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Moving in the Dark: Enlightening the Spatial Population Ecology of European Cave Salamanders

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ABSTRACT

Space use and movement are fundamental aspects of organisms' ecology, mirroring individual fitness, behavior, and life-history strategies. These mechanisms are shaped by environmental heterogeneity, which often makes it difficult to understand these dynamics. Subterranean habitats are simplified and understudied environments, potentially ideal for evaluating the fine-scale spatial ecology of organisms. Spatial capture–recapture (SCR) methods allow us to investigate animal space use and movement, including spatially explicit observation data on individuals, to assess the relationship between population dynamics and landscape ecology. In this context, we assessed individual interactions, movement ecology, and activity patterns of a subterranean population of the cave salamander *Speleomantes strinatii*, applying SCR modeling to a photographic capture–recapture dataset of 104 identified individuals (43 males, 35 females and 26 subadults). Analysis of overlap indices showed that the proportion of males' home range (HR) was more covered by females' HR than vice versa ($U = 25$, $p = 0.038$). During the monitoring, females traveled longer distances in less time than males (0.60 vs. 0.22 m/day). Finally, salamanders tended to be more active during summer periods, showing a preference for the inner sectors of the cave and rougher cave walls. Combining the analysis of spatially explicit capture history (overlap and movement estimates) and SCR (activity pattern, sex-specific density and space use), we enlightened new features and confirmed previous knowledge of the spatial ecology of *S. strinatii*. Our study demonstrates how structured capture–recapture data can be used to infer individual interactions and movement in low-complexity habitats and is open to the application of SCR methods in more complex environments for revealing finer-scale ecological variation with important conservation implications.

1 | Introduction

Spatial heterogeneity within the environment significantly influences individual fitness, behavior, or life-history traits' selection, and has the potential to impact populations through co-evolutionary mechanisms, operating not solely in time but also across space (Levin 1992). Consequently, comprehending ecological processes necessitates not only temporal but also spatial focus, to effectively quantify the effect of spatial heterogeneity on ecological and behavioral dynamics. However, the extensive variability in abiotic and biotic conditions throughout the environment can pose challenges to detecting and measuring ecological and behavioral mechanisms (Mackey and Lindenmayer 2001). In this context, subterranean environments represent a simplified habitat, when compared to epigean ones, with low environmental variability, presenting a valuable opportunity to investigate space use mechanisms of the species living therein, which usually display a narrow ecological niche (Culver and Pipan 2019; Mammola 2019).

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Although reduced compared to epigean environments, microhabitat and seasonal variations in cave environments are still present, affecting the distribution and activity of organisms in space and time (Lunghi, Manenti, and Ficetola 2015; Mammola 2019; Salvidio et al. 2019). Indeed, subterranean habitats harbor a unique and diversified fauna, characterized by multiple ecological and functional traits, which reflect the adaptations of organisms to these particular environments (Culver and Pipan 2019). Among the many troglophile (i.e., organisms that are particularly adapted and reproduce in cave environment but may also be found in other environments, Mammola 2019) and troglobiont species (i.e., organisms that are strictly dependent and reproduce only in cave environment, generally endowed with morphological and physiological adaptations such as depigmentation or anophthalmia) well suited to study small-scale drivers of space use, Plethodontids, a family of lungless salamanders (Plethodontidae), could primarily (e.g., *Speleomantes* sp. is mostly found in caves and karst systems) or exclusively (e.g., *Eurycea spelaea* and *Gyrinophilus palleucus* are troglobiotic species adapted to life in complete darkness) inhabit caves and represent a key component of the subterranean environment, acting as top predators on the invertebrate communities of caves (Salvidio et al. 2019).

Plethodontid salamanders, and in particular terrestrial species, generally possess low dispersal rates and display a relative ease of repeated captures, which makes them particularly well-suited as a study subject to evaluate fine-scale variation in demographic and activity patterns (e.g., Petranka 1998; Jaeger 1980). The activity of these salamanders is also known to be highly constrained by their physiological requirements, to narrow temperature and humidity conditions (Wake 1987). For this reason, the spatial ecology of Plethodontids, and in particular of North American ones, has been widely investigated, revealing important details into movement patterns, territoriality, and habitat preferences (Kleeberger and Werner 1982; Marvin 1998; Mathis 1991; Sutherland et al. 2019). The ecology and the demography of European cave salamanders of genus *Speleomantes* has been deeply investigated, both in surface and subterranean environments (Costa et al. 2020; Ficetola et al. 2018; Lunghi, Manenti, and Ficetola 2015; Salvidio et al. 2019). In underground habitats, distribution of individuals seems influenced by both abiotic and biotic features (e.g., illuminance, temperature, prey distribution and social processes), also showing a clear spatial segregation related to age (Ficetola et al. 2013; Salvidio 2002; Salvidio et al. 2019). Despite this, fine-scale studies of space use remain limited, especially in subterranean environments and no study, except for that carried out by Salvidio (2013), has been conducted on *Speleomantes* species using spatially explicit approaches such as spatial capture–recapture (SCR). Furthermore, few SCR application investigated amphibians' ecology in subterranean environments (Balázs et al. 2020), entailing a lack of valuable information (i.e., spatial ecology of animals of great conservation interest, with particular reference to what factors drive their space use, activity and density), since hypogean (i.e., subterranean) habitats are often exploited by various species of amphibians, both occasionally and exclusively (Balogová et al. 2017; Lunghi and Bruni 2018). SCR methods allow to assess simultaneously population dynamics and spatial distribution of herptiles, providing important information regarding their ecology and demography, with valuable insights from the conservation point of view (Cisternas et al. 2022; Kervellec et al. 2023; Schmidt et al. 2017).

