Original Research Article

Yearly variation in individual diet specialization: Evidence from cave salamanders

Enrico Lunghi [a], Gentile Francesco Ficetola [b,c,*], Raoul Manenti [b], Giorgio Mancinelli [d,e]

[a] Dipartimento di Medicina clinica, Sanità pubblica, Scienze della Vita e dell'Ambiente (MeSVA), Università degli Studi dell'Aquila, Piazzale Salvatore Tommasi 1, L'Aquila 67100, Italy
[b] Dipartimento di Scienze e Politiche Ambientali, Università degli Studi di Milano, via Celoria 26, Milano 20133, Italy
[c] Laboratoire d'Écologie Alpine (LECA), Université Grenoble Alpes, CNRS, CS 40700, Grenoble 38058, France
[d] Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali (DiSTeBA), Università del Salento, Centro Ecotekne, S.P. Lecce-Monteroni, Lecce 73100, Italy
[e] CoNISMa, Consorzio Nazionale Interuniversitario per le Scienze del Mare, Piazzale Flaminio, 9, Roma 00196, Italy

A B S T R A C T

Individual diet specialization is a key behavioral trait, with multifaceted impacts on population dynamics. Long-term studies are fundamental to properly understand such dynamics and their main causes. Indeed, individuals may vary from specialism to generalism in response to multiple biological and environmental factors, thus snapshot studies can have limited relevance for the overall biology of species. Yet, so far longitudinal studies testing the potential variation of diet specialization through time are lacking. Here we present the results of a study on individual diet specialization performed over a four-year period. Using three populations of the Ambrosi's cave salamander *Speleomantes ambrosii* we tested: *i*) whether seasonal variation in the occurrence of specialized individuals is maintained through different years, and *ii*) if the degree of specialization observed in a single season is constant through different years. Our results showed that the species maintains a high proportion of specialized individuals in spring and more generalist in autumn during different years, confirming a consistent seasonal variation of trophic specialization through time. On the other hand, the proportion of specialized individuals of the same period can vary up to ten-fold across years, suggesting that a single study may be not enough to properly understand the dynamics of a species' trophic niche. This study highlights the need of repeated surveys to accurately understand this behavioral trait, especially for species that are strongly dependent on local environmental conditions.

1. Introduction

The study of individual diet specialization has drawn a renewed interest in researches focused on species trophic niche (Ingram et al., 2018; Costa-Pereira et al., 2019) and has improved our understanding of the importance of personality in non-human animals (Gosling, 2008; Toscano et al., 2016). Specialized individuals are those that forage on a particular subset of the population's trophic

---

resources independently from age, sex or size (Bolnick et al., 2003; Svanbäck and Persson, 2004; Araújo et al., 2011). Therefore, the total niche width of a population (TNW) can be considered as the sum of the contributions of both specialized (between individual component; BIC) and generalist individuals (within individual component; WIC) (Bolnick et al., 2003). The occurrence of specialized individuals can be determined by different, non-exclusive conditions. On the one hand, specialization may be the consequence of intraspecific competition, where more competitive individuals are able to target the most profitable prey, while the others are compelled to select alternative prey (Araújo et al., 2008; Costa-Pereira et al., 2019). On the other hand, the increase of ecological opportunity, in terms of prey richness and abundance, can trigger individuals' preference allowing a higher selectivity according to their own taste, needs or experience (Cloyed and Eason, 2016, 2017). Nonetheless, environmental conditions are key drivers of the occurrence of specialized individuals, as shown by studies performed at both broad and fine spatial scales (Araújo and Costa-Pereira, 2013; Lunghi et al., 2020e).

The study of individual diet specialization is generally based on the analysis of gut contents, sometimes complemented by additional analyses (e.g., stable isotopes) (Araújo et al., 2008; Cloyed and Eason, 2017; Rosumek et al., 2018) and, in the best case, involves multiple conspecific populations (Lunghi et al., 2020e). Data collection has been often replicated on different seasons to clarify the seasonality of foraging habits and potential variation in the overall diet of the populations (Costa et al., 2015; Lunghi et al., 2018a, 2022b); however, to the best of our knowledge, no study has assessed if trophic specialization remains consistent over longer time periods (e.g. across different years). Short-time surveys certainly are important to assess the occurrence of diet specialization (e.g., Salvidio et al., 2015; Carvalho-Rocha et al., 2018) and would offer a clear overview of the population if such attribute was genetically determined and can be therefore considered stable (Stanczyk et al., 2010). However, given the large variability of factors potentially affecting individual specialization (Araújo et al., 2011; Lunghi et al., 2020e), it is unlikely that within populations the proportions of specialized and generalist individuals can remain consistent through time. Therefore, short-period surveys likely provide only a snapshot of the dynamics of a population trophic ecology (Johnson, 2000; Brose, 2010; Pille et al., 2023). For example, fluctuation in populations densities may trigger a variation in intraspecific competition (e.g., Lejeune et al., 2023), promoting niche portioning and significantly affecting diet specialization of individuals (Araújo et al., 2008). Another influencing factor may be the availability and/or the possibility to access the best foraging areas, where ecological opportunity is the highest and individuals can specialize on their preferred prey, or target the most profitable ones (Denoël et al., 2007; Cloyed and Eason, 2016). Assessing factors determining the temporal variation of individual diet specialization can be particular important for amphibians such as salamanders, which are key components of both aquatic and terrestrial habitats. By preying on a diverse array of invertebrates, salamanders play central roles in resources flow across and within ecosystems (Semlitsch et al., 2014; Barzaghi et al., 2017). Knowledge on feeding ecology and diet variation of salamanders is therefore pivotal to understanding the ecological functions they provide.

