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Trophic niche of invasive murid rodents in urban and peri-urban areas in South Africa: insights from stable isotope analysis

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Abstract

There are three invasive *Rattus* species (*R. norvegicus*, *R. rattus*, *R. tanezumi*) that are known to occur in South Africa. The three *Rattus* species are widespread in urban and peri-urban areas where they have had a significant impact on human health, infrastructure, and biodiversity. There is, however, limited information on their trophic ecology and on how factors that affect access to, and utilization of, food resources facilitate successful establishment. This study applied stable isotope analysis (SIA) to assess the trophic niches of the three *Rattus* species across selected urban and peri-urban landscapes in Gauteng Province. The three *Rattus* species occurred allopatrically: *R. norvegicus* in urban areas (Alexandra and Tembisa townships), *R. rattus* in the peri-urban University of Pretoria Experimental Farm, and *R. tanezumi* in peri-urban areas near Hammanskraal. *Rattus tanezumi* and *R. rattus* exploited broader food resources in peri-urban areas that are used for agricultural activities, while *R. norvegicus* had a more restricted niche in urban environments. Niche breadth expanded in summer, reflecting seasonal food availability, while sex- and age-related differences suggested subtle differences in feeding strategies, although the results were inconsistent across species and sites. Although some limitations, such as the fact that the three *Rattus* species were sampled in allopatry and missing baseline resources constrained interpretation, the study provides insights into how invasive rats adapt to different environments. Future research combining stable isotope analysis with long-term monitoring data will be essential to clarify niche partitioning and guide effective management of invasive rat populations.

Keywords *Rattus norvegicus*, *Rattus rattus*, *R. tanezumi*, Isotopic niche, Gauteng Province, South Africa

Introduction

Three invasive murid rodent species of *Rattus* (*R. norvegicus*, *R. rattus*, and *R. tanezumi*) occur in South Africa, and are particularly prevalent in urban and peri-urban informal settlements [1]. Informal urban and peri-urban settlements are often ideal for rat populations to proliferate because they are characterised by high human population densities and a lack of basic infrastructure, such as access to clean water, sanitary infrastructure, waste management, and adequate housing [2–5]. Rat infestations in these informal settlements have been implicated in causing major impacts through the transmission of diseases

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and parasites, threats to food security, damage to infrastructure, and threats to biodiversity [6–12].

Rattus species are known to occur in both sympatry and syntopy in their native range [13]. Sympatric species co-occur in the same region, while syntopic species are found within the same habitat [14]. For example, in our study, the three *Rattus* species occurred within Gauteng province (sympatry), however, they did not co-occur in their respective habitats (allopatry). The spatial scale at which they interact determines whether they can be referred to as being sympatric, syntopic, or allopatric species [15]. There are relatively few studies on the behaviour of *Rattus* species to allow insights into how they partition their resources (e.g., food and locality) when they occur in syntopy, e.g [16–18]. , . This is largely due to their neophobic behaviour and their use of subterranean tunnel systems [19]. The three *Rattus* species have broad distributional ranges in their native and invasive ranges [20]. While the three species may be able to co-exist through niche partitioning, information on their patterns of resource use in invaded areas is limited. Niche partitioning is the process by which, due to differential resource allocation, competition is reduced in species that utilize the same resources/niches, resulting in their coexistence [21, 22]. The co-occurrence of different *Rattus* species in the same habitat may lead to niche shifts, displacement through competition [23, 24], or niche complementarity due to differences in resource use, e.g [25]. , . For example, *R. norvegicus* has been reported to out-compete and displace *R. rattus* because of its relatively larger body size and aggressive nature [26], while *R. tanezumi* can displace other *Rattus* species through scent-marking, which is strong enough to repulse even the larger and more aggressive *R. norvegicus* [27]. *Rattus* species can also co-exist in syntopy through niche complementarity for food resources and habitats. For example, *R. rattus* prefers elevated locations such as the upper floors of buildings, roofs, and ceilings that are not preferred by *R. norvegicus* [2, 28], while *R. tanezumi* can utilise both indoor and outdoor habitats such as agricultural fields and forests [29].

A broad and flexible generalist diet has been suggested as key to the invasion success of *Rattus* species [30, 31]. Invasive *Rattus* species are omnivores that feed on a wide range of food items, which include invertebrates, vertebrates, and plants, e.g [17, 32–34]. , . They are also known to adjust their feeding niche to opportunistically feed on what is available. For example, the invasive *R. rattus* has been shown to have a broad and flexible diet that changes seasonally and differs among habitats, indicating trophic plasticity [31, 35]. Similar plasticity in feeding niches has also been noted for *R. norvegicus*, where populations in modified habitats such as urban areas had constricted and less diverse diets than in natural habitats [36]. *Rattus norvegicus*, *R. rattus*, and *R. tanezumi* have been widely introduced globally and co-occur in many areas across their invasive ranges [20]. It is likely that these three *Rattus* species can co-exist through niche complementarity of resources such as food, e.g [17, 31, 37]. , .

This study applied trophic niche analysis to assess patterns of food resource use among *Rattus* species in selected urban and peri-urban areas in South Africa. 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 areas of introduction, e.g [31, 38]. , . We used stable isotope analysis (SIA) to identify and quantify niche dimensions (niche width and size) and to elucidate trophic interactions (niche overlap and complementarity). SIA of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) can be used to assess resource partitioning by determining the isotopic composition of various tissues to trace food assimilated over time [39]. $\delta^{15}N$ is enriched by ~ 3.4‰ per trophic level and can be used to determine the trophic position of a consumer, while $\delta^{13}C$ is enriched by ~ 1‰ and can be used to identify food sources [40, 41]. The premise that $\delta^{13}C$ and $\delta^{15}N$ values obtained from a consumer reflect those of its diet has been widely applied to assess the trophic structure of ecological communities [39, 42–44].

This study used stable isotope analysis to infer the trophic ecology of invasive *Rattus* species found in peri-urban and rural areas in Gauteng Province, South Africa. We predict that the generalist and flexible feeding strategy of *Rattus* species allows them to shift the variety and type of food items utilized by altering their feeding strategy in response to differences in food availability among habitats and seasons, and food selectivity linked to life history traits (e.g., sex and age).