We aimed to assess, for the first time, the extent of space use, evaluating eventual differences between individuals and sexes, and how Strinati's cave salamanders exploit the subterranean environment. We assume that females, due to the huge reproductive investment, are more mobile than males, who are expected to show a greater site fidelity. To achieve this, we carried out a multi-year SCR study to investigate the fine-scale spatial ecology and demography of a population of *Speleomantes strinatii* (Aellen, 1958) in a subterranean environment. In particular, we assessed the spatial distribution and interactions of adult individuals, together with population activity patterns, and how these dynamics are affected by different environmental features. The results of this SCR study will contribute to a better understanding of the spatial ecology of an endangered amphibian and to evaluate the applicability of the SCR approach for monitoring other species of the genus *Speleomantes* both in underground and surface habitats.

2 | Material and Methods

2.1 | Study Site and Species

The study site is the Biospeleological Station "Arturo Issel," an artificial tunnel situated at 369 m a.s.l. in the municipality of Savignone (province of Genova, Liguria, NW Italy). This cave is a U-shaped 40-m tunnel with one entrance (the shelter originally had two entrances but one collapsed soon after the construction), excavated in calcareous siltstones and used as an air-raid shelter during World War II. The Biospeleological Station has been naturally colonized by a population of Strinati's cave salamanders (*S strinatii*) and is dedicated to the ecology and demographic study of this species (Salvidio et al. 1994; Costa et al. 2024). *S strinatii* is a medium-sized (maximum total length < 115 mm) plethodontid distributed from the southeast of France to the northwest of Italy, recently listed as Endangered (IUCN SSC Amphibian Specialist Group 2022). The species is found in the leaf litter of temperate forests along streams, on humid rock faces, and in subterranean habitats (Lanza 2007). *S. strinatii* is active during humid time periods with mild temperatures, while it retreats underground during dry and hot seasons and the coldest months of the year (Salvidio 1993). Females lay clutches containing 8–13 eggs on the humid soil or in rock crevices and actively guard and defend them (Oneto et al. 2010). In order to analyze the spatial distribution and demographic dynamics of the resident population of *S. strinatii*, the Biospeleological Station walls have been equipped with air thermometers, a psychrometer, and a permanent grid with a 1 × 1 m mesh (Salvidio 2002; Figure 1). Microclimatic conditions of the cave, described by Salvidio et al. (2019), show annual variations very similar to those measured in natural single-entrance caves located in the same geographic area (Isaia et al. 2011; Ravbar and Kosutnik 2014).

2.2 | Sampling Design

Salamanders were sampled monthly (one sampling occasion per month) from July 2021 to July 2023 through the photographic capture–mark–recapture method. Adult and subadult salamanders were captured by hand and photographed on the

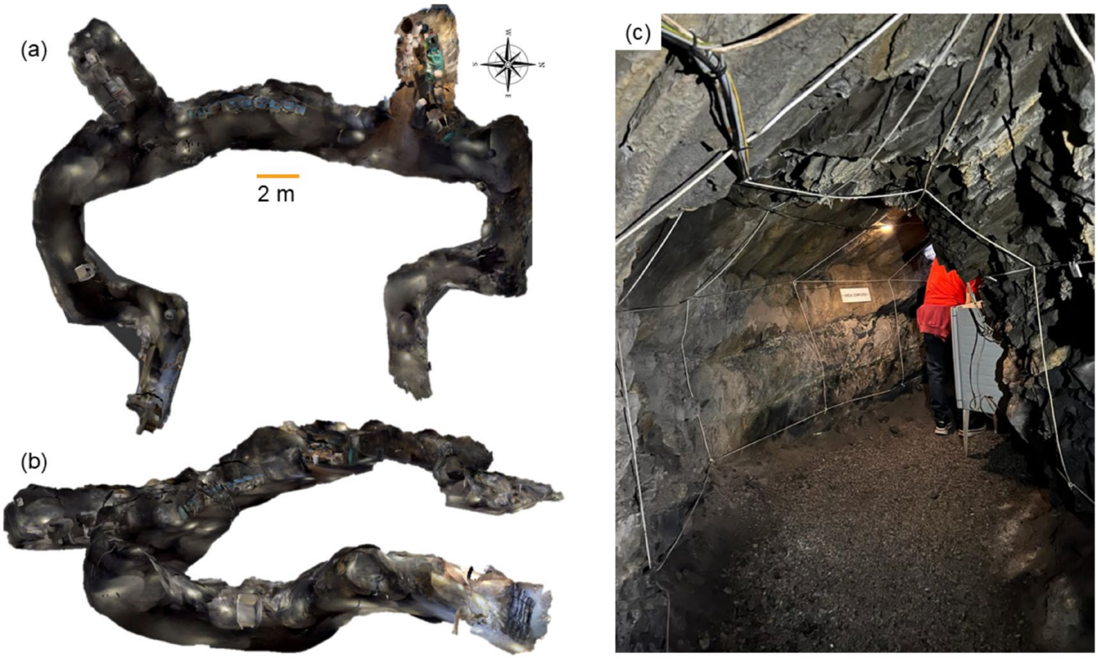


FIGURE 1 | View from above (a) and a side view (b) of the Biospeleological Station "Arturo Issel", cave (3D planimetry of cave, realized by Antonio Travi, is available at https://scaniverse.com/scan/odvoikodupff5t7p), together with a photo of part of the study grid and the study habitat (c).

