

Spatial segregation among age classes in cave salamanders: habitat selection or social interactions?

Gentile Francesco Ficetola · Roberta Pennati ·
Raoul Manenti

Received: 21 April 2012 / Accepted: 23 October 2012 / Published online: 18 November 2012
© The Society of Population Ecology and Springer Japan 2012

Abstract Within species, individuals with different sexes, morphs and age classes often show spatial segregation. Both habitat selection and social processes have been proposed to explain intraspecific spatial segregation, but their relative importance is difficult to assess. We investigated spatial segregation between age classes in the cave salamander *Hydromantes (Speleomantes) strinatii*, and used a hypothetico-deductive approach to evaluate whether social or ecological processes explain segregation pattern. We recorded the location and age class of salamanders along multiple caves; we measured multiple microhabitat features of different sectors of caves that may determine salamander distribution. We assessed age-class segregation, and used generalized mixed models and an information-theoretic framework, to test if segregation is explained by social processes or by differences in habitat selection. We found significant age-class segregation, juveniles living in more external cave sectors than adults. Multiple environmental features varied along caves. Juveniles and adults showed contrasting habitat selection patterns: juveniles were associated with sectors having high invertebrate abundance, while adults were associated with scarce invertebrates and low temperature. When the effect of environmental features was taken into account, the relationship between juveniles and adults was non negative. This suggests that different habitat preferences,

related to distinct risk-taking strategies of age classes, can explain the spatial segregation. Juveniles require more food and select more external sectors, even if they may be risky. Conversely, adults may trade off food availability in favour of safe areas with stable micro-climate.

Keywords A priori inference · Predation risk · Spatial pattern · Spider abundance · Trade-off

Introduction

The identification of processes determining the distribution of organisms is a major challenge of spatial ecology. Patterns of spatial segregation, defined here as differences in spatial organization among individuals, can occur at both the interspecific and the intraspecific level. At the interspecific level, the segregation at fine spatial scale allows the coexistence of species occupying similar niches, and can be an important driver of biodiversity patterns (Firth and Crowe 2010; Darmon et al. 2012). However, spatial segregation can also occur at the intraspecific level among morphs, sexes or age classes (Formica et al. 2004; Field et al. 2005; Ruckstuhl 2007; Main 2008; van Toor et al. 2011; Bjørneraas et al. 2012). For instance, in several vertebrates, sexes segregate in groups showing distinct spatial organization and resource exploitation (Ruckstuhl 2007; Main 2008). Two types of processes have been proposed to explain intraspecific spatial segregation patterns: the social and the habitat segregation hypotheses (HSH). The social segregation hypotheses (SSH) suggest that social processes (selection for neighbours with similar features, intraspecific competition, cannibalism) are the major drivers of segregation. In contrast, the HSH suggest that segregation is mostly driven by habitat-selection

G. F. Ficetola
Dipartimento di Scienze dell'Ambiente e del Territorio,
Università di Milano-Bicocca, Piazza della Scienza 1,
20126 Milan, Italy

G. F. Ficetola (✉) · R. Pennati · R. Manenti
Dipartimento di Bioscienze, Università degli Studi di Milano,
Via Celoria 26, 20133 Milan, Italy
e-mail: francesco.ficetola@gmail.com

processes, such as the selection of sites on the basis of foraging quality or risk of predation (Ruckstuhl 2007; Main 2008). Under habitat-selection processes, social segregation may occur as a simple by-product of differences in habitat selection (Bowyer et al. 2002).

In organisms with complex life cycles, such as many insects and amphibians, the age classes correspond to discrete, morphologically distinct phases that often exploit distinct habitats. It has been proposed that the evolution of complex life cycles can be explained ecologically by the reduction of intraspecific competition (Istock 1966; Wilbur 1980; Moran 1994), with mechanisms somehow analogous to those proposed to explain spatial segregation. Complex life cycles are extremely successful life-history strategies, suggesting that organisms minimizing interactions between life-history stages (which usually correspond to age classes) have strong advantages (Wilbur 1980; Moran 1994). The similarity between this ecological hypothesis for the evolution of complex life cycles, and the HSH, suggests that spatial segregation among age classes (hereafter, age-class segregation) may widely occur also in organisms without complex life cycles. However, most analyses on intraspecific segregation have focused on sexual segregation in a few groups of vertebrates (Ruckstuhl 2007; Main 2008). Very few studies investigated the causes of age-class segregation in organisms with direct development (see Cransac et al. 1998; Bon et al. 2001; Ruckstuhl and Festa-Bianchet 2001 for analyses with ungulates).

The identification of processes determining spatial segregation is challenging: for example, multiple explanations have been suggested after the observation of sexual segregation in the same species of ungulates (Ruckstuhl 2007). The HSH has been proposed as the most likely explanation of sexual segregation (Main 2008; but see Singh et al. 2010). The few studies that assessed the factors determining age-class segregation hypothesized both ecological differences (Labée-Lund et al. 1993; Field et al. 2005) and aggressive/social interactions (Cransac et al. 1998; Salvidio and Pastorino 2002; Galvan 2004; Harvey et al. 2008), but we are not aware of explicit tests to distinguish the hypotheses. The hypothetico-deductive reasoning is an emerging approach to infer processes from distribution patterns (McIntire and Fajardo 2009), and may help to identify the causes of spatial segregation. This requires well distinct a priori hypotheses, formulated on the basis of biological knowledge (Ruckstuhl 2007; McIntire and Fajardo 2009; Dochtermann and Jenkins 2011), and the application of information-theoretic statistical models, explicitly testing the support of alternative hypotheses on causal processes (McIntire and Fajardo 2009; Ficetola et al. 2010; Dochtermann and Jenkins 2011; Symonds and Moussalli 2011). Spatial segregation determines clear distribution patterns of individuals, and the different underlying

processes (i.e., SSH vs. HSH) are expected to produce distinct patterns. If segregation is mostly determined by social mechanisms (SSH), we predict a negative relationship between the distribution of different classes of individuals (age, sexes, or morphs; Fig. 1a) but, when these social relationships are taken into account, classes should show similar habitat selection patterns (Fig. 1b). Conversely, if segregation is mostly determined by ecological differences (HSH), we predict different habitat selection patterns between classes (Fig. 1d) and, when environmental features are controlled for, no negative relationships between them (Fig. 1c).