With the present paper, we performed a repeated assessment of diet specialization of three populations of the Ambrosi's cave salamander (*Speleomantes ambrosii*) in different years to test two hypotheses related to the potential drivers affecting the incidence of specialized individuals within populations through time. Considering the trophic niche of individuals as a dynamic trait, we expected to observe within the same population seasonal variations (Lunghi et al., 2018b, 2020a), but also differences in the proportion of specialized individuals during the same season but in different years. For example, multiple environmental conditions showing a seasonal cycle (e.g. climate, resource availability) can directly or indirectly affect the occurrence of specialized individuals within a population (Lunghi et al., 2020e); therefore, a consistent divergence between seasons in individuals diet specialization is expected.

Table 1

The data analyzed in this paper. Data for years 2016 and 2017 is retrieved from Lunghi et al. (2018a), while that for year 2020 from Lunghi et al. (2021b). Within season, sampling performed in late June/early July were coded as spring (S), while those performed in September as autumn (A). Populations are coded following Lunghi et al. (2020); below each population code, information of the explored area is provided. The explored area was estimated through the measurement of the explored inner cave space (length × width of the ground floor + length × height of explored cave walls on both sides). Coordinates of sampled sites are omitted due to conservation concerns Lunghi et al. (2019). For each sex we provide the number of individuals used into diet specialization analysis (i.e., individuals from which stomach contents were recognized; in bold), along with the total number of captured individuals (in brackets). We also report the percentage of individuals in each combination population/year/season characterized by only a single prey item in their stomachs (% single prey).

Population	Year	Season	Females	Males	Juveniles	Total	% single prey
S_ambrosii2	**2016**	A	**3**(5)	0	**6**(15)	**9**(20)	77.8
(342.3 m²)		S	**6**(9)	**7**(10)	**16**(21)	**30**(40)	90.0
	2017	S	**9**(12)	**3**(9)	0	**12**(21)	75.0
	2020	A	**6**(38)	**3**(19)	**3**(41)	**12**(98)	75.0
		S	**18**(39)	**19**(30)	**5**(39)	**42**(108)	81.0
S_ambrosii3	**2016**	A	**4**(5)	**4**(4)	**2**(4)	**10**(13)	54.5
(635.31 m²)		S	**1**(3)	**3**(3)	**1**(3)	**5**(9)	33.3
	2017	S	**7**(9)	**2**(3)	0	**9**(12)	71.4
	2020	A	**4**(12)	**2**(6)	**3**(33)	**9**(51)	66.7
		S	**7**(8)	**1**(1)	**4**(12)	**12**(21)	91.7
S_ambrosii4	**2016**	A	**6**(11)	**4**(9)	**6**(10)	**16**(30)	68.8
(951.84 m²)		S	**5**(7)	**6**(6)	**6**(12)	**17**(25)	88.2
	2017	S	**6**(9)	**8**(9)	**2**(3)	**16**(21)	75.0
	2020	A	**18**(45)	**3**(10)	**8**(59)	**29**(114)	86.2
		S	**20**(23)	**6**(7)	**3**(29)	**29**(59)	89.7
TOTAL			**119**	**71**	**65**		

Nevertheless, climate does not only shows an annual cycle, but also presents fluctuations that can affect the abundance and diversity of species (Kazadi and Kaoru, 1996; Kröncke et al., 1998) and, potentially, also diet specialization. The degree of individual diet specialization can be affected by the intensity of competition (only intraspecific in the present study due to the lack of heterospecific competitors; Lanza et al., 2006), thus we expect that changes in populations abundance through time can impact specialization. Ambrosi's cave salamander are facultative cave species that use subterranean environments to avoid unsuitable outdoor microclimate, i.e., they require high humidity and relatively cold temperatures (Lunghi et al., 2014; Ficetola et al., 2018). After some days from their occurrence, external environmental influences can reflect into subterranean environments (Badino, 2010), mostly affecting the shallowest areas, where the abundance of trophic resources is the highest (Lunghi et al., 2015; Manenti et al., 2015). Unsuitable microclimate can limit the access of *Speleomantes* to near-surface areas, confining them into relatively deep but food-poor subterranean spaces (Lunghi et al., 2018d; Culver and Pipan, 2019). Consequently, when *Speleomantes* can forage in the shallowest cave areas they have more opportunities to meet their trophic requirements and preferences, increasing the proportion of specialized individuals within the population.

2. Material and methods

We analyzed the data from three subterranean populations of *Speleomantes ambrosii*. The diet of these populations has been first investigated in 2016 and 2017, before (late June/beginning of July; hereafter spring) and after (late September; hereafter autumn) the warm season, period in which salamanders reduce their activity and estivate in the deepest cave areas (Lunghi et al., 2015, 2020a). These populations were surveyed again in 2020 (i.e., more than three years later) during the same periods, in order to collect comparable data and to avoid seasonal pseudoreplication (Lunghi et al., 2021b). These populations were monitored during day time (10 am – 5 pm) within the same subterranean space (i.e., first 45 m of the caves) (Lunghi et al., 2020a), therefore we can use population density as a proxy for the intensity of intraspecific competition. Salamanders where collected throughout the explored environment (Table 1), and each individual was weighted and photographed prior to undergo stomach flushing. Stomach flushing is a harmless technique that involves the injection of water through salamander's mouth to induce regurgitation of the remains of their last foraging activity (Crovetto et al., 2012; Lunghi et al., 2021a). When possible, the distance of each salamander from the cave entrance was also recorded. Images were used to estimate salamanders snout-vent length (SVL) and total length (TL) (Lunghi et al., 2020c). Presence/absence of secondary sexual characters (mental glands) was used to identify adult males (Lanza et al., 2006). SVL was then used to distinguish between juveniles and adult females; individuals with SVL $\geq$ 50 mm were considered adult females (Lunghi et al., 2018c; Lunghi, 2022). Consumed prey were recognized and counted following Lunghi et al. (2018a). Briefly, prey categories where mainly defined based on their taxonomic order; when specific morphology and/or different ecology (including larval stages) could be recognized with high confidence, they were considered as separate prey category.