ventral side, which is characterized by a unique and recognizable pattern in each individual (Figure S1). Salamanders were sexed by means of secondary sexual characters: Mature males possess a conspicuous mental gland (Lanza 2007); females that lack a gland become reproductive when possessing a snout-vent length (SVL) > 57 mm (Salvidio 1993) and subadults are those individuals lacking a mental gland and whose SVL is comprised between 47 and 57 mm (Salvidio 2002). Individuals possessing an SVL < 47 mm are considered juveniles and were discarded from the capture mark–recapture experiment. Once photographed, each salamander was released at the exact site of capture, which corresponds to one of the 210 squares of the permanent grid. Before and during each sampling occasion, we followed the procedures for mitigating the spreading of fungal diseases (Garner et al. 2016). The comparison of images was then carried out through the software Wild-ID (Bolger et al. 2012), which has already successfully been used for the monitoring of various species of amphibians, including *S. strinatii* (Renet et al. 2019). To avoid potential identification errors in the database, such as false negatives (i.e., a photographed individual is considered as a new capture, although it was previously captured) or false positives (i.e., a photographed individual is considered as a recapture, although it was not previously captured), one experienced observer (GR) visually compared all the images in the database. The resulting dataset consisted of a matrix reporting the detection site (i.e., one of the 210 squares of the permanent grid) of captured and identified individuals during each sampling occasion.

Microhabitat features were measured for each mesh square of the study cave and assessed using three different measurements: air temperature, distance from entrance, and wall complexity. During the sampling period, the temperature of the cave was registered (°C) every hour using four dataloggers (Elitech, RC-51H), placed at 1, 8, 18, and 40 m from the entrance. These measurements were then used to model the air temperature of each 1-m section inside the tunnel. The linear distance of capture sites from the entrance was recorded in meters based on the permanent grid. Cave wall's complexity was calculated by summing the linear distance attained by a 1 m string pressed vertically on side walls (Camp and Jensen 2007; Lunghi, Manenti, and Ficetola 2015). These measures were then subtracted from 200, the value obtained if both cave walls were completely smooth. In this way, a substrate with minimum complexity (i.e., completely smooth) would score 0, while increasing values indicate a gradual increase in complexity.

3 | Data Analysis

The multi-year capture–recapture data were used to carry out a SCR analysis at individual and population level. Individual home range (HR) and spatial interactions between animals were assessed using R package *adehabitat* (Calenge 2006). HRs were obtained by the estimation of the utilization distribution (UD; i.e., the probability density that an animal is found at a point according to its geographical coordinates) through 50% kernel density estimation (KDE), for those individuals with at least four recapture events. Given the limited area of the cave, we used only KDE 50%, defining HR as the minimum area in which an animal has some specified probability (in this case KDE 50%) of being located (Calenge 2006). Individual interactions were analyzed using three different overlap indices (Fieberg and Kochanny 2005): (i) Method "HR" ("percent of home range overlap") calculates the proportion of the UD of one animal covered by the UD of another one (Figure S2 and Table S1); (ii) method "PHR" ("proportional home range") calculates the volume under the

