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ARTICLE
Animal Ecology

The rhythm of the night: Assessing temporal variation of activity patterns in arhythmic environments from field data

Elia Lo Parrino[1] | **Stefano Lapadula**[1,2] | **Damiano Brognoli**[1] | **Giorgia Terraneo**[1] | **Benedetta Barzaghi**[1,2] | **Sarah Boulamail**[3] | **Valentina Balestra**[4,5] | **Matteo Galbiati**[1] | **Jacopo Luciano Gandini**[1] | **Giorgio Grassi**[1] | **Mattia Falaschi**[1] | **Gentile Francesco Ficetola**[1,6] | **Salvatore Inguscio**[7,8] | **Valeria Messina**[1] | **Edgardo Mauri**[9] | **Francesco Passoni**[1] | **Veronica Zampieri**[1] | **Francesco Cozzoli**[3,10,11] | **Raoul Manenti**[1,2]

[1]Department of Environmental Science and Policy, Università degli studi di Milano, Milan, Italy

[2]Laboratorio di Biologia Sotterranea "Enrico Pezzoli", Parco Regionale del Monte Barro, Galbiate, Italy

[3]Research Institute on Terrestrial Ecosystems (IRET) – National Research Council of Italy (CNR) URT Lecce, Lecce, Italy

[4]Department of Environment, Land and Infrastructure Engineering, Politecnico di Torino, Turin, Italy

[5]Biologia Sotterranea Piemonte – Gruppo di Ricerca, Piedmont, Italy

[6]Grenoble Alpes University, CNRS, University of Savoie Mont Blanc, Laboratory of Alpine Ecology (LECA), Grenoble, France

[7]Laboratorio Ipogeo salentino di Biospeleologia "Sandro Ruffo", Nardò, Italy

[8]Gruppo Speleologico Tricase, Lecce, Italy

[9]Speleovivarium "Erwin Pichl", Trieste, Italy

[10]Department of Biological and Environmental Sciences and Technologies, Lecce, Italy

[11]National Biodiversity Future Center (NBFC), Palermo, Italy

Correspondence
Stefano Lapadula
Email: stefano.lapadula.1999@gmail.com

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Abstract

Ecologists and zoologists have worked for centuries to understand the spreading, extent, and regulation of biological rhythms among eukaryotes living in arhythmic ecosystems. Investigating whether the long-standing hypothesis that biological clocks went lost in species inhabiting arhythmic environments can thus have broad implications in assessing evolutionary and ecological pathways allowing colonization of new and physiologically challenging environments. Subterranean habitats generally lack temporal variation in environmental conditions. However, chronobiological research in such communities remains hindered by methodological, ethical, and logistical constraints, particularly for field-based studies. Here, we propose a flexible, noninvasive framework to assess temporal variation in the activity of animals using detection

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probability as a proxy for biological rhythms. Our approach is based on Bayesian occupancy and abundance models, which can incorporate quantitative (count), qualitative (presence/absence), or semiquantitative (abundance classes) data derived from repeated surveys. This framework is adaptable to a variety of taxa, monitoring strategies, and environmental conditions. It is particularly well-suited for sensitive ecosystems, as it minimizes disturbance and allows for standardized, repeatable analysis of activity patterns. We illustrate the utility of the framework through three case studies (*Proteus anguinus* Laurenti 1768, *Niphargus* sp. Schiødte 1849, and *Spelaeomysis bottazzii* Caroli 1924) encompassing different data types and showing its potential to detect rhythmicity even in stable subterranean environments. By facilitating robust in situ assessments of temporal activity, our method provides a novel and accessible tool for exploring biological rhythms in arhythmic environments, and can thus support the conservation of highly specialized, often vulnerable, fauna.