For each population, we calculated the index of individual specialization as:

$$IS = \frac{\sum_i PS_i}{N}$$

(Bolnick et al., 2002) where N = number of i individuals in a population, while PS_i values were estimated using the revised version of Schoener's (1968) proportional similarity index PS proposed by Bolnick et al. (2002):

$$PS_i = 1 - 0.5 \sum_j |p_{ij} - q_j|$$

where p_{ij} is the frequency of a prey category j in the individual i's diet, and q_j is the frequency of prey category j in the entire population. IS approximates 0 when individual specialization is high and 1 when there is no individual specialization and provides a measure of individual specialization averaged over the whole population. For the sake of clarity, we used the measure $V = 1$- IS (Bolnick et al., 2007), so that larger V values corresponded with a higher degree of individual specialization.

For each combination of population/year/season, the estimation of V values was performed using the whole pool of individuals showing recognizable prey in their stomachs, without excluding those having items belonging to a single prey taxon (see Table 1 in Results). The inclusion of monophagous individuals may determine an overestimation of the degree of individual specialization, yet IS (and thus V) is among the indices that are less sensitive to monophagy (Bolnick et al., 2002). To confirm that this effect does not bias our conclusions, we assessed the relationship between estimated V values vs. the % contribution of monophagous individuals in each combination population/season/year.

A preliminary PERMANOVA performed on individual specialization V index with "population" (3 levels), "season" (2 levels), "sex/ontogenetic stage" (3 levels) as fixed factors and "year" (3 levels) as a random factor, showed negligible effects for "sex/ontogenetic stage", alone or interacting with other factors. Sex or ontogenetic stage of salamanders were thus excluded from further analyses.

The different surveys (combination population/season/year) were characterized by a number of analyzed individuals ranging between 6 and 42 (Table 1). To confirm that estimates of individual specialization are not biased by small sample size, we simulated V values by randomly subsampling n individuals from the total pool of each combination population/season/year (4999 replicates), with n varying between 2 and the total pool of analyzed individuals in the combination minus one. Local regression models (LOESS) fitted to the simulated data were used to qualitatively inspect the dependency of estimated V values on sample numerosity. The procedure was repeated on the four sampling occasions characterized by the largest pools of individuals. We calculated population densities using the number of captured individuals as a proxy of abundance. Previous assessments using removal sampling showed that the number of

individuals captured during the first capture session is an excellent proxy of the actual population abundance ($R^2 > 0.9$; (Ficetola et al., 2018). We used Generalized Linear Mixed Models to assess whether salamanders' density and/or distance from the cave entrance affect the diet specialization of individuals (Douglas et al., 2015). The *V* index of each salamander was used as the dependent variable, while the distance from the cave entrance, population density, year and season of survey were the independent variables; population identity was used as random factor. We did not include into the model sex and size of salamanders as previous analyses showed that these factors do not affect individual diet specialization (Salvidio et al., 2015; Lunghi et al., 2020a, 2020e). Likelihood ratio test was used to assess the significance of variables (Kuznetsova et al., 2016).

3. Results

The full dataset includes 642 records of salamanders (126 males, 235 females, 281 juveniles), 451 of which were collected in 2020 (Table 1). Overall, 365 individuals were discarded because they had empty stomach, or the food residuals were not recognizable because of their advanced stage of digestion. Among the others, we did recognize 708 prey items belonging to the following categories: Pulmonata, Sarcoptiformes, Trombidiformes, Araneae, Pseudoscorpiones, Opiliones, Lithobiomorpha, Geophilomorpha,
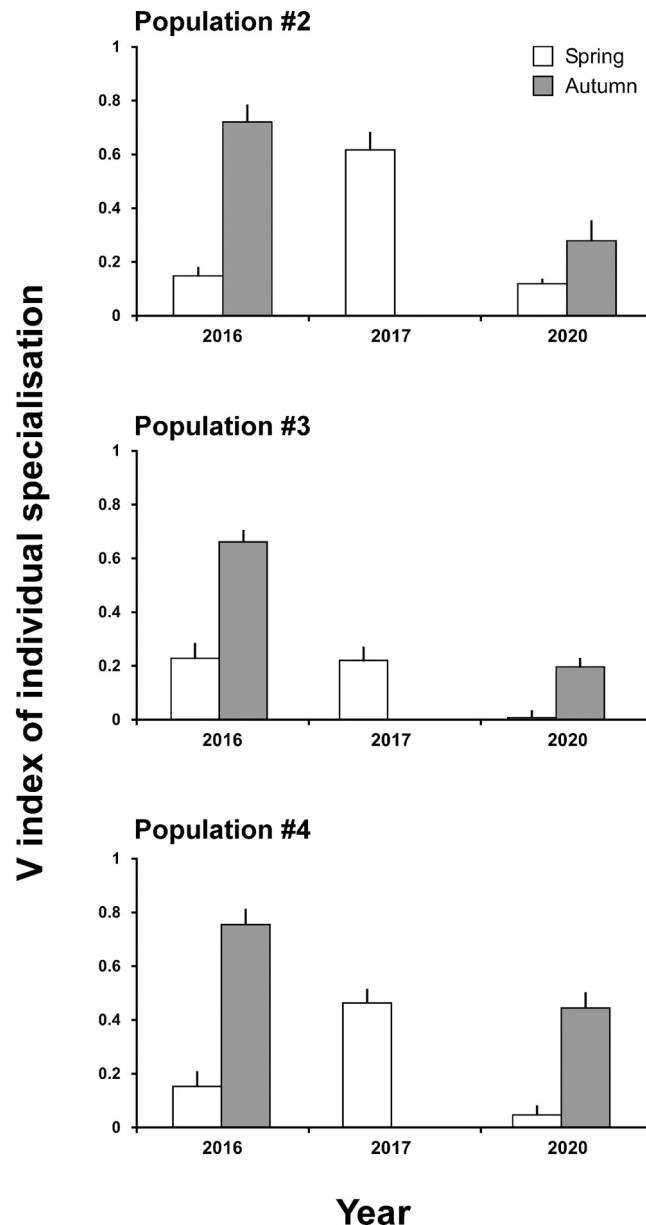

