank, with an account of *D. crockeri*, new species from South Carolina. *Proceedings of the Biological Society of Washington* 96:420–428
- Hobbs, H. H., Jr. and P. H. Carlson. 1985. A new member of the genus *Distocambarus* (Decapoda: Cambaridae) from the Saluda Basin, South Carolina. *Proceedings of the Biological Society of Washington* 98:81–89
- House, G. J., 1989. Soil arthropods from weed and crop roots of an agroecosystem in a wheat-soybean-corn rotation: impact of tillage and herbicides. *Agriculture, Ecosystems and Environment* 25: 233–244. [https://doi.org/10.1016/0167-8809\(89\)90054-6](https://doi.org/10.1016/0167-8809(89)90054-6).
- Jackson, A. L., R. Inger, A. C. Parnell & S. Bearhop, 2011. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* 80: 595–602. <https://doi.org/10.1111/j.1365-2656.2011.01806.x>.
- Jackson, M. C. & J. R. Britton, 2014. Divergence in the trophic niche of sympatric freshwater invaders. *Biological Invasions* 16: 1095–1103. <https://doi.org/10.1007/s10530-013-0563-3>.
- Jakobsson, S., C. Bernes, J. M. Bullock, K. Verheyen & R. Lindborg, 2018. How does roadside vegetation management affect the diversity of vascular plants and invertebrates? A Systematic Review. *Environmental Evidence* 7: 17. <https://doi.org/10.1186/s13750-018-0129-z>.
- Johnston, K., B. J. Robson & P. G. Fairweather, 2011. Trophic positions of omnivores are not always flexible: evidence from four species of freshwater crayfish. *Austral Ecology* 36: 269–279. <https://doi.org/10.1111/j.1442-9993.2010.02147.x>.
- Kratina, P., E. Hammill & B. R. Anholt, 2010. Stronger inducible defences enhance persistence of intraguild prey. *The Journal of Animal Ecology* 79: 993–999. <https://doi.org/10.1111/j.1365-2656.2010.01705.x>.
- Kreps, T. A., E. R. Larson & D. M. Lodge, 2016. Do invasive rusty crayfish (*Orconectes rusticus*) decouple littoral and pelagic energy flows in lake food webs? *Freshwater Science* 35: 103–113. <https://doi.org/10.1086/683358>.
- Kuznetsova, A., P. B. Brockhoff, & R. H. B. Brockhoff, 2015. Package ‘lmerTest’: Tests in linear mixed effects models. R package versions 2.0.
- Lancaster, J., D. C. Bradley, A. Hogan & S. Waldron, 2005. Intraguild omnivory in predatory stream insects. *Journal of Animal Ecology* 74: 619–629. <https://doi.org/10.1111/j.1365-2656.2005.00957.x>.
- Larson, E. R., J. D. Olden & N. Usio, 2010. Decoupled conservatism of Grinnellian and Eltonian niches in an invasive arthropod. *Ecosphere* 1: 1–13. <https://doi.org/10.1890/ES10-00053.1>.
- Larson, E. R., L. A. Twardochleb & J. D. Olden, 2017. Comparison of trophic function between the globally invasive crayfishes *Pacifastacus leniusculus* and *Procambarus clarkii*. *Limnology* 18: 275–286. <https://doi.org/10.1007/s10201-016-0505-8>.
- Letourner, Y., P. Fey, J. Dierking, R. Galzin & V. Parravicini, 2024. Challenging trophic position assessments in

- complex ecosystems: calculation method, choice of baseline, trophic enrichment factors, season and feeding guild do matter: a case study from Marquesas Islands coral reefs. *Ecology and Evolution* 14: e11620. <https://doi.org/10.1002/ece3.11620>.
- Lorman, J. G. & J. J. Magnuson, 1978. The role of crayfish in aquatic systems. *Fisheries* 3: 8–10.
- Loughman, Z. J. 2010. Ecology of *Cambarus dubius* (Upland Burrowing Crayfish) in North-Central West Virginia. *Southeastern Naturalist* 9: 217–230.
- Loughman, Z. J., R. F. Thoma, J. W. J. Fetzner & G. W. Stocker, 2015. *Cambarus (Jugicambarus) pauleyi*, a new species of crayfish (Decapoda: Cambaridae) endemic to southcentral West Virginia, USA, with a re-description of *Cambarus (J.) dubius*. *Zootaxa* 3980: 526–546. <https://doi.org/10.11646/zootaxa.3980.4.4>.