KEYWORDS
chronobiology, imperfect detection, N mixture, occupancy model, phenology

INTRODUCTION

Biological rhythms of various kinds have independently evolved multiple times across eukaryotes (Arendt, 1998; Yerushalmi & Green, 2009). These rhythms occur at different biological scales, from the cellular to the supracellular level, and span a wide range of periods, from fractions of a second to several decades (Goldbeter, 2008). In many cases, biological rhythms, especially circadian rhythms, are driven by endogenous oscillatory processes known as "biological clocks," which are synchronized by periodic environmental cues referred to as "zeitgebers" (Aschoff & Pohl, 1978). Common zeitgebers include sunlight, temperature fluctuations, food availability, and social interactions (Collins & Blau, 2007; Friedrich, 2013).

Maintaining circadian rhythms would represent an unnecessary energetic cost for animals inhabiting arrhythmic oligotrophic environments (Moran et al., 2014), such as caves or abyssal depths, where geophysical cycles (circadian, seasonal, lunar, tidal) are absent. Caves, for instance, are considered stable environments, characterized by constant temperature and relative humidity throughout the year, and by permanent darkness, which eliminates daily light–dark cycles and reduces environmental cyclicity (Mammola, 2019). Because of these metabolic costs, it has been hypothesized that biological clocks would be lost in cave-dwelling animals (Friedrich, 2013). While this appears to be true for some organisms, a growing body of evidence indicates that many hypogean (i.e., subterranean) species retain their biological clocks (Abhilash et al., 2017; Beale et al., 2013; de Souza et al., 2024; Soriano-Morales et al., 2013; Stringer & Meyer-Rochow, 1997).

Biological rhythms may facilitate species coexistence by promoting temporal niche partitioning, a mechanism predicted by coexistence theory whereby competing species avoid direct overlap in activity, thus reducing competition (Chesson, 2000; Kronfeld-Schor & Dayan, 2003). Similarly, temporal segregation of activity patterns can lower predation risk, as preys reduce the probability of encountering predators by being active at different times (Kronfeld-Schor & Dayan, 2003). Both mechanisms have been reported in cave animals, underlining the importance of biological rhythmicity in hypogean species (de Souza et al., 2024; Galbiati et al., 2023).

Moreover, several phenomena may serve as potential zeitgebers in caves, possibly influencing the expression of circadian and circannual rhythms. Natural caves are typically divided into three zones, each inhabited by distinct ecological communities: the photic zone, closest to the entrance, where direct light reaches; the twilight zone, which receives indirect light; and the aphotic zone, characterized by total darkness (Reis-Venâncio et al., 2024; Tobin et al., 2013). In the photic and twilight zones, the microclimate may be unstable, with fluctuations in temperature, humidity, and light levels (Lunghi et al., 2017; Romero, 2012). Additionally, in anchialine caves, salinity can exhibit both horizontal and vertical gradients and may vary seasonally (Sket, 1996). Moreover, it has been observed that some organisms associated with subterranean environments are capable, under certain conditions, of exploiting epigean habitats, particularly springs

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(Manenti & Barzaghi, 2021; Manenti & Pezzoli, 2019; Manenti & Piazza, 2021; Manenti, Riccardo, et al., 2024; Manenti, Zampieri, et al., 2024), and in these environments they may exhibit a different rhythmic behavior, because of both plasticity (Bilandžija et al., 2020; Walasek et al., 2025) and, arguably, true reversal (Elmer & Clobert, 2025). For cave-dwelling organisms living near the entrance, facing seasonal variations in salinity or other environmental factors and/or exploiting surface habitats, circadian and circannual rhythmicity may provide significant adaptive advantages. Additionally, even hypogean species living under constant climatic conditions may experience periodic phenomena, such as bat activity patterns or nutrient inputs driven by precipitation cycles, which can potentially act as zeitgebers (Moldovan et al., 2018; Soriano-Morales et al., 2013; Stringer & Meyer-Rochow, 1997).

