Trophic niche of an invasive generalist consumer: Australian redclaw crayfish, *Cherax quadricarinatus*, in the Inkomati River Basin, South Africa

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Abstract Trophic niche analysis can be used to assess ecological opportunities available to alien species in areas of introduction that might aid their establishment, define their functional role and inform on their potential impacts. This study assessed the trophic niche utilized by an invasive population of the Australian redclaw crayfish, *Cherax quadricarinatus*, in the Inkomati River Basin, South Africa. It evaluated the hypothesis that the euryphagous feeding strategy of redclaw crayfish may allow it to shift its niche width and niche position by altering its feeding strategy in response to fluctuations in resource availability. Gut content and stable isotope analyses were used to determine trophic niche and trophic interactions. Redclaw crayfish were shown to be omnivores and their diet consisted mainly of algae, plant material and invertebrates. Small-sized individuals had a constricted niche width and fed primarily on invertebrates, whereas larger individuals expanded their niche width to include larger proportions of plant material. Crayfish caught from lotic environments had a higher proportion of invertebrates in their diet than crayfish from lentic environments, and the species exploited a wider niche in summer than in winter. These differences are likely related to differences in productivity amongst habitats and fluctuations in resource availability. There was significant niche overlap (>60%) between redclaw crayfish and Sidney's river crab (*Potamonautes sidneyi*), but not with other native invertebrates. Both species are omnivores and have similar functional roles, possibly making redclaw crayfish functionally redundant in this ecosystem. Even though both species mainly feed on resources (plant material and invertebrates) that are not normally limiting, the redclaw crayfish invasion might be an accelerator of ecosystem processes such as shredding and decomposition rates of plant material. There is, therefore, a need for further studies to examine potentially altered ecosystem functions caused by redclaw crayfish invasion in the Inkomati River system.

Key words: niche complementarity, niche overlap, niche position, niche width, stable isotopes.

INTRODUCTION

The development of conceptual frameworks that can predict community invasibility based on traits of potential invaders and those of the invaded community has been a major goal of many studies in biological invasions (see Wilson *et al.* 2020). The ecological niche concept (*sensu* Hutchinson 1957) has been central to our understanding of species interactions and community structure (Polechová & Storch 2008). The concept synthesizes three main tenets: the

ecological space that is required by a species to sustain and maintain its population (Grinnell 1917), its functional role within an ecosystem in relation to trophic structure (Elton 1927), and its position within such a community as determined by its environmental (multidimensional) requirements and interactions with other species (Hutchinson 1957).

Trophic niche analysis has been used to assess the ecological opportunities available to alien species in terms of food resources and how factors that affect access to, and utilization of, food resources facilitate successful establishment and adverse impacts in a recipient ecosystem (e.g. Copp *et al.* 2017; Tonella

et al. 2018). This can be linked to the emergence of new analytical and computational tools such as stable isotopes analysis that are able to identify and quantify niche dimensions (niche width, trophic position) and elucidate trophic interactions (niche overlap and complementarity) (Newsome *et al.* 2007; Boecklen *et al.* 2011; Layman *et al.* 2012; Cucherousset & Villéger 2015). For example, alien species can utilize unexploited resources and this may facilitate their successful establishment by minimizing trophic interactions with native species (Shea & Chesson 2002). Conversely, alien and native species can utilize similar food resources and this can either lead to competition or niche complementarity (Lombard *et al.* 2018). Competition often leads to niche shifts by the weaker competitor to minimize competition or to its exclusion from an ecosystem when resources are limited (Hardin 1960; Kramer & Drake 2014). Niche complementarity allows species utilizing the same resources to co-exist through niche differentiation along important niche axes such as diet or habitat (e.g. MacArthur & Levins 1967; Olsson *et al.* 2009; Jackson & Britton 2014; Magoulick & Piercey 2016).

Trophic niche analysis can also be used to illustrate how adaptive life history traits such as ontogenetic diet shifts and trophic plasticity can confer an advantage for the establishment of alien species in areas that experience fluctuations in food resource availability (Pettitt-Wade *et al.* 2015). In addition, the trophic niche concept can also be used to assess the impacts of alien species on community structure and function (see Jackson *et al.* 2016). Such impacts include competition for food and space that can lead to niche shifts, decreases in abundance and extirpation of native species, habitat destruction, alteration of food web structures and changes in habitat coupling, as well as overall ecosystem-level effects (for reviews of impacts of invasive crayfish see Lodge *et al.* 2012; and Twardochleb *et al.* 2013). Quantifying the trophic niche of alien species is therefore important to aid our understanding of how their feeding strategies might aid establishment, to define their functional role and identify possible impacts they might cause in areas of introduction (Gozlan *et al.* 2010; Copp *et al.* 2017).

Redclaw crayfish, *Cherax quadricarinatus* (Von Martens, 1868), is native to north-west Queensland and the Northern Territory of Australia and Papua New Guinea (Riek 1967), but it has been widely introduced worldwide for aquaculture and the aquarium trade (Lodge *et al.* 2012). This species is well suited to aquaculture, as it is hardy and can tolerate a wide range of trophic and ecological adaptations (Masser & Rouse 1997). It also has adaptive life history traits such as multiple spawning events, high fecundity, fast growth rates and parental care (Ghanawi & Saoud 2012). Redclaw crayfish were introduced into South Africa in 1988, initially to assess their suitability for aquaculture, but their subsequent use in aquaculture was restricted because of concerns about potential adverse impacts (Nunes *et al.* 2017a). However, they were introduced in neighbouring Swaziland, where they eventually escaped from aquaculture facilities, and spread into adjacent river systems in South Africa and Mozambique (Nunes *et al.* 2017a, 2017b; Weyl *et al.* 2020). Redclaw crayfish have also been introduced and are naturalized in other countries in Southern Africa, namely Zambia and Zimbabwe (Marufu *et al.* 2014; Nunes *et al.* 2016, 2017b; Madzivanzira *et al.* 2021). In Southern Africa, only a limited number of studies have been performed to elucidate factors that might have aided redclaw crayfish establishment and to study their potential impacts (see Nunes *et al.* 2017a). Given that there are no native freshwater crayfish species in Africa (except for Madagascar), the presence of established redclaw crayfish populations in several river systems in South Africa may present an ecological risk because invasive crayfish can cause strong alterations at multiple trophic levels of invaded ecosystems (Reynolds *et al.* 2013), act as novel predators, competitors and vectors of pathogens (Lodge *et al.* 2012; Twardochleb *et al.* 2013).

Research on the trophic ecology of this species in the wild is limited to a recent study in a large man-made impoundment in Zimbabwe (Marufu *et al.* 2018) and none have focussed on lotic environments. The study by Marufu *et al.* (2018) found that redclaw crayfish were facultative omnivores that consumed mainly detritus, macrophytes and invertebrates. Omnivory allows generalist feeders to survive food resource fluctuations, and this might be an essential life history trait required to successfully establish in highly seasonal aquatic systems. Gathering information on the trophic ecology of the redclaw crayfish in the full extent of its invaded habitats is essential because it aids our understanding on how the crayfish feeding strategies might have aided establishment, its functional role in the ecosystem, and allows for the assessment of potential impacts.