European cave salamanders (genus *Hydromantes*, subgenus *Speleomantes*) are ideal organisms for the study of spatial segregation. Cave salamanders have direct development. They are not obligate cave-dwellers but, when external conditions would be too harsh (e.g., dry, hot, and particularly during warm seasons) for lungless terrestrial salamanders, they retreat to underground environments where they find a more suitable microclimate (Cimmaruta et al. 1999; Camp and Jensen 2007; Vignoli et al. 2008; Ficetola et al. 2012). When in the cave environment, they are easily detectable (Ficetola et al. 2012) and show extremely limited displacements; for instance, in *H. strinatii*, each individual usually occupies areas $\leq 8 \text{ m}^2$ (Salvidio et al. 1994). Therefore, observed distribution patterns reflect the actual occupancy of the cave environment. Furthermore, cave environments have relatively simple habitat features that can be well described by a limited number of parameters representing abiotic and biotic features.

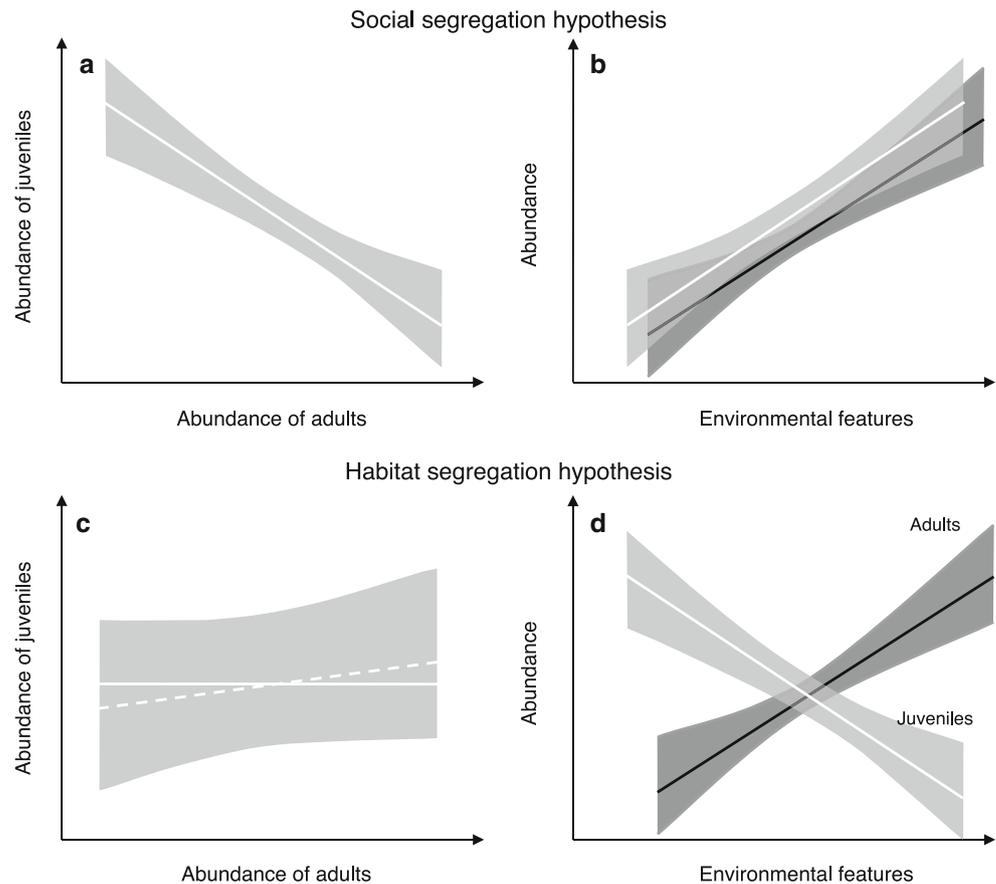
Previous studies observed spatial age-class segregation in cave salamanders, with juveniles living more close to the cave entrance (Salvidio and Pastorino 2002). It has been proposed that segregation may occur because juveniles avoid cannibalism, or are losers during competition for the best home ranges (Salvidio and Pastorino 2002), in accordance with the SSH (avoidance of aggression hypothesis; Ruckstuhl 2007). However, the processes determining segregation have not been assessed. Here, we analyzed spatial segregation among age classes in the cave salamander, *Hydromantes (Speleomantes) strinatii*, and tested whether the distribution pattern supports the predictions of the SSH or of the HSH (Fig. 1).

Methods

Study species and area

Hydromantes strinatii is a small (up to 13 cm) plethodontid cave salamander endemic of a small area between NW Italy and SE France. In Mediterranean regions it can be

Fig. 1 Distribution patterns predicted by different hypotheses proposed to explain spatial segregation. The social segregation hypothesis **a** predicts a negative relationship between the distribution of the different classes but, **b** when this relationship is taken into account, similar responses to environmental gradients. The habitat segregation hypothesis predicts **c** a non-negative relationship between the abundance of different classes, but **d** different responses to environmental gradients. Different classes are here exemplified by age classes [juveniles (*white lines*) vs. adults (*black lines*)], but sex, morph or other classes can be similarly considered



active throughout the year: it is a hygrophilic species, and can be found in epigeous environments from autumn to early spring, when outdoor conditions are cool and wet. Conversely, in late spring and summer it usually inhabits caves, crevices and other cavities (Bologna and Salvidio 2006; Lanza et al. 2006). Our study focused on age-class segregation within caves, therefore we performed surveys during early summer, when the importance of underground environments is maximum. We surveyed the natural cavities in two nearby small valleys of Western Liguria: Valle Perti and Valle Ponci (approximately 42.20°N, 8.35°E). Additional details on the study area are provided elsewhere (Ficetola et al. 2012).