Fig. 1. Variation in the individual specialization index *V* (mean ± SE) across populations/years/seasons.

Scolopendromorpha, Julida, Glomerida, Isopoda, Symphypleona, Entomobryomorpha, Orthoptera, Blattodea, Psocodea, Hemiptera, Hymenoptera, Hymenoptera_Formicidae, Coleoptera, Coleoptera_Staphylinidae, Lepidoptera, Lepidoptera_larva, Diptera, Diptera_larva, Archaeognatha, *Speleomantes*, *Speleomantes*_skin, Tricladida, Gordea, Haplotaxida.

The highest *V* value was observed in population#4 in autumn 2016 (0.76 ± 0.04, mean $\pm$ SE) while individuals from population#3 in spring 2020 were characterized by the lowest dietary specialization (0.01 ± 0.005). Similarly, also the remaining populations showed a significant seasonal and inter-year variability in *V* values (Fig. 1; Table 2). Variations in the degree of individual specialization were not related to the number of analyzed individuals (Pearson's correlation: $r = -0.38$, $P = 0.16$, d.f. $= 13$) nor to the % contribution of monophagous individuals in each combination population/season/year ($r = -0.33$, $P = 0.15$, d.f. $= 13$), suggesting no biases due to samples size or monophagy. The subsampling procedure implemented on the combinations with the most numerous pools of individuals (population#2: Spring 2016 and 2020; population#4: Autumn and Spring 2020; Table 1) showed that reliable estimation of *V* values are already achieved with a sample size of 8–10 individuals (Appendix A, Fig. A). This suggests that *V* values can be reliable also for the sampling occasions with less individuals (Table 1). In both 2016 and 2020 the individual specialization of the three populations was generally higher in autumn than spring (Fig. 1; PERMANOVA, post-hoc comparisons: max $P_{MC} = 0.003$ for population#3 in 2020). In autumn, all populations showed the same inter-year trends of individual dietary specialization, with values significantly higher in 2016 compared with 2020 (max $P_{MC} = 0.001$ for population#4). In contrast, in spring the three populations were characterized by different yearly patterns, with populations #2 and #4 showing a significantly higher specialization in 2017 (max $P_{MC} = 0.002$ for the comparison 2016 vs. 2017 in population#4), while population#3 maintained similar *V* values between 2016 and 2017 ($P_{MC} = 0.49$) and decreased significantly in 2020 (max PMC $= 0.0001$, population#3 for the comparison 2017 vs. 2020).

The individual diet specialization of salamanders was significantly related to their density ($F_{1,95.66} = 11.50$, $P = 0.001$), year ($F_{1,128} = 27.9$, $P < 0.001$) and season ($F_{1,173.98} = 105.52$, $P < 0.001$), while distance from the cave entrance was not significant ($F_{1,173.81} = 3.14$, $P = 0.078$) (Fig. 2). The proportion of specialized individuals increased with density and was higher in autumn compared to spring. Furthermore, the frequency of specialized individuals decreased significantly from 2016 to 2020.

4. Discussion

Using the salamander *Speleomantes ambrosii* as model species, we demonstrated that the frequency of generalist vs. specialized individuals within a population strongly changes through time, and is likely affected by both endogenous (e.g. density) and exogenous (e.g. seasonality, differences between years) factors. Although it is known that, within the same population, a different proportion of specialized individuals may arise according to the season in which the sampling is performed (Araújo et al., 2009; Lunghi et al., 2020e), there is little quantification of how such variability may occur within the same season across different years. Indeed, our results showed that, for a given period (e.g., a single season) the proportion of specialized individuals can show a ten-fold variation across years (Fig. 1). This suggests that short-time studies may not provide exhaustive information on the degree of individual specialization that characterizes the population, but just a snapshot of a more dynamic trajectory of variation of dietary habits. Climatic variability, and its effects on individual specialization, is therefore not limited to the four seasons (Kazadi and Kaoru, 1996), but should be also considered in long-term studies.

