

Behavioral observations of the olm (*Proteus anguinus*) in a karst spring via direct observations and camera trapping

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Abstract

The olm (*Proteus anguinus*), an endemic amphibian of the Dinarides' underground waters (Europe), is one of the world's most widely known subterranean species. Although various aspects of olm biology have been extensively studied, the data on their behavior in the wild remain scarce mostly due to inaccessibility of their natural habitat. Yet, olms also occur in several karstic springs during nighttime. These are easier to access and present an exciting opportunity to study olm behavior in nature. Here, we report on systematic observations of olms in one such spring in Slovenia, where we observed them for nine consecutive summer nights, coupling direct on-site observations with IR camera trap recordings. We used IR camera trap recordings to construct simple ethograms, as well as to quantify olm movement activity by video-tracking. Olms regularly occurred on the surface during the night, and dawn appeared to be a key stimulus for their retreat underground. They were constantly active, but rarely swam far from the spring. Despite the short-term nature of the study, we collected new occurrence and movement data, and at the same time tested the usability of IR cameras for surveying olm presence and behavior in nature. Experience gained through the study may prompt long-term and more complex behavioral studies using similar approaches.

Keywords

Behavioral conservation, cave salamander, co-occurrence, ethogram, habitat choice, IR cameras, movement activity, remote sensing, video-tracking

Introduction

The olm (*Proteus anguinus* Laurenti, 1768) is one of the most charismatic and widely known subterranean species, with a more than 300 years long history of research (Aljančič 2019). It is an endemic of the underground waters of the Dinarides, distributed from Italy on the northwest to Bosnia and Herzegovina and Montenegro on the southeast (Sket 1997; Gorički et al. 2017), with over 500 localities known to date (SubBioDB 2022). The olm is classified as a vulnerable species on the IUCN Red List (Arntzen et al. 2009), protected by several national legislations, and listed in Annex II of the Habitats Directive (The Council of European Communities 1992). Its populations are at risk throughout its distribution range with the main threats being habitat destruction by hydroengineering and construction (Koller Šarić 2019), as well as agricultural and industrial water pollution (Bressi 2004; Hudoklin 2011). More locally the olm's populations are threatened also by the presence of toxins in the environment and even poaching (Pezdiric et al. 2011; Năpăruș-Aljančič et al. 2017). Environmental conditions at many of the olm's localities remain unfavorable (Sket 1997; Hudoklin 2011), calling for an urgent development and implementation of conservation plans.

Over the last decades, the emergence of new tools and methods in the conservation biology already provided first steps towards its effective conservation. For example, molecular tools were developed and used to assess the genetic structure of olm populations (Gorički and Trontelj 2006; Zakšek et al. 2018; Vörös et al. 2019), providing a basis for future identification of sites and populations of the highest conservation priority. Further, the application of environmental DNA approaches facilitated the detection of olm presence and supplemented its previously known geographical distribution (Gorički et al. 2017; Vörös et al. 2017). Significant progress in olm's developmental biology, essential for sustainable captive breeding has been made in the last years (Gredar et al. 2019), and even sanctuaries for injured animals and individuals washed-out from their subterranean habitat were established (Aljančič et al. 2014; Lewarne 2018). Finally, deciphering the olm's genome is underway, promising great potential for further progress in olm conservation (Kostanjšek et al. 2022). Nevertheless, assessing the status of olm populations remains challenging (Trontelj and Zakšek 2016), largely due to habitat inaccessibility and incomplete knowledge on the biology, life history, ecology, and behavior of this species.

Most of the current knowledge on olm biology is based on observations in the laboratory or semi-natural conditions. Yet, the success of conservation actions heavily depends on our understanding of species' behavior in their natural habitats (Caro 1999; Blumstein and Fernandez-Juricic 2010). As observing olms in their natural habitats is difficult and commonly requires cave diving performed by specially trained researchers using expensive equipment, such knowledge is scarce. The most comprehensive work on olm behavior in the natural habitat has been done by Briegleb (1962) and the most recent finding of this approach is that olms show extremely high site-fidelity (Balázs et al. 2020). On the other hand, easily accessible sites, such as karst springs, offer a unique

opportunity for *in-situ* behavioral observations of otherwise inaccessible populations using relatively cheap equipment. Although most records of olms in epigeal habitats are only accidental, they occur in several perennial springs during nighttime (Sket and Arntzen 1994; Bressi et al. 1999; Bressi 2004; Kordiš 2016). However, there is currently no data on the regularity or fraction of olm's populations occurring in springs, and no systematic observations were conducted at such sites so far.