The objective of this study was to identify and quantify trophic niche dimensions (niche width, niche position) of redclaw crayfish in relation to factors that might affect resource use and availability such as ontogeny, habitat, season, and trophic interactions with native species of molluscs and crustaceans, such as red-rimmed melania snail *Melanoides tuberculata* (Müller, 1774), Sidney's river crab *Potamonautes sidneyi* (Rathbun, 1904), freshwater shrimp *Caridina nilotica* (P. Roux, 1833), and hairy river prawn *Macrobrachium rudi* (Heller, 1862) that are known to occur in the Inkomati River Basin. It was hypothesized that the euryphagous feeding strategy of redclaw crayfish may allow it to shift its niche width (the variety of food items utilized by a species) and

niche position (type of food resources utilized) by altering its feeding strategy in response to fluctuations in resource availability. In addition, the trophic plasticity of redclaw crayfish may allow it to co-exist with native species that feed on similar food sources through niche complementarity.

METHODS

Study site and sampling

The study area encompassed the known invaded range of redclaw crayfish in the Inkomati River Basin in Mpumalanga Province, South Africa (Fig. 1). A detailed description of the invaded range of redclaw crayfish in the region is given in Nunes *et al.* (2017b). Briefly, the Inkomati River rises west of Carolina in Mpumalanga, South Africa, and flows for 480 km in a north-easterly direction through three riparian countries: South Africa, Swaziland and Mozambique (Fig. 1). It has a catchment area of about 50 000km² that is divided into two distinct topographical areas by the Great Escarpment: a temperate plateau area in the west (elevation >2000 m above sea level) and a sub-tropical lowland in the east. Both areas receive summer rainfall that varies from 400–1000 mm on the lowlands to 1500 mm on the escarpment. The lowland area is extensively utilized for agriculture, and as a result, the river's flow has been highly modified through the construction of dams and river weirs to retain water for irrigation. In addition, a lot of small farm dams have also been constructed off channel on adjacent farmlands to act as transient reservoirs for irrigation water that is pumped from the river main stem.
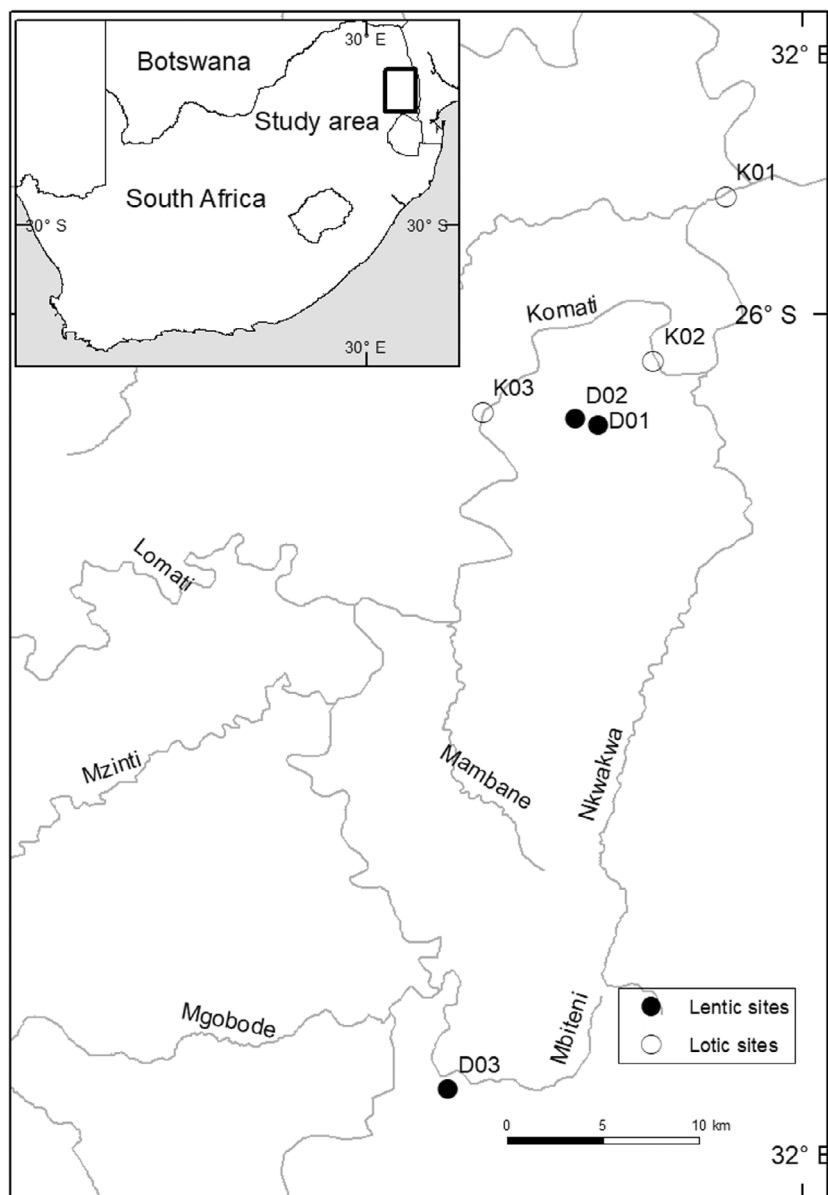

Fig. 1. Location of sampling sites along the Inkomati River Basin, South Africa. Habitats are indicated by symbols: (●) = lotic sites (K01–K03) and (○) = lentic sites (D01–D03).

Sampling was done at six sites that represented lotic and lentic habitats. Sites K01–K03 were located on the river main stem and were characterized by deep pools formed behind river weirs (lotic habitats). Sites D01–D03 were located on off channel irrigation dams that varied in size (surface area in hectares) from small farm dams (D01 = 10 ha. and D02 = 23 ha.) to the medium-sized Mazibekela Dam (D03 = 206 ha.) (lentic habitats).

Samples were collected during seven sampling events between July 2015 and July 2016 in winter (May–July) and summer (September–December). Crayfish were caught using collapsible crayfish traps (Model #TR-101, © Promar Gardena). Ten traps baited with dog food pellets were set overnight at each site and collected the following morning. Crayfish were then sorted according to sex, measured using callipers [occipital carapace length (OCL) to the nearest mm] and weighed using a scale (to the nearest 0.01 g).

Determination of diet and trophic interactions

Gut content and stable isotope analyses were used to determine trophic niche and patterns of trophic interactions. Gut content analysis was used to identify possible crayfish food items, and the observed food matrix was then used to inform stable isotope analysis. Stable isotope data were also used to differentiate food niches amongst species in terms of niche space, niche size and degree of niche overlap.