The data analyzed here were collected before and after the warm season during three different years (Table 1), although for the second one (2017) data collection was performed in spring only. The prevalence of specialized individuals was generally higher in autumn compared to spring (Fig. 1), in agreement with what was already observed in this and other *Speleomantes* species (Salvidio et al., 2015; Lunghi et al., 2018b, 2020a). Such variation in diet composition is correlated to the seasonal climatic conditions affecting the phenology of both *Speleomantes* and their prey. At the end of spring, the high temperatures and low precipitations confine *Speleomantes* within their subterranean refuges, where suitable microclimatic conditions occur all year round (Lunghi et al., 2015; Ficetola et al., 2018). In these periods, prey such as crane flies (Diptera, Tipuloidea) also seek refuge in subterranean environments during hot season, where they can aggregate and reach very high densities (Lunghi et al., 2020b). Such aggregations of prey within a restricted environment offers *Speleomantes* the opportunity to easily capture multiple prey with a minimum effort (i.e., optimal foraging theory; (Stephens and Krebs, 1986), making these flies preponderant in their diet (usually between 58% and 94%; (Cianferoni and Lunghi, 2023). These conditions allow to a large proportion of individuals to forage primarily on this prey (Salvidio, 1992; Lunghi et al., 2018a, 2022a; see also Fig. 1). On the other hand, both *Speleomantes* and crane flies can leave subterranean environments when, in autumn, conditions become milder and wetter, and individuals can reach surface environments for foraging. Compared to caves, in surface environments prey availability for *Speleomantes* is higher; the access to broader foraging areas and the availability of a more diverse set

Table 2

Results of PERMANOVA analysis on *V* values, using season and population as fixed factors and year as random factor.

Source	d.f.	SS	MS	Pseudo-F	P(MC)
year (1)	2	5.94	2.97	329.23	**0.0001**
season (2)	1	4.79	4.79	32.50	0.13
population (3)	2	0.35	0.17	1.24	0.37
1 ×2**	1	0.15	0.15	16.35	**0.0001**
1 ×3	4	0.70	0.17	19.31	**0.0001**
2 ×3	2	0.33	0.16	0.60	0.62
1 ×2×3**	2	0.54	0.27	29.99	**0.0001**

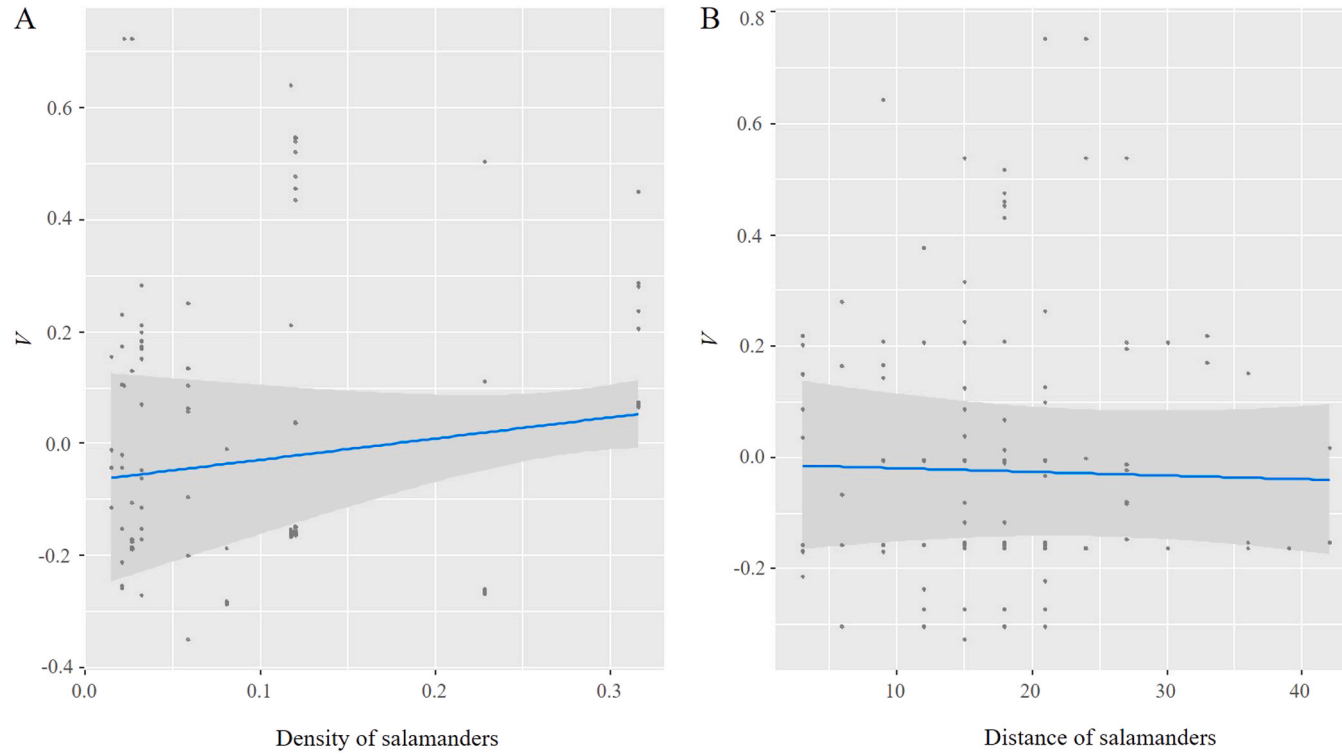** Term has one or more empty cells.

Fig. 2. Plots showing results of GLMM analyses. The individual diet specialization index *V* for each salamander is plotted against A) the density of active salamanders (individuals/m^2) observed during samplings and B) the linear distance (m) from the cave entrance of salamanders. Shaded areas indicate confidence intervals.

of prey allow individuals to target the most preferred ones (Salvidio et al., 2015; Lunghi et al., 2018a, 2018d), ultimately increasing their specialization.