UD of an animal, that is, inside the UD of another one (i.e., the probability of one animal being located in the HR of another one); (iii) method "VI" (volume of intersection index) calculates the volume of intersection between two UD, where values range from 0 (i.e., no overlap) to 1 (i.e., identical UDs). Mann–Whitney non-parametric rank test was used to assess possible differences among and between sexes for overlap indices. The state-space was defined as a 5×42 m grid symmetric to the permanent one installed in the cave. A buffer (i.e., fixed distance added around the trapping grid to define the area over which individuals' activity centers are assumed to be distributed) of 2 m was selected using the function *suggest.buffer* of R package *secr* (Efford and Fewster 2013). Movements of individuals and related metrics (i.e., the distance moved by each salamander between sampling occasions) were analyzed using R package *secr* (Efford and Fewster 2013) and selecting salamanders with at least three encounters. This allowed also to quantify the displacement rates of salamanders, expressed as the maximum straight-line distance reached by each individual between two subsequent capture occasions divided by the number of days elapsed between the two events in which salamanders moved this distance. Finally, population activity patterns, space use and density were assessed using the R package *oSCR* (Sutherland et al. 2019), analyzing the data as a single temporal session (i.e., the data were not analyzed by distinguishing different sampling times, such as seasons and years, but by considering a single study period) while assuming that potential biases from the violation of closure assumption (i.e., no animals are entering or leaving the population via births, deaths, immigration, or emigration) were negligible. Our approach (i.e., analyze data as single temporal session and assume closure assumption) is based on the following previously established characteristics of the study species: (i) the demographic structure (in terms of abundance, age structure and sex ratio) of the studied population was relatively stationary over 27 years (mean abundance 103 ± 6 to 20.18 and four age groups; Salvidio et al. 2015; Costa et al. 2024); (ii) cave salamanders are long-living amphibians (i.e., 25 years old, Lanza 2007; Lunghi 2022) and the adult mortality rate of a population monitored for only 2 years is likely to be very low; (iii) our monitoring focused only on adult salamanders, therefore it did not take into consideration newborns that have entered the population; (iv) juveniles growth rates of *S. strinatii* during the first 2 years of life after hatching are estimated to vary from 10 to 13 mm/year (Salvidio 1993), consequently the proportion of juveniles (i.e., individuals with an SVL < 47 mm) who may have become subadults during the monitoring is negligible; (v) given the limited movements of plethodontids (Sutherland et al. 2016, 2019), it is unlikely that adult individuals from the outside have entered in the studied population; (vi) the trend of temperatures recorded inside the cave during this biennial monitoring was constant, showing negligible fluctuations of environmental conditions (Figure S3 and Table S2). In the spatial detection model, the probability of detecting an individual at a given trap decreases as the distance between the trap and the individual's activity center increases. The baseline detection probability ($p0$), that is, the probability of detecting an individual at its activity center, was modeled as a function of sex, air temperature, and distance from the cave entrance, cave wall complexity and day of the year. A quadratic effect of day of year was included to account for potential non-linear seasonal trends in detection probability (see Section 2 below for model specification). The activity of *S strinatii* is often influenced by seasonal environmental factors (e.g., temperature, humidity, and reproductive cycle), which may not change linearly throughout the year. A quadratic term allows to identify peak and deflection in detectability during the year (e.g., the optimal environmental conditions and at the end of the activity season). Separate space use parameters (σ) were estimated for males and females to account for potential differences in movement ecology between sexes. Salamanders' density was modeled as constant across the state-space, assuming a uniform distribution across grid cells without spatial heterogeneity. Given the structural and environmental homogeneity of the study area, ecological gradients that typically justify spatially explicit density models (e.g., vegetation cover, elevation, or temperature gradients) are minimal or absent. Therefore, a uniform density model provides a baseline while still allowing for sex-specific variation in space use and detection parameters (σ and $p0$). Although density was modeled as uniform, the estimated number of individuals of each sex can differ, depending on the detection function parameters $p0$ and σ (which determines how detection probability declines with distance from an individual's activity center). Differences of these parameters between sexes can lead to different estimated number of individuals of males and females, even if density is constant (i.e., if $p0$ or σ differ by sex, the expected number of captures for each sex will be different, making sex ratio a derived parameter). The baseline detection probability ($p0$) was modeled using a logit link, space use (σ, the spatial scale parameter) was modeled on the log scale A total of 10 SCR models was fitted using a half-normal detection function, including the null model and models incorporating covariates on $p0$ and sex-specific effects on σ. The former was modeled as function of day of the year, modeled as a quadratic term ($\text{Day} + \text{Day}^2$) to capture potential seasonal variation in detection, wall complexity, temperature, and distance from the cave entrance, modeled as linear effects. The full model for detection probability was:

$$\begin{aligned}\operatorname{logit}(p0_i) &= \beta 0 + \beta 1 \cdot \text{Day}_i + \beta 2 \cdot \text{Day}_i{}^2 + \beta 3 \cdot \text{complexity}_i \\ &\quad + \beta 4 \cdot \text{temperature}_i + \beta 5 \cdot \text{distance}_i \text{ and } \log(\sigma_i) = \alpha 0 + \alpha 1 \cdot \text{sex}_i\end{aligned}$$

where the state-space density D was modeled as constant.

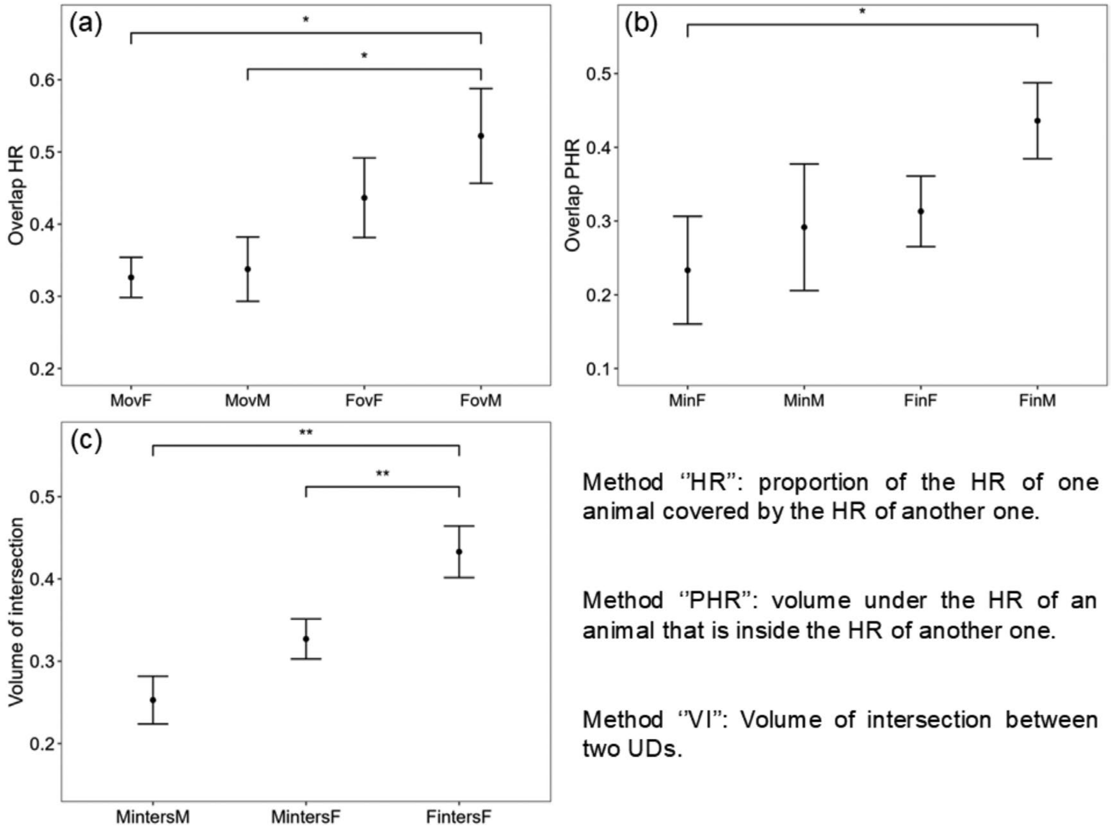
Models were ranked by Akaike information criterion (AIC), considering that when $\Delta\text{AIC}_c > 2$ models show substantially different support (i.e., indicating less support for the model with the higher AIC_c). All analyses were performed in (R Core Team 2021).