Crayfish gut contents were examined using a microscope and identified to the lowest possible taxonomic level and later combined into broad taxonomic categories for quantitative comparisons. These categories included algae (cyanophyta, green algae and periphyton), plant material (detritus, macrophytes), aquatic insects, crayfish and molluscs. The importance of each prey item species was determined using frequency of occurrence (% F) (Hyslop 1980). The approach provides estimates of the number of stomachs containing a specific prey item as a percentage of all analysed stomachs. It has been shown to provide a more robust and interpretable measure of diet composition than other quantitative approaches, especially when it is difficult to quantify macerated gut contents (Baker *et al.* 2014; Buckland *et al.* 2017; Amundsen & Sánchez-Hernández 2019). The food items identified in the gut contents analysis were then collected from the environment to delineate the food web structure in the river system. The premise was that crayfish are likely to be better samplers of the food items available in the environment than humans collecting putative samples. In addition, mixed isotopic models were used to estimate the food items assimilated by crayfish and the food matrix used to train the models should ideally be informed by a prior analysis of food items likely utilized by the consumer (see Phillips *et al.* 2014). Aquatic insects were collected using the kick-net method on all available substrates at each site, whereas algae and plant material were collected by hand. There are several native species of molluscs (snails and mussels) and crustaceans (crabs and shrimps) that occur in the Inkomati system that might utilize similar food resources to redclaw crayfish. To assess for possible patterns of trophic interactions between the redclaw crayfish and native molluscs and crustaceans, several

species were selected from each respective taxon. These included the red-rimmed melania snail *Melanoides tuberculata* (Müller, 1774), Sidney's river crab *Potamonautes sidneyi* Rathbun, 1904 and the freshwater shrimp *Caridina nilotica* (P. Roux, 1833), which were collected from the environment using collapsible crayfish traps, active searches and a kick-net. The hairy river prawn *Macrobrachium rudi* (Heller, 1862), although also known to occur in the system, was not found.

Stable isotope analysis

Stable isotope values were obtained from muscle tissue, which provide a longer time-averaged estimate (weeks to months) of food assimilated by crayfish (Carolan *et al.* 2012; Glon *et al.* 2016; Viozzi *et al.* 2021). Therefore, the observed stable isotope values may reflect dietary patterns over similar time scales or longer. Muscle tissue was excised from all invertebrate samples except for aquatic insects, which were analysed whole. Aquatic insects were combined so that they included representatives from the four functional groups: predators (Coenagrionidae and Notonectidae), scrapers (Baetidae), filterers (Hydropsychidae and Culicidae) and gatherers (Caenidae, Oligochaeta and Chironomidae). Algae, plant material, insect samples and muscle tissue were oven-dried at 70°C for 12 h. Samples were then ground into a fine powder before lipid extraction was made using a 2:1 chloroform-ethanol solution (Søreide *et al.* 2006). Inorganic carbons (i.e. $CaCO_3$) were removed from all invertebrate and plant samples with 1 mol HCL (Søreide *et al.* 2006). Samples were then re-dried for 12 h before aliquots of approximately 0.6–0.7 mg were weighed into tin capsules that were pre-cleaned in toluene. Isotopic analysis was made on a Flash EA 1112 Series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (all equipment supplied by Thermo Fisher, Bremen, Germany), housed at the Stable Isotope Laboratory, Mammal Research Institute (MRI), University of Pretoria, South Africa. A laboratory running standard (Merck Gel: $\delta^{13}C = -20.57‰$, $\delta^{15}N = 6.8‰$, C% = 43.83, N% = 14.64) and blank sample were run after every 12 samples. The standards were Vienna Pee-Dee Belemnite for $\delta^{13}C$ (Craig 1957) and atmospheric nitrogen for $\delta^{15}N$ (Ehleringer & Rundel 1989). Stable isotope results are expressed in delta notation using a permil scale using the standard equation:

$$\delta X(‰) = \left[\left(R_{sample} - R_{standard} \right) / R_{standard} \right] \times 1000.$$

Where X = ^{15}N or ^{13}C and R represents $^{15}N/^{14}N$ or $^{13}C/^{12}C$, respectively. Analytical precision was <0.05‰ for $\delta^{13}C$ and <0.09‰ for $\delta^{15}N$.

Isotopic baseline corrections

Stable isotope values for basal resources can vary considerably along spatial and temporal gradients, and to correct for these differences, $\delta^{15}N$ values were converted to trophic position (TP) and $\delta^{13}C$ values were corrected to Ccorr

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following the approaches recommended by Olsson *et al.* (2009) and Jackson and Britton (2014). The stable isotope correction equations were:

$$TP = \left(\frac{N_{consumer} - N_{baseline}}{2.3} \right) + 2$$

$$C_{corr} = \frac{\delta^{13}C_{consumer} - \delta^{13}C_{meaninv}}{CR_{inv}}$$

Where TP is the trophic position of each consumer, $N_{consumer}$ is the isotopic ratio of each consumer and $N_{baseline}$ is the isotopic ratio of a long-lived filter feeding primary consumer (Van der Zanden *et al.* 1999; Post 2002). Where C_{corr} = the corrected carbon of each consumer, $\delta^{13}C_{consumer}$ is the isotopic ratio of each consumer, $\delta^{13}C_{meaninv}$ is the mean invertebrate isotope ratio and CR_{inv} is the invertebrate carbon range ($\delta^{13}C_{max} - \delta^{13}C_{min}$) (Olsson *et al.* 2009). This study used the mean nitrogen values of aquatic insects because the filter feeding African porcelain mussel, *Corbicula fluminalis* (Müller, 1774) ($\delta^{15}N = 9.91 \pm 1.79‰$) that is native to the Inkomati river basin was scarce and was only collected at one site. The study, therefore, used the mayfly grazer (Baetidae) to estimate the nitrogen baseline ($\delta^{15}N_{baseline}$) and correct for differences in basal resources for carbon (C_{corr}) because it was sampled at all the sites and comparisons amongst sites revealed no significant spatial difference for $\delta^{15}N$ (Welch: F = 1.12, df = 12.92, $P > 0.05$) and $\delta^{13}C$ values (Welch: $F = 0.02$, df = 13.62, $P > 0.05$) (Table 1). The nitrogen trophic enrichment factor was set at 2.3 following the meta-analysis of McCutchan *et al.* (2003).

Mixing models

A Bayesian mixing model (simmr, Parnell *et al.* 2010; R Core Team 2020) was used to estimate the relative proportion of basal food resources that were assimilated by redclaw crayfish and native species of molluscs and crustaceans. The mixing model was calibrated using food sources that were identified from the gut content analysis. Food items were grouped into three broad taxonomic categories: algae, plant material and invertebrates. Models were constructed using uncorrected stable isotope data. The trophic enrichment factors were set at $2.3 \pm 0.28\%$ for nitrogen and $0.4 \pm 0.17\%$ for carbon following the meta-analysis of McCutchan *et al.* (2003). Model performance and fit were checked using the convergence diagnostic tool and by visually checking the posterior probability distributions.