One of our hypotheses explaining the divergence in diet specialization between different years was related to the accessibility of the most profitable foraging sites (Pille et al., 2023). Specifically, we expected to observe a more specialized diet in individuals closer to the cave entrance, and more generalist in those captured in the deepest cave area. Although results were marginally not significant ($P =$ 0.08), the observed trend provides a partial support to our hypothesis (Fig. 2B), allowing to speculate that additional factors may have affected our analysis. For example, the lack of data during autumn 2017, and the relative low number of salamanders with recognizable stomach contents probably reduced the statistical power of this test. On the other hand, we do not have information on where salamanders foraged. It is possible that most salamanders exited the cave to forage, and individuals from deeper cave areas just foraged earlier than those closer to the entrance, meaning that they had the time to partially digest the prey and consequently reduce the diversity observed within their stomach contents. Testing these hypotheses would require tracking individuals both inside and outside the cave, a challenging activity to be performed on cave salamanders, even though controlled conditions trials (performed e.g., in mesocosms) might help to answer these questions (Mammola et al., 2021).

Our second hypothesis, predicting a decrease of specialized individuals when density is low because of the relaxation from intraspecific competition, was supported by the data. Intraspecific competition is often density-dependent (Araújo et al., 2008), therefore populations with lower density likely have less specialized individuals. Indeed, during our surveys, when the number of individuals was higher (i.e., more salamanders foraging at the same time) we observed a higher diet specialization. During the study period (2016 – 2020), we did not detect a decrease in population abundances (Table 1), meaning that a variation in the trophic availability might have had a major role in determining the reduction of specialized individuals (Fig. 1). *Speleomantes* are ectothermic vertebrates that fully depend from the environment to regulate body temperature and water homeostasis (Spotila, 1972; Lunghi et al., 2016). Variation in local temperatures and precipitations determines the possibility of *Speleomantes* to exit and forage in surface environments, where the trophic supply is higher and therefore individuals can specialize on their preferred prey (Araújo et al., 2011; Lunghi et al., 2018a, 2021b). Considering the high temperatures recorded through the study period (Table S1), the variation of precipitations may represent the key factor that triggered *Speleomantes* foraging activity. For example, the intense precipitations characterizing spring 2017 (Table S1) may have allowed a higher number of individuals to forage outside the cave (Lunghi et al., 2018d), while in spring 2016 and 2020 individuals were probably confined to subterranean environments where diversity of prey is lower (Culver and Pipan, 2019). Having dietary data also from autumn 2017 would have provided an additional seasonal comparison, allowing to produce more robust data in support to this hypothesis.

Our study showed clear evidence that multiple factors can determine the variation of specialization, still it shows some limitation that may require attention in future researches. Here we did not consider potential recaptures of the same individuals through different years, an event that may help explaining the divergence of specialized individuals observed during different years. First, these populations are quite abundant and the recapture rates of individuals are very low (Ficetola et al., 2018; Lunghi et al., 2020a). Consequently, during different years we mostly sampled different populations' subsets, which might represent different proportion of specialized individuals. On the other hand, longitudinal studies on diet specialization of these salamanders are lacking, and it would be extremely interesting testing whether single individuals change their diet preference and specialization through the time.

In conclusion, this study provided novel information on the dynamism of the individual diet specialization and how it should be carefully interpreted from studies performed on a short time, and also provided insights for future researches. Indeed, our data highlighted the potential role that local environmental conditions may have in determining the occurrence of specialized individuals, meaning that long-term studies are needed to properly understand foraging tactics of species which physiology is highly dependent on local environmental conditions.

CRediT authorship contribution statement

Giorgio Mancinelli: Formal analysis, Software, Writing – review & editing. **Raoul Manenti:** Investigation, Writing – review & editing. **Gentile Francesco Ficetola:** Investigation, Writing – review & editing. **Enrico Lunghi:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data for years 2016 and 2017 are available from Lunghi et al. (2018a), while that for year 2020 from Lunghi et al. (2021b)

Acknowledgments

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2024.e02864.

References

Araújo, M.S., Bolnick, D.I., Martinelli, L.A., Giaretta, A.A., dos Reis, S.F., 2009. Individual-level diet variation in four species of Brazilian frogs. J. Anim. Ecol. 78, 848–856.

Araújo, M.S., Bolnick, D.L., Layman, C.A., 2011. The ecological causes of individual specialisation. Ecol. Lett. 14, 948–958.

Araújo, M.S., Costa-Pereira, R., 2013. Latitudinal gradients in intraspecific ecological diversity. Biol. Lett. 9, 20130778.

Araújo, M.S., Guimarães, P.R.J., Svanbäck, R., Pinheiro, A., Guimarães, P., Reis, S.F. dos, Bolnick, D.I., 2008. Network analysis reveals contrasting effects of intraspecific competition on individual vs. population diets. Ecology 89, 1981–1993.

Badino, G., 2010. Underground meteorology - "what's the weather underground?". Acta Carsol. 39, 427–448.

Barzaghi, B., Ficetola, G.F., Pennati, R., Manenti, R., 2017. Biphasic predators provide biomass subsidies in small freshwater habitats: a case study of spring and cave pools. Freshw. Biol. 62, 1637–1644.

Bolnick, D.I., Svanbäck, R., Araújo, M.S., Persson, L., 2007. Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. Proc. Natl. Acad. Sci. USA 104, 10075–10079.

Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D., Forister, M.L., 2003. The ecology of individuals: incidence and implications of individual specialization. Am. Nat. 161, 1–28.

Bolnick, D.I., Yang, L.H., Fordyce, J.A., Davis, J.M., Svanbäck, R., 2002. Measuring individual-level resource specialization. Ecology 83, 2936–2941.

Brose, U., 2010. Body-mass constraints on foraging behaviour determine population and food-web dynamics. Funct. Ecol. 24, 28–34.