To complement the existing knowledge on the olm occurrence and behavior in karst springs, we conducted a nine-day systematic survey during nighttime in a spring in southeastern Slovenia. Besides direct on-site observations, we employed infrared (IR) cameras for indirect observations of olms. The importance of IR cameras for detecting remote wildlife and rare species has been widely acknowledged (Cutler and Swann 1999; Swann and Perkins 2014). IR cameras are often used to follow the presence and abundance of species and to study different aspects of animal behavior in their natural environments. As such, they are increasingly important in wildlife monitoring and conservation (Swann and Perkins 2014).

Thus far, IR cameras have been used to observe olms in the laboratory or under semi-natural conditions, as well as in the natural cave habitat (Balázs and Lewarne 2017; Lewarne 2018). However, IR cameras have not been used before to monitor olms in karst springs. In the present study, we aimed to evaluate whether the usage of IR cameras enables acquiring reliable data on olm behavior. Additionally, we explored possibilities to analyze the recordings from IR cameras to obtain quantitative data on olm presence and movement in the epigeal part of the spring during nighttime.

Materials and methods

Site description

We observed the olms in a perennial karst spring located in southeast Slovenia. Due to potential threats to the population and internal research policy in Slovenia, we do not provide exact name and location of the spring.

Groundwater surfaces from two main springs and fills a shallow pool (hereafter referred to as "surface pool"; Fig. 1A) which continues as a small brook after approximately 10 meters. The bottom of the pool is covered mostly with fine gravel intermixed with silt, and a few larger rocks. Treetops in the immediate surroundings shade the pool throughout the day.

The water level of the surface pool fluctuates seasonally. In summer it is the lowest and thus with the best visibility, making summertime the most appropriate season for observing olms at this location. The surface pool was approximately 20 cm deep at the time of our observations. Visibility remained the same throughout all days of observations and was not affected by rainfall. The temperature in the spring and surface pool was constant, 11.5 ± 0.5 °C.