Surveys and environmental features

We performed preliminary surveys in >30 cavities of the study area, to identify those with presence of cave salamanders. On the basis of these surveys, we selected 11 natural caves with presence of salamanders for more in-depth surveys. We used visual encounter surveys (Crump and Scott 1994) to assess the distribution of salamanders. Each cave was surveyed in June 2011. To minimize variation in outdoor conditions, caves were visited in consecutive days between 11.00 am and 3.00 pm; all days had

similar temperature and were sunny and dry, as it is characteristic of Mediterranean summer. In each survey, up to six trained people actively searched salamanders over the floor and all the walls of the cave; we explored the caves as deeply as possible, compatibly with our equipment. Previous analyses showed that using these approaches the per-visit detection probability of *H. strinatii* is high (91 %), thus surveys allow a reliable assessment of the distribution of the species (Ficetola et al. 2012). We recorded the location (distance from the cave entrance and height above the cave floor) and measured total length of all salamanders observed. We identified age classes on the basis of secondary sexual characters and size: salamanders above 58 mm or with male sexual characters (mature males have mental glands and premaxillary teethes) were considered adults; the remaining individuals were considered juveniles (Salvidio 1993; Lanza et al. 2006).

Each cave was subdivided in 3-m longitudinal intervals (hereafter, sectors) for the measurement of environmental features. The size of sectors approximately corresponds to the usual size of home ranges of *H. strinatii* (4–8 m²) (Salvidio et al. 1994), even if larger home ranges are possible (Pastorelli et al. 2005; Lanza et al. 2006). The number of sectors in a cave depended on cave size and distribution of salamanders. If at least one salamander was

detected in the deepest sectors of the cave, sectors covered the whole cavity. If salamanders were not detected at the end of the cave, sectors covered the whole cave until the position of the last salamander, plus one additional, deeper 3-m sector. For instance, if the deepest salamander in a cave was at 23 m from the entrance, and the cave was deeper than 24 m, we considered nine 3-m sectors for that cave. We recorded the number of adults and juveniles per each sector. Furthermore, in each sector we measured four environmental variables that are known to be the major determinants of salamander distribution in underground environments (Briggler and Prather 2006; Camp and Jensen 2007; Vignoli et al. 2008; Ficetola et al. 2012). Three parameters represented abiotic conditions: air temperature (°C), relative humidity (%) and illuminance (i.e., intensity of incident light, measured in lux). These parameters were measured using a EM882 multi-function thermo-hygrometer and light-meter (PCE Instruments). The minimum illuminance recordable by the light-meter was 0.01 lux. Furthermore, as biotic parameter, we counted the number of adult *Meta menardi* spiders in each sector. In the study area, *M. menardi* is the most abundant large spider living in the twilight zone of caves (Ficetola et al. 2012; R. Manenti et al., unpublished data). *Meta menardi* spiders can be predated by *Hydromantes* salamanders (Lanza et al. 2006). Furthermore, both salamanders and spiders are predators of arthropods found in underground chambers (Salvidio et al. 1994; Smithers 2005; Vignoli et al. 2006; Novak et al. 2010), and spiders are likely positively related to the overall abundance of invertebrates. Therefore, we considered spider abundance as a measure of the abundance of invertebrates (Ficetola et al. 2012).

Statistical analyses

We used the “sexual segregation and aggregation statistic” (SSAS, Bonenfant et al. 2007) to test for spatial segregation among age classes. The SSAS has been developed to assess patterns of segregation and aggregation between sex classes; it is based on well known chi-square statistics and its approach is general and can be applied to investigate segregation also in other situations. SSAS provides an estimate of the distance between the observed and the expected distributions of age classes, under the null hypothesis of independence of the distributions of age classes among the groups. Segregation occurs when the ratio n adults: n juveniles in each group deviates strongly from the ratio observed in the whole population (i.e., in all the considered groups). Similarly, aggregation occurs when the ratio in each group is close to the ratio of the population (Bonenfant et al. 2007; Singh et al. 2010). In our study, we considered all the individuals occurring in the same 3-m sector as a “group”. We used a randomization procedure

(50,000 replicates) to identify the 95 % confidence intervals of SSAS expected under random association between age classes. SSAS varies between 0 (no segregation) and 1 (complete segregation), nevertheless the SSAS values cannot be considered as an absolute measure of segregation, and are meaningful only if compared to confidence intervals of expected SSAS (Bonenfant et al. 2007).

We used generalized linear mixed models (GLMMs; normal error distribution) to test whether the position of each observed salamander within the caves (distance from the entrance, height above the cave floor) was different between adults and juveniles, and to test whether the environmental variables recorded in each cave sectors were affected by the distance of the sector from the entrance of the cavity. In all GLMMs, we included cave identity as a random factor.

Subsequently, we used GLMMs within an information-theoretic approach, to assess whether segregation was most likely explained by the SSH or by the HSH. First, we built a model representing the SSH (social model), relating the abundance of juveniles to the abundance of adults. Second, we built a model representing the HSH (habitat model), relating the abundance of juveniles to the four environmental variables. We used Akaike’s information criterion (AIC) to assess the relative support of the two models. AIC trades-off explanatory power versus number of predictors; parsimonious models explaining more variation have the lowest AIC values. We considered the model with the lowest AIC as the “best AIC” model, and calculated Δ AIC, which is the AIC difference between the models. We then calculated the evidence ratio of the models, which provides a measure of the relative likelihood of one hypothesis versus another (Burnham and Anderson 1998; Lukacs et al. 2007; Symonds and Moussalli 2011). Using AIC corrected for small sample size instead than AIC would lead to identical results. We also built a model combining social and environmental independent variables, to assess the possibility of a joint effect of environmental and social factors. We then repeated the same procedure for adults, considering the abundance of adults as dependent variable and the abundance of juveniles as the social independent variable. In these models, the dependent was a count (number of individuals), therefore we used a Poisson error distribution. In all models we also reported significance values of independent variables, to facilitate interpretation of their role (Stephens et al. 2007). Estimation of fit of the GLMMs used maximum likelihood; we used likelihood ratio to assess the significance of variables in Poisson models. In all the best AIC GLMMs, residual deviance was similar to residual degrees of freedom, indicating that our models were not affected by overdispersion.