Materials and methods

Study species and study area

The three *Rattus* species occurred allopatrically at each study site (Table 1). *Rattus norvegicus* was sampled at Alexandra township (-26.1038° S; 28.0962° E) and Tembisa (-26°S; 28.214°E) - just outside the City of Johannesburg (Fig. 1). The townships are characterized by high-density housing units that are interspaced with informal settlements. *Rattus rattus* was sampled from the University of Pretoria (UP) Experimental Farm (-25.749°S 28.238°E), located 5 km to the east of the City of Pretoria, and is used for agricultural activities such as animal husbandry and crop production. *Rattus tanezumi* was sampled on a small holding property in the peri-urban area just outside of Hammanskraal township (-25° 23' 59.99" S, 28° 16' 60.00" E), 46 km to the north of the City of Pretoria. Smallholdings are parcels of land measuring

Table 1 Composition of trap catches of three *Rattus* species from urban (Alexandra and Tembisa) and peri-urban areas (University of Pretoria experimental farm and Hammanskraal) of Gauteng Province, South Africa

Species	Sampling site	Sex		Age			Season			
		Female	Male	Adult	Juvenile	Subadult	Autumn	Spring	Summer	Winter
Rattus norvegicus	Alexandra	132	135	150	27	90				267
	Tembisa	42	48	54	15	21	30		60	
Rattus rattus	UP Experimental Farm	24	57	30	27	24	21	3	54	3
Rattus tanezumi	Hammanskraal	15	18	15	9	9	3	6	15	9

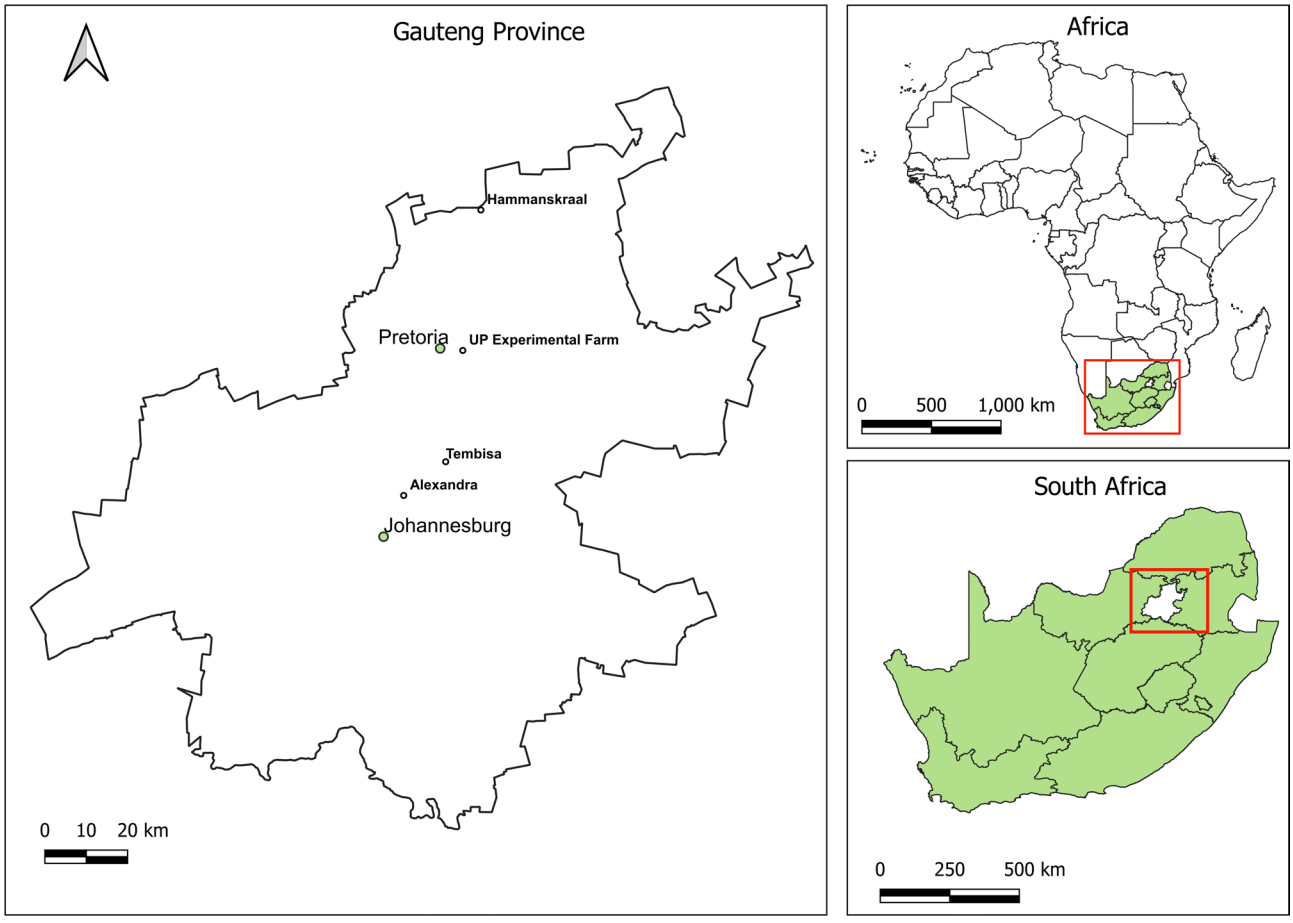

Fig. 1 A map (with an insert of Africa and South Africa) showing the sampling sites of *Rattus norvegicus* (Alexandra and Tembisa Townships), *R. rattus* (University of Pretoria (UP) Experimental Farm), and *R. tanezumi* (Hammanskraal) in Gauteng Province, South Africa

less than 20 hectares in size that are used for small-scale agricultural activities, such as animal husbandry and limited crop production. The study sites were selected based on confirmed species occurrence data sourced from previous studies [1, 10, 45, 46].

Ethics approval

Rattus norvegicus, *R. rattus*, and *R. tanezumi* samples were sourced between December 2010 and June 2012 and then genetically identified (through Cyt *b* mtDNA sequencing) in previous studies [1, 10, 45, 46]. These studies used a variety of methods to obtain samples, including Sherman traps (H.B. Sherman Traps Inc., Florida, U.S.A.) and snap traps, as well as donations received opportunistically from members of the public, pest control companies, or through an invasive synanthropic rodent extermination program instituted by the Johannesburg Metropolitan Municipality, Gauteng Province, South Africa. Live-trapped samples were collected under a permit from the Gauteng`s Provincial Department of Nature Conservation, Johannesburg, South Africa (Permit number CPF6 0032), and the approval of animal capture and care procedures of the University of Pretoria Animal Ethics Committee, Pretoria, South Africa (Ethics approval number ECO25–10) and were euthanized using either isoflurane (administered by a

veterinary professional) or CO_2 inhalation. Permission to conduct this study was granted in terms of Sect. 20 of the Animal Diseases Act (Act no. 35 of 1984) by the South African Department of Forestry, Fisheries, and the Environment (Permit number 13788). All specimens are currently housed in the Department of Zoology and Entomology at the University of Pretoria, Pretoria, South Africa, and after which they will be deposited in the Ditsong Museum of Natural History, Pretoria, South Africa.