4 | Results

During 24 sampling occasions, we performed 276 captures, identifying 104 salamanders (43 males, 35 females and 26 subadults). Of these, we obtained individual UD of 21 adults (11 females and 10 males; Figure S4). Mean HR of the sampling population was $14.44\,\text{m}^2$ ($\text{SD} = 3.56$), with no differences between sexes (females: $15.56\,\text{m}^2$, $\text{SD} = 3.85$; males: $13.21\,\text{m}^2$ $\text{SD} = 2.9$; Mann–Whitney: $U = 33.5$, $p = 0.14$). The analysis of overlap indices showed that the proportion of male HRs covered by female HRs was higher than the proportion of female HRs covered by male HRs (Mann–Whitney: $U = 25$, $p = 0.038$; Figure 2a) and the probability of finding a female in the HR of a male was higher than that of finding a male in the HR of a female (Mann–Whitney: $U = 24$, $p = 0.032$; Figure 2b). Furthermore, females shared more volume of their respective HR with other females than with males (Mann–Whitney: $U = 128$, $p = 0.02$; Figure 2c).

Movement analysis, conducted using *secr*, focused on 41 individuals (20 males, 15 females and 6 subadults; Figure S5), that is, those with at least two recapture events. Mean distance moved between sampling occasion was 6.88 m (range: 2.3–14.62), while mean maximum distance was 13.22 m (range: 0–35.13), without any significant difference between sexes (Table 1). However, females showed significant higher displacement rates (i.e., moved longer distance in less time) than males ($U = 74$; $p = 0.01$).

Analysis of population activity pattern and spatial ecology with *oSCR* included 56 individuals (27 males, 18 females and 11 subadults), that is, those with at least one recapture event. Model selection using AIC showed that baseline detection (i.e., the probability of detecting an individual at its activity center) was best explained using a quadratic effect of the day of sampling, wall complexity, distance from entrance, and air temperature (Table 2). In particular, day of sampling, wall complexity, and distance from entrance had a significant effect on the probability of detecting an individual at its activity center. The maximum



Method "HR": proportion of the HR of one animal covered by the HR of another one.

Method "PHR": volume under the HR of an animal that is inside the HR of another one.

Method "VI": Volume of intersection between two UDs.

FIGURE 2 | Overlap indices among and between sexes. Bars represent standard error of the mean. In (a), "ov" indicates the proportion of the home range (HR) of one sex covered by the other one or by the other individuals of the same sex (e.g., MovF: proportion of the HR of females covered by males) based on the home range (HR) method. In (b), "in" indicates the volume of the HR of one sex inside the HR of the other one or inside that of other individuals of the same sex (e.g., MinF: volume of the HR of males, that is, inside the HR of females) based on the proportional home range (PHR) method. In (c) the term "inters" indicates the volume shared between two different utilization distributions (UDs) or volume intersection (VI) method (e.g., MintersF: volume of HR that males share with females). Lines on top show significant statistical differences between plotted means: * indicates p value < 0.05, whereas ** indicates a p value < 0.01.

TABLE 1 | Mean movement data of cave salamanders with at least three encounters.

	Mean distance	Max distance	Encounters	Displacement rate
Population	6.88 m (2.3–14.6)	13.22 m (1–35.13)	5 (3–11)	0.38 (0–1.77)
Females	6.63 m (2.3–14.6)	16.25 m (1–35.13)	5 (3–10)	0.60* (0–1.77)
Males	7.71 m (2–19.9)	11.64 m (3–28.28)	5 (3–11)	0.22* (0–0.79)

Note: Range values (i.e., maximum and minimum values measured during the monitoring) are between brackets.
*Significant difference between sexes ($p < 0.05$).

TABLE 2 | AIC table for the spatial capture–recapture models fit.

