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Trophic niche changes associated with the eradication of invasive mammals in an insular lizard: an assessment using isotopes

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Abstract

Invasive species are a major threat to island biodiversity, and their eradications have substantially contributed to the conservation of island endemics. However, the consequences of eradications on the trophic ecology of native taxa are largely unexplored. Here, we used the eradication of invasive black rats *Rattus rattus* and European rabbits *Oryctolagus cuniculus* from the Berlenga Island, in the western coast of Portugal, as a whole-ecosystem experiment to investigate the effects of the eradication of invasive mammals on the trophic niche and body dimensions of the island-restricted Berlenga wall lizard *Podarcis carbonelli berlengensis* over a 2-year period. Our results suggest an expansion of the isotopic niche and an intensification of the sexual dimorphism of the lizard following mammal eradication. Additionally, we found considerable variability in isotopic niche across the island and detected evidence of sex-specific and season-modulated nutritional requirements of this threatened reptile. Our findings support that the eradication of 2 of the planets most problematic invasive vertebrates led to changes in the lizard trophic niche and sexual dimorphism in just 2 years. This suggests that the ecological pressuresfor example, prey availability and habitat structureto which lizards are exposed have substantially changed post-eradication. Our study emphasizes the scientific value of island eradications as experiments to address a wide range of ecological questions and adds to the increasing body of evidence supporting substantial conservation gains associated with these restoration interventions.

Key words: food-webs, invasive species eradication, *Oryctolagus cuniculus*, *Podarcis carbonelli berlengensis*, *Rattus rattus*, trophic seasonality

Islands often harbor a considerable diversity of endemisms, resulting from evolutionary processes to which insular species are subjected over time (Jansson 2003; Kier et al. 2009; Jønsson and Holt 2015). Yet, island ecosystems are particularly vulnerable to biological invasions (Sakai et al. 2001; Courchamp et al. 2003) and due to unprecedented rates of human-aided transport of species to areas outside their native ranges, many formerly isolated islands are now hotspots of established non-native species (Dawson et al. 2017; Silva-Rocha et al. 2019).

Invasive species impact native island taxa through multiple direct and indirect mechanisms, including competition and niche

displacement (Clout and Russell 2008), acting as disease vectors (Blackburn et al. 2010), altering plant communities (Herrera-Giraldo et al. 2019) and by changing habitat structure (Davis 2009). Although invasive species can greatly affect key trophic community properties such as food-web structure and food-chain length (Feit et al. 2020), the effects of invasive species on the trophic processes of insular ecosystems remain poorly studied (but see e.g., Graham et al. 2018; Pringle et al. 2019).

Because the nutrients from preys are incorporated into the tissues of consumers (Bond and Jones 2009), the analysis of stable isotopes offers a viable option to investigate invasive species-mediated changes in food webs (Bond and Jones 2009). It is possible to ascertain the main source of organic carbon and the trophic level of a species by quantifying the fractionation of the ^{13}C and ^{15}N isotopes (Post 2002). For instance, marine carbon sources have an isotopic ratio (^{13}C) more depleted in ^{13}C (and therefore ^{13}C values closer to zero) when compared with continental carbon sources (Briggs et al. 2012), whereas ^{15}N allows to assess the trophic level of a consumer and its trophic relationships (higher values indicate higher trophic levels; Barrett et al. 2005; Jackson et al. 2011; Layman et al. 2012). These 2 stable isotopes can be used to infer the trophic niche width of a species, which is related to the diversity of resources consumed (Fink et al. 2012). For instance, Barrett et al. (2005) found that ^{15}N values in tissues of arthropodivorous lizards were higher in islands with seabirds and this was due to guano-mediated nitrogen input into the soil, which was incorporated into plants, that were in turn consumed by insects, the lizards' main prey (Barrett et al. 2005).

Mammalian invaders, such as rats *Rattus* spp. and the European rabbit *Oryctolagus cuniculus* have contributed to the rarefaction and extinction of numerous island-restricted species by a multitude of factors that include direct predation and changes to habitat structure and/or to the abundance and availability of feeding resources (Courchamp et al. 2003; Clout and Russell 2008; Rodriguez-Cabal et al. 2009; Tershy et al. 2015; Bellard et al. 2016; Rocha et al. 2017; Holmes et al. 2019). Accordingly, over the last decades, invasive mammals have been targeted by an increasing number of island restoration projects that involve their eradication (Courchamp et al. 2003; Buckley and Jetz 2007; Olivera et al. 2010; Courchamp et al. 2014). As islands are usually isolated systems, the probability of re-establishment of mammalian invaders is reduced when compared with mainland and as such eradications are a particularly effective conservation intervention in insular ecosystems (Courchamp et al. 2003; Blackburn et al. 2010; Holmes et al. 2016).