Sample preparation

Specimens were weighed, liver and gastrocnemius muscle dissected out, and hair samples clipped from the postero-dorsal part of the animal, near the base of the tail, in a Level 2 biosecurity laboratory in the Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa. The categorization of relative age classes in both species was based on craniometric analysis [47] that grouped tooth wear classes into juveniles (tooth wear class I), subadults (II–III), and adults (IV–V) based on maxillary molar eruption and wear. A list of the *Rattus* species sampled is grouped by relative tissue type, locality, sex, age, and season, and indicated in Table S3.

Liver and gastrocnemius muscle tissue samples were dried for 24 h at 70° C in a drying oven after which they were pulverised either with a mortar and pestle or a homoginiser (Quigen Tissuelyser II, Quigen, Venlo, Netherlands). Hair samples were pre-treated in a solution with a ratio of 2:1 of chloroform: ethanol to remove residues before being dried and stored in Eppendorf tubes [48]. Minute quantities of each sample were transferred into tin capsules and weighed using a Mettler Toledo MX5 microbalance (Mettler-Toledo GmbH Laboratory and Weighing Technologies, Greifensee, Switzerland) with an accuracy of 3 decimal places. The ideal weight of each sample contained in each tin capsule was between 0.600 and 0.800 mg (Thermo Fischer, Bremen, Germany).

Stable isotope analysis

SIA was conducted on a Flash Elemental Analyser 1112 Series coupled to a delta V plus stable light isotope ratio mass spectrometer via a ContFlo IV system (all the equipment was supplied by Thermo Fischer, Bremen, Germany) at the Stable Isotope Laboratory, Mammal Research Institute (MRI), University of Pretoria, Pretoria, South Africa. A laboratory running standard (Merk Gel: $\delta^{13}C$ = – 20.6‰; $\delta^{15}N$ = 6.8‰; C% = 43.8%; N% = 14.6%) and a blank sample were run after every 12 unknown samples. All the results were referenced to Vienna Pee–Dee Belemnite (VPDB; [49]) for carbon isotope values and to air [50] for nitrogen isotope values. The results were expressed in delta notation using a per mille scale (Eq. 1):

$$\delta \mathrm{X}\,(‰) = \left[\left(R_{sample} - R_{standard}\right)/R_{standard}\right] \times 1000 \quad (1)$$

Where, X = ^{15}N or ^{13}C and R represents $^{13}C/^{12}$ or ^{15} N/^{14}N, and analytical precision was < 0.2‰ for both δ^{15}N and δ^{13}C.

Statistical analysis

The stable isotope data generated for *R. norvegicus*, *R. rattus*, and *R. tanezumi* were summarised using independent box plots of δ^{15}N and δ^{13}C values with reference to tissue type, locality, sex, age, and season. The δ^{15}N and δ^{13}C data were tested for normality and homogeneity of variances by means of Shapiro-Wilk and Bartlett tests, respectively, and then subjected to independent analysis of variance (ANOVA) to assess the effect of tissue type, locality, sex, age, and season. Where statistically significant differences were detected ($\alpha < 0.05$), Tukey's *post hoc* tests were used to identify non-significant differences between subsets ($\alpha > 0.05$) in factors with more than two categories that included tissue type and age class. A Welch *F* test was used in cases of unequal variances. Stable isotope values can be influenced by several factors, such as tissue type [37], life history traits (e.g., age and sex) [38], and location [39].

A preliminary analysis indicated that stable isotope values varied among tissue types; therefore, subsequent analysis to assess the effect of locality, sex, age, and season on the isotopic niches of the rats was restricted to hair samples (Appendix S1). Hair integrates the isotopic composition of the food consumed over long periods (weeks to months) relative to other body tissues, such as muscle and liver tissues (hours to days), making it ideal for assessing dietary changes that may persist over similar time scales or longer [51–54].

The package, Stable Isotope Bayesian Ellipses R (SIBER); [55] was used to estimate the isotopic niche size utilised by the *Rattus* species in relation to tissue type, locality, sex, age, and season. The standard ellipse area (SEA) was calculated from the variance and covariance of the sample data and converted into a sample-size corrected standard ellipse area (SEAc) that encompassed 40% of the sample data. SEAc is robust and less sensitive to the effects of sample size. The actual niche width of the sampled population can potentially be underestimated due to small sample sizes, resulting in the promulgation of inaccurate data when comparing between populations that have different sample sizes. Niche overlap among ellipses was calculated following (Eq. 2):

$$\%\ \text{overlap} = \frac{Area\,of\,Overlap}{(SEAc1 + SEAc2 - Area\,of\,Overlap)} \times 100 \quad (2)$$

Where SEAc 1 and 2 represented the isotopic niche area utilised by each population and/or species, and area of

overlap represented the area overlapped by SEAc 1 and 2. The extent of overlap was classed as either low (< 40%), moderate (40–60%), or high (>60%). Niche overlap values >60% were regarded as being statistically significant [56, 57]. All statistical analyses were undertaken using algorithms in Palaeontological Statistics (PAST) version 3.15 [58] and in R version 3.4.3 [59].

Results

The three *Rattus* species occurred allopatrically, *R. norvegicus* was sampled in Alexandra and Tembisa Townships, *R. rattus* at UP Experimental Farm, and *R. tanezumi* in Hammanskraal. Sex, age and season had disparate effects on isotopic niches utilized by *Rattus* species across the four sites. Sex had no statistically significant effect on the mean $\delta^{15}N$ and $\delta^{13}C$ values of *R. norvegicus* from Alexandra, but there were significant differences for *R. norvegicus* from Tembisa, where males were $\delta^{15}N$-enriched than females, and females were $\delta^{13}C$-enriched than males (Fig. 2, Table S2). Sex had a significant effect (SIBER: $p < 0.05$) on the isotopic niche space utilised by *R. norvegicus* at both sites, where males had a larger isotopic niche space than females (Table 2).