In a large number of sectors we detected zero salamanders. To ensure that our results were not affected by

zero-inflation, we repeated our analyses three times. First, we run the analyses by excluding sectors where zero salamanders (either adults and juveniles) were present, and by excluding sectors with zero or one salamander. Furthermore, we re-run our analyses using Bayesian Markov Chain Monte Carlo GLMMs (MCMCglmm) (Hadfield 2010). With MCMCglmm, we built the models corresponding to the different hypotheses using zero-inflated Poisson models. We then used the deviance information criterion (DIC) to compare the zero-inflated models with Bayesian MCMCglmm assuming Poisson error (i.e., the same error distribution used in standard GLMMs). DIC is a parameter estimating the performance of a Bayesian model on the basis of deviance and of the effective number of parameters; it gives particular emphasis to the random effects. Models having lower deviance and less effective parameters show lower DIC, and are considered to be the models most appropriate to the dataset (Spiegelhalter et al. 2002, 2008). For MCMCglmm, we performed 30,000 iterations as burn-in, followed by 300,000 runs with a thinning interval of 100.

If needed, variables were transformed using logarithms (distance from cave entrance, illuminance). Correlation between pairs of environmental variables was weak (in all pairwise correlations, $|r| < 0.3$), indicating that multicollinearity did not pose problems to our models. Analyses were performed using packages LME4, nlme (Pinheiro and Bates 2000; Bates and Maechler 2010; Pinheiro et al. 2010) and MCMCglmm (Hadfield 2010) under the R statistical environment (R Development Core Team 2010).

Results

Pattern of spatial segregation

We detected salamanders in all the 11 caves considered. Adults and juveniles cohabited in seven caves; in two caves we detected adults only, in two caves we detected juveniles only. Adults were found at distances of 3–49 m from the cave entrance, while juveniles were found at distances of 1–12 m (Fig. 2). The SSAS showed significant segregation of age classes among the sectors (observed SSAS = 0.048, 95 % CI of expected SSAS = 0.021–0.042). Overall, juveniles were detected closer to the cave entrance than adults (mixed model, $F_{1,64} = 31.0$, $P < 0.0001$). Conversely, height from the cave floor was not different between adults and juveniles ($F_{1,64} = 0.19$, $P = 0.67$).

Most environmental features significantly varied with the distance from the cave entrance: in sectors far from the cave entrance humidity was higher ($B \pm SE = 1.7 \pm 0.6$; $F_{1,49} = 7.71$, $P = 0.008$), while abundance of spiders and

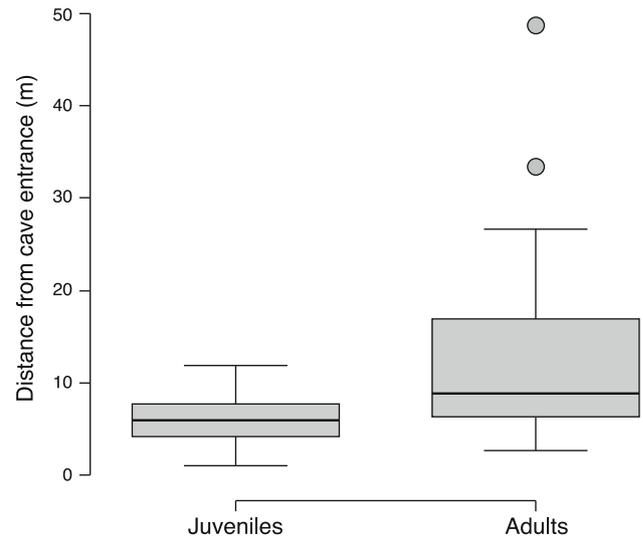


Fig. 2 Box-plot representing the distance of juvenile and adult salamanders from the cave entrance. **Bold lines** represent the median of groups. The **circles on top right** for adults represent outliers

illuminance were lower ($B = -0.3 \pm 0.1$, $P = 0.004$ and $B = -0.10 \pm 0.04$, $P = 0.009$, respectively). The relationship between distance and temperature was not significant ($B = -0.05 \pm 0.12$; $F_{1,49} = 0.16$, $P = 0.690$).

Distribution of juveniles

The abundance of juveniles across sectors was not related to the abundance of adults (Table 1a). However, this is not in contrast with the SSAS analysis: this result probably occurred because of the presence of sectors in which the abundance of both adults and juveniles was very low. If sectors with zero or only one individual (either adults or juveniles) were excluded from the analysis, the abundance of juveniles was negatively related to the abundance of adults ($B = -0.25$, $\chi^2_1 = 4.1$, $P = 0.041$).

The habitat model, explaining the distribution of juveniles on the basis of environmental features, had a lower AIC than the social model (Table 1b; $\Delta AIC = 8.8$). On the basis of evidence ratio, the habitat model was 77 times more likely to be the best model than the social model. This model suggested association between juveniles and cave sectors with high spider abundance (Table 1b; Fig. 3a).

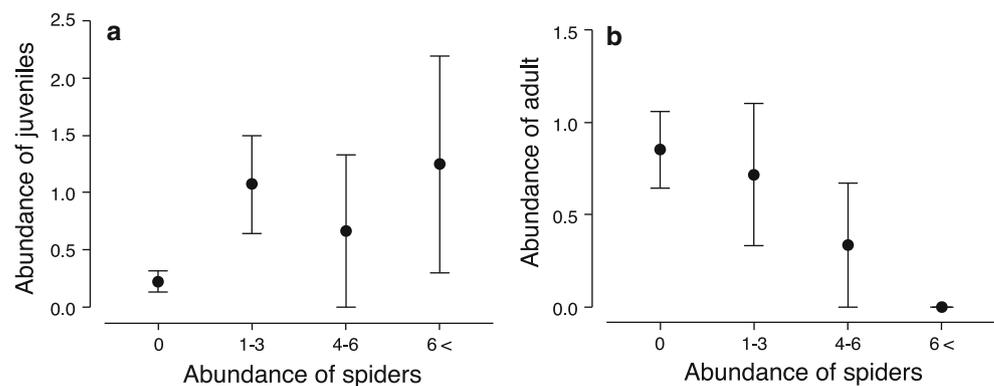
A combined model, considering both environmental variables and presence of adults, showed a lower AIC than the habitat model. However, contrary to the expectations of the SSH, the relationship between the abundance of juveniles and adults was positive, when taking into account the relationship between the abundance of juveniles and the environmental variables. Also in this model, juveniles were strongly associated with sectors with abundant spiders (Table 1).