Research on chronobiology in hypogean species is still in its early stages and is affected by significant geographical and taxonomic biases, with freshwater taxa being particularly underrepresented (de Souza et al., 2024). Assessing the expression of circadian rhythms in the laboratory remains challenging. Differences in the experimental protocols, the duration and presence of entrainment, the type of free-running conditions, and the selection of zeitgebers to test can lead to contrasting results, complicating the interpretation and comparison of studies (Beale et al., 2016; Pasquali & Sbordoni, 2014). Field studies, on the other hand, may represent a complementary strategy to laboratory experiments, identifying patterns that do not emerge from the latter (Manenti et al., 2026), but require substantial sampling effort and must consider the vulnerability of species inhabiting small and sensitive environments, such as isolated subterranean ponds (de Souza et al., 2024). Moreover, a plethora of monitoring strategies have been applied to study animals in hypogean environments over the years due to differences between monitored species, environmental conditions in the focal caves, heterogeneity within and between caves, and other factors (Hunt & Millar, 2001; Wynne et al., 2019). For instance, collected data may be qualitative (e.g., observation data), semiquantitative (e.g., abundance classes), or quantitative (e.g., count data).

There is thus a pressing need to develop standardized, noninvasive techniques for assessing variation in activity patterns in hypogean species, and for investigating the relationships between these patterns and potential zeitgebers. In this study, we aimed to establish a methodological framework for assessing daily and seasonal changes in the activity patterns of cave-dwelling animals by analyzing variation in detection probabilities, showing different analytical approaches suitable for qualitative, semiquantitative, and quantitative data within a Bayesian framework. To this end, we investigate three case studies regarding subterranean aquatic species chronobiology: (1) circadian and circannual activity patterns in *Proteus anguinus* from Friuli-Venezia Giulia, Northeastern Italy; (2) circadian and circannual activity patterns in *Niphargus* sp. from Lombardy, Northern Italy; (3) circadian and circannual activity patterns in *Spelaeomysis bottazzii* from Apulia, Southern Italy.

MATERIALS AND METHODS

Framework development

Our framework uses Bayesian hierarchical models to analyze the detection probability of the focal species (Kéry & Schmidt, 2008). For this purpose, we use the package "nimble" (de Valpine et al., 2017) as integrated in the software R (R Core Team, 2021).

The modeling approach changes with the type of ecological data available, which may be qualitative data (detection data), semiquantitative data (abundance classes), and quantitative data (count data). Each model is able to inform about within-species variation, but posterior parameters of different models built using different data sources or different species are not comparable between them. In the following section, we provide a case study for each scenario.

For each of these case studies, we performed repeated surveys throughout time in subterranean transects. We conducted 10-min visual surveys to count individuals, and, for the crustacean species (i.e., case study 2 and 3), it was followed by 5 min of dip-netting. The number of observers varied between two and four per transect, and the same observers performed every repeated survey in the same transect within the same sampling year. Moreover, to minimize biases due to different observers, all observers were trained by the same person (Raoul Manenti) and received the same information about the sampling protocol, and the same standard protocol was followed throughout the years. If necessary, the beginning and the end of each transect were marked to ensure the same surface was covered throughout repeated surveys, as groundwater habitats can be highly variable across time.

Case study

Case study 1: *P. anguinus*

P. anguinus is a neotenic cave salamander endemic to the Dinaric Karst Region. It is the largest troglobiont vertebrate in the world and the only stygobiont amphibian in

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the Palearctic (Balazs et al., 2020; Manenti, Zampieri, et al., 2024). Moreover, it is one of the first cave animals to have ever been studied. Even though it is strictly cave-dwelling, it can be found in springs and "estavelle" where it actively looks for trophic resources (Manenti, Zampieri, et al., 2024). Despite decades of research on this animal, both in laboratory and in natural environments, knowledge about its rhythmicity is still at an embryonic phase.

Surveys in the Italian sector of the Classic Karst Region were conducted between 2020 and 2025. We monitored 16 hypogean transects in caves and wells within 5 localities, and each transect was surveyed between 1 and 11 times each year to count individuals of *P. anguinus*. We tested the effect of daytime, season, and lunar phase on detection probability while accounting for the locality identity as a random factor. Two approaches can be used to account for the fact that the surveys span over multiple years: the first one is to keep a static N-mixture model and add a further hierarchical level for the year (year stratification); the second one is to use a dynamic model. In this case, we opted for the latter, and we built a Ricker model, which describes population dynamics by relating the population size in 1 year to that in the previous year.

Case study 2: *Niphargus* sp.