Carvalho-Rocha, V., Cortês, L.B., Neckel-Oliveira, S., 2018. Interindividual patterns of resource use in three subtropical Atlantic Forest frogs. Austral Ecol. 43, 150–158.

Cianferoni, F., Lunghi, E., 2023. Inferring on Speleomantes foraging behavior from gut contents examination. Animals 13, 2782.

Cloyed, C.S., Eason, P.K., 2016. Different ecological conditions support individual specialization in closely related, ecologically similar species. Evolut. Ecol. 30, 379–400.

Cloyed, C.S., Eason, P.K., 2017. Niche partitioning and the role of intraspecific niche variation in structuring a guild of generalist anurans. R. Soc. Open Sci. 4, 170060.

Costa-Pereira, R., Araújo, M.S., Souza, F.L., Ingram, T., 2019. Competition and resource breadth shape niche variation and overlap in multiple trophic dimensions. Proc. R Soc. B Biol. Sci. 286, 20190369.

Costa, A., Salvidio, S., Posillico, M., Matteucci, G., De Cinti, B., Romano, A., 2015. Generalisation within specialization: inter-individual diet variation in the only specialized salamander in the world. Sci. Rep. 5, 13260.

Crovetto, F., Romano, A., Salvidio, S., 2012. Comparison of two non-lethal methods for dietary studies in terrestrial salamanders. Wildl. Res. 39, 266–270.

Culver, D.C., Pipan, T., 2019. The Biology of Caves and Other Subterranean Habitats, second ed. Oxford University Press, New York.

Denoël, M., Whiteman, H.H., Wissinger, S.A., 2007. Foraging tactics in alternative heterochronic salamander morphs: trophic quality of ponds matters more than water permanency. Freshw. Biol. 52, 1667–1676.

Douglas, B., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48.

Ficetola, G.F., Barzaghi, B., Melotto, A., Muraro, M., Lunghi, E., Canedoli, C., Lo Parrino, E., Nanni, V., Silva-Rocha, I., Urso, A., Carretero, M.A., Salvi, D., Scali, S., Scarì, G., Pennati, R., Andreone, F., Manenti, R., 2018. *N*-mixture models reliably estimate the abundance of small vertebrates. Sci. Rep. 8, 10357.

Ficetola, G.F., Lunghi, E., Canedoli, C., Padoa-Schioppa, E., Pennati, R., Manenti, R., 2018. Differences between microhabitat and broad-scale patterns of niche evolution in terrestrial salamanders. Sci. Rep. 8, 10575.

Gosling, S.D., 2008. Personality in non-human animals. Soc. Personal. Psychol. Compass 2, 985–1001.

Ingram, T., Costa-Pereira, R., Araújo, M.S., 2018. The dimensionality of individual niche variation. Ecology 99, 536–549.

Johnson, K., 2000. Trophic-dynamic considerations in relating species diversity to ecosystem resilience. Biol. Rev. 75, 347–376.

Kazadi, S.-N., Kaoru, F., 1996. Interannual and long-term climate variability over the Zaire River Basin during the last 30 years. J. Geophys. Res. 101, 21351–21360.

Kröncke, I., Dippner, J.W., Heyen, H., Zeiss, B., 1998. Long-term changes in macrofaunal communities off Norderney (East Frisia, Germany) in relation to climate variability. Mar. Ecol. Prog. Ser. 167, 25–36.

Kuznetsova, A., Brockhoff, B., Christensen, H.B., 2016. lmerTest: Tests in Linear Mixed Effects Models. R Package Version 2.0-2.9.

Lanza, B., Pastorelli, C., Laghi, P., Cimmaruta, R., 2006. A review of systematics, taxonomy, genetics, biogeography and natural history of the genus *Speleomantes* Dubois, 1984 (Amphibia Caudata Plethodontidae). Atti Mus. Civ. Stor. Nat. Trieste 52, 5–135.

Lejeune, B., Sturaro, N., Lepoint, G., Denoël, M., 2023. Facultative paedomorphosis as a mechanism promoting intraspecific niche differentiation. Oikos 127, 427–439.

Lunghi, E., 2022. Doubling the lifespan of European plethodontid salamanders. Ecology 103, e03581.

Lunghi, E., Bacci, F., Zhao, Y., 2021. How can we record reliable information on animal colouration in the wild? Diversity 13, 356.

Lunghi, E., Cianferoni, F., Ceccolini, F., Mulargia, M., Cogoni, L., Avitabile, D., Veith, M., Manenti, R., Ficetola, G.F., Corti, C., 2018. Field-recorded data on the diet of six species of European *Hydromantes* cave salamanders. Sci. Data 5, 180083.

Lunghi, E., Cianferoni, F., Ceccolini, F., Veith, M., Manenti, R., Mancinelli, G., Corti, C., Ficetola, G.F., 2018. What shapes the trophic niche of European plethodontid salamanders? PLoS One 13, e0205672.

Lunghi, E., Cianferoni, F., Ceccolini, F., Zhao, Y., Manenti, R., Corti, C., Ficetola, G.F., Mancinelli, G., 2020. Same diet, different strategies: variability of individual feeding habits across three populations of Ambrosi's cave salamander *(Hydromantes ambrosii)*. Diversity 12, 180.

Lunghi, E., Cianferoni, F., Corti, C., Zhao, Y., Manenti, R., Ficetola, G.F., Mancinelli, G., 2022. The trophic niche of subterranean populations of *Speleomantes italicus*: a multi-temporal analysis. Sci. Rep. 12, 18257.

Lunghi, E., Cianferoni, F., Giachello, S., Zhao, Y., Manenti, R., Corti, C., Ficetola, G.F., 2021. Updating salamander datasets with phenotypic and stomach content information for two mainland *Speleomantes*. Sci. Data 8, 150.