Table 1 Results of generalized linear mixed models describing the abundance of juveniles and adults on the basis of the social (a, e), habitat (b, f) and combined hypotheses (c, g)

Dependent: abundance of juveniles					Dependent: abundance of adults				
Independent	<i>B</i>	χ^2_1	<i>P</i>	AIC	Independent	<i>B</i>	χ^2_1	<i>P</i>	AIC
Social models									
(a) <i>N</i> adults	0.12	1.86	0.172	97.54	(e) <i>N</i> juveniles	0.13	1.17	0.280	99.51
Habitat models									
(b) Temperature	0.02	0.017	0.896	88.86	(f) Temperature	−0.31	6.23	0.013	88.25
Humidity	−0.04	2.65	0.104		Humidity	−0.04	1.88	0.170	
Illuminance	−0.38	0.44	0.506		Illuminance	−2.14	3.39	0.066	
Spider abundance	0.58	12.03	0.002		Spider abundance	−0.45	4.51	0.034	
Combined models									
(c) <i>N</i> adults	0.47	9.31	0.002	81.55	(g) <i>N</i> juveniles	0.40	6.01	0.014	84.24
Temperature	0.27	3.78	0.052		Temperature	−0.30	6.74	0.009	
Humidity	−0.02	0.84	0.360		Humidity	−0.03	1.07	0.300	
Illuminance	−0.08	0.02	0.887		Illuminance	−2.67	4.25	0.039	
Spider abundance	0.83	19.32	<0.001		Spider abundance	−0.70	8.01	0.005	
(d) Best MCMCglmm			MCMC <i>P</i>		(h) Best MCMCglmm			MCMC <i>P</i>	
<i>N</i> adults	0.77		0.010		<i>N</i> juveniles	0.47		0.045	
Temperature	0.41		0.114		Temperature	−0.36		0.012	
Humidity	−0.02		0.619		Humidity	−0.01		0.601	
Illuminance	−0.82		0.615		Illuminance	−3.20		0.043	
Spider abundance	1.26		0.002		Spider abundance	−0.87		0.007	

We also report the results of Bayesian Markov Chain Monte Carlo models (MCMCglmm) with lowest DIC (d, h; see Table 3 for DIC values). Significant values are in bold

Fig. 3 Relationship between the abundance of spiders and the abundance of **a** juvenile cave salamanders; **b** adult cave salamanders. Error bars are standard errors; the scales of the vertical axes differ between the panels



Distribution of adults

The abundance of adults across sectors was not related to the abundance of juveniles (Table 1e). The habitat model, explaining the distribution of adults on the base of environmental features, showed lower AIC than the social model (Table 1f; Δ AIC = 11.3). On the basis of evidence ratio, the habitat model was >270 times more likely to be the best model than the social model. This model suggested association between adults and the sectors with less spiders and with cold temperature and limited light (Table 1f). It is

remarkable that the relationship between the abundance of adults, spiders and temperature was opposite to the relationship between juveniles and the same variables (Table 1; Fig. 3b).

A combined model, considering both environmental variables and the presence of juveniles, showed a lower AIC than the habitat model but, contrary to the expectations of the SSH, the relationship between the abundance of juveniles and adults was positive. Also in this model, adults were associated with cold sectors without spiders (Table 1).

Table 2 (a) Comparison of Akaike information criterion (AIC) values of generalized linear mixed models describing the abundance of juveniles and adults on the basis of the social, ecological and combined hypotheses, considering only sectors in which at least one salamander was present (either adults or juveniles), (b) comparison of

AIC values of models considering only sectors in which at least two salamanders (either adults or juveniles) were present, (c) coefficients and significance of individual variables for the model with lowest AIC in Table 2a

	Dependent: abundance of juveniles			Dependent: abundance of adults		
(a) Comparison among models: sectors with at least one salamander	AIC			AIC		
Social models	58.88			49.79		
Combined models	50.76			41.53		
Habitat models	49.92			40.47		
(b) Comparison among models: sectors with at least two salamanders						
Social models	30.93			30.12		
Combined models	31.87			26.55		
Habitat models	29.99			24.55		
	<i>B</i>	χ^2_1	<i>P</i>	<i>B</i>	χ^2_1	<i>P</i>
(c) Coefficients of independent variables						
Temperature	0.02	0.02	0.896	−0.33	8.88	0.003
Humidity	−0.01	0.08	0.775	−0.01	0.41	0.525
Illuminance	1.50	1.29	0.257	−0.30	0.03	0.868
Spider abundance	0.58	7.65	0.006	−0.49	4.45	0.035

Significant values are in bold

Table 3 Comparison of deviance information criterion (DIC) between MCMCglmm built using a zero-inflated Poisson family, and MCMCglmm built using a Poisson family

	Dependent: abundance of juveniles		Dependent: abundance of adults	
	Zero-inflated DIC	Poisson DIC	Zero-inflated DIC	Poisson DIC
Social model	112.8	97.9	145.1	133.5
Habitat model	106.4	97.9	139.9	133.3
Combined model	104.8	93.3	139.6	131.5

The independent variables included in the social, in the ecological and in the combined models are listed in Table 1

Analyses using alternative models

If the sectors with zero salamanders (either adults or juveniles) are excluded from analyses, the habitat model showed much higher support than the social model (Table 2), confirming the results of previous analyses. For juveniles, evidence ratios suggested that the habitat model was about 90 times more likely than the social model; for adults, the habitat model was about 105 times more likely than the social model. In this case the combined models showed lower support than the habitat model. Overall, the coefficients of the best models were in agreement with the analysis considering all sectors (Tables 1, 2c). The habitat model is the best model even if sectors with

zero or one salamander are excluded. In this analysis, AIC differences among models are limited, probably because of small sample size (Table 2b).

For all candidate models, the zero-inflated MCMCglmm had higher DIC than the respective Poisson models (Table 3), suggesting that our results are not affected by zero-inflation. For both juveniles and adults, the MCMCglmm with lowest DIC was nearly identical to the best-AIC GLMMs (Table 1d, h).