Species of the genus *Niphargus* are distributed over many karst areas across Europe and Asia (Borko et al., 2021). It is quite a specious genus that thrives in the majority of underground waters and it is commonly found also in cave entrances, springs, and ecotonal habitats, such as Po Plain's "fontanili," where they show a diel fluctuation in abundance (Manenti & Barzaghi, 2021). In laboratory experiments, *Niphargus* spp. showed a photophobic response (Borowsky, 2011; Fišer et al., 2016; Manenti et al., 2023), but no test on the circadian rhythmicity in cave populations has been conducted.

Between April and November 2024, we monitored nine caves in the Larian Triangle and Bergamo Prealps (Lombardy, Italy), hosting *Niphargus* populations, for a total of 30 transects. Each transect was visited from 3 to 11 times. Detection/non-detection data were recorded, and we used a simple site occupancy model to assess the detection probability as a function of daytime (night = 1, day = 2), season, and lunar phase (expressed as the proportion of lunar surface at light during the survey), while accounting for sampling method (visual survey vs. dip-netting). The transect was used as the sampling unit; thus, we used cave identity as a random factor to account for nonindependence of transects within caves.

Case study 3: *S. bottazzii*

S. bottazzii is a cave-dwelling crustacean mysid, endemic to Apulia (SE Italy), and is considered the only representative of its genus in Europe (Ariani & Wittmann, 2010). It is a euryhaline and eurythermal species that inhabits groundwaters in caves and wells, even highly polluted ones (Ariani & Wittmann, 2010; Inguscio et al., 2006; Rossi & Inguscio, 2003). It has also been observed in surficial waters, in correspondence of sinkholes, locally known as "spunnulate" or "spundulate" (Boulamail, Inguscio, et al., 2025; Rose & Parise, 2002). Moreover, it is able to exploit the photic zone (Ariani & Wittmann, 1997). For this reason, we decided to test whether the activity rates of this crustacean display rhythmic patterns.

We monitored 20 hypogean transects within five localities, here defined as areas encompassing multiple caves and sinkholes that are likely connected through the same aquifer, in the Salento peninsula (Apulia) during February, May, and October 2024. Each transect was visited between one and 18 times, during both day and night when possible. Both for visual surveys and dip-netting, *S. bottazzii* individuals were counted. Data were classified into two abundance classes (small population ≤ 20 individuals; large population > 20 individuals) and were analyzed using a multistate occupancy model, modeling detection probabilities as functions of daytime (night = 1, day = 2), season, and lunar phase, while accounting for sampling method. We used locality identity as a random factor to account for the nonindependence of transects within each locality.

RESULTS

Framework

Our framework (Figure 1) assumes that variations in activity patterns are reflected by changes in the detection probability of the focal species. Detection probability, that is, the probability of observing the species/individual when it is present in the monitored environment, is generally <1 (Kéry & Schmidt, 2008). This results in what is called "imperfect detection," which, if not properly accounted for, may affect the reliability of estimates of occupancy, colonization, extinction, and abundance (Hostetler & Chandler, 2015; MacKenzie et al., 2002, 2003; MacKenzie & Kendall, 2002). Peaks of activity linked to phenology may increase detection probability (Ficetola, Barzaghi, et al., 2018); thus, it is possible to use detection probability as a proxy for activity levels (Falaschi, 2021; Strebel et al., 2014). By evaluating temporal changes in detection probability using occupancy