- Loughman, Z. J., S. A. Welsh & R. F. Thoma, 2019. *Cambarus fetzneri* sp. nov., a new species of burrowing crayfish (Decapoda: Cambaridae) from the Allegheny Mountains of Virginia and West Virginia, USA. *Zootaxa* 4651: 038–050. <https://doi.org/10.11646/zootaxa.4651.1.2>.
- McCormack, R. B. & J. Coughran, 2011. Taxonomy, distribution and ecology of the Setose Yabby, *Cherax setosus* (Riek 1951). *Crustacean Research* 40: 1–11. https://doi.org/10.18353/crustacea.40.0_1.
- McCutchan, J. H., Jr., W. M. Lewis Jr., C. Kendall & C. C. McGrath, 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102: 378–390. <https://doi.org/10.1034/j.1600-0706.2003.12098.x>.
- Momot, W. T., 1995. Redefining the role of crayfish in aquatic ecosystems. *Reviews in Fisheries Science* 3: 33–63. <https://doi.org/10.1080/10641269509388566>.
- Olsson, K., P. Nyström, P. Stenroth, E. Nilsson, M. Svensson & W. Granéli, 2008. The influence of food quality and availability on trophic position, carbon signature, and growth rate of an omnivorous crayfish. *Canadian Journal of Fisheries and Aquatic Sciences* 65: 2293–2304. <https://doi.org/10.1139/F08-137>.
- Parkyn, S. M., J. K. Collier & B. J. Hicks, 2001. New Zealand stream crayfish: functional omnivores buttrophic predators? *Freshwater Biology* 46: 641–652. <https://doi.org/10.1046/j.1365-2427.2001.00702.x>.
- Parnell, A., & R. Inger, 2016. Simmr: a stable isotope mixing model R package version 0.3. R.
- Pecor, K. W. & B. A. Hazlett, 2003. Frequency of encounter with risk and the tradeoff between pursuit and antipredator behaviors in crayfish: a test of the risk allocation hypothesis. *Ethology* 109: 97–106. <https://doi.org/10.1046/j.1439-0310.2003.00834.x>.
- Phillips, D. L., 2012. Converting isotope values to diet composition: the use of mixing models. *Journal of Mammalogy* 93: 342–352. <https://doi.org/10.1644/11-MAMM-S-158.1>.
- Post, D. M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83: 703–718. [https://doi.org/10.1890/0012-9658\(2002\)083\[0703:USITET\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2).
- Qi, H., T. B. Coplen, H. Geilmann, W. A. Brand & J. K. Böhlke, 2003. Two new organic reference materials for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements and a new value for the $\delta^{13}\text{C}$ of NBS 22 oil. *Rapid Communications in Mass Spectrometry* 17: 2483–2487. <https://doi.org/10.1002/rcm.1219>.
- Reynolds, J. B., C. Souty-Grosset & A. M. M. Richardson, 2013. Ecological roles of crayfish in freshwater and terrestrial habitats. *Freshwater Crayfish* 19: 197–218.
- Richman, N. I., M. Bohm, S. B. Adams, F. Alvarez, E. A. Bergey, J. J. Bunn, Q. Bumham, J. Cordeiro, J. Coughran, K. A. Cradall, K. L. Dawkins, R. J. Distefano, N. E. Doran, L. Edsman, A. G. Eversole, L. Fureder, J. M. Furse, F. Gherardi, P. Hamr, D. M. Holdich, P. Horwitz, K. Johnston, C. M. Jones, J. P. G. Jones, R. L. Jones, T. G. Jones, T. Kawai, S. Lawler, M. Lopezmejia, R. M. Miller, C. Pedraza-Lara, J. D. Reynolds, A. M. M. Richardson, M. B. Schultz, G. A. Schuster, P. J. Sibley, C. Souty-Grosset, C. A. Taylor, R. F. Thoma, J. G. Walls, T. S. Walsh & B. Collen, 2015. Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). *Philosophical Transactions of the Royal Society B* 370: 20140060. <https://doi.org/10.1098/rstb.2014.0060>.
- Roth, B. M., C. L. Hein & M. J. Vander Zanden, 2006. Using bioenergetics and stable isotopes to assess the trophic role of rusty crayfish (*Orconectes rusticus*) in lake littoral zones. *Canadian Journal of Fisheries and Aquatic Sciences* 63: 335–344. <https://doi.org/10.1139/f05-217>.
- Sánchez-Hernández, J. & P. A. Amundsen, 2018. Ecosystem type shapes trophic position and omnivory in fishes. *Fish and Fisheries* 19: 1003–1015. <https://doi.org/10.1111/faf.12308>.
- Santrock, J., S. A. Studley & J. M. Hayes, 1985. Isotopic analyses based on the mass spectrum of carbon dioxide. *Analytical Chemistry* 57: 1444–1448. <https://doi.org/10.1021/ac00284a060>.