Discussion

When spatial segregation occurs, differences of distribution patterns between categories of individuals may be striking. In our study case, we observed strong age-class segregation in the cave salamander: juveniles were close to the cave entrance, while adults were in deepest sectors of caves (Fig. 1). Multiple environmental features of caves strongly varied with the distance from the entrance. The combination of a priori hypotheses with an information-theoretic approach allowed to assess the relative likelihood of processes potentially determining segregation of age classes (habitat suitability vs. social interaction), and suggested that segregation was most likely caused by different habitat selection patterns (HSH). First, the models assuming that the distribution of age classes is mostly affected by ecological features (HSH; models b and f in Table 1) have much stronger support by the data than those assuming that

the distribution of one age class is affected by the distribution of the other class (SSH; social models a and e in Table 1). Furthermore, if the distribution of the other age class is added to the HSH model, the relationship between age classes become positive (Table 1). This pattern is opposite to the expectations under the SSH, which would predict negative relationships between age classes (Fig. 1a).

The relationship between the abundance of juveniles and adults was positive, when differences in habitat selection are taken into account (Table 1c, d). This result corresponds to the converse of the prediction of the SSH, yet it is a pattern non predicted by the HSH (Fig. 1). This positive association might occur because both age classes are affected in a similar way by some unmeasured environmental feature. This hypothesis is supported by the analysis excluding sectors without salamanders (Table 2). If sectors without salamanders were not considered, the HSH remains the most supported by the data, but the combined model showed higher AIC than the habitat model. The results of the model considering all sectors (Table 1) are probably affected by certain areas having environmental features unsuitable for both adults and juveniles. For instance, both age classes may be very scarce in cave sectors characterized by walls with limited heterogeneity (Camp and Jensen 2007). Finally, we cannot rule out the occurrence of unknown social process, determining some positive response to the presence of conspecifics (Gautier et al. 2004); additional studies are required to test these hypotheses.

Age classes had a contrasting response to major environmental gradients: juveniles were associated with the cave sectors inhabited by abundant spiders, while adults were associated with scarce spiders and lower temperature (Tables 1, 2; Fig. 3). *Meta* spiders are among the major predators of arthropods in caves, therefore we considered spider abundance as a proxy of the abundance of invertebrates (Smithers 2005; Novak et al. 2010; Ficetola et al. 2012). Cave food webs mostly depend on external inputs: sectors close to the surface receive more inputs and usually host a richer invertebrate fauna (Hills et al. 2008; Culver and Pipan 2009; Novak et al. 2010; Manenti et al. 2011; Schneider et al. 2011). During summer, both juveniles and adult salamanders feed within caves, and the small home range size (about 4–8 m²) suggests that they feed approximately in the sectors where they have been observed (Salvidio et al. 1994; Lanza et al. 2006). For salamanders, selecting shallow sectors may have the advantage of a higher food availability. On the other hand, inhabiting shallow sectors may determine multiple disadvantages, including micro-climatic conditions more similar to the external ones (e.g., lower humidity) and a highest risk of predation either from invertebrates or from vertebrates

entering in the first meters of caves (Culver and Pipan 2009). Actually, some large cave invertebrates (e.g., large spiders) have been observed preying on *Hydromantes* juveniles (Lanza et al. 2006; Pastorelli and Laghi 2007). Furthermore, the most external sectors often had humidity <75 %, and this may pose physiological problems to salamanders. Humidity influences dehydration rate: inhabiting more humid sectors can allow better maintaining water balance. Plethodontid cave salamanders are lungless and skin humidity/dehydration is also related to respiratory exchanges (Spotila 1972; Briggler and Prather 2006; Hillman et al. 2009). Conversely, selecting the deepest, coldest sectors would reduce predation risk and provide the most suitable microclimate (Hutchinson 1958; Spotila 1972), but food items become more scarce there. This suggests the existence of a trade-off between the selection of most external sectors (abundant prey, but unstable microclimate and high risk of predation) and the deepest ones (lack of predators, suitable microclimate but scarce prey). We propose that the habitat selection patterns may be explained by different risk-taking strategies between age classes. For juveniles, accessing to relatively abundant food is necessary for growth and development, therefore they choose external sectors, even if this may be risky.

In cave salamanders, adult survival is the most important determinant of population growth rate (Lindstrom et al. 2010). This suggests that, after attaining sexual maturity, adults may trade off food availability in favour of safe areas with stable micro-climate. A spatial distribution pattern maximising survival after reaching sexual maturity would thus provide the highest fitness to individuals. Overall, the age class found at the riskiest location may be not the one that experiences the lowest risk of mortality there: segregation probably occurs because the mortality risk of inhabiting the riskier habitat is offset by increased foraging requirements (Rochette and Grand 2004). The interplay between predation risk and resource availability has been observed multiple times as a cause of intraspecific segregation. For instance, a similar strategy determines sexual segregation in several polygynous ungulates, in which males need high-quality diet to be successful in competition for mates, and thus select areas with superior food availability even if predation risk may be high. Conversely, females maximise fitness by increasing offspring survival, and therefore select the less risky areas, even if they have limited foraging quality (Main 2008). It should also be remarked that our study was not manipulative, and segregation among age classes may be caused by other factors, including different optima for microclimate or additional unmeasured features.

The occurrence of intraspecific spatial segregation has led to a flourishing number of hypotheses (Ruckstuhl 2007). In many cases, potential causes of spatial segregation have

been proposed only after segregation was observed (inductive reasoning) (McIntire and Fajardo 2009). Comprehensive analyses, particularly in the last years, allowed identifying the processes most likely determining sexual segregation, and highlighted the importance of trade-offs between resources and predation risk (Main and Coblenz 1996; see also Bon et al. 2001; Main 2008; Bjorneraas et al. 2012). Conversely, less attention has been devoted to age-class segregation. A hypothetico-deductive reasoning, coupled with the comparison of explicit hypotheses, can allow inferring the processes most likely determining distribution and segregation on the basis of distribution patterns (McIntire and Fajardo 2009; Dochtermann and Jenkins 2011), thereby boosting our understanding of age-class segregation. Our results are in agreement with studies highlighting the importance of the trade-off between resources and predation risk: these trade-offs may be important also for age-class segregation. Nevertheless, age-class segregation remains a much less studied topic, if compared to sexual segregation: further analyses are required to better understand the costs and benefits of different strategies of spatial distribution.