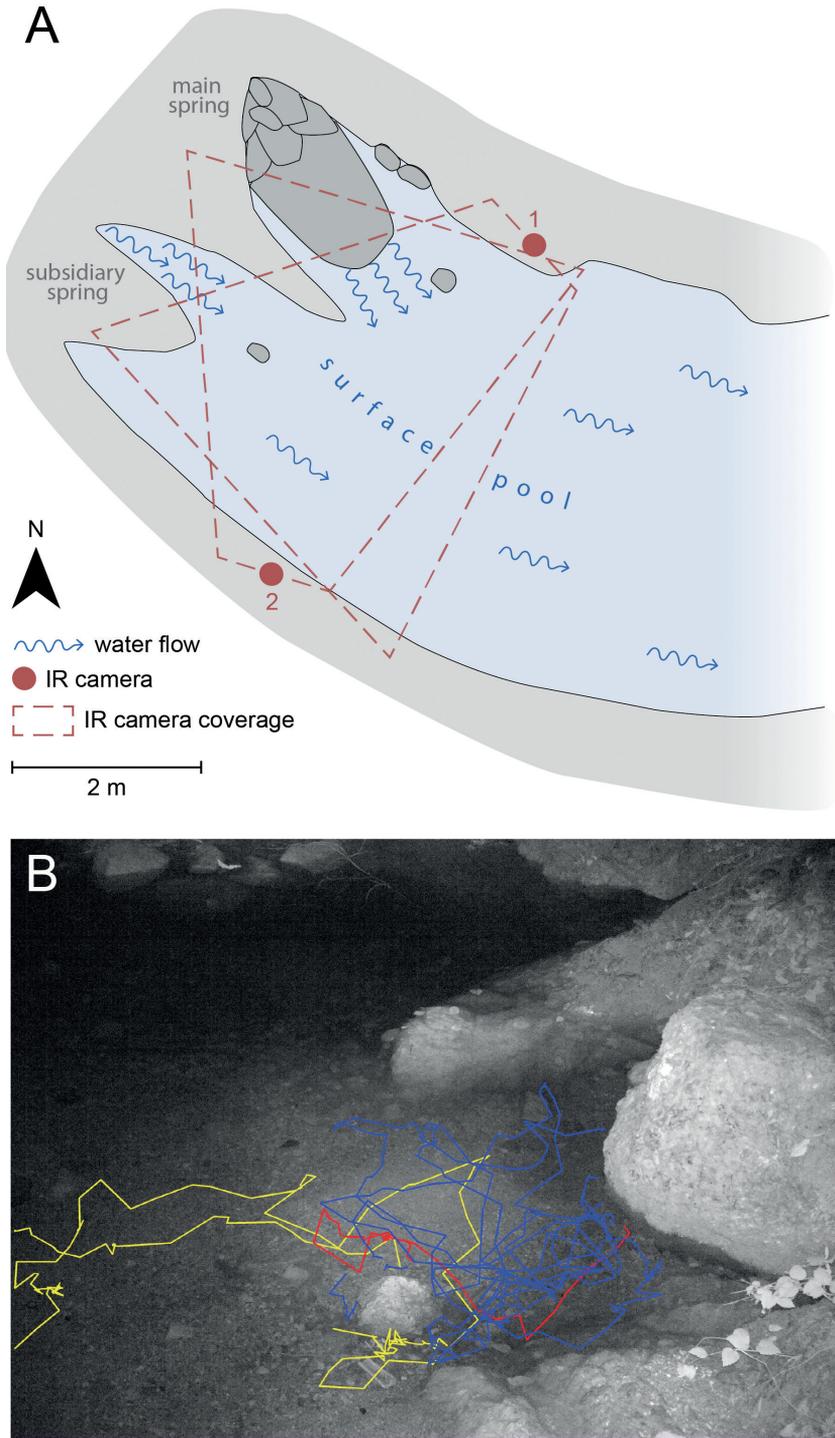


Figure 1. **A** ground plan of the karst spring in which olms (*Proteus anguinus*) were observed **B** an example of trajectories of three olms (different colors correspond to different individuals) during night 8, obtained by processing the images from the IR camera 1.

Fieldwork and camera setting

We observed occurrence and behavior of olms in the surface pool for nine consecutive nights, between 17th and 27th July 2019. For the first five nights, we observed olms directly on-site, while for the last four nights we coupled the direct observations with infrared (IR) camera recordings.

We carried out the direct on-site observations in three time periods per night. The first visit started at sunset (approx. 20:40 Central European Summer Time (CEST)) and lasted 1–3 hours. We returned to the spring after midnight (approx. between 00:00 and 2:00 CEST) for 20–30 minutes. The third and last visit was in the morning, one hour before sunrise (approx. 5:30 CEST). We provide exact observation times for each night in Suppl. material 1. During the direct on-site observations, we illuminated the surface pool every 10 minutes, counted the olms, and noted their position. To avoid disturbing the olms with too bright lights, we used only brief illumination time and dimmed red LED lights to which they appear to be less responsive according to observations of captive specimens (Schlegel et al. 2009).

Two IR cameras (Magonin WK 4HDW) were positioned at the opposing banks of the surface pool to cover an area as large as possible (Fig. 1A). The cameras were set to capture one frame per minute, starting one hour after sunset and finishing at sunrise. In total, direct observations lasted for 2–3 hours per night, while cameras were set to record for 7–8 hours per night.

Using both observation methods, we identified the holes through which olms emerged to the surface or retreated underground (hereafter referred to as “surface-subterranean corridors”) and determined the number of olms simultaneously present in the surface pool. For each olm we also noted its first and last occurrence in the surface pool during the night. The methods used did not enable reliable distinction of individual olms between nights.

Analysis of images recorded with IR cameras

We used images recorded with IR cameras to construct simple ethograms for each olm for the time of its presence in the surface pool. Although image quality did not allow recognition of complex behaviors, such as e.g., food searching, feeding, and agonistic behavior, we could clearly define and quantify the duration of four basic behaviors related to olms’ position in the spring, named “emerging”, “outside”, “retreating” and “inside”, which are described in detail in Table 1. The cameras covered the entire olms’ movement in all but one case, when one olm swam further downstream. This event was treated as behavior “outside” until the animal reached the field of view again.