Age had a statistically significant effect on the mean $\delta^{15}N$ and $\delta^{13}C$ values of *R. norvegicus* from Alexandra but not for individuals from Tembisa (Fig. 3). In Alexandra, juveniles had enriched $\delta^{15}N$ and $\delta^{13}C$ values relative to the other age classes. Age, however, had a statistically significant effect (SIBER: $p < 0.05$) on the isotopic niche space utilised by *R. norvegicus* across the two sites (Table 2). In Alexandra, juvenile *R. norvegicus* had a larger isotopic niche than sub-adults and adults. In contrast, adults had the largest niche size in Tembisa. *Rattus norvegicus* from Alexandra were only sampled in winter; therefore, no seasonal analysis was done. *Rattus norvegicus* from Tembisa were sampled in autumn and summer, but season had no significant effect on the mean $\delta^{15}N$ and $\delta^{13}C$ values (Fig. 4). However, season had a significant effect (SIBER: $p < 0.05$) on isotopic niche size. Niche size was significantly higher in summer than in autumn

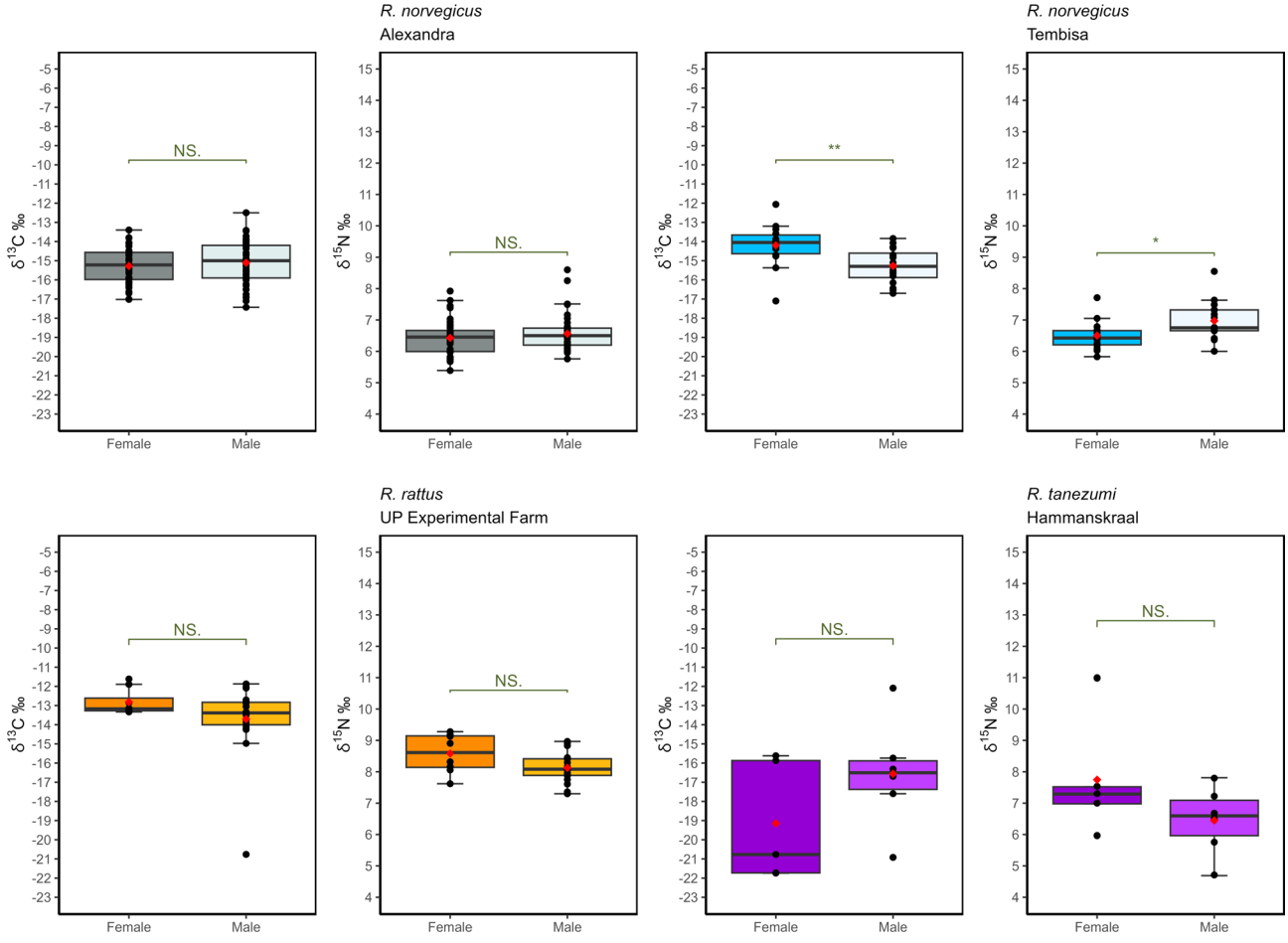

Fig. 2 Box plots of stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) values with reference to sex for populations of *Rattus norvegicus* from Alexandra (grey) and Tembisa Townships (blue), *R. rattus* from the University of Pretoria Experimental Farm (orange), and *R. tanezumi* from Hammanskraal Township (purple) in Gauteng Province, South Africa. Statistically significant differences are indicated by the asterisks above each pair being compared (level of significance: *** = 0.001, ** = 0.01, or * = 0.05), while non-significant differences are indicated by NS

Table 2 Estimates of isotopic niche area (sample size-corrected standard ellipse area) of populations of *Rattus norvegicus* from Alexandra and Tembisa Townships, *R. rattus* from the University of Pretoria experimental Farm, and *R. tanezumi* from Hammanskraal Township in Gauteng Province, South Africa

Species	Locality	Sex		Age			Season	
		Female	Male	Juvenile	Sub-adult	Adult	Summer	Autumn
R. norvegicus	Alexandra	1.5	1.9	3.1	1.3	1.4		
	Tembisa	1.8	1.9	0.9	1.8	2.6	2.6	1.4
R. rattus	UP Experimental Farm	1.2	2.8	0.7	4.9	0.9	3.6	0.7
R. tanezumi	Hammanskraal	23.6	12.3	23.5	6.1	4.7		