The Berlenga Island is part of the Berlengas archipelago, located on the continental shelf of the Iberian Peninsula west coast. It is home to multiple endemic plant species (Gomes et al. 2004; Nascimento et al. 2019) and is an important nesting site for several seabird species (Oliveira et al., unpublished data). The island was until recently inhabited by 2 species of reptiles, the Berlenga wall lizard *Podarcis carbonelli berlengensis* and the ocellated lizard *Timon lepidus*. The latter is now presumed locally extinct, as it has not been detected for over 10 years (Oliveira et al., unpublished data). European rabbits were introduced to the island in the 18th century and, alongside black rats *Rattus rattus* (accidentally introduced during the middle ages), were the only 2 invasive vertebrates established in the island (Amado et al. 2007). Both species were targeted by an eradication intervention, which started in September 2016. Despite intensive monitoring efforts, no rats have been detected since 2017 and the last live rabbit was seen in June 2019 (Fagundes et al., unpublished data).

Here, we use the eradication of European rabbits and black rats as a whole-ecosystem manipulation experiment to investigate the effects of the eradication of both mammal species (hereinafter simply "eradication") on the trophic niche of the Berlenga wall lizard. Through stable-isotope analysis, we investigated how the width of the trophic niche of the Berlenga wall lizard has changed between (1) late summer 2016 (pre-eradication) and late summer 2018 (post-eradication) and (2) late summer 2018 (end of the dry season characterized by limited food availability) and spring 2019 (period with abundant food resources). Additionally, we (3) contrasted the trophic niche width of male and female lizards and (4) compared the body size of lizards on the pre- and post-eradication periods and, post-eradication, between seasons. We predicted that the isotopic niche of lizards would expand post-eradication as a consequence of increased availability of feeding resources, with the population becoming more generalist. However, it is also possible that a more selective diet may be adopted when resources become abundant, as it will be easier to select preferential prey. The outcome of these 2 contrasting predictions is interesting and can be tested with the spring/summer comparison: if lizards may afford to become selective when resources are abundant, their isotopic niche should shrink in the spring when compared with summer. We also predicted that the larger-bodied males would exhibit a broader isotopic niche than females. Finally, and regardless of sex, we predicted that the body size of lizards would increase between the pre- and post-eradication periods as result of the higher availability of food resources, allowing a higher investment in body growth and/or survival.

Materials and Methods

Study area and focal species

Fieldwork was conducted in Berlenga Island, the largest (79 ha) of the archipelago of Berlengas (comprised Berlenga, Farilhões, and Estelas), with a maximum elevation of 92-m above sea level. The island is located 10 km off Peniche, in the western coast of Portugal (Figure 1A), and it is formed by a calc-alkaline granite outcrop with a nearly flat plateau, and steep slopes that often end in cliffs. Despite the island's small size, it exhibits characteristics of both Atlantic (slopes facing north) and Mediterranean (slopes facing south) climate regimes (Almada et al. 1995) and is home to over 120 plant species (see Gomes et al. 2004 for a detailed description of its flora). The archipelago is a protected area (Nature Reserve) since 1981, and it is also classified under the European Natura 2000 network as a Special Area of Conservation and a Special Protection Area. It is also part of the World Network of Biosphere Reserves (UNESCO 2011).

Our focal taxon was the Berlenga wall lizard *P. carbonelli berlengensis*, an endemic subspecies of theIUCN (The International Union for Conservation of Nature's Red List of Threatened Species) Endangered Carbonell's wall lizard (Figure 1B). The Berlenga wall lizards are larger than most populations of their mainland counterparts (medium snout to vent length [SVL] of males: 62.8 mm; Vicente et al. 1995; Sá-Sousa et al. 2000; Harris and Sá-Sousa 2002; Kaliontzopoulou et al. 2010) and previous studies based on stomach contents suggested a generalist diet, with Coleoptera, followed by Heteroptera and Homoptera as preferential preys, with seasonal variations (Vicente et al. 1995). Although herbivory has not yet been described in *P. carbonelli berlengensis* it cannot be excluded, as some insular populations of *Podarcis* are known to consume plant matter (Pérez-Mellado and Corti 1993). During spring, males favor mating and territory defense over actively searching for food, whereas
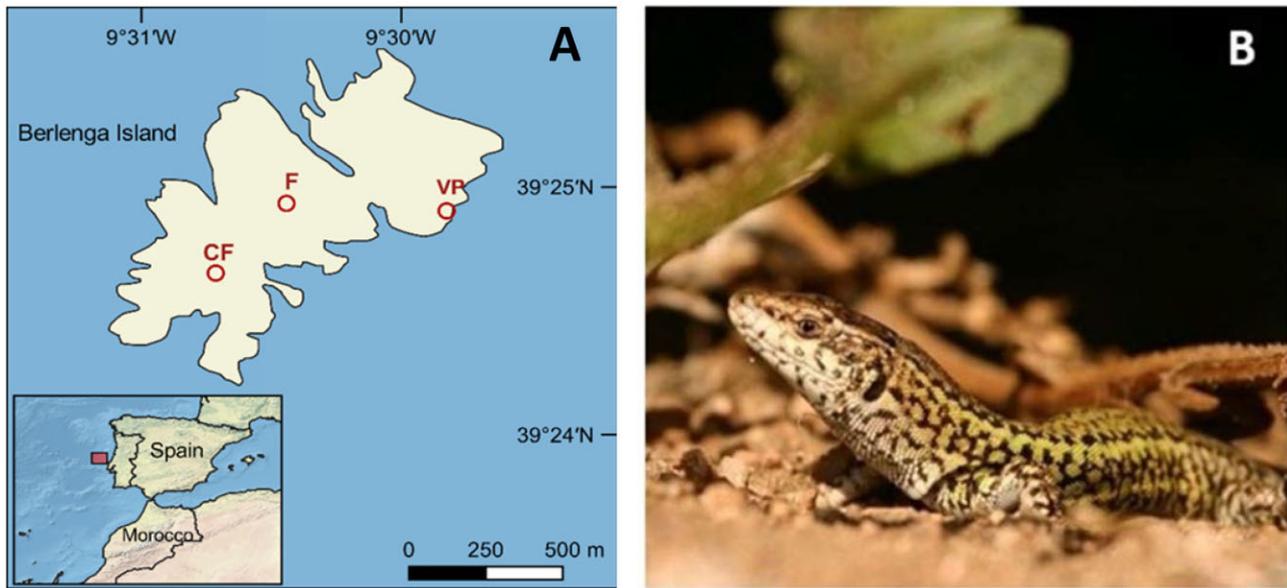
Figure 1. (A) Location of the Berlengas archipelago and of the 3 sites sampled for lizards andarthropods (CF; F and VP); (B) Berlenga wall lizard (male). Photo by Ricardo Rocha.