Niche position and niche width

Measures of central tendency (centroids) and dispersion (niche width) were used to identify and quantify the niche position and niche size utilized by redclaw crayfish in the Inkomati River Basin in relation to life history traits (ontogeny) and environmental variability (habitat and season) following the approach of Turner *et al.* (2010) and Jackson *et al.* (2011). Central tendency was a test for differences in the location (Euclidean distance) of centroids (C_{corr} and TP bivariate mean) between respective groups of isotope samples. The significance of the test statistic for differences in niche position was then evaluated using null distributions generated from nested linear models and a residual

Table 1. Abundance of redclaw crayfish (*Cherax quadricarinatus*) and Sydney's river crab (*Potamonautes sidneyi*) in relation to habitat and season in the Inkomati River Basin

Habitat	Sampling site	Season	Species	CPUE (N/trap/night)
Lotic	K01	Summer	*Cherax quadricarinatus*	2.2 ± 1.39
		Winter		3.2 ± 5.1
		Summer	*Potamonautes sidneyi*	0.7 ± 0.1
		Winter		0.7 ± 0.3
	K02	Summer	*Cherax quadricarinatus*	3.1 ± 4.4
		Winter		1.4 ± 1.8
		Summer	*Potamonautes sidneyi*	2.6 ± 1.6
		Winter		1.3 ± 0.5
	K03	Summer	*Cherax quadricarinatus*	9.4 ± 7.7
		Winter		4.4 ± 5.0
		Summer	*Potamonautes sidneyi*	
		Winter		
Lentic	D01	Summer	*Cherax quadricarinatus*	15.3 ± 20.0
		Winter		1.8 ± 2.1
		Summer	*Potamonautes sidneyi*	
		Winter		
	D02	Summer	*Cherax quadricarinatus*	3.3 ± 2.83
		Winter		1.9 ± 4.1
		Summer	*Potamonautes sidneyi*	
		Winter		
	D03	Summer	*Cherax quadricarinatus*	7.4 ± 8.9
		Winter		0.9 ± 1.1
		Summer	*Potamonautes sidneyi*	0.2 ± 0.2
		Winter		0.5 ± 0.1

permutation procedure (Turner *et al.* 2010). *Post hoc* analysis of significant differences was done using one-way analysis of variance (ANOVA) (for normally distributed data) or Kruskal–Wallis test followed by Mann–Whitney pairwise *post hoc* test (when data departed from normality).

The trophic niche size and overlap were estimated using size-corrected standard ellipse areas (SEAc) that were constructed using the *Stable Isotope Bayesian Ellipses* (SIBER) package in *R* (Jackson *et al.* 2011). The niche space utilized by a species is often delineated by a convex hull drawn around the most extreme outliers in each isotope biplot (Layman *et al.* 2007). This inherently makes it susceptible to changes in sample size and spatio-temporal variation in isotopic values. To minimize these potential sources of variability, the SEAc were constructed using variance and covariance of the isotope biplot that contained only 40% of the data, which represents the core isotopic niche and typical resources used by a species. For each analysed group, a Bayesian estimate of the SEAc and its area was then calculated using a Markov chain Monte Carlo simulation with 10^4 iterations, which provides 95% confidence limits of the isotopic niche size (Jackson *et al.* 2011). The extent of niche overlap (%) between groups was then estimated using the maximum likelihood fitted SEAc. Overlap between groups was expressed as the proportional area that overlapped between two SEAc divided by the total non-overlapping area of both ellipses. Extent of overlap was classified as low (<40%), intermediate (40–60%) or high (>60%) (Langton 1982).

To assess for ontogenetic diet shifts, redclaw crayfish samples were grouped into five incremental size classes of 10 mm, from the smallest size class of <10 mm OCL to the largest individuals of >40 mm OCL, based on Leland *et al.* (2015). Assessment of potential bias from ontogenetic effects was restricted to samples from Mazibekela Dam (D03) because it was the only site where all five size classes of crayfish were caught. Crayfish were further grouped according to habitat (lentic and lotic environments) and seasons (winter and summer). Comparisons of crayfish diet using gut content and stable isotope analyses from different habitats, sex and seasons were restricted to two size classes (31–40 mm and >40 mm OCL) to minimize bias from ontogenetic effects on diet. In addition, the analysis of the effect of season on crayfish diet was restricted to three sites (K01, K02, D03) and of the effect of sex to two sites (K02 and D03) that had samples caught across each treatment. It is possible that the observed variation in crayfish niche width between habitats and season may be linked to differences in food resource availability, but this was not quantified in this study.

A preliminary analysis to assess potential niche overlap between redclaw crayfish and native molluscs and crustaceans revealed minimal overlap between redclaw crayfish and snails or shrimps. Therefore, comparisons of patterns of resource use and overlap were restricted to redclaw crayfish and Sidney's river crab. In addition, the analysis was restricted to two sites (K02 and D03) that had samples of both species caught across habitats and seasons.

RESULTS

Species abundance

Redclaw crayfish abundance was generally higher in lentic habitats (0.9–15.3 ind. per trap $night^{-1}$) than in lotic habitats (1.4–9.4 ind. per trap $night^{-1}$), but the differences were not statistically significant (ANOVA, $F_{1,10} = 0.03$, $P > 0.05$) (Table 1). Similarly, there was no significant seasonal difference in the abundance of redclaw crayfish caught from lotic habitats (mean CPUE = 4.9 ind. per trap $night^{-1}$ in summer and 3 ind. per trap $night^{-1}$ in winter) (ANOVA, $F_{1,4} = 0.48$, $P > 0.05$), but the seasonal differences in redclaw crayfish abundance in lentic habitats (mean CPUE = 8.67 ind. per trap $night^{-1}$ in summer and 1.53 ind. per trap $night^{-1}$ in winter) were significant (ANOVA, $F_{1,4} = 11.1$, $P < 0.05$). Sydney's river crabs were sparse (found in 3 out of 6 sampling sites) and occurred in lower abundance than redclaw crayfish in both lotic habitats (mean CPUE = 1.65 ind. per trap $night^{-1}$ in summer and 1 ind. per trap $night^{-1}$ in winter) (ANOVA, $F_{1,10} = 11.3$, $P < 0.03$) and lentic habitats (mean CPUE = 0.2 ind. per trap $night^{-1}$ in summer and 0.5 ind. per trap $night^{-1}$ in winter) (ANOVA, $F_{1,10} = 40.91$, $P < 0.05$).

Gut content analysis

Gut content analysis indicated that crayfish were omnivores and their diet consisted mainly of algae, plant material and invertebrates (Table 2). Invertebrates mainly consisted of aquatic insects, and skeletal fragments of crayfish and snails. Crayfish

Table 2. Frequency of occurrence (% numbers) of food sources utilized by redclaw crayfish (*Cherax quadricarinatus*) in relation to habitat and season in the Inkomati River Basin

Habitat	Site	Season	Algae	Plant matter	Aquatic insects	Crayfish	Snails
Lotic	K01	Summer	75	95	10	65	35
		Winter	92	100	23	23	8
	K02	Summer	90	100		70	30
		Winter	71	100	14	14	
Lentic	D03	Summer	94	100	22	39	22
		Winter	28	100	11		6

diet varied across habitats and season. Crayfish caught from lotic environments had a higher proportion of invertebrates (crayfish and snails) than crayfish from lentic environments. There was a higher frequency of occurrence of algae and invertebrates in summer than in winter in lentic environments. In lotic environments, crayfish diet generally remained similar across seasons, but there was a higher occurrence of invertebrates (crayfish and snails) in summer.