Lunghi, E., Corti, C., Biaggini, M., Merilli, S., Manenti, R., Zhao, Y., Ficetola, G.F., Cianferoni, F., 2022. Capture-mark-recapture data on the strictly protected *Speleomantes italicus*. Ecology 103, e3641.

Lunghi, E., Corti, C., Manenti, R., Barzaghi, B., Buschettu, S., Canedoli, C., Cogoni, R., De Falco, G., Fais, F., Manca, A., Mirimin, V., Mulargia, M., Mulas, C., Muraro, M., Murgia, R., Veith, M., Ficetola, G.F., 2018. Comparative reproductive biology of European cave salamanders (genus *Hydromantes*): nesting selection and multiple annual breeding. Salamandra 54, 101–108.

Lunghi, E., Corti, C., Manenti, R., Ficetola, G.F., 2019. Consider species specialism when publishing datasets. Nat. Ecol. Evol. 3, 319.

Lunghi, E., Ficetola, G.F., Zhao, Y., Manenti, R., 2020. Are the neglected Tipuloidea crane flies (Diptera) an important component for subterranean environments? Diversity 12, 333.

Lunghi, E., Giachello, S., Manenti, R., Zhao, Y., Corti, C., Ficetola, G.F., Bradley, J.G., 2020. The post hoc measurement as a safe and reliable method to age and size plethodontid salamanders. Ecol. Evol. 10, 11111–11116.

Lunghi, E., Giachello, S., Zhao, Y., Corti, C., Ficetola, G.F., Manenti, R., 2020. Photographic database of the European cave salamanders, genus *Hydromantes*. Sci. Data 7, 171.

Lunghi, E., Manenti, R., Canciani, G., Scarì, G., Pennati, R., Ficetola, G.F., 2016. Thermal equilibrium and temperature differences among body regions in European plethodontid salamanders. J. Therm. Biol. 60, 79–85.

Lunghi, E., Manenti, R., Cianferoni, F., Ceccolini, F., Veith, M., Corti, C., Ficetola, G.F., Mancinelli, G., 2020. Interspecific and inter-population variation in individual diet specialization: do environmental factors have a role? Ecology 101, e03088.

Lunghi, E., Manenti, R., Ficetola, G.F., 2014. Do cave features affect underground habitat exploitation by non-troglobite species? Acta Oecol. 55, 29–35.

Lunghi, E., Manenti, R., Ficetola, G.F., 2015. Seasonal variation in microhabitat of salamanders: environmental variation or shift of habitat selection? PeerJ 3, e1122.

Lunghi, E., Manenti, R., Mulargia, M., Veith, M., Corti, C., Ficetola, G.F., 2018. Environmental suitability models predict population density, performance and body condition for microendemic salamanders. Sci. Rep. 8, 7527.

Mammola, S., Lunghi, E., Bilandzija, H., Cardoso, P., Grimm, V., Schmidt, S.I., Hesselberg, T., Martínez, A., 2021. Collecting eco-evolutionary data in the dark: impediments to subterranean research and how to overcome them. Ecol. Evol. 11, 5911–5926.

Manenti, R., Lunghi, E., Ficetola, G.F., 2015. Distribution of spiders in cave twilight zone depends on microclimatic features and trophic supply. Invertebr. Biol. 134, 242–251.

Pille, F., Pinto, L., Denoël, M., 2023. Functional and temporal facets of predation by marsh frogs across the aquatic–terrestrial ecotone of ponds and implications in the context of biological invasions. Freshw. Biol. 68, 2184–2196.

Rosumek, F.B., Blüthgen, N., Brückner, A., Menzel, F., Gebauer, G., Heethoff, M., 2018. Unveiling community patterns and trophic niches of tropical and temperate ants using an integrative framework of field data, stable isotopes and fatty acids. PeerJ 6, e5467.

Salvidio, S., 1992. Diet and food utilization in the European plethodontid *Speleomantes ambrosii*. Vie Milieu 42, 35–39.

Salvidio, S., Oneto, F., Ottonello, D., Costa, A., Romano, A., 2015. Trophic specialization at the individual level in a terrestrial generalist salamander. Can. J. Zool. 93, 79–83.

Schoener, T.W., 1968. The *Anolis* lizards of Bimini: resource partitioning in a complex fauna. Ecology 49, 704–726.

Semlitsch, R.D., O'Donnell, K.M., Thompson, F.R., 2014. Abundance, biomass production, nutrient content, and the possible role of terrestrial salamanders in Missouri Ozark forest ecosystems. Can. J. Zool. 92, 997–1004.

Spotila, J.R., 1972. Role of temperature and water in the ecology of lungless salamanders. Ecol. Monogr. 42, 95–125.

Stanczyk, N.M., Brookfield, J.F.Y., Ignell, R., Logan, J.G., Field, L.M., 2010. Behavioral insensitivity to DEET in Aedes aegypti is a genetically determined trait residing in changes in sensillum function. Proc. Natl. Acad. Sci. 107, 8575–8580.

Stephens, D.W., Krebs, J.R., 1986. Foraging Theory. Princeton University Press, Princeton, N.J.

Svanbäck, R., Persson, L., 2004. Individual diet specialization, niche width and population dynamics: implications for trophic polymorphisms. J. Anim. Ecol. 73, 973–982.

Toscano, B.J., Gownaris, N.J., Heerhartz, S.M., Monaco, C.J., 2016. Personality, foraging behavior and specialization: integrating behavioral and food web ecology at the individual level. Oecologia 182, 55–69.