females tend to invest in foraging in order to store energy for reproduction (Vicente et al. 1995).

Invasive mammal eradication

The eradication of introduced black rats and European rabbits from Berlenga Island began in September 2016 (Fagundes et al., unpublished data). Black rats were baited using ca. 1,030 baiting stations (rigid plastic boxes 230 200 95 mm), placed along a 25 25 m grid. Additional baiting stations were placed in areas with high human pressure (e.g., near the fisherman settlement, the lighthouse, and camping area). Initially, baiting stations were baited with 4 rodenticide blocks, 0.005% brodifacoum (Talon and Racumim). After frequent monitoring, and checking the reduction of rodenticide consumption, baiting was reduced to 2 blocks per station. In December 2016, 1 month after the detection of the last signs of rats, all the baits were removed and replaced with paraffin and peanut butter blocks. Motion sensor cameras and lethal snap-traps with different baits (cheese or chorizo) were also installed. Monitoring was carried out, quarterly, until June 2017. From then on and until the end of 2019, snap-traps were installed in 5 lines (10 traps in each line), in places with a higher probability of acting as gateways for the arrival of invasive rodents to the island (e.g., around the island's docks) or in places where there was a high consumption of rodenticide. As for rabbits, poison baits were placed in all previously identified burrows, and these were regularly checked and baited until December 2016. Tomahawk traps, baited with vegetables or fruit, were also set to capture individuals who were not attracted by poison baits. Captured animals were euthanized and between March 2017 and September 2018 detected rabbits were shot. Rabbit eradication results were monitored through night transects, camera trapping, and prospection of latrines. No signs of rats and rabbits were detected since November 2016 and June 2019, respectively (Fagundes et al., unpublished data). No bait-associated lizard mortality was detected. Post-eradication, herbaceous, and shrub cover has mostly increased across the island and the reproductive success of the 2 monitored colonies of Cory's shearwater *Calonectris*

borealis was higher than in 2015–2016 (Fagundes et al. unpublished data; SPEA, unpublished data).

Study sites

Three sampling sites were defined along the island based on the perceived availability of resources for the lizards. We chose a resource-rich area, a resource-poor area, and the area most impacted by human presence: Vale Paínhos (VP; 39.41467, 9.50425), Cisternas do Forte (CF; 39.41220, 9.51300), and Farol (F; 39.41495, 9.51033), respectively (Figure 1A). VP (resource-rich) is located in the east slope of the island, has abundant cover of native vegetation, and includes an area with artificial nests for seabirds that are used as refuges by the Berlenga wall lizards (Supplementary Figure S1A). CF (resource-poor) is located in the island's plateau, near a pathway frequently used by tourists. It has sparse and low herbaceous vegetation cover and it is heavily used by yellow-legged gulls *Larus michahellis* (Supplementary Figure S1B). The human-impacted F is also located in the island's plateau but next to the accommodation of the nature reserve and lighthouse staff, the only area of the island with round the year human presence. It has a sparse vegetation cover and is heavily impacted by organic waste (Supplementary Figure S1C).