In addition, we used the acquired images to quantify olms’ movement activity by video-tracking analysis. With both IR cameras, we captured 2713 images in total. Due to camera lens fogging or people in front of the camera, we discarded 424 (16%) images. We then carefully checked the remaining 2289 (84%) images for the presence of olms and detected them on 996 (37%) images. Due to the low quality of the raw images, we manually marked individual animals on these images by adding a 45-pixel

colored circle on olms' heads in Adobe Photoshop CS6. To distinguish between individuals, we used circles of different colors.

Next, we converted the modified image sequences to videos and performed video-tracking in Bonsai 2.5.2. (Lopes et al. 2015) (Fig. 1B). We tracked the olms for the whole time that their heads were visible in the surface pool, disregarding the previously defined behaviors (Table 1). We retrieved the coordinates of the olms' positions from each camera separately, from which we calculated the total path covered and movement speed. We report video-tracking results from images obtained by camera 1 (Fig. 1A), as it better captured the olms' movement around the surface pool and provided more data. We used Pearson's correlation coefficient to assess the relationship between time spent in the surface pool and total path covered. Since shooting-angle of cameras prevented accurate distance measurements, we expressed the covered distances and movement speed using olm body length (bl) as a relative distance unit, or as an approximation of metric units. For the relative distance unit, we measured the olms' length in pixels multiple times (3–5 repeated measurements) in up to six images where they were well visible and used mean value in all analyses. For approximation in metric units, we assumed that the body length of the olms ranged between 15 and 25 cm. Lastly, when multiple individuals were simultaneously present in the surface pool, we analyzed their interactions by quantifying their mutual distance. We analyzed four pairs of co-occurring olms (one from night 6 and three from night 8) and measured the distance between their heads using body length of the larger olm in the pair as a relative distance measure.

The methods used did not enable reliable distinction of individual olms between different nights. Thus, results presented in Table 3 and Fig. 3 might be replicated observations of the same individuals during different nights. While we cannot completely exclude the chance of misidentifying the olms during same night, we argue that distinction of individuals based on their positions and body size was reliable.

Video-tracking data analyses and result visualizations were carried out in R 4.0.3 (R Development Core Team 2022) using the packages ggplot2 (Wickham 2016), dplyr (Wickham et al. 2019), scales (Wickham and Seidel 2020), and ggpubr (Kas-sambara 2020).

Table 1. Description of the four basic behaviors used to construct simple ethograms and quantify the olms' presence in the surface pool.

Behavior	Description
emerging	The animal is emerging from the subterranean part of the spring to the surface pool. The behavior starts when the head of the animal first appears from the subterranean corridor and stops when the whole animal emerges to the surface pool or retreats underground.
outside	The whole animal is present in the surface pool. The animal is still or moving around the pool.
retreating	The animal is retreating from the surface pool to the subterranean part of the spring. The behavior starts when the head of the animal first disappears into the subterranean corridor and stops when the whole animal retreats underground or returns to the surface pool.
inside	The animal is not present in the surface pool, the whole body is underground.

Results

Olm presence in the spring

During the first five nights, when olms were observed only directly, their presence in the surface pool was recorded on three nights. Their number was either one or at most two simultaneously present olms in the surface pool. During the next four nights, when we observed the olms also via IR cameras, we recorded up to three simultaneously present olms in the surface pool (Table 2).

Via direct observations, we identified two surface-subterranean corridors that olms used to transition from underground to the surface or vice versa. An additional, third corridor was discovered from IR cameras recordings. Olms first emerged in the surface pool one hour after sunset (Fig. 2). First emergences were equally distributed across almost four consecutive hours, i.e., from approximately 21:30 to 1:00 CEST (approx. 1–4.5 hours after sunset). On the other hand, last retreats of olms to the underground mostly clustered around 5:00 CEST (approx. 0.5–1 hour before sunrise). In three observations, animals retreated distinctively earlier (Fig. 2). Both the timing of first emergence and last retreat suggest sunlight as the most probable factor the olms use to guide their presence in the surface pool. At the time of their first emergence, at approx. 21:30, we had to use red LED lights to illuminate the surface pool, while in the morning, from approx. 4:30, we were able to observe olms without any additional lights. Connecting the first emergence and last retreat timing of the same individuals (grey lines in Fig. 2) showed considerable variation in the timing of olm presence in the surface pool. With more data at hand and ideally with known individual identity, such analysis could potentially test the presence of distinct behavioral types, e.g., “early bird” and “night owl” chronotypes or shy and bolder individuals.