Acknowledgments The comments of K. Ruckstuhl and one reviewer improved early versions of this manuscript. We thank S. Salvidio for constructive discussions. E. Massa (Savonese Speleo Club) kindly provided cave location data. We thank also M. Merazzi (Erba CAI Speleo Club). Data were collected during the Herpetological field work of Natural Sciences students at the Università degli Studi di Milano. We thank N. Santo, F. Basso, F. Belluardo, L. Borrelli, F. Barazzetta, E. Crenna, R. Gavazzi, L. Limongi, E. Lunghi, L. Morotti, E. Orlando, F. Pantuso, M. Palombelli, A. Seriola, G. Soldà, S. Virtuani, D. Zani and M. De Nicola for help during fieldwork. GFF was funded by a scholarship of Univ. Milano-Bicocca. This paper is dedicated to Matteo.

References

- Bates D, Maechler M (2010) lme4: linear mixed-effects models using Eigen and Eigen. R package version 0.999375-37. <http://www.r-project.org>
- Bjorneraas K, Herfindal I, Solberg EJ, Sther BE, van Moorter B, Rolandsen CM (2012) Habitat quality influences population distribution, individual space use and functional responses in habitat selection by a large herbivore. *Oecologia* 168:231–243
- Bologna MA, Salvidio S (2006) *Speleomantes strinatii* (Aellen, 1958). In: Sindaco R, Doria G, Razzetti E, Bernini F (eds) Atlas of Italian amphibians and reptiles. Polistampa, Firenze, pp 258–261
- Bon R, Rideau C, Villaret JC, Joachim J (2001) Segregation is not only a matter of sex in Alpine ibex, *Capra ibex ibex*. *Anim Behav* 62:495–504
- Bonenfant C, Gaillard JM, Dray S, Loison A, Royer M, Chessel D (2007) Testing sexual segregation and aggregation: old ways are best. *Ecology* 88:3202–3208
- Bowyer RT, Stewart KM, Wolfe SA, Blundell GM, Lehmkühl KL, Joy PJ, McDonough TJ, Kie JG (2002) Assessing sexual segregation in deer. *J Wildl Manage* 66:536–544
- Briggler JT, Prather JW (2006) Seasonal use and selection of caves by plethodontid salamanders in a Karst area of Arkansas. *Am Midl Nat* 155:136–148
- Burnham KP, Anderson DR (1998) Model selection and inference. Springer, New York
- Camp CD, Jensen JB (2007) Use of twilight zones of caves by plethodontid salamanders. *Copeia* 2007:594–604
- Cimmaruta R, Forti G, Nascetti G, Bullini L (1999) Spatial distribution and competition in two parapatric sibling species of European plethodontid salamanders. *Ethol Ecol Evol* 11:383–398
- Cransac N, Gerard JF, Maublanc ML, Pépin D (1998) An example of segregation between age and sex classes only weakly related to habitat use in mouflon sheep (*Ovis gmelini*). *J Zool* 244:371–378
- Crump ML, Scott NJ (1994) Visual encounter surveys. In: Heyer WR, Donnelly MA, McDiarmid RW, Hayek LC, Foster MS (eds) Measuring and monitoring biological diversity: standard methods for Amphibians. Smithsonian Institution Press, Washington, pp 84–92
- Culver DC, Pipan T (2009) The biology of caves and other subterranean habitats. Oxford University Press, Oxford
- Darmon G, Calenge C, Loison A, Jullien J-M, Maillard D, Lopez J-F (2012) Spatial distribution and habitat selection in coexisting species of mountain ungulates. *Ecography* 35:44–53
- Dochtermann NA, Jenkins SH (2011) Developing multiple hypotheses in behavioral ecology. *Behav Ecol Sociobiol* 65:37–45
- Ficetola GF, Scali S, Denoël M, Montinaro G, Vukov TD, Zuffi MAL, Padoa-Schioppa E (2010) Ecogeographical variation of body size in the newt *Triturus carnifex*: comparing the hypotheses using an information-theoretic approach. *Global Ecol Biogeogr* 19:485–495
- Ficetola GF, Pennati R, Manenti R (2012) Do cave salamanders occur randomly in cavities? An analysis with *Hydromantes strinatii*. *Amphib Reptil* 33:251–259
- Field IC, Bradshaw CJA, Burton HR, Sumner MD, Hindell MA (2005) Resource partitioning through oceanic segregation of foraging juvenile southern elephant seals (*Mirounga leonina*). *Oecologia* 142:127–135
- Firth LB, Crowe TP (2010) Competition and habitat suitability: small-scale segregation underpins large-scale coexistence of key species on temperate rocky shores. *Oecologia* 162:163–174
- Formica VA, Gosner RA, Ramsay S, Tuttle EM (2004) Spatial dynamics of alternative reproductive strategies: the role of neighbors. *Ecology* 85:1125–1136
- Galvan I (2004) Age-related spatial segregation of great cormorants in a roost. *Waterbirds* 27:377–381
- Gautier P, Léna JP, Miaud C (2004) Responses to conspecific scent marks and the ontogeny of territorial marking in immature terrestrial salamanders. *Behav Ecol Sociobiol* 55:447–453
- Hadfield JD (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J Stat Softw* 33:1–22
- Harvey V, Cote SD, Hammill MO (2008) The ecology of 3-D space use in a sexually dimorphic mammal. *Ecography* 31:371–380
- Hillman SS, Whithers PC, Drewes RC, Hillyard SD (2009) Ecological and environmental physiology of amphibians. Oxford University Press, New York
- Hills N, Hose GC, Cantlay AJ, Murray BR (2008) Cave invertebrate assemblages differ between native and exotic leaf litter. *Austral Ecol* 33:271–277
- Hutchinson VH (1958) The distribution and ecology of the cave salamander, *Eurycea lucifuga*. *Ecol Monogr* 28:1–20
- Istock CA (1966) The evolution of complex life cycle phenomena: an ecological perspective. *Evolution* 21:592–605