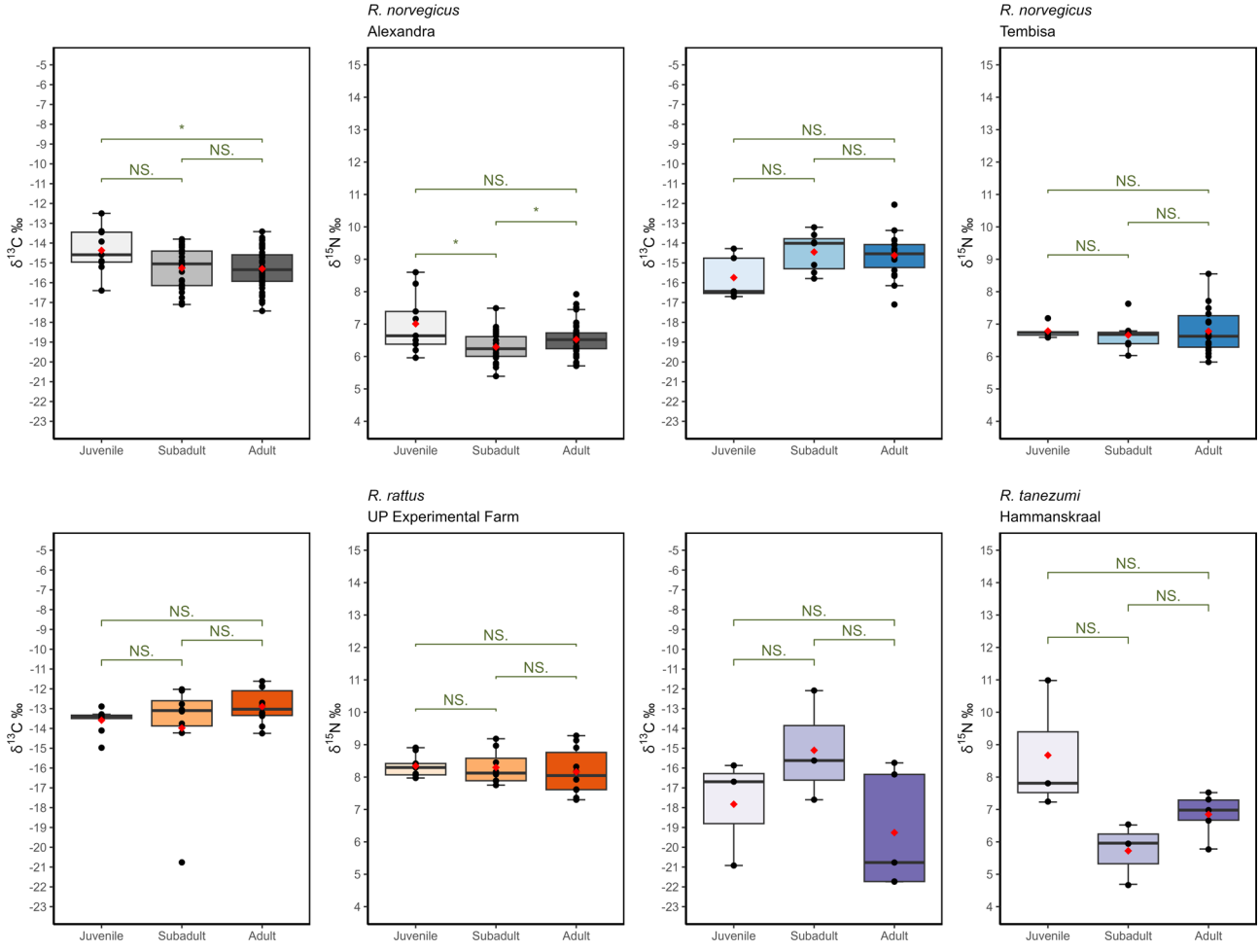

Fig. 3 Box plots of stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) with reference to age for populations of *Rattus norvegicus* from Alexandra (grey) and Tembisa Townships (blue), *R. rattus* from the University of Pretoria Experimental Farm (orange), and *R. tanezumi* from Hammanskraal Township (purple) in Gauteng Province, South Africa. Statistically significant differences are indicated by the asterisks above each pair being compared (level of significance: *** = 0.001, ** = 0.01, or * = 0.05), while non-significant differences are indicated by NS

(Table 2). There were no significant interactions among factors (sex, age, and season) for *R. norvegicus* from Alexandra and Tembisa.

Sex had a significant effect on the mean $\delta^{15}N$ values of *R. rattus* from UP Experimental Farm, where females were $\delta^{15}N$-enriched relative to males (Fig. 2). However, sex had no significant effect on the mean $\delta^{13}C$ values. Sex had a statistically significant effect (SIBER: $p < 0.05$) on the isotopic niche space of *R. rattus*, where males had a larger isotopic niche space than females (Table 2). Age and season had no effect on $\delta^{15}N$ and $\delta^{13}C$ values of *R. rattus* (Figs. 3 and 4); however, both had a significant effect (SIBER: $p < 0.05$) on the isotopic niche size (Table 2). Sub-adults had larger niche sizes than other age classes, and niche size was significantly higher in summer than autumn. There were no significant interactions among factors (sex, age, and season).