- Shurin, J. B., D. S. Gruner & H. Hillebrand, 2006. All wet or dried up? Real differences between aquatic and terrestrial food webs. *Proceedings of the Royal Society B: Biological Sciences* 273: 1–9. <https://doi.org/10.1098/rspb.2005.3377>.
- Shurin, J. B., E. T. Borer, E. W. Seabloom, K. Anderson, C. A. Blanchette, B. Broitman, S. D. Cooper & B. S. Halpern, 2002. A cross-ecosystem comparison of the strength of trophic cascades. *Ecology Letters* 5: 785–791. <https://doi.org/10.1046/j.1461-0248.2002.00381.x>.
- Stenroth, P., N. Holmqvist, P. Nyström, O. Berglund, P. Larsson & W. Granéli, 2006. Stable isotopes as an indicator of diet in omnivorous crayfish (*Pacifastacus leniusculus*): the influence of tissue, sample treatment, and season. *Canadian Journal of Fisheries and Aquatic Sciences* 63: 821–831. <https://doi.org/10.1139/f05-265>.
- Stites, A. J., C. A. Taylor & E. J. Kessler, 2017. Trophic ecology of the North American crayfish genus *Barbicambarus* Hobbs, 1969 (Decapoda: Astacoidea: Cambaridae): evidence for a unique relationship between body size and trophic position. *Journal of Crustacean Biology* 37: 263–271. <https://doi.org/10.1093/jcbiol/rux019>.
- Sullivan, T. P. & D. S. Sullivan, 2003. Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems. *Environmental Reviews* 11: 37–59. <https://doi.org/10.1139/a03-005>.

- Sun, X., H. Wang, F. Wanf, Y. Zhau, H. Wang, J. Zhu, S. Wei & H. Chen, 2023. Effects of different fertilization patterns on the dietary composition of *Procambarus clarkii* in a rice-crayfish coculture system. *Aquaculture Reports* 33: 101801. <https://doi.org/10.1016/j.aqrep.2023.101801>.
- Taylor, C. A. & D. J. Soucek, 2010. Re-examining the importance of fish in the diets of stream-dwelling crayfishes: implications for food web analyses and conservation. *American Midland Naturalist* 163: 280–293. <https://doi.org/10.1674/0003-0031-163.2.280>.
- Thoma, R. F., & B. J. Armitage. 2008. Burrowing crayfish of Indiana. Indiana Department of Natural Resources <https://doi.org/10.13140/RG.2.2.25838.28488>.
- Usio, N. & C. R. Townsend, 2004. Roles of crayfish: consequences of predation and bioturbation for stream invertebrates. *Ecology* 85: 807–822. <https://doi.org/10.1890/02-0618>.
- Usio, N. & C. R. Townsend, 2008. Functional significance of crayfish in stream food webs: roles of omnivory, substrate heterogeneity and sex. *Oikos* 98: 512–522. <https://doi.org/10.1034/j.1600-0706.2002.980316.x>.
- Usio, N., 2000. Effects of crayfish on leaf processing and invertebrate colonization of leaves in a headwater stream: decoupling of a trophic cascade. *Oecologia* 124: 608–614. <https://doi.org/10.1007/s004420000422>.
- Vander Zanden, M. J. & J. B. Rasmussen, 2001. Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation: implications for aquatic food web studies. *Limnology and Oceanography* 46: 2061–2066. <https://doi.org/10.4319/lo.2001.46.8.2061>.
- Vander Zanden, M. J. and W. W. Fetzer. 2007. Global patterns of aquatic food chain length. *Oikos* 116: 1378–1388.
- Vannote, R. L., & R. Ball, C, 1972. Community productivity and energy flow in an enriched warmwater stream. Technical Report No. 27, Michigan State University, East Lansing, MI.
- Welch, S. M. & A. G. Eversole, 2006. The Occurrence of Primary Burrowing Crayfish in Terrestrial Habitat. 130: 458–464. <https://doi.org/10.1016/j.biocon.2006.01.007>.
- Wilson, A. L., D. S. Ryder, R. J. Watts & M. M. Stevens, 2005. Stable isotope analysis of aquatic invertebrate communities in irrigated rice fields cultivated under different management regimes. *Aquatic Ecology* 39: 189–200. <https://doi.org/10.1007/s10452-004-7085-0>.
- Wootton, K. L., 2017. Omnivory and stability in freshwater habitats: does theory match reality? *Freshwater Biology* 62: 821–832. <https://doi.org/10.1111/fwb.12908>

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