Model	Logl.	*K*	AIC	ΔAIC	Weight
D(~1) *p*(~Day + Day^2 + Compl + Temp + Dist) sig(~sex)	1680	10	3380	0	1.0e+00
D(~1) *p*(~Day + Day^2 + Compl + Temp) sig(~sex)	1682	9	3382	2	1.5e−05
D(~1) *p*(~Day + Day^2) sig(~sex)	1694	7	3402	22	3.8e−05
D(~1) *p*(~Day + Day^2 + Temp) sig(~sex)	1694	8	3403	23	2.0e−05
D(~1) *p*(~Day + Day^2 + Compl) sig(~sex)	1711	8	3439	59	3.4e−13
D(~1) *p*(~Compl) sig(~sex)	1724	6	3460	80	1.0e−17
D(~1) *p*(~Temp) sig(~sex)	1732	6	3475	99	4.6e−21
D(~1) *p*(~1) sig(~sex)	1736	5	3482	102	1.5e−22
D(~1) *p*(~1) sig(~1)	1737	4	3482	103	1.5e−22
D(~1) *p*(~Dist) sig(~sex)	1789	6	3590	210	6.5e−46

Note: Logl. represents the log-likelihood of the model; *K* is the number of estimated parameters; AIC is the Akaike Information Criterion; ΔAIC is the difference in AIC score between the best model and the model being compared, and weight represents the relative likelihood of each model given the data. Abbreviations: Compl, cave wall complexity; *D*, density; Day, day of year; Dist, distance from entrance; *p*, probability of detecting an individual at its activity center; sig, space use; Temp, air temperature.

probability of detection was around the 200th day of the year (19 July), increasing with wall complexity and decreasing slightly as the distance from the cave entrance increased (Figure 3). Males had higher densities per quadrat (i.e., m^2) than females (0.36 salamanders/m^2; 95% CI = 0.25–0.51 vs. 0.22 salamanders/m^2; 95% CI = 0.14–0.34). Finally, females showed larger space use area than males (10.01 m^2; 95% CI = 7.83–12.8 vs. 8.01 m^2; 95% CI = 6.74–9.54).

5 | Discussion

Our findings describe the spatial ecology and the intraspecific spatial interactions occurring in a salamander population living in an underground habitat. In general, our results support previous reports of plethodontid salamanders generally having small HRs and limited movements (Olson and Kluber 2014; Sutherland et al. 2016). Plethodontids are known to be highly constrained by their narrow physiological requirements and reliant on specific environmental conditions (Wake 1987). Furthermore, the suitable conditions (i.e., constant humidity, low temperature variation and trophic availability almost constant during highest activity periods) and the limited surface of the study site may further influence the low displacement of salamanders. SCR analyses conducted over two consecutive years showed that adult male and female salamanders had similar HRs, but females moved significantly faster and could cover longer distances compared to males. HR equivalence between sexes has already been reported for plethodontids (Mathis 1991; Kleeberger and Werner 1982; but see Marvin 1998). Indeed, our HR size estimates obtained with *oSCR* were smaller but still consistent with those obtained with *adehabitat* (Figure 4). Overlap indices showed that females tended to occupy more surface area and be more frequently in the HRs of males than vice versa and that they shared more volume of their UD with other females than with males (Figure 5). Higher co-occurrence between females has also been found in *Plethodon cinereus* and *P. kentucki* in deciduous forests (Gade et al. 2023; Marvin 1998). These intersexual differences may be due to the environment and to the combination of multiple biological traits of the species, in particular territoriality and different investment in parental care. Terrestrial salamanders are known to be able to evict conspecific intruders from a defended area, displaying strong territoriality (Jaeger et al. 1982). A reflection of this territoriality could be the very low volume of UD shared between males (Figure 2c). Similarly, as they are not forced to move and find suitable territories (i.e., the study cave represents an optimal habitat with abundant resources), males may display higher fidelity, possessing and defending defined HRs and allowing females to enter inside to enhance reproductive success (Jaeger et al. 1982). *S. strinatii* has a high site fidelity, showing a tendency to return to the site of origin when moved to a new site, even if the latter possesses suitable living conditions for plethodontids (Salvidio 2013). Moreover, plethodontids tend to be more tolerant toward invaders of the opposite sex than toward invaders of the same sex (Jaeger et al. 1982; Mathis 1991). This pattern is reflected also by our overlap indices, which show how females are found more frequently in males' HRs than vice versa. Finally, males had also higher densities, which may reduce space use of individuals (i.e., the more individuals, the less space for all; Gade et al. 2023). On the other hand, females can attain larger sizes and be very aggressive with conspecifics while they are guarding eggs and newborns, especially with male intruders (Oneto et al. 2014). Our results show that females tend to share a greater amount of territory with other females than with males. The high investment in parental care could also explain the larger distances covered by females, which may search for specific microclimatic conditions for hatching or to favor eggs development (Oneto et al. 2014; Lunghi, De Falco, et al. 2015; Lunghi, Manenti, and Ficetola 2015). This hypothesis is supported also by the study of Lunghi and Bruni (2018), who observed different females of *Speleomantes imperialis* using the same nesting site in different years, suggesting nesting site selection in this species. Furthermore, laying eggs in sites difficult to access (e.g., deep crevices) might also improve egg protection. Finally, females of plethodontid salamanders are known to colonize vacated suitable habitats faster than males and move more, actively selecting potential mates and their territories (Mathis 1991).