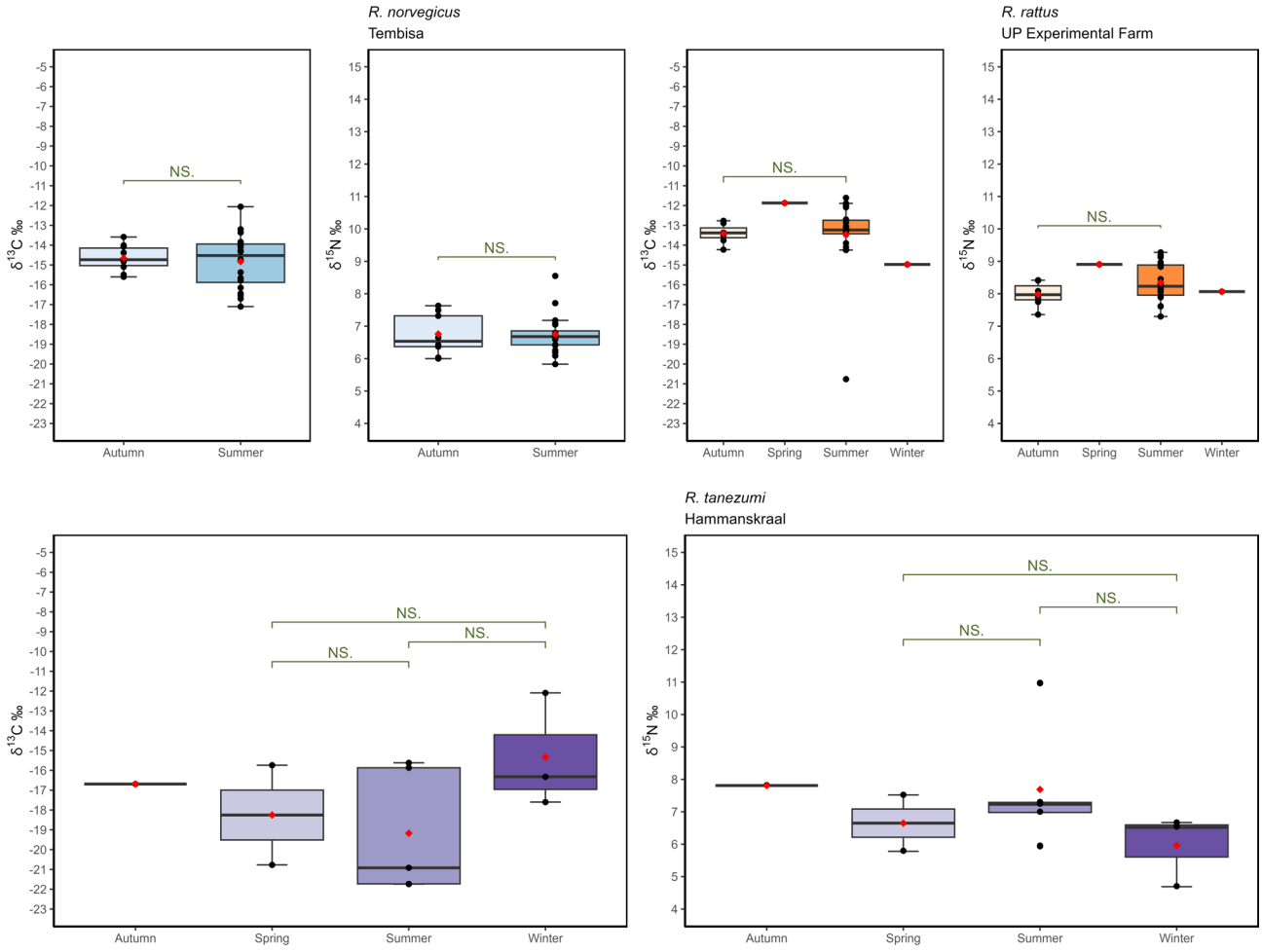

Fig. 4 Box plots of stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) with reference to season for populations of *R. norvegicus* from Tembisa Township (blue), *R. rattus* from the University of Pretoria Experimental Farm (orange), and *R. tanezumi* from Hammanskraal Township (purple) in Gauteng Province, South Africa. There were no statistically significant differences between boxplots; *Rattus norvegicus* from Alexandra township was only sampled in winter and is not shown. Non-significant differences are indicated by NS

Sex, age, and season had no significant effect on the mean $\delta^{15}N$ and $\delta^{13}C$ values of *R. tanezumi* from Hammanskraal (Figs. 2, 3 and 4). However, both variables had a significant effect (SIBER: $p < 0.05$) on the isotopic niche size. Females utilised a larger isotopic niche space than males; niche size was significantly higher in summer than in spring and winter, and juveniles had a larger niche than sub-adult and adults (Table 2). There were no significant interactions among factors (sex, age, and season).

Location had a significant effect on the mean $\delta^{15}N$ and $\delta^{13}C$ values of the three species across the four sampling sites (Fig. 5). *Rattus rattus* from the UP Experimental Farm had enriched-$\delta^{15}N$ values relative to the other two species. $\delta^{13}C$ values of *R. norvegicus* from Tembisa and Alexandra were similar but significantly different from *R. rattus* from the UP Experimental Farm and *R. tanezumi* from Hammanskraal. Similarly, location had a statistically significant effect (SIBER: $p < 0.05$) isotopic niche. *Rattus tanezumi* from Hammanskraal had the largest isotopic niche, followed by *R. rattus* from the UP Experimental Farm, and then *R. norvegicus* from Tembisa and Alexandra (Fig. 6).

Discussion

This study employed stable isotope analysis to infer the trophic ecology of invasive *Rattus* species found in urban and peri-urban areas of Gauteng Province, South Africa. The three *Rattus* species are omnivorous generalists that feed on a wide variety of food items, such as invertebrates, vertebrates, and plants [31, 33, 35, 36]. The three *Rattus* species occurred allopatrically and had different isotopic niches, which implies that they might be utilising different food resources. *Rattus tanezumi* and *R. rattus* were sampled in peri-urban areas and had relatively larger isotopic niches than *R. norvegicus*, which was sampled in urban areas of Alexandra and Tembisa. Food resources are likely to be more diverse in peri-urban landscapes that are utilized for agriculture;