Ontogenetic changes in crayfish diet

The simmr mixing model confirmed that the diet of redclaw crayfish in Mazibekela Dam was omnivorous with an ontogenetic shift towards invertebrates (Table 3). Small crayfish size classes (<10 mm and 10–20 mm OCL) fed mostly on invertebrates (aquatic insects, crayfish and snails), whereas larger size classes (>31 mm OCL) had a higher proportion of plant material and crayfish in their diet. All five size classes were, however, classified in one trophic group (TP = 2.1–2.8), but there were significant differences in centroid positions indicative of the ontogenetic diet shifts (Fig. 2a). Centroid positions of the smallest size classes [<10 mm OCL (TP = 2.8 ± 0.1, Ccorr = 0.57 ± 0.3), 10–20 mm (TP = 2.6 ± 0.3, Ccorr = 0.47 ± 0.2)] occupied apex positions on the biplot that were significantly different from all the other larger size classes. These differences were largely driven by higher TP values and Ccorr-enriched values of these two small size classes, relative to the larger crayfish size classes (Mann–Whitney pairwise test, $P < 0.05$). The 21–30 mm OCL size class occupied a basal position on the biplot because it had the lowest TP (2.1 ± 0.5) and Ccorr (0.24 ± 0.2) values.

Niche width generally increased with crayfish size, where smaller sized crayfish had smaller niche sizes than larger crayfish (Fig. 3a). The only significant differences in niche size were cross-comparisons between the smaller size classes (<10 mm and 10–20 mm OCL) and the largest size class (<40 mm OCL). The extent of overlap varied from low to intermediate with the most pronounced overlap observed amongst the medium to large size classes

[21–30 mm and 31–40 mm OCL (43%), 21–30 mm and >40 mm OCL (51%) and 31–40 mm and >41 mm OCL (51%)] (Fig. 3a).

Habitat effect on crayfish diet

Habitat had a significant effect on crayfish centroid positions ($P < 0.05$) (Fig. 2b). Generally, crayfish caught in lotic habitats had a higher TP (4.19 ± 0.3) and Ccorr-depleted values (0.10 ± 0.19) than crayfish caught from lentic habitats (TP = 3.04 ± 0.9 and Ccorr = 0.38 ± 0.2), except for crayfish from lentic site D01 (Mann–Whitney pairwise test, $P < 0.05$). In addition, all crayfish utilized similar sized niches across habitats, except for crayfish from lentic site D03 that utilized a significantly larger niche (Fig. 3b). The simmr mixing model and gut content analysis both indicated that crayfish caught from lotic environments had a higher proportion of invertebrates in their diets than crayfish from lentic environments (Table 4).

Cross-comparisons within sites from lentic habitats indicated that the centroid positions of crayfish caught from the three dams were significantly different from each other ($P < 0.05$). Post hoc tests indicated that TP values of crayfish from the smaller sized irrigation dams (D01 = 3.92 ± 0.3, D02 = 3.75 ± 0.2) were similar, but significantly higher than TP values of crayfish from the larger dam (D03 = 2.34 ± 0.6) (Kruskal–Wallis $\chi 2 = 24.88$, $P < 0.05$). In contrast, Ccorr values for crayfish from dams (D01 = 0.17 ± 0.3and D03 = 0.40 ± 0.2) were similar, but significantly lower than those from D02 (0.54 ± 0.2) (ANOVA, $F_{2,35} = 8.59$, $P < 0.01$).

Cross-comparisons within sites from lotic habitats indicated that the centroid positions of crayfish caught from riverine sites were also significantly different from each other ($P < 0.05$). Post hoc test indicated that trophic values of crayfish from upstream sites (K02 = 4.29 ± 0.19 and K03 = 4.53 ± 0.34) were similar and significantly higher than at downstream site K01 (3.97 ± 0.15) (ANOVA, $F_{2,39} = 15.22$, $P < 0.01$). However, the Ccorr values for crayfish from all the three sites were similar (K01 = 0.10 ± 0.11, K02 = 0.07 ± 0.2, K03 = 0.2 ± 0.1) (ANOVA, $F_{2,39} = 0.75$, $P > 0.05$).

Size class	Algae	Plant material	Aquatic insects	Crustaceans	Molluscs
<10 mm	10.3 ± 7.3	13.8 ± 10.7	18.8 ± 13.8	30.9 ± 19.5	26.2 ± 17.1
10–20 mm	10.9 ± 7.2	15.2 ± 10	17.1 ± 11.8	36.3 ± 18.3	20.5 ± 13.2
21–30 mm	9.4 ± 7.1	30.3 ± 14.7	10.5 ± 8.5	42.4 ± 18.8	7.4 ± 5.7
31–40 mm	9.1 ± 6.7	20 ± 13.5	13.6 ± 11.6	46.8 ± 20.8	10.6 ± 8.7
>40 mm	10.4 ± 8.5	22.5 ± 17.2	15.8 ± 14.3	38.4 ± 23.5	12.9 ± 10.8

Table 3. Proportion (% of isotopic value) of food sources utilized by redclaw crayfish (*Cherax quadricarinatus*) in relation to occipital carapace length in Mazibekela Dam (D03), Inkomati River Basin