Behavior and movement activity of olms

We used IR camera recordings from three nights to quantitatively describe the behavior and movement activity of olms in the surface pool. We excluded the seventh night from the analyses due to the camera lens fogging and consequent unreliable detection of olms.

Table 2. Summary of observation methods and number of olms observed in the surface pool for each night.

Night	Observation method	Number of olms observed
1	Direct	1
2	Direct	0
3	Direct	0
4	Direct	2
5	Direct	2
6	Direct and IR cameras	3
7	Direct and IR cameras	3
8	Direct and IR cameras	3
9	Direct and IR cameras	1

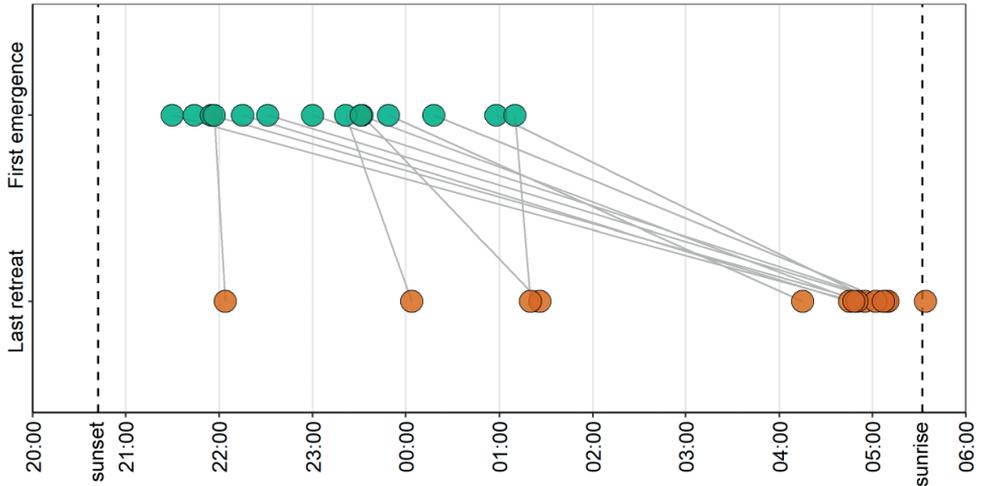


Figure 2. Observations of the first emergence (green dots) and last retreat (orange dots) of olms in the surface pool based on direct and IR camera observations. Grey lines connect the first emergence and last retreat time of the same animal within one night.

Table 3. Total duration of behaviors, path covered, and movement speeds for individual olms. The individual ID numbers (Ind.) correspond to those in Fig. 3. Note that numbers 1–7 represent the number of observations and do not imply the number of individuals, as we could not reliably distinguish them between different nights. Path and speed are given in body length (bl) units.

Night	Ind.	Behavior duration			Path covered [bl]	Speed [bl/min]		
		Emerging	Outside	Retreating		Minimum	Average	Maximum
6	1	NA	8 min	NA	2.4	0.04	0.34	0.68
6	2	13 min	1 h 41 min	1 min	37.3	0.01	0.30	2.28
6	3	14 min	3 h 21 min	18 min	87.1	0.01	0.36	1.42
8	4	50 min	2 h 5 min	3 min	36.7	0	0.22	1.10
8	5	4 min	37 min	2 min	17.1	0.03	0.38	1.28
8	6	22 min	4 h 19 min	19 min	104.2	0	0.34	2.24
9	7	14 min	2 h 47 min	1 min	66.1	0.004	0.36	1.69
	Mean ± SD	20 ± 16 min	128 ± 89 min	7 ± 9 min	50 ± 37	0.01 ± 0.01	0.33 ± 0.05	1.53 ± 0.59