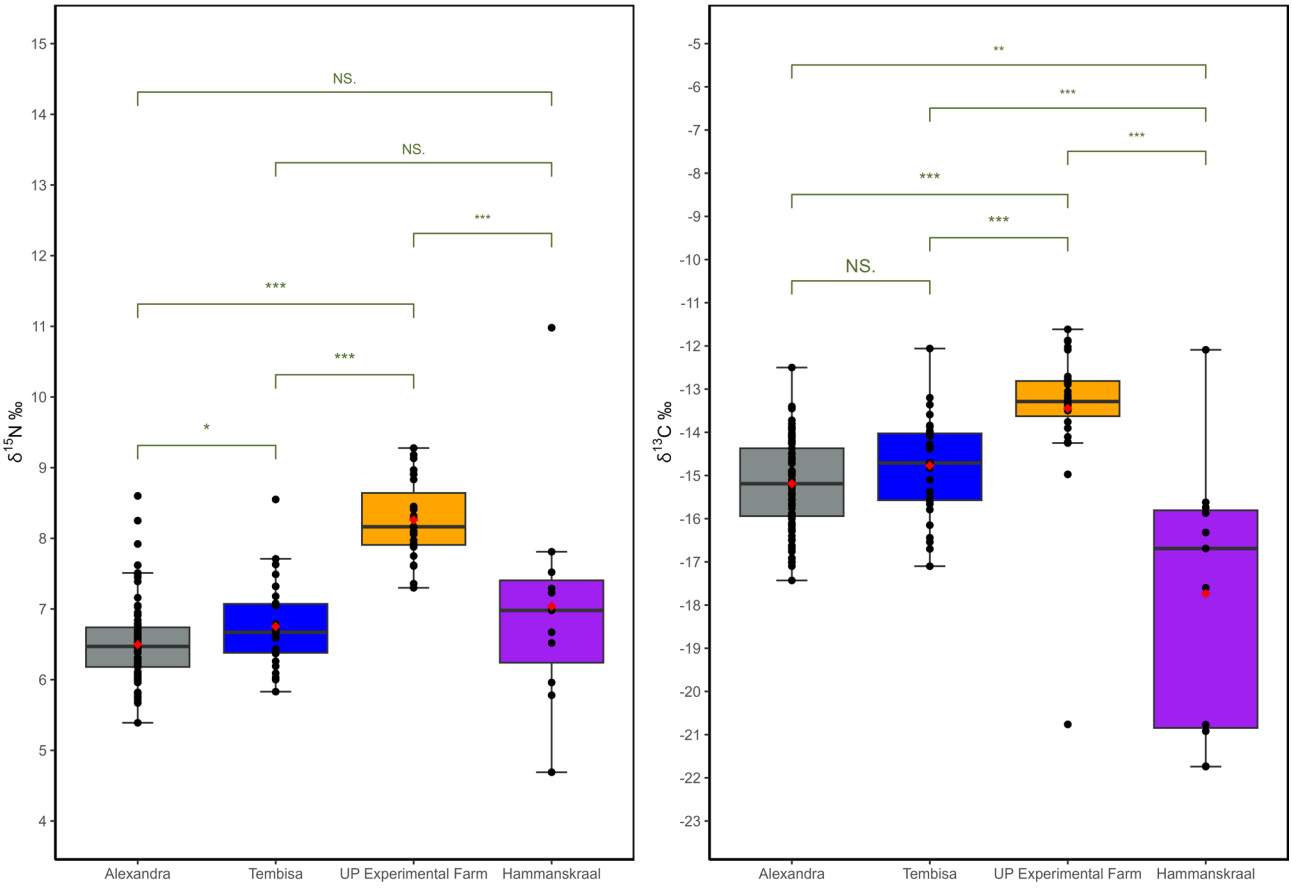

Fig. 5 Box plots of stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) for populations of *Rattus norvegicus* from Alexandra (grey) and Tembisa Townships (blue), *R. rattus* from the University of Pretoria Experimental Farm (orange), and *R. tanezumi* from Hammanskraal Township (purple), Gauteng Province, South Africa. Significant differences are indicated by the asterisk above each pair being compared (level of significance: *** = 0.001, ** = 0.01, or * = 0.05), while non-significant differences are indicated by NS

therefore, *R. tanezumi* and *R. rattus* might subsist on a wider range of food sources [31, 60]. The differences in niche size between *R. tanezumi* and *R. rattus* might be related to different feeding preferences and habitats found between the two peri-urban areas. *Rattus rattus* was sampled at the University of Pretoria Experimental Farm, which is dominated by cultivated land with a few scattered buildings that are used for animal husbandry. In contrast, *R. tanezumi* was caught on a smallholding property in Hammanskraal, which is used for small-scale farming and lifestyle purposes. Enriched mean $\delta^{13}C$ values (13.4‰ ± 1.7) of *R. rattus* from the UP Experimental Farm suggest a diet based on C_4 plants, likely from crops or animal feed [46]. In contrast, the depleted $\delta^{13}C$ values (-17.7‰ ± 3.1) of *R. tanezumi* suggest a diet based on C_3 plants, likely from natural vegetation found around the small holding. This is similar to observations in other locations, where *Rattus* species exhibit a broader stable isotope niche in natural environments compared to agricultural fields [20]. The restricted niche size of *R. norvegicus* is more typical of high-density urban areas such as Tembisa and Alexandra, where rats likely feed opportunistically on a limited range of food resources obtained from refuse material and pilfering from households [4]. Similarly, a recent study found that the trophic niche of urban rats was more homogenous in urban rats relative to their rural counterparts likely as a result of rats largely consuming human-derived food in urban areas; in comparison, rural rats, where food was scarcer tend to feed on a wider range of food items and as such had a more heterogenous trophic niche [36].

Season did not affect the mean isotopic values, but it did significantly influence niche size. *Rattus* species have a flexible generalist diet and can switch their diet to feed on what is seasonally available [31]. Sex and age also influenced mean isotopic values and niche size, although patterns were inconsistent across species and sites. For example, males of *R. norvegicus* and *R. rattus* had a larger isotopic niche space than females; in contrast, females of *R. tanezumi* had a larger isotopic niche space than males. Research elsewhere on the influence of sex on the trophic niche of *Rattus* species is equally confounding. Some