Results regarding baseline detection probability confirmed the previous knowledge about the activity of European plethodontids

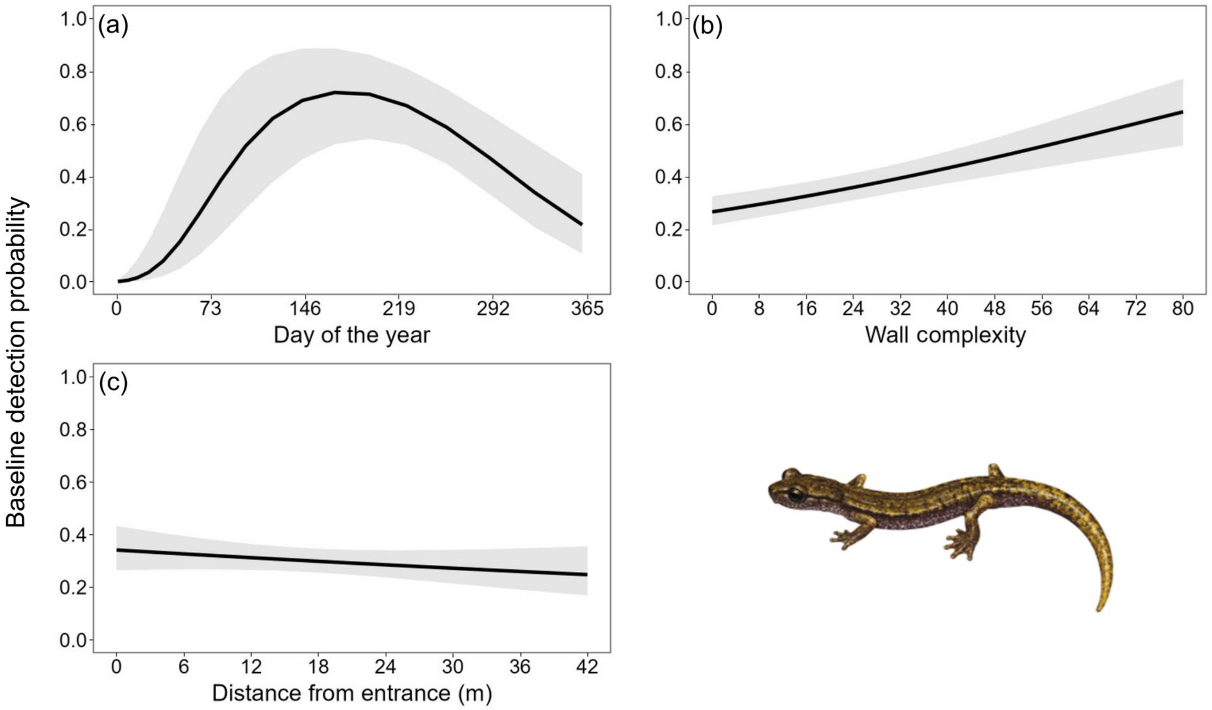


FIGURE 3 | Estimated effect of the day of sampling (a), the complexity of the walls (b) and distance from entrance in meters (c) on the probability of detecting an individual at its activity center. The gray shade area represents the 95% confidence interval. Image of *Speleomantes strinatii* partially adapted from Speybroeck et al. (2016).

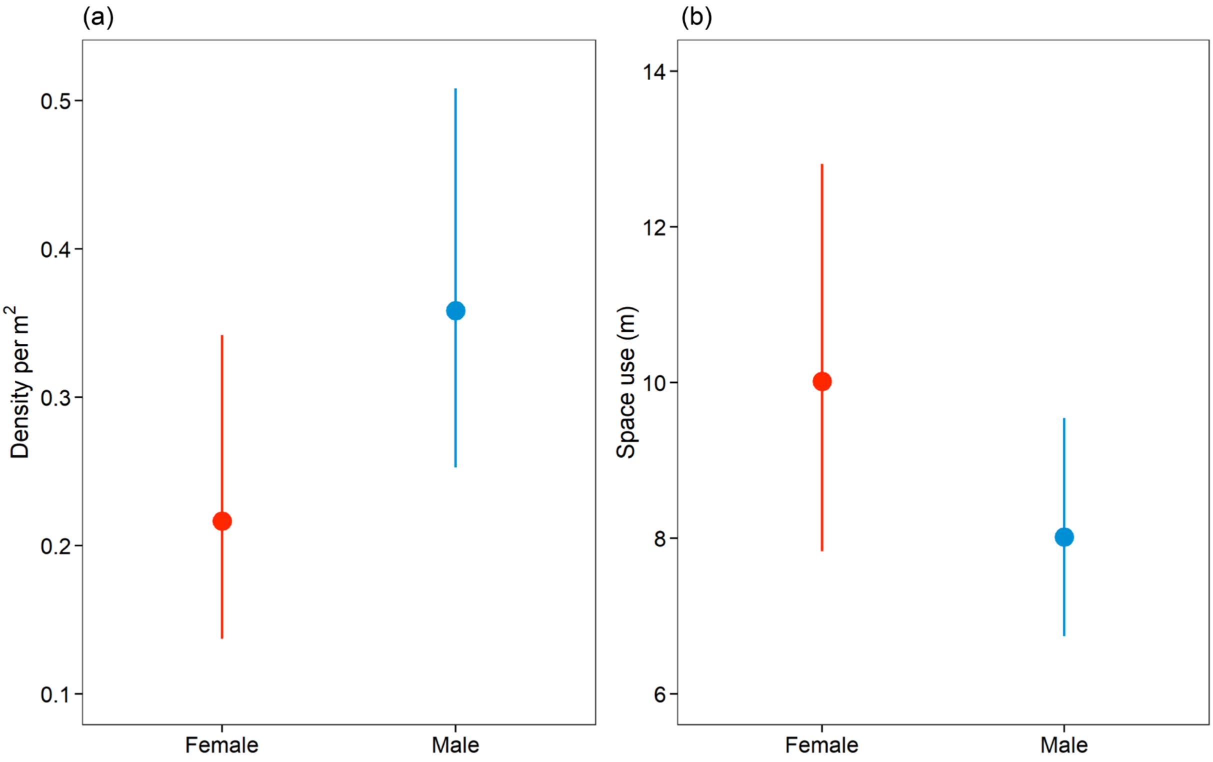


FIGURE 4 | Population density (a) and space use (b) (i.e., the extent of space used by an individual) estimates, with 95% confidence intervals, for both sexes.