Sampling and stable isotope quantification

Sampling occurred in 3 periods: late summer 2016 (pre-eradication, 10th to 13th September; Pre-16), late summer 2018 (post-eradication, 29th August to 5th October; Post18); spring 2019 (post-eradication, 11th May to 14th June; Post19). Although rabbits were still present on the island during the Post-18 and Post-19 periods, their numbers were assumed to be near zero. The time taken for the nutrients from prey to be incorporated into the tissues of consumers varies depending on the type of tissue and taxonomic group; prey nutrients take around 90 days to be incorporated into the tissues of reptiles (Warne et al. 2010). Therefore, our late summer samples probably encompass the lizard's diet during the entire summer, just as our late spring samples encompass the entire spring.

Lizards were captured using 3 traps per site baited with canned tuna, and adults (identified based on the presence and development

state of femoral pores) were measured (SVL, mm) and weighed with an electronic scale (0.1 g). Sex was attributed by the observation of the femoral pores (well developed in males and poorly developed in females).

In each sampling period and for each site, we collected tail tips (2 cm) from 6 to 15 randomly selected lizards, for stable isotope analysis (Supplementary Table S1). As for arthropods, one species of Cicadellidae and *Messor* sp., a Formicid, both found on the 3 sites were collected using 6 pitfall traps with water, left for 24 h at each site. *Messor* sp. is mainly a primary consumer that feeds on seeds and other plant material but also consumes dead invertebrates (Segev et al. 2014; Saar et al. 2018), whereas Cicadeliidae are strictly primary consumers, feeding on plant sap (Denno et al. 1995; Ma et al. 2017). Isotopic signatures of both insects were used as reference values to assess the trophic level of lizards in the trophic web. All the samples were stored dry at 20°C.

In the laboratory, lizard muscle tissue and arthropods (whole body) were dried at 60°C from 24 to 48 h and grounded to a fine powder. Lipids can cause variation in the isotopic ratio with effects on ^{13}C (Bond and Jones 2009). As such, all samples were delipidified by immersion in Chloroform–Methanol (2:1), followed by centrifugation (4,000 r.p.m., 2 min). The process was repeated until a translucent supernatant, without any trace of yellow tones, was obtained. Lastly, samples were dried at 60 °C.

Samples were loaded to tin capsules and isotopic ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were determined in a Sercon Hydra 20-22 mass spectrometer (Sercon, UK), coupled to a EuroEA Elemental Analyzer (EuroVector, Italy), which performs automatic preparation of the samples by Dumas combustion. Protein Standard OAS (Elemental Microanalysis, UK), Sorghum Flour Standard OAS (Elemental Microanalysis, UK), and IAEA-N1 (IAEA, Vienna, Austria) were used as reference materials. The carbon standard was Vienna Peedee Belemnite, and the standard for nitrogen was atmospheric air. The precision of the isotope ratio measurements was ±0.2 ‰. Sorghum Flour Standard OAS was the reference material for the animal samples. These analyses were performed at SIIAF, Stable Isotopes, and Instrumental Facility—Plant Department, FCUL. Isotopic ratios () were calculated using the isotopic ratio between the heavy isotope (higher molar mass) and the light isotope (lower molar mass) according to the formula described in Michener and Lajtha (2007).

Data Analyses

All biometric data were initially tested for normality using Shapiro–Wilk tests and homoscedasticity using Bartlett tests. Based on the lizard's weight and SVL we calculated a body condition index, the scaled mass index (Peig and Green 2009). For both the scaled mass index and SVL, we constructed generalized linear models with site, period, and the interaction between the 2 variables as fixed factors. To facilitate model interpretation, we analyzed males and females separately.

The position of the Berlenga wall lizard isotopic signatures in the $^{13}C/$ ^{15}N biplots was compared with the isotopic signature of the insects used as reference in each site. Niche width was compared, in each site, between the pre- and post-eradication (Pre-16/Post-18) periods and, seasonally, between late summer and late spring, in the post-eradication years (Post-18/Post-19). Isotopic niche metrics were estimated using the SIBER package—Stable Isotope Bayesian Ellipses in R (Jackson et al. 2011). Two isotopic parameters were calculated: total area (TA) of the occupied niche and corrected

standard ellipses area (SEAc). Although both parameters provide an indication of niche width, TA is sensitive to sample size and prone to bias in the direction of larger TA values with larger sample sizes. SEAc, on the other hand, is insensitive to bias associated with sample size, allowing for more reliable comparisons when sample sizes differ between sites or study periods (Jackson et al. 2011). Comparisons of isotopic niches between the different sites and periods, as well as calculus of niche overlap, were conducted with SEAc estimates (Jackson et al. 2011; Layman et al. 2012; Sebastián et al. 2015; Grundler et al. 2017).

Overlap between 2 SEAc estimates was calculated using the formula: % overla $P = [S (A S) + B]$ 100, where A and B represent the 2 SEAc and S the overlap between them. Differences in SEAc between periods were tested using a Wilcoxon test (Sebastián et al. 2015). We used *t*-tests to compare differences between the values of ^{13}C and ^{15}N, separately per site, and between periods.

All statistical analyses were performed using software R version 3.4.3.