We analyzed the behavior of three olms on the first two nights and a single olm on the last night, resulting in a maximum of seven olms. Nevertheless, the actual number of animals in the study might as well be lower, due to our inability to reliably discriminate the individuals occurring on different nights. Most olms were active for several hours during the night. On average, they took almost three times longer to appear from the underground (“emerging” behavior) than to retreat underground from the surface pool (“retreating” behavior) (Table 3, Fig. 3). This indicates that the olms are likely more cautious when emerging to the surface from their primary subterranean habitat. Five out of seven olms emerged to the surface and retreated underground several times during the night before they finally retreated at sunrise (Fig. 3). The total path covered by animals strongly positively correlated with the duration of their presence in the surface pool: the olms which were outside for longer also covered

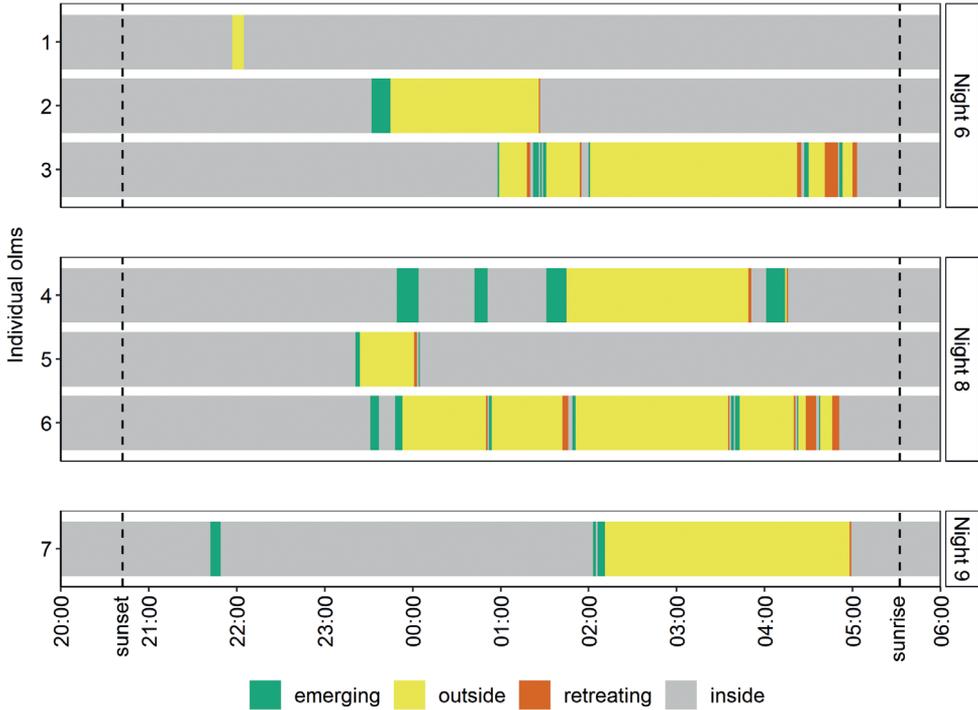


Figure 3. Simple ethograms of the seven olms observed in the karst spring with IR cameras. Individual olms are marked with numbers which correspond to those in Table 3. Note that numbers 1–7 represent the number of observations and do not imply the number of individuals, as we could not reliably distinguish them between different nights.

the longest path ($\rho = 0.986$, $p < 0.001$) (Table 3). Assuming a body length of 15 and 25 cm, paths ranged from 0.4 m to 15.6 m (mean \pm SD: 7.5 ± 5.6 m) and from 0.6 m to 26.1 m (mean \pm SD: 12.5 ± 9.3), respectively.

Average moving speeds were similar among all olms, with a mean of 0.33 ± 0.05 bl/min and a range of 0.22–0.38 bl/min. On the other hand, olms differed in their maximum speeds, with a mean of 1.53 ± 0.59 bl/min and ranged from 0.68 to over 2.2 bl/min. Interestingly, their minimum speeds were only rarely zero, implying a rather constant movement within the surface pool after their emergence to the surface. Assuming body lengths of 15 cm and 25 cm, their average speed was 4.9 ± 0.8 cm/min and 8.2 ± 1.3 cm/min, respectively. Their maximum speed was 22.9 ± 8.8 cm/min (assuming 15 cm body length) and 38.2 ± 14.7 cm/min (assuming 25 cm body length).