- Labée-Lund JH, Langeland A, Jonsson B, Ugedal O (1993) Spatial segregation by age and size in Arctic Charr—a trade-off between feeding possibility and risk of predation. *J Anim Ecol* 62:160–168
- 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 St Nat Trieste* 52 (Suppl):5–135
- Lindstrom J, Reeve R, Salvadio S (2010) Bayesian salamanders: analysing the demography of an underground population of the European plethodontid *Speleomantes strinatii* with state-space modelling. *BMC Ecol* 10:4
- Lukacs PM, Thompson WL, Kendall WL, Gould WR, Doherty PF, Burnham KP, Anderson DR (2007) Concerns regarding a call for pluralism of information theory and hypothesis testing. *J Appl Ecol* 44:456–460
- Main MB (2008) Reconciling competing ecological explanations for sexual segregation in ungulates. *Ecology* 89:693–704
- Main MB, Coblenz BE (1996) Sexual segregation in Rocky Mountain mule deer. *J Wildl Manage* 60:497–507
- Manenti R, Ficetola GF, Marieni A, De Bernardi F (2011) Caves as breeding sites for *Salamandra salamandra*: habitat selection, larval development and conservation issues. *North West J Zool* 7:304–309
- McIntire EJB, Fajardo A (2009) Beyond description: the active and effective way to infer processes from spatial patterns. *Ecology* 90:46–56
- Moran NA (1994) Adaptation and constraint in the complex life cycle of animals. *Annu Rev Ecol Syst* 25:573–600
- Novak T, Tkavc T, Kuntner M, Arnett AE, Delakorda SL, Perc M, Janzekovic F (2010) Niche partitioning in orbweaving spiders *Meta menardi* and *Metellina merianae* (Tetragnathidae). *Acta Oecol* 36:522–529
- Pastorelli C, Laghi P (2007) Predation of *Speleomantes italicus* (Amphibia: Caudata: Plethodontidae) by *Meta menardi* (Arachnida: Araneae: Metidae). In: Bologna MA, Capula M, Carpaneto GM, Luiselli L, Marangoni C, Venchi A (eds) *Atti del 6 Congresso Nazionale Societas Herpetologica Italica, Belvedere, Latina*, pp 45–48
- Pastorelli C, Laghi P, Scaravelli D (2005) Spacing of *Speleomantes italicus* (Dunn, 1923): application of a geographic information system (G.I.S.) (Amphibia, Plethodontidae). In: Salvadio S, Poggi R, Doria G, Pastorino MV (eds) *Atti del Primo Convegno Nazionale Biologia dei geotritoni europei Genere Speleomantes, Annali del Museo Civico di Storia naturale “G. Doria”, Genova*, 97, pp 169–177
- Pinheiro P, Bates D (2000) *Mixed-effect models in S and S-PLUS*. Springer, New York 528 pp
- Pinheiro P, Bates D, DebRoy S, Sarkar D (2010) *Linear and nonlinear mixed effects models*. R package version 3.1-97. <http://cran.r-project.org/web/packages/nlme>
- R Development Core Team (2010) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. <http://www.r-project.org>
- Rochette R, Grand TC (2004) Mechanisms of species coexistence: a field test of theoretical models using intertidal snails. *Oikos* 105:512–524
- Ruckstuhl KE (2007) Sexual segregation in vertebrates: proximate and ultimate causes. *Integr Comp Biol* 47:245–257
- Ruckstuhl KE, Festa-Bianchet M (2001) Group choice by subadult bighorn rams: trade-offs between foraging efficiency and predator avoidance. *Ethology* 107:161–172
- Salvadio S (1993) Life history of the European plethodontid salamander *Speleomantes ambrosii* (Amphibia, Caudata). *Herpetol J* 3:55–59
- Salvadio S, Pastorino MV (2002) Spatial segregation in the European plethodontid *Speleomantes strinatii* in relation to age and sex. *Amphibia-Reptilia* 23:505–510
- Salvadio S, Lattes A, Tavano M, Melodia F (1994) Ecology of a *Speleomantes ambrosii* population inhabiting an artificial tunnel. *Amphibia-Reptilia* 15:35–45
- Schneider K, Christman MC, Fagan WF (2011) The influence of resource subsidies on cave invertebrates: results from an ecosystem-level manipulation experiment. *Ecology* 92:765–776
- Singh NJ, Bonenfant C, Yoccoz NG, Cote SD (2010) Sexual segregation in Eurasian wild sheep. *Behav Ecol* 21:410–418
- Smithers P (2005) The diet of the cave spider *Meta menardi* (Latreille 1804) (Araneae, Tetragnathidae). *J Arachnol* 33:243–246
- Spiegelhalter DJ, Best NG, Carlin BR, van der Linde A (2002) Bayesian measures of model complexity and fit. *J R Stat Soc Ser B Stat Methodol* 64:583–616
- Spiegelhalter D, Thomas A, Best N, Lunn D (2008) WinBUGS 1.4.3. Imperial College and MRC, UK. <http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/contents.shtml>
- Spotila JR (1972) Role of temperature and water in the ecology of lungless salamanders. *Ecol Monogr* 42:95–125
- Stephens PA, Buskirk SW, Hayward GD, Del Rio CM (2007) A call for statistical pluralism answered. *J Appl Ecol* 44:461–463
- Symonds MRE, Moussalli A (2011) A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike’s information criterion. *Behav Ecol Sociobiol* 65:13–21
- van Toor ML, Jaberg C, Safi K (2011) Integrating sex-specific habitat use for conservation using habitat suitability models. *Anim Conserv* 14:512–520
- Vignoli L, Caldera F, Bologna MA (2006) Trophic niche of cave populations of *Speleomantes italicus*. *J Nat Hist* 40:1841–1850
- Vignoli L, Caldera F, Bologna MA (2008) Spatial niche of the Italian cave salamander, *Speleomantes italicus* (Dunn, 1923) (Plethodontidae, Amphibia), in a subterranean system of Central Italy. *Ital J Zool* 75:59–65
- Wilbur HM (1980) Complex life cycles. *Annu Rev Ecol Syst* 11:67–93