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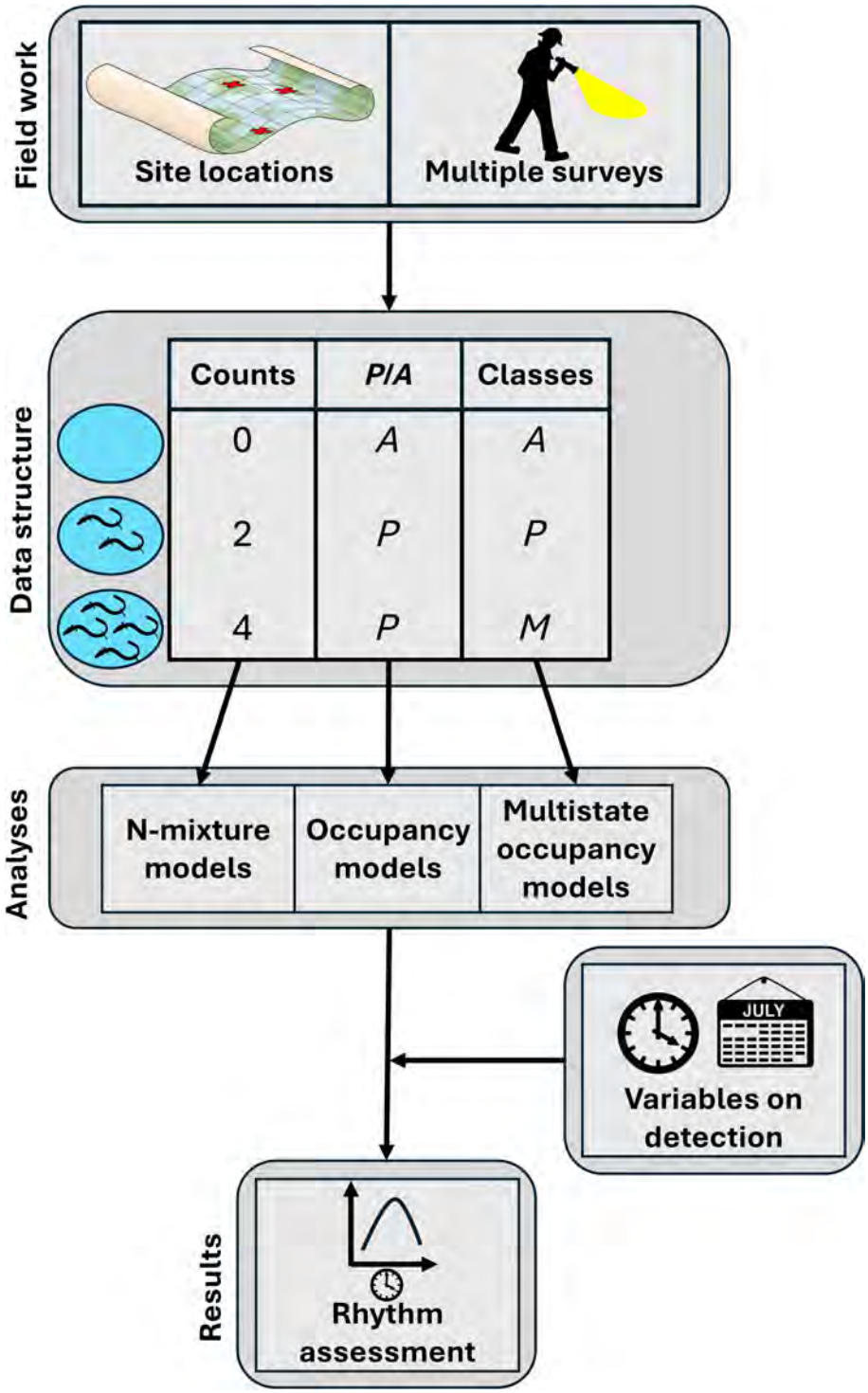

FIGURE 1 Schematic representation of the proposed framework. *A*, absence; Classes, class abundance; Count, Count data; *M*, locally abundant; *P*, presence; *P*/*A*, presence–absence data. Illustration credits: Jose Carlos Arenas-Monroy, Natasha Sinegina, hamdolii, geralt, centroacademico, UnboxScience, and JoyPixel.

models, previous studies have assessed seasonal variations of activity levels in epigean animals (e.g., Lo Parrino et al., 2024; Ramellini et al., 2024). To our knowledge, this strategy has never been applied to hypogean animals, and even detection probability of cave species has rarely been considered (Lunghi et al., 2017; Mouser et al., 2022).

Here, we provide a description of the modeling approach in case of qualitative data (detection data),

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semiquantitative data (abundance classes), and quantitative data (count data).