Co-occurrence and interaction of olms

We observed four pairs of olms simultaneously present in the spring: pair 1 (individuals 2 and 3) during night 6, and pair 2 (individuals 5 and 6), pair 3 (individuals 4 and 5), pair 4 (individuals 4 and 6) during night 8 (Fig. 3). The duration of individual co-occurrence differed between pairs. It was roughly 20 minutes in three pairs, but it

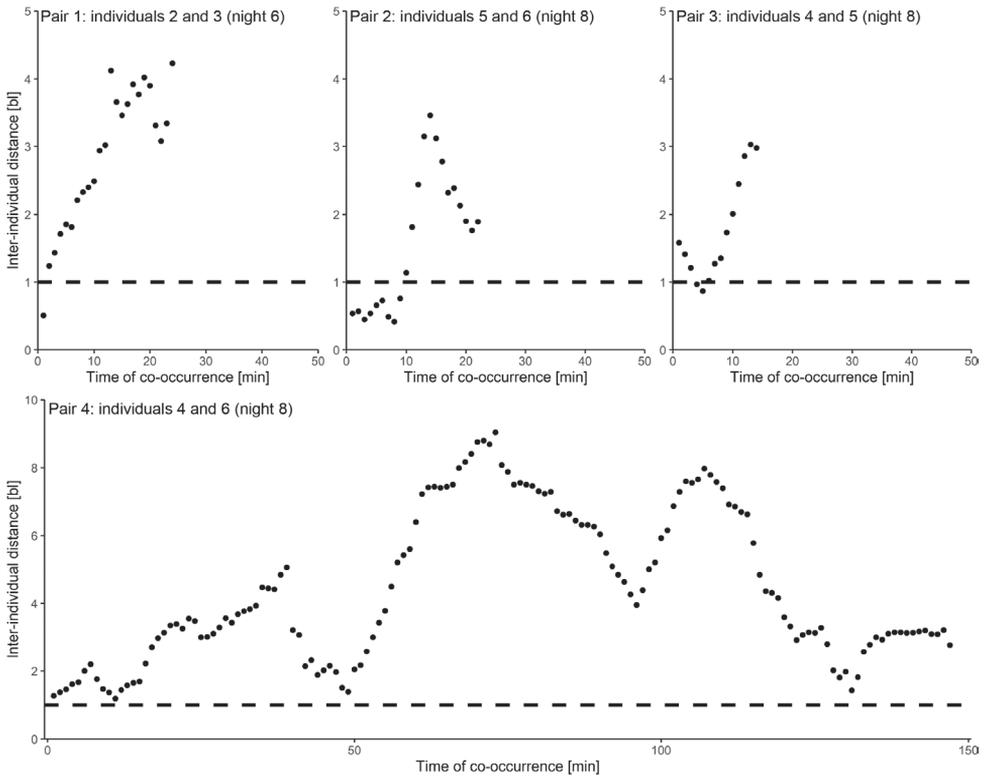


Figure 4. Four cases of an interaction between a pair of olms co-occurring in the surface pool. Individual olm’s numbers match those in Fig. 3. The plotted distance is the distance between both olms’ heads and expressed in body lengths [bl] of the larger olm in the pair. One body length distance is indicated with a dashed line. Time 0 represents the start of co-occurrence, i.e., the moment when the second olm of the pair emerged to the surface pool while the first was already present.

lasted almost 2.5 hours in one pair (Figs 3 and 4). Most of the time, the olms were much more than one body length distance apart, and when they came close, they soon moved away from each other again (Fig. 4). This suggests that when multiple olms are present in the surface pool, they might avoid each other.

Discussion

The combination of direct and indirect observations of olms provided valuable data on their occurrence and movement activity in a karst spring and associated surface pool. By conducting the first systematic observation of olms and analysis of their behavior in a spring, we complement the existing knowledge of this enigmatic species in its natural habitat. Olm behavior in their natural environment is an important, but unfortunately rarely studied aspect of the species’ biology and Briegleb’s (1962) classic work remains

the most comprehensive source of relevant information. Karst springs may provide an opportunity to further explore possibilities for assessing olm behavior and collecting behavioral data. Below, we summarize our observations and discuss potential challenges, improvements, and advantages of the methods used.