Results

Body size differences between pre- and post-eradication and between seasons

We captured a total of 277 adult Berlenga wall lizards (111 females and 166 males; Supplementary Table S2). Forty-six were captured in Pre-16 and 116 were captured in both Post-18 and Post-19. By site, most lizards were captured in CF (112), followed by VP (93), and lastly F (72). The mean SVL of captured individuals was 58.60 mm (sd = 4.69) and ranged between 41.20 and 69.90 mm, whereas the mean weight was 4.73 g (sd = 1.43) and ranged between 1.68 and 9.34 g. Males were usually longer than females (Supplementary Table S2 and Figure S2). For females, we found significant differences in neither SVL nor in scaled mass index values between any combination of sampling periods and sites (Supplementary Tables S3 and S4). However, for males, we found significant differences between both sampling periods and sites for both SVL and scaled mass index (Supplementary Tables S3 and S4). Males captured in CF and F were smaller before mammal eradication (Supplementary Table S3 and Figure S2). The body condition of males captured in CF was higher before mammal eradication than after it (Supplementary Table S4 and Figure S3).

Isotopic niche variation

Isotopic analyses were conducted for 100 tail tips of Berlenga wall lizards (27 collected in 2016 [pre-eradication]; 28 collected in late summer 2018 [post-eradication]; 45 collected in late spring 2019; representing 54 males, 33 females, 7 juveniles, and 6 individuals with undetermined sex;

Supplementary Table S1). Within sampling period, isotopic niche widths were constantly higher in the humanized area F when compared with the 2 other sampling sites (Table 1).

Isotopic niche variation between the pre- and post-eradication periods

The isotopic niche width of the lizards captured in both resource-rich and resource-poor areas (VP and CF) was larger in the post-than in the pre-eradication period, considering either TA or SEAc (Table 1 and Figure 2). This increase in the niche width in the post-eradication period was larger in resource-rich VP. The isotopic niche width of the lizards captured in the humanized area F exhibited an

Table 1. TA and SEAc of *P. carbonelli berlengensis* captured in the 3 sampling sites (VP, CF, and F) and periods

Site	Pre16		Post-18		Post-19	
	TA	SEAc	TA	SEAc	TA	SEAc
VP	0.61	0.37	3.72	2.26	4.93	1.85
CF	0.85	0.71	1.25	0.88	1.54	0.61
F	6.17	3.34	4.14	2.36	9.54	4.28

inverse pattern, with smaller TA and SEAc values in the post- than in the pre-eradication periods (Table 1 and Figure 2). No isotopic niche overlap between the pre- and post-eradication periods was detected in F, whereas in VP and CF, the overlap was 10.7 and 3%, respectively (Table 2 and Figure 2).

Post-eradication, there was a significant increase in the trophic level of the lizards from resource-poor CF, translated into an increase in the average values of ^{15}N (t-test: $t = 2.71$, $P < 0.05$). On the other hand, in the humanized area F, there was a significant decrease in ^{15}N values (t-test: $t = 8.28$, $P < 0.001$). When it comes to ^{13}C, there was a significant depletion in the isotopic signature of lizards from resource-rich VP in the post-eradication period (t-test: $t = 2.29$, $P < 0.05$), whereas the 2 other sampling sites did not differ pre- and post-eradication (CF: t-test: $t = 0.71$, $P = 0.49$; F: t-test: $t = 1.84$, $P = 0.09$).

Isotopic niche variation between seasons

During the post-eradication period, there was an increase in the TA from Post-18 (late summer) to Post-19 (late spring), being the increase more accentuated in the humanized area F, followed by resource-rich VP and then resource-poor CF (Table 1). Yet, as denoted by SEAc, when differences in sample size are considered, we observed that although the isotopic niche doubled in F between Post-18 and Post-19, it decreased in the 2 other sampling sites, being this reduction more evident in resource-rich VP. There was no SEAc overlap between the 2 seasons in VP, whereas in F and CF, the overlap was 5 and 26%, respectively (Table 2).

The ^{15}N signatures were higher in Post-19 than in Post-18 (suggesting that lizards were consuming prey of higher trophic levels in spring than in summer) in 2 sites (the exception was resource-poor CF). This enrichment in ^{15}N during Post-19 was significant in VP (t-test: $t = 5.04$; $P < 0.001$). The isotopic signature of lizards in all the sites presented a shift in ^{13}C toward more negative values, being significant in CF (t-test: $t = 2.49$; $P < 0.05$; Figure 2).

Isotopic niche differences between sexes

The comparison of isotopic niches of both sexes in the 3 periods (Pre-16, Post-18, and Post-19) was possible only in resource-rich VP, due to limitations associated with sample size. We used tail tips of 7 males and 3 females in Pre-16, 5 males and 5 females in Post-18, 7 males and 4 females in Post-19.