Fig. 2. Centroid positions (corrected carbon (Ccorr) and trophic positions (TP)) of red-claw crayfish (*Cherax quadricarinatus*) in relation to (a) occipital carapace length in Mazibekela Dam (D03) and (b) habitat (lotic and lentic environments) in the Inkomati River Basin. Sites K01–K03 were located in lotic habitats and sites D01–D03 in lentic habitats.
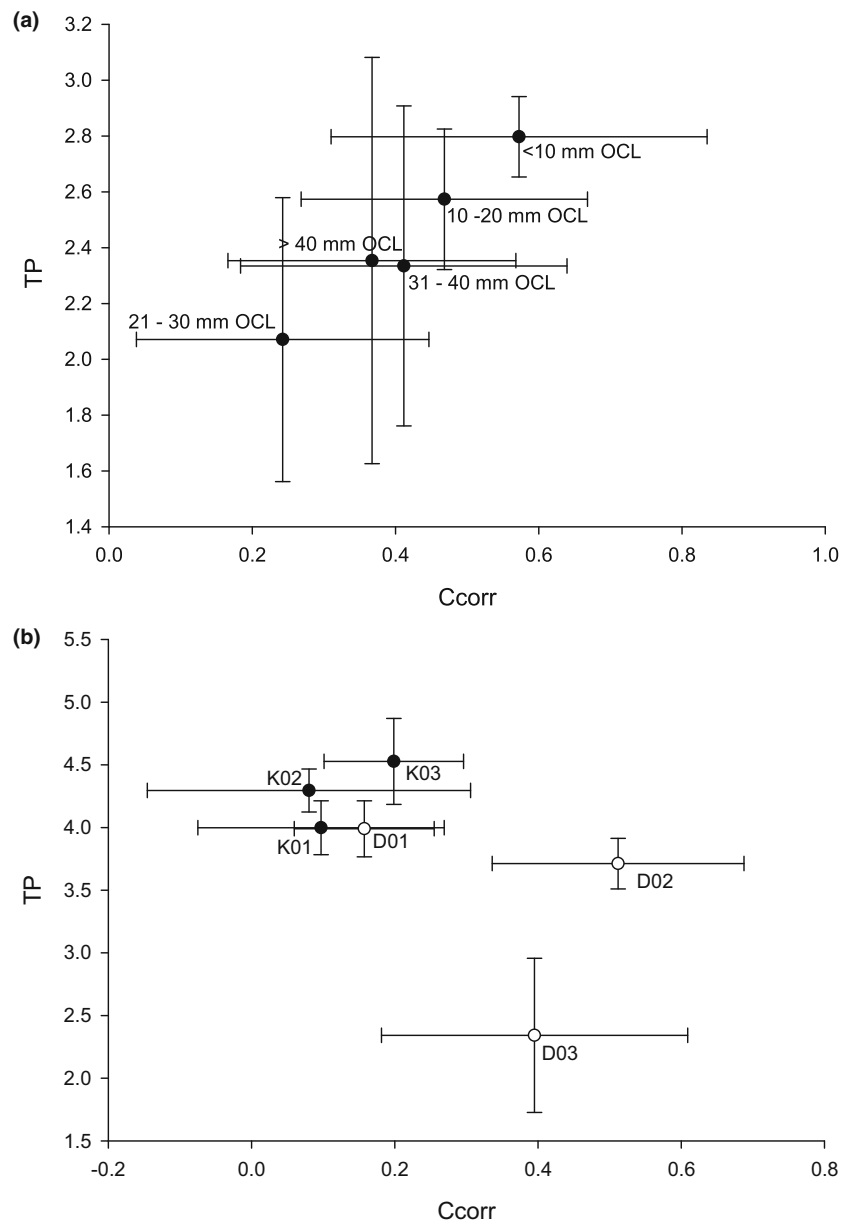
Seasonal and sex effect on crayfish diet

Season had no significant effect on crayfish centroid positions, but had a significant effect on niche size ($P< 0.05$). Niche size was generally larger in summer than in winter at all sites, but the differences were only significant at D03 (Appendix S1). The simmr mixing model indicated that, in both lentic and lotic environments, crayfish diet generally remained similar across seasons, with a higher occurrence of invertebrates in summer (Table 4). Crayfish sex had no significant effect ($P > 0.05$) on the centroid positions and niche width of female and male crayfish, irrespective of sampling site (Appendix S2). Niche overlap between female and male crayfish varied from

low (site K02 = 32%) to intermediate (sites K02 = 44% and D03 = 58%) (Fig. 3c).

Trophic interrelationships between crayfish and crabs

The simmr mixing model indicated that Sydney's river crabs were also omnivores and their diet also consisted mainly of algae, plant material and invertebrates (Table 4). Habitat had a significant effect on centroid positions of crabs ($P < 0.05$). Crabs from lotic habitats had significantly higher TP values (K02 = 3.24 ± 0.97) than crayfish from the lentic habitat (D03 = 1.43 ± 0.55), but Ccorr values were
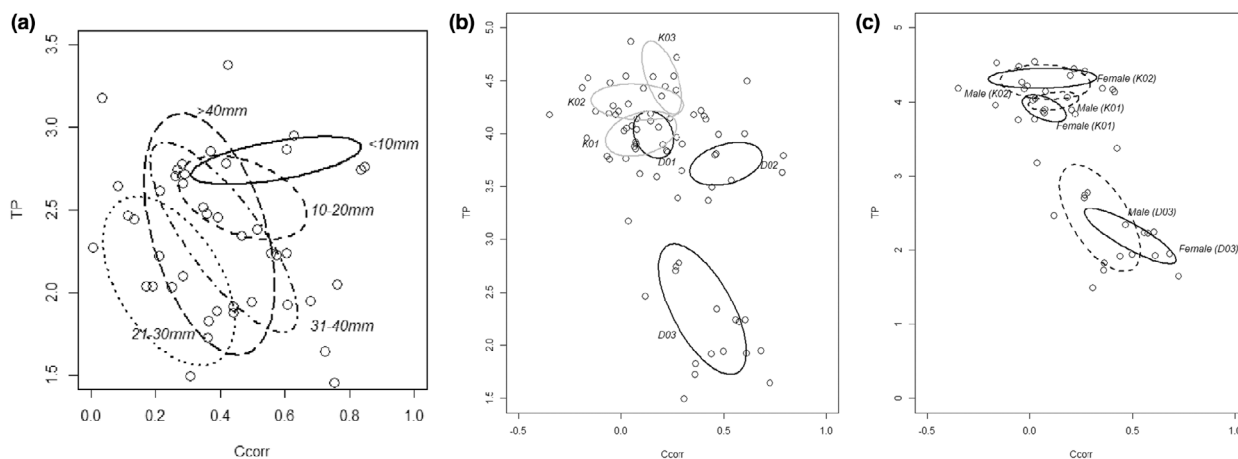
Fig. 3. Estimates of niche overlap using sample size-corrected standard ellipse areas (SEAc) of redclaw crayfish (*Cherax quadricarinatus*) in relation to (a) occipital carapace length (b) habitats (K01–K03 = lotic and D01–D03 = lentic environments) and (c) sex in the Inkomati River Basin.

Table 4. Proportion (% of isotopic value) of food sources utilized by redclaw crayfish (*Cherax quadricarinatus*) and Sydney's river crab (*Potamonautes sidneyi*) in relation to habitat (lotic and lentic environments) and season (summer and winter) in the Inkomati River Basin