Our observations indicate that olms regularly occur in the epigeal habitat at night during the summer, and that sunlight represents the most likely stimulus for olms to retreat to the subterranean habitats. The reasons for the occurrence of olms in karst springs are not yet clear. The springs may simply serve as an extension of the olms' primary habitat during the night. The emergence of olm at the surface could simply be the result of their nocturnal movement along the subterranean-surface corridors when both habitats are not clearly divided by sunlight. However, some evidence suggests deliberate presence on the surface. First, olms quickly evade the potential threats (e.g., strong light and water disturbance), by swimming directly to holes leading underground (unpublished personal observations at several sites), suggesting exceptional orientation and/or spatial memory. Second, it has been suggested that olms emerge to the surface to feed in a food-rich environment, a prediction supported by a few observations (Sket and Arntzen 1994; Bressi et al. 1999; Recknagel et al. 2022). Olms are apex predators underground (Briegleb 1962), but might become a prey of larger epigeal animals in or close to epigeal habitats. This might further explain their movement in the close vicinity of the surface-subterranean corridors and retreat at sunlight.

In the future, several other aspects of the olm behavior might be addressed using the same observation methods, such as seasonality and interactions between animals. We observed the olms only in summer. If sunlight is the only factor keeping them underground, they should spend a relatively larger amount of time on the surface in the fall and winter when nights are long, and days are short. However, if they emerge to the surface mainly to feed, we might expect to see this behavior more often in parts of the year when food is usually more abundant at the surface or when food is particularly scarce underground. Our dataset did not allow a thorough analysis of olm interactions, but we were still able to detect and distinct four events of a co-occurring pair of olms. Most of the time, the two olms were much more than a body length apart and quickly moved away from each other when they got closer. To determine if they are actually avoiding each other, exhibit territorial behavior, or perhaps compete for food, more data and further studies are needed.

Compared to direct on-site observations, those via IR cameras provided more data on olms' emergence to and retreat from the surface, the use of surface-subterranean corridors, and their movement activity. At the same time, direct observations were valuable as notes on olm positions within the spring enabled easier identification of the animals on camera images. Our results show that the images captured by IR cameras are useful for detection and monitoring of olms and can be further analyzed to obtain behavioral data. For the first time, we have extended the use of IR camera recordings of olms beyond descriptive results by providing quantitative data on their movement. On the other hand, there are some limitations associated with the extraction of the data from raw IR camera images. First, we only tested the cameras in good

weather conditions, and cannot provide any information about their usability in bad weather, e.g., fog or rain. Second, the images required additional processing to obtain movement and behavior data due to poor contrast between olms and the background. Our approach to this issue was relatively simple, yet probably too time-consuming for manually processing images in longer behavioral studies. There are several possible solutions for such cases, including better IR illumination, optimized camera positions (e.g., from above rather than from the side) and using more complex methods to process the images. Despite some limitations, we conclude that IR camera recordings are reliable and appropriate for the extraction of both qualitative and quantitative data.

In the future, our approach could be improved by placing more cameras to cover as wide an area as possible. It could further be improved by employing remote-controlled IR cameras capable of remote live streaming. These would avoid possible disturbances caused by our presence at the study site, which might have affected the olms' behavior. Wider coverage and no direct disturbance combined with accurate distinction of individual olms would allow the detection and recognition of more complex behaviors and consequently the construction of more detailed and informative ethograms. This would enable more in-depth studies on olm movement and use of epigeal space, feeding, and predator-prey related behaviors, as well as studies on intraspecific interactions – all of which are key behavioral domains to consider in conservation efforts (Berger-Tal et al. 2011). Lastly, well-placed IR cameras could help detecting and monitoring harmful human activities like disturbance and even poaching at olm localities. We conclude that studies such as ours, although short-term and small-scale, represent a step toward integrating knowledge on olm behavior into plans of its conservation.

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Supplementary material I

Figure S1

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Data type: Png image.

Explanation note: Overview of the methods used and related observation durations for all nine nights of the study.

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