in cave environments, which reach their peak during summer periods (Salvidio et al. 1994; Ficetola et al. 2013). The preference of salamanders for walls with higher complexity is also straightforward: Rough walls with creeks are easier to climb and may provide more suitable shelters for individuals. Lower detection probabilities in the more internal part of the tunnel near the collapsed entrance could be related to the lower availability of external prey or to the higher climate variability of this area.

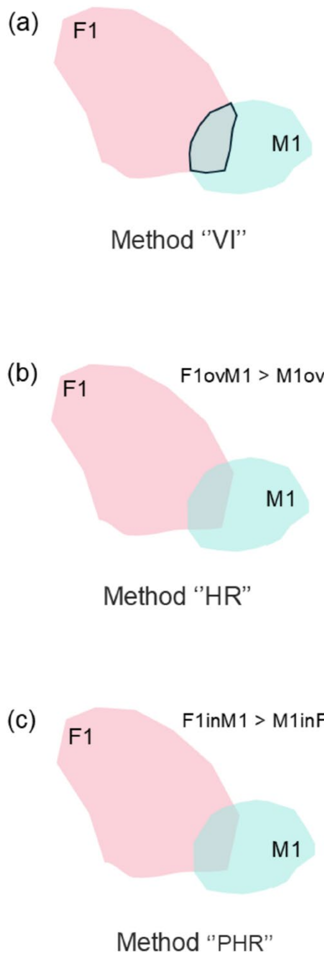


FIGURE 5 | Graphic representation of the three overlap indices used in this study. Pink polygon represents the utilization distribution (UD) of individual F1, whereas the blue one represents that of individual M1. (a) Brown area is the volume of intersection between two UDs calculated by method "VI" (volume intersection). (b) Method "HR" (home range), the proportion of the home range (HR) of M1 covered by the HR of F1 is higher than the proportion of the HR of F1 covered by the HR of M1. (c) Method "PHR" (proportional home range), the probability of finding F1 in the HR of M1 is higher than that of finding M1 in the HR of F1.

The prevalence of adult salamanders in the median sectors of the cave (Figure S5) is probably due to the interaction of multiple environmental factors, in particular humidity, illuminance, and prey availability (Ficetola et al. 2013; Salvidio et al. 2019). The combination of these variables and their homogeneity through this gradient would make these sectors more suitable for these amphibians. Contrary to our expectation, temperature did not affect salamanders' activity as previously reported for this species (Salvidio et al. 2019). However, activity patterns and space use of organisms may be shaped by other effects, different from the investigated environmental features (Hernández-Pacheco et al. 2019).

Our study deepened the understanding of the spatial ecology of *S. strinatii* in a cave environment. Application of SCR methods, albeit time-effort expensive, is truly valuable to assess the effects of ecological variables on the distribution of animals and helped us quantify sex-specific differences in density and space use in a simplified environment. Similar approaches should be applied to congeneric *Speleomantes* species, all threatened by extinction (Luedtke et al. 2023). Indeed, increasing the knowledge of the spatial ecology of these species is critical to improve the implementation of conservation and management actions. Our study was conducted in a relatively homogeneous cave system, but cave salamanders also occur in more complex surface habitats, where SCR could be a useful tool for disentangling the roles of environmental heterogeneity in shaping movement, detectability, and distribution. Future SCR studies should be conducted also in these habitats to understand the plasticity in space use between two environments with dramatic differences in microhabitat heterogeneity. Given the continuing declines of amphibian populations, improved characterization of spatial ecology and demography of cave salamanders may help to properly address conservation efforts, limiting the negative effect of anthropic action and climate change on these emblematic organisms.

Author Contributions

G.R., A.C., and S.S. conceived the research idea. G.R. performed field work and SCR analyses. All authors contributed to the draft of the manuscript.

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Ethics Statement

Permits for capture, handling, and stomach flushing of salamanders in the present study were issued by the Italian Ministry of Environment—authorization #0039130 of 15 April 2021.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** CMR methodology used for monitoring *S. strinatii* inside the Biospeleological Station “Arturo Issel.” 3D planimetry of Biospeleological Station is available at the following link https://scaniverse.com/scan/odvoikodupff5t7p. **Figure S2:** Example of the possible interactions between sexes of salamanders. Home range shapes are those estimated for a certain individual (i04, i12, i40 are salamanders' identifiers) using *adehabitat*. **Table S1:** Meaning of each overlap index on the basis of the selected method. **Figure S3:** Trend of temperatures recorded outside and inside the cave at different depths, during the 2 years monitoring. **Table S2:** Mean temperature (°C) recorded with dataloggers inside the Biospeleological station at different depths for each sampling occasion. Data of external temperatures were recorded by the meteo-station of Busalla. **Figure S4:** HRs estimated through KDE for the 21 salamanders with at least five encounters. Individuals' identifiers and sex are above kernels, whereas HR size in square meters is showed inside boxes. **Figure S5:** Simplified graphic representation of salamanders' movements inside the cave.