In the pre-eradication period (Pre-16), SEAc was very similar between sexes, with 19.6% of isotopic niche overlap (Table 3). Post-eradication (Post-18), both female and male lizards expanded their isotopic niche in relation to the pre-eradication period (Pre-16; Figure 3). However, whereas the SEAc of females increased ca. 3-fold, for males it increased over 10-fold (Table 3). The isotopic niche of males in Post-18 depicted less negative ^{13}C values than in Pre-16 (Figure 3), and the overlap in the SEAc of female and male lizards in Post-18 was only 12%.
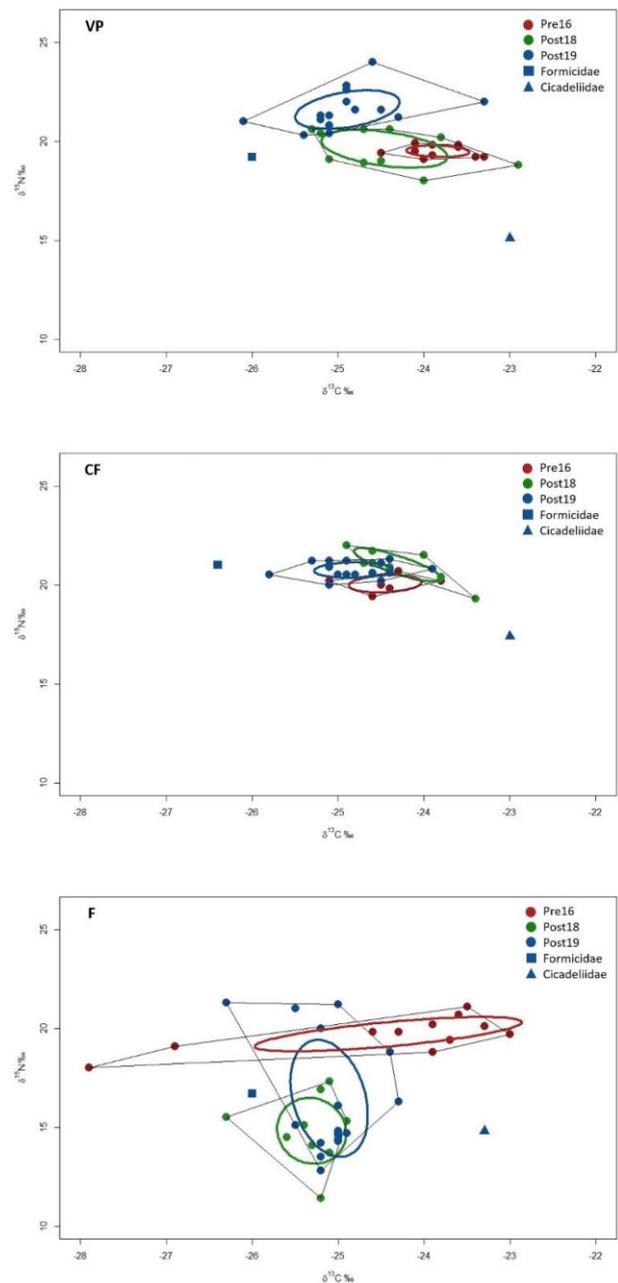

Figure 2. Isotopic biplots of Berlenga wall lizards in VP, CF, and F, as given by the TA (in black) and SEAc. Red, green, and blue denote SEAc in the pre-eradication sampling in 2016 (Pre16), post-eradication sampling in late summer 2018 (Post-18), and post-eradication sampling in late spring 2019 (Post-19), respectively.

Table 2. SEAc overlap of each sampling site between periods (pre-[Pre16] and post-eradication [Post18] periods and between late summer [post-18] and late spring [post-19])

Site	SEAc overlap Pre16/post-18 (%)	SEAc overlap Post-18/post-19 (%)
VP	10.70	0
CF	2.99	4.82
F	0	25.90

Table 3. TA, SEAc, and SEAc overlap of females and males of Berlenga wall lizards captured in VP in the pre-eradication sampling in 2016 (Pre-16), post-eradication sampling in late summer 2018 (post-18), and post-eradication sampling in late spring 2019 (post-19)