Species	Habitat	Season	Site	Algae	Plant material	Aquatic insects	Crustaceans	Molluscs
Cherax quadricarinatus	Lotic	Summer	K01	5.9 ± 4.3	10.3 ± 8.6	12.6 ± 10.7	55.4 ± 16.6	15.8 ± 12.1
		Winter		8.1 ± 6.4	14.3 ± 11.9	15.0 ± 12.9	46.0 ± 20.4	16.6 ± 13.5
		Summer	K02	7.6 ± 6.0	13.4 ± 12.0	16.9 ± 14.6	41.7 ± 21.6	20.4 ± 17.0
		Winter		7.9 ± 6.1	14.7 ± 12.5	14.7 ± 12.7	47.5 ± 20.7	15.2 ± 12.8
	Lentic	Summer	D03	9.4 ± 7.2	20.7 ± 14.3	14.1 ± 12.1	44.9 ± 21.5	10.9 ± 8.7
		Winter		10 ± 8	21.4 ± 16.4	15 ± 13.2	41.3 ± 23.2	12.3 ± 10.5
Potamonautes sidneyi	Lotic	Summer	K02	5.9 ± 5.2	10.7 ± 11.0	11.6 ± 12.2	57.1 ± 24.7	14.7 ± 15.7
		Winter		3.2 ± 2.6	5.6 ± 5.4	5.7 ± 5.5	78.8 ± 13.4	6.8 ± 7.7
	Lentic	Summer	D03	11.6 ± 9.5	16.7 ± 14.40	20.1 ± 16.5	28.0 ± 20.8	23.5 ± 18.5
		Winter		9.1 ± 6.2	16.7 ± 10.2	14.7 ± 11.0	46.0 ± 18.5	13.4 ± 10.2

similar (K02 = 0.40 ± 0.13, D03 = 0.40 ± 0.17). The niche size utilized by crabs also varied across sites, where crabs from the lotic habitat (K02) had a larger niche size than crabs from the lentic environment (D03). Season had no significant effect on crab centroid positions, but had a significant effect on niche size ($P < 0.05$). Crabs from both sites (K02 and D03) utilized a larger niche size in winter than in summer (Appendix S1).

Cross-comparisons between crayfish and crabs indicated that they occupied similar centroid positions in both lotic and lentic habitats (K02 and D03) ($P > 0.05$). Crabs generally had a larger niche size than crayfish, but these differences were only significant at site K02 ($P < 0.05$). The most pronounced niche overlap between crayfish and crabs was at site D03 (63%), but at site K02, overlap was low (10%) (Fig. 4). Crabs generally utilized larger niche sizes than crayfish across seasons, except at site D03 where crayfish had a larger niche size than crabs in

summer (Appendix S1). Niche overlap between crabs and crayfish varied significantly with season at D03 (winter = 62% and summer = 13%), but remained low across seasons for site K02 (<12%).

DISCUSSION

Effect of ontogeny, habitat and seasonality on redclaw crayfish diet

This study assessed the ecological opportunities available to redclaw crayfish in the Inkomati River Basin in terms of food resources and how factors that affect access to and utilization of such food resources might have facilitated its successful establishment in the system. We found evidence to support the hypothesis that the euryphagous feeding strategy of redclaw crayfish may allow it to shift its niche width and alter
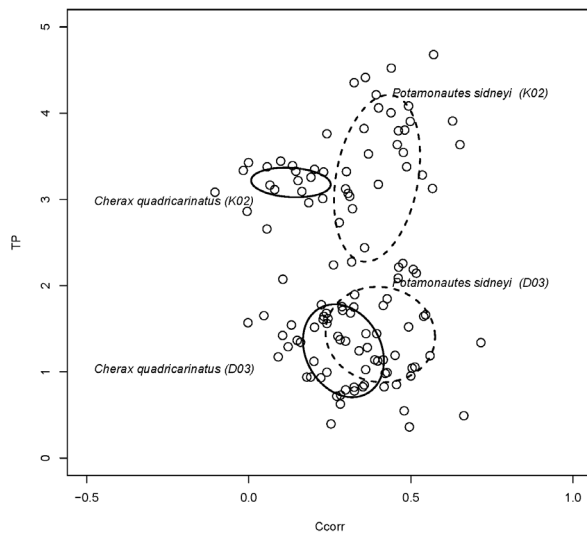
Fig. 4. Estimates of niche overlap using sample size-corrected standard ellipse areas (SEAc) of redclaw crayfish (*Cherax quadricarinatus*) and Sydney's river crab (*Potamonautes sidneyi*) in relation to habitat (K02 = lotic and D03 = lentic environments) in the Inkomati River Basin.

its trophic position in response to ontogeny and fluctuations in resource availability. Redclaw crayfish were facultative omnivores that consumed mainly algae, macrophytes and invertebrates. This is in agreement with observations elsewhere, where redclaw crayfish have been shown to consume a wide variety of prey across multiple trophic levels (Jones 1990; Saoud *et al.* 2012; Marufu *et al.* 2018). The high frequency of occurrence of skeletal fragments of crayfish and snails observed in the gut contents may indicate consumption of the invertebrate prey through predation or scavenging of exoskeletons from moulted or dead individuals. Cannibalism is common amongst many decapod groups including members of the genus *Cherax* (Polis 1981; Romano & Zeng 2017). Cannibalism is often caused by the presence of vulnerable stages of recently moulted individuals amongst populations and density-dependent intraspecific competition for food and habitat (Elgar & Crespi 1992). Crayfish moult to grow and, during moulting, individuals are more vulnerable to predation because they are unable to defend themselves until the new exoskeleton has calcified (Romano & Zeng 2017). Crayfish are also known to bury and later feed on their own exuviae (shed exoskeleton) soon after moulting (Buřič *et al.* 2016). This behaviour might be a two-pronged strategy, self-protective to hide recently moulted individuals that are vulnerable to predation, as well as a foraging strategy to catch and retain minerals that are expanded during the moulting process (Buřič *et al.* 2016).

Changes in niche width and trophic position amongst the different size classes indicated

ontogenetic diet shifts. Small-sized individuals had a small niche size that consisted of prey with high TP values, relative to larger size classes that had larger niche sizes and wider range of TP values. This likely indicates a constrained diet largely consisting of invertebrate prey for the juvenile crayfish, whereas larger individuals expanded their diet breadth to include a wider range of prey items. Evidence of ontogenetic diet changes amongst crayfish species from elsewhere is inconsistent. Some observations indicate that juveniles of some crayfish species feed mostly on invertebrates, whereas adults have a wider niche breadth that consists of plant material and invertebrates (e.g. Goddard 1988; France 1996; Marufu *et al.* 2018). In contrast, other studies have however found no evidence of ontogenetic shift in crayfish diet (e.g. Bondar & Richardson 2009; Usio *et al.* 2009; Ercoli *et al.* 2021). These disparities might partly be the result of differences in how samples were collected and analysed. For example, studies that found no evidence of ontogenetic diet shifts likely classified some crayfish as juveniles, but their diet had already shifted towards food consumed by adults. In this study, crayfish were grouped into incremental size classes of 10 mm OCL that represented 0–3+ age classes, following Leland *et al.* (2015), and this allowed for an ontogenetic diet shift to be quantitatively tracked with growth.