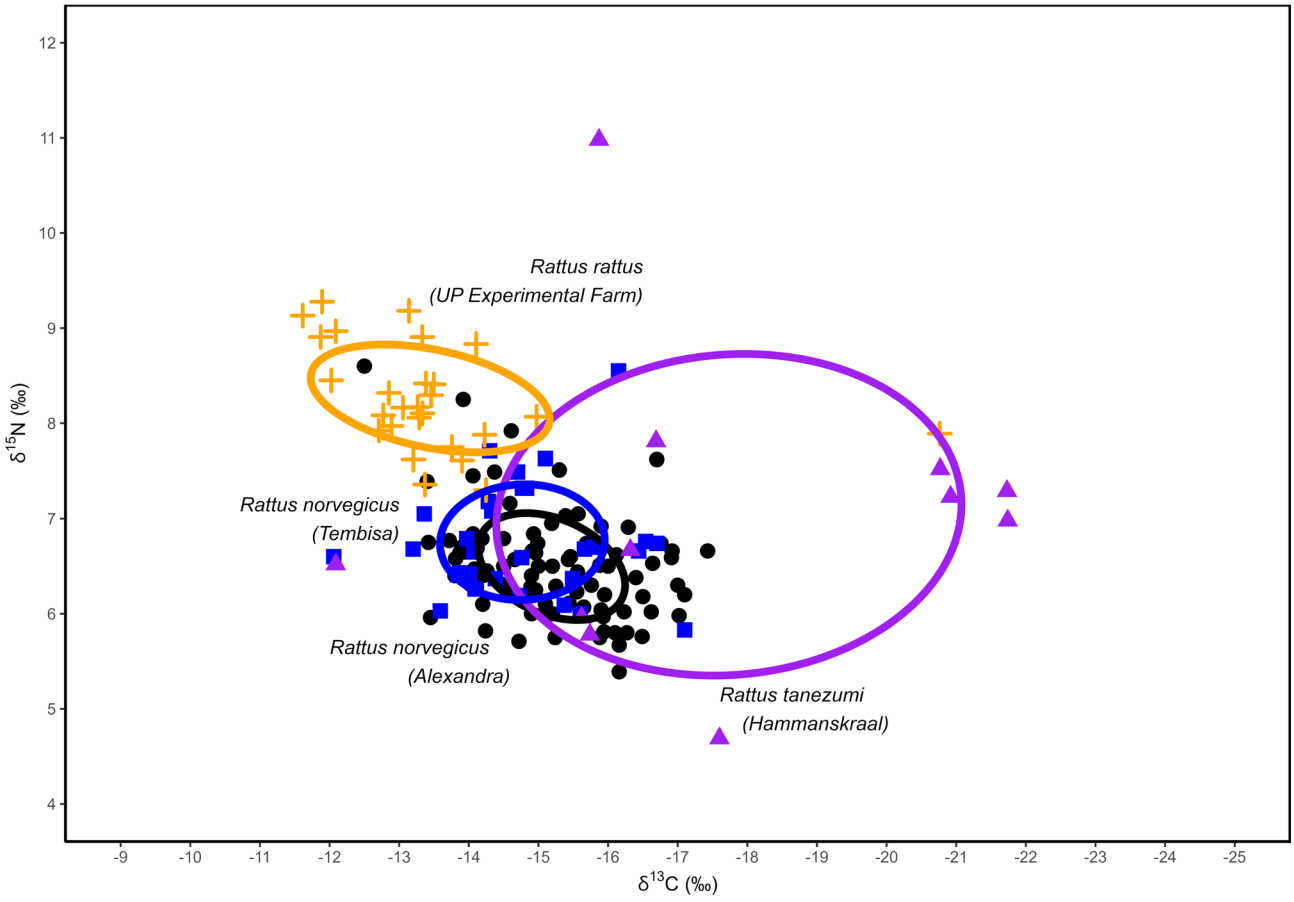

Fig. 6 Stable isotopic biplot of stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) for individuals of *Rattus norvegicus* from Alexandra (black circle) and Tembisa (blue square) Townships, *R.rattus* from the University of Pretoria Experimental Farm (orange cross), and *R. tanezumi* from Hammanskraal (purple triangle), Gauteng Province, South Africa

studies have found no differences based on sex on the trophic niche utilised by *R. tanezumi* and *R. rattus*, e.g [32]. , , but others have noted differences between sexes that were attributed to differential growth patterns [38, 48], behavioural differences in dispersal patterns [49], and foraging strategies [50]. Similarly, age-related differences may reflect subtle differences in feeding preferences among age classes, such as juveniles consuming more protein-rich food to meet their growth requirements [61, 62]. One possibility is that some *R. norvegicus* juveniles from Alexandra were in the process of being weaned off milk from their mothers, which could explain the elevated $\delta^{15}N$ and $\delta^{13}C$ values relative to both subadults and adults. Since suckling young acquire nutrients from their mother's milk, their isotopic signatures tend to be higher than those of their mothers [63].

Several limitations constrain the interpretation of the results from this study. Although the three *Rattus* species were sampled in sympatry, they were allopatric in their habitats, which limited insights into patterns of potential niche partitioning. This is despite us sampling sites such as Tembisa and Hammanskraal, where previous studies have found the species to co-occur [1]. Small sample sizes, likely due to the stochastic nature of trapping success, may also have obscured interactions among sex, age, and season, as catch data were largely skewed across the three factors. Future studies could also include additional sites where the *Rattus* species have been found to co-occur syntopically, e.g [1]. , , and implement a regular monitoring regime through repeated trapping efforts that could help increase the sample size and counteract catch variability, which could reveal more robust estimates of the trophic niche utilised by the three *Rattus* species. Basal resources were not collected to help infer the stable isotope values. This is partly due to the sampling strategy we used, where some of the samples were obtained opportunistically from pest control companies and from previous studies that assessed other aspects of the rat`s biology. It was therefore not possible to collect food items from the environment to help delineate the food web structure. The dietary preferences of wild rats are difficult to discern due to the environment that they inhabit, as well as their behaviour. Gut content analysis could be used to circumvent this, as the rats are likely to be better

samplers of the food items available in the environment than humans collecting putative samples. Gut content analysis was attempted; however, this proved difficult due to the high percentage of empty stomachs and macerated food items that were difficult to identify. This, again, could be improved on by increasing the sample size and augmenting gut content analysis with molecular techniques to help identify ingested prey items, e.g [52, 53]. , . Furthermore, when combined with other techniques, such as telemetry, information about the trophic niche can provide a more comprehensive understanding of the foraging behaviour of rats in urban and peri-urban areas. This knowledge could have implications for developing long-term control strategies, not just within the province, but also in other regions.

In summary, this study demonstrates that the trophic niches of the three *Rattus* species in Gauteng Province differ across habitats. *Rattus tanezumi* and *R. rattus* exploited broader food resources in peri-urban areas that are used for agricultural activities, while *R. norvegicus* had a more restricted niche in urban environments. Niche breadth expanded in summer, reflecting seasonal food availability, while sex- and age-related differences suggested subtle differences in feeding strategies, although these results were inconsistent across species and sites. Although some limitations, such as the fact that the three *Rattus* species were not sampled in syntopy, as well as the fact that missing baseline resources constrained interpretation, the study provides insights into how invasive rats adapt to different environments. Future research combining stable isotope analysis with long-term monitoring data will be essential to clarify niche partitioning and guide effective management of invasive rat populations.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40850-025-00243-1.

Supplementary Material 1

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Author contributions
All authors contributed to the study conception and design, material preparation, data collection and analysis. The first draft of the manuscript was written by Gordon Ringani, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability
All experiments were performed in accordance with relevant guidelines and regulations. Live-trapped samples were collected under a permit from the Gauteng`s Provincial Department of Nature Conservation, Johannesburg, South Africa (Permit number CPF6 0032), and the approval of animal capture and care procedures of the University of Pretoria Animal Ethics Committee, Pretoria, South Africa (Ethics approval number ECO25–10) and were euthanized using either isoflurane (administered by a veterinary professional) or CO2 inhalation. Permission to conduct this study was granted in terms of Sect. 20 of the Animal Diseases Act (Act no. 35 of 1984) by the South African Department of Forestry, Fisheries and the Environment (Permit number 13788). All specimens are currently housed in the Department of Zoology and Entomology at the University of Pretoria, Pretoria, South Africa, and, after which, they will be deposited in the Ditsong Museum of Natural History, Pretoria, South Africa.

Declarations

Ethical approval
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Competing interests
The authors declare no competing interests.

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