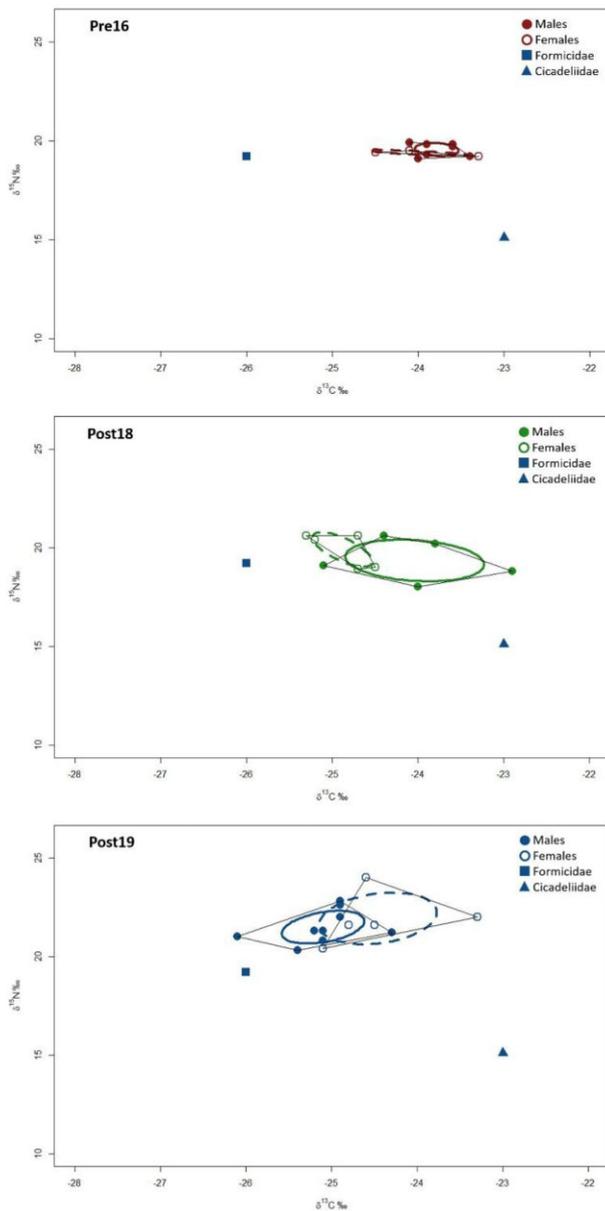| Period	Males		Females		SEAc overlap (%)
	TA	SEAc	TA	SEAc	
Pre-16	0.39	0.31	0.10	0.36	19.6
Post-18	3.04	3.51	0.68	0.91	12.2
Post-19	2.20	1.33	2.84	3.65	20.3

Figure 3. Isotopic niche width of female (dashed lines) and male (continuous lines) of Berlenga wall lizards, as given by the TA (black) and SEAc (color). Pre16, Post-18, and Post-19 denote, respectively, pre-eradication in 2016, post-eradication sampling in late summer 2018, and post-eradication sampling in late spring 2019.

The isotopic niche of female lizards was larger in spring 2019 (Post-19) than in summer 2018 (Post-18), whereas the isotopic niche of males contracted over the same period. During this period, the isotopic niche of female lizards was displaced toward less negative ^{13}C values (Figure 3) and the overlap in the SEAc of female and male lizards increased to 20%.

Discussion

Using the eradication of invasive black rats and European rabbits as a whole ecosystem manipulation experiment, we observed a probable eradication-induced expansion of the isotopic niche of an island-restricted lizard. Additionally, we found a considerable variability in lizard isotopic niche across a relatively small island and detected evidence of sex-specific seasonality in nutritional requirements. In both resource-rich and resource-poor areas, the isotopic niche of lizards expanded after the eradication of invasive mammals and contracted from late summer to the following spring. Interestingly, in the human settlement, the pattern was the opposite.

Post-eradication, we found a marked increase in the isotopic niche width of lizards inhabiting resource-rich VP (Table 1). Owing to its deep soils, this area had a particularly high density of rabbits that probably limited the food resources available to the lizards due to considerable damage to vegetation and soil erosion (Oliveira et al., unpublished data). Our results indicate that mammal eradication has allowed lizards in VP to explore a higher diversity of food resources, leading to a generalization of their diet. This trophic niche expansion was unsurprising, considering the generalist and often opportunistic food habits of *Podarcis* spp. (Pérez-Mellado 1983; Pérez-Cembranos et al. 2016; Santamaría et al. 2020). A similar result was found by Pringle et al. (2019) in an experimentally manipulated lizard community in the Bahamas, where the trophic niche width of 2 native lizards was also restricted by competition with a non-native species.

In the island's only permanently human-inhabited area (F), changes in the lizard's isotopic niche after mammal eradication were different: the niche width shrunk and the ^{15}N values decreased, suggesting a reduction in trophic level (lizards likely became more secondary and less tertiary consumers; Table 1 and Figure 2). In F, there is a much higher availability of organic waste discarded by humans than anywhere else on the island. Although black rats in Berlenga were mostly herbivorous (94% of their winter and spring diet consisted of plants, mostly seeds of native species), they also consumed organic waste and were particularly abundant in area F (Oliveira et al., unpublished data; Fagundes et al., unpublished data; Nascimento et al. 2019). Pre-eradication, these human waste-associated resources were probably largely exploited by rats, leading to a competitive exclusion of the lizards. Post-eradication, lizards, free of competition from rats, could more easily explore the resources discarded by humans, taking advantage of its accessibility as a way of saving energy due to the reduced foraging time involved. This post-eradication shift toward waste-associated resources in detriment of a diet mostly composed by arthropods not associated with human waste is likely responsible for the observed post-eradication reduction of niche width and decrease in the trophic level of the consumed prey, and therefore of the lizards.