The diet of the redclaw crayfish was also affected by other factors, such as habitat and season. Crayfish caught from lotic sites had enriched TP values and depleted Ccorr values relative to crayfish from lentic sites. This suggests that crayfish from the two different habitats exploited different food resources. Gut content analysis and stable isotope mixing models indicated that crayfish from lotic sites had a larger proportion of invertebrates in their diet than crayfish from lentic habitats that fed mainly on plant material, and this could account for the higher TP values in the former. It is possible that the observed variation in crayfish niche width between the two habitats may be linked to differences in food resource availability, although this is yet to be quantified. For example, stream hydrology has been shown to have a strong influence on the productivity and diversity of food sources in aquatic systems (Winemiller & Winemiller 2003). The differences in the trophic niche utilized by crayfish between the lentic and lotic habitats were mainly evident between the larger Mazibekela Dam (D03) and the lotic sites (K01–K03), than between the smaller irrigation dams (D01 and D02). The smaller irrigation dams (< 25 ha.) have a short water residency period because water pumped from the river main channel for irrigation is largely transient. It is therefore more likely that productivity in the two small dams is similar to the Komati River main channel. In contrast, Mazibekela Dam has a larger surface area (~ 206 ha.)

and a longer water residency that provides a relatively more stable environment for the establishment of aquatic macrophytes, as observed elsewhere in man-made impoundments (e.g. Zengeya & Marshall 2008). The larger niche size and lower TP values of crayfish from Mazibekela Dam likely reflect that crayfish are feeding predominantly on macrophytes, compared to crayfish in the main river channel and smaller irrigation dams. In addition, Mazibekela Dam was built to store water pumped from the Komati River in the rainy season, for irrigation in the dry season when river flows are low, but the dam is filling up naturally from its own catchment without the need to pump infill water from the Komati River (Tapela *et al.* 2015). This could lead to differences in productivity amongst habitats within the system related to different nutrient inflow dynamics.

Seasonality also influenced crayfish diet. Niche positions did not differ across seasons, but crayfish caught in summer had a wider niche size than those caught in winter. Food resource availability varies throughout the year in tropical rivers, but crayfish fed largely on vegetative detritus, which is often the most abundant food resource and it is usually available throughout the year (Winemiller & Winemiller 2003). They, however, expanded their niche to include a relatively greater proportion of invertebrate prey in summer, likely to take advantage of increased secondary productivity. Crayfish are known to opportunistically change their diet to take advantage of an increase in animal biomass that is often associated with summer periods (Gherardi *et al.* 2011; Dekar *et al.* 2009; Ercoli *et al.* 2021).

Patterns of resource use and overlap between redclaw crayfish and native species

The are no freshwater crayfish native to South Africa, and the successful establishment of redclaw crayfish in the Inkomati system implies that the species is either utilizing a previously vacant trophic niche or that it can coexist with native species by competitively displacing ecologically similar species. There were significant differences in centroid positions between the redclaw crayfish and three native macroinvertebrate species (African porcelain mussels, red-rimmed melania snails and freshwater shrimps), which indicates that niche partitioning was likely achieved through feeding on different food items. African porcelain mussels are filter feeders (Vaughn & Hakenkamp 2001), red-rimmed melania snails are microphages that feed on benthic algae, detritus and periphyton (Raw *et al.* 2016) and freshwater shrimps are primarily detritivores that feed on benthic algae and detritus (Hart *et al.* 2003).

However, native Sidney's river crabs had significant niche overlap (>60%) with redclaw crayfish.

Sidney's river crabs are omnivores, where adults are mainly herbivores and detritivores, whereas juveniles appear to favour a carnivorous diet (Peer *et al.* 2015). This omnivorous feeding behaviour is well supported by results from this study, where Sidney's river crabs were polytrophic and had a large niche size, inferring a broad-based diet. The broad food niches of redclaw crayfish and Sidney's river crabs likely lead to the observed high niche overlap between the two species. This indicates that the redclaw crayfish is not using a vacant trophic niche in this system, but is instead using similar food resources to native crabs, potentially competitively displacing it. This also indicates that the redclaw crayfish and native *Potamonautes* crabs might have similar functional roles, and the redclaw crayfish could be functionally redundant (see Dunoyer *et al.* 2014). Functional redundancy is the degree to which species perform similar ecosystem functions such that one species may substitute for another (Rosenfeld 2002; Fetzer *et al.* 2015). Functionally similar species can have different impacts on ecosystems (Ercoli *et al.* 2015; Larson *et al.* 2017). The advent of redclaw crayfish in the Inkomati system might lead to adverse ecological impacts on ecosystem structure and function, if they replace the native *Potamonautes* species. For example, in Kenya, invasive red swamp crayfish (*Procambarus clarkii*) have been implicated in the extirpation of a functionally similar native crab species (*Potamonautes loveni*), decline in the abundance of invertebrate species and increased decomposition rates (Jackson *et al.* 2016). There is no evidence yet of similar changes to ecosystem structure and function in the Inkomati system because of the redclaw crayfish invasion, but the species has already managed to achieve greater abundances than native *Potamonautes* crabs. However, the impacts of invasive species can be complex and context-specific (e.g. Usio *et al.* 2009; Ruokonen *et al.* 2014; Kreps *et al.* 2016) and the impact of redclaw crayfish may not increase linearly with higher densities (Kornis *et al.* 2014; Jackson *et al.* 2015). There is, therefore, a need for further studies to examine potential ecological redundancy of redclaw crayfish in the Inkomati system, as it could cause reduced ecosystem function post-invasion. There is also a need to assess the role of intraguild predation in the establishment and persistence of redclaw in the Inkomati system. Populations of crayfish that live in environments that experience fluctuations in food resources often use cannibalism as a 'life boat strategy' to decrease the probability of extirpation, and to increase the long-term persistence of the population (Polis 1981). This is especially pertinent in the Inkomati River, where seasonal patterns of precipitation and hydrology can lead to fluctuations in the availability of habitats and food resources.

AUTHOR CONTRIBUTIONS

Tsungai Alfred Zengeya: Conceptualization (equal); formal analysis (lead); investigation (lead); methodology (lead); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Reuhl Jan-Hendrik Lombard:** Formal analysis (supporting); investigation (supporting); visualization (supporting); writing – review and editing (equal). **Vhutali Ernest Nelwamondo:** Formal analysis (supporting); investigation (supporting); visualization (supporting); writing – review and editing (equal). **Ana Luisa Nunes:** Conceptualization (equal); formal analysis (supporting); investigation (supporting); visualization (supporting); writing – review and editing (equal). **John Measey:** Conceptualization (equal); formal analysis (supporting); investigation (supporting); visualization (supporting); writing – review and editing (equal). **Olaf Weyl:** Conceptualization (equal); formal analysis (supporting); investigation (supporting); visualization (supporting); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may/can be found online in the supporting information tab for this article.

Appendix S1. Centroid positions (corrected carbon (Ccorr) and trophic positions (TP)) and estimates of niche size utilised by redclaw crayfish (*Cherax quadricarinatus*) and Sydney`s river crab (*Potamonautes sidneyi*) in relation to (a) habitat (lotic

and lentic environments) and season (summer and winter) in the Inkomati River Basin.

Appendix S2. Centroid positions (corrected carbon (Ccorr) and trophic positions (TP)) and estimates of niche size utilised by redclaw crayfish (*Cherax quadricarinatus*) in relation to (a) habitat (lotic and lentic environments) and sex in the Inkomati River Basin.