After the mammal eradication, the vegetation is recovering but, subject to the Mediterranean climate of the region, the differences in the island's greenness between the rainy spring and the dry, hot summer, have been particularly evident. We predicted that if lizards become selective when resources are abundant, their isotopic niche should shrink in the spring when compared with summer. Indeed, this contraction of the lizard isotopic niche between summer and spring was pronounced in resource-rich VP (Table 1). Here, there

was no niche overlap between seasons, corresponding to a total change in diet, and there was also an increase in ^{15}N values, indicating a rise in trophic level in the spring (Table 2 and Figure 2). These results align with previous studies indicating seasonally modulated responses of insular reptiles toward resource availability (e.g., Penado et al. 2015; Santamaría et al. 2020). Preferential predation of favorable prey will then be perceived as trophic specialization (Sagonas et al. 2015, 2019). Once more, and contrary to the results observed in the other areas, the subpopulation living in the humanized area F showed a high niche overlap between seasons and an increase in the amplitude of the isotopic niche from spring to summer (Table 2). This might be explained by the generalist feeding habits of *Podarcis* spp., which might have allowed lizards living in F to benefit from the availability of organic waste (and its associated arthropods) throughout the year, thus reducing the seasonal dietary shifts in this human-impacted section of the island.

Spring corresponds to the reproductive season of the Berlenga wall lizard (Vicente 1989) and therefore is an energy-demanding period for females (Hierlihy et al. 2013). Based on the size classes of arthropods found in the stomach contents of male and female individuals sampled throughout the 4 seasons, Vicente et al. (1995) observed sex-specific differences in the diet of Berlenga wall lizards prior to the eradication of invasive mammals, namely that males fed more during autumn, whereas females fed on a higher diversity of arthropods during spring. Here we found that the summer trophic niche of male lizards in VP was much wider than that of females, indicating that males fed on a greater diversity of resources (Table 3 and Figure 3). However, and according to the findings of Vicente et al. (1995), in spring females were the sex with the wider isotopic niche. Female lizards might favor energy-rich prey during spring, due to the high energetic requirements associated with reproduction (Galán 1996; Padilla et al. 2009; Hierlihy et al. 2013; Sagonas et al. 2019). This prey selection can be sex-specific and might occur on the basis of prey size (e.g., by selecting larger arthropods with higher energy input) or be associated for instance with reductions in foraging movements (Díaz and Carrascal 1990; Carretero 2004). Sex-specific seasonal responses were also observed for *Lacerta trilineata* in several Greek islands, in which males showed a more active foraging behavior during summer (Sagonas et al. 2019). Higher activity in a season characterized by food scarcity was suggested to represent a way to ensure a richer diet so as to invest in body growth (Sagonas et al. 2019). Notwithstanding the different methodological approaches and our reduced sample size for addressing sex-related changes, our results corroborate the persistence of sex-specific trophic differences after the eradication of rats and rabbits (Table 3).

In the pre-eradication period, males from F and CF had the shortest SVL values among the 3 subpopulations (Supplementary Figure S2). F was one of the island's areas with highest rat density before the eradication (Fagundes et al., unpublished data; Nascimento et al. 2019). High level of interaction with rats might have limited the access of lizards to food resources, thus limiting growth or survival to larger sizes. There could be also an effect of lower male survival in F and CF, as sexual dimorphism in some lizard species increases with age (e.g., Stewart 1985). A shift toward larger sized individuals after the eradication of black rats, similar to that we found at Berlenga, was observed in European Leaf-toed gecko *Euleptes europaea* in a Mediterranean island (Delaugerre et al. 2019). These authors suggested that, in addition to competition for food, the shift might also have been driven by fear-induced physiological stress and by rat-related habitat degradation and its associated effects on features such as thermoregulation opportunities and gecko social structure.

These same factors might have influenced the changes in body size detected in Berlenga. On the other hand, CF was overall the area less vegetated and with less pronounced post-eradication changes in vegetation cover (Fagundes et al., unpublished data). Interestingly, we found that males (but not females) had an overall better body condition in pre- than post-eradication (Supplementary Table S4). The observed post-eradication increase in male size alongside a proportional decrease in body condition may be associated with a higher investment in growth to larger sizes (e.g., due to demands associated with the defense of territory or access to females) or be associated with inter-annual climatic fluctuations (rainfall was considerably higher in the 4 months prior to sampling in 2016 than in 2018; Supplementary Table S5).

We found some evidence that the eradication of invasive black rats and European rabbits, 2 of the planet's most problematic invasive vertebrates, might be leading to an expansion of the trophic niche and an intensification of the sexual dimorphism of the Berlenga wall lizards. This suggests that the ecological pressures to which lizards were exposed are likely to have been affected by the eradication of invasive mammals, and that the Berlenga wall lizards are starting to benefit from a richer habitat, with a wider availability of food resources. Our results probably reflect short-term responses that are likely to continue to change with time post-eradication. As such, it is important to continue monitoring this lizard population to investigate possible changes in the long-term.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

Conflict of Interest

The authors declare no competing or financial interests.

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