Case 1: Count data

Hierarchical N-mixture models enable obtaining reliable approximations of species abundance from spatially replicated counts (Royle, 2004). These models account for imperfect detection by modeling two processes: the ecological process (i.e., actual abundances) and the observation process (i.e., individual detection probability) (de Valpine & Hastings, 2002; Mathieu-Bégné et al., 2019). Given the low impact that replicated counts have on cave systems, this approach has already been used to assess the local abundance of animals in hypogean environments (Ficetola, Barzaghi, et al., 2018), but not to test variation of activity levels using variation in detection probabilities. In N-mixture models, the modeled probability is the detection probability of the individuals, meaning that we can infer the variation of activity rate at a resolution comparable to what can be obtained in laboratory experiments. By assessing the effects of the time of the day or of the year on detection probability, it is possible to assess whether and how activity rates change, assuming that populations are closed during the monitoring season. Alternatively, it can be tested whether and how factors potentially acting as zeitgebers (light conditions, air or water temperature, salinity, etc.) affect activity levels.

In case of count data, we can define the abundance of the species at site i as:

$$N_i \sim \text{Poisson}(\Lambda_i)$$

or, alternatively, as:

$$N_i \sim \text{NegBinomial}(\Lambda_i, \rho)$$

where Λ is the expected abundance at site i. In Poisson case, the assumption is that the mean of N_i is equal to its variance, while in negative binomial case, the variance can exceed the mean, with the parameter ρ representing the amount of overdispersion. The observation model, on the other hand, is defined as:

$$X_i \sim \text{Binomial}(N_i, p_i)$$

where X_i is the observed state at site i and p_i is the individual detection probability at site i. In this case, testing for the effects of potential zeitgebers can be done by modeling p_i as:

$$\text{logit}\left(p_{ij}\right) \sim \alpha_p + \beta_{p[1]} \times x_{1[ij]} + \beta_{p[2]} \times x_{2[ij]} \ldots + \beta_{p[n]} \times x_{n[ij]}$$

where α is the intercept and x_1, $x_2 \ldots x_n$ are variables at site i and survey j potentially affecting the activity rate.

To test for circadian rhythmicity, the hour of the survey may be used as a predictor of the model. Alternatively, a factorial day–night variable may be used if the hour of the surveys is strongly unevenly distributed. The same can be done for circannual rhythms, with day of the year as a continuous variable—simply numeric or using Fourier cycles—or a factorial season variable. Other rhythms can be tested, such as the circalunar rhythm, using the lunar phase as a predictor, the tidal rhythm, using the variation in water levels, or rhythms linked to interactions with other species. Lastly, in the detection part of the models, parameters to test interactions can be added, for instance to address the interaction between a site feature and a rhythm that is being tested. Site features, like habitat heterogeneity or transect area, may be tested in the ecological part of the models; however, when the focus is fauna's rhythmicity, between-transects variability is addressed with the hierarchical structure of the model itself, while between-sites variability can be simply modeled with a random factor.

Case 2: Detection data

Observation data, that is, 0–1 data representing the non-detection and the detection of the focal species during a survey, are relatively easy to collect and are among the most common data available in historical sources, particularly concerning subterranean environments (Ficetola et al., 2024). The suggested modeling choice to assess temporal variations in detection probability from observation data is to use site occupancy models. Site occupancy models constitute a class of species distribution models designed to estimate species presence using detection and non-detection data, while explicitly accounting for imperfect detection. This is achieved by simultaneously modeling the ecological process (i.e., actual occupancy) and the observation process (i.e., probability of detection of at least one individual), similarly to N-mixture models (Guillera-Arroita et al., 2010; Kéry et al., 2013; MacKenzie et al., 2002). By doing so, these models can provide insights into how external factors affect changes in activity rates at the subpopulation level.

Specifically, in the ecological part of the models, the true state z (i.e., present or absent) of the species of a site i is defined as:

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$$z_i \sim \text{Bernoulli}(\psi_i)$$

where ψ_i is the occupancy probability in site i. Concerning the observational model, observations in site i at survey j at time t were defined as:

$$y_{ij} \mid z_i \sim \text{Bernoulli}\left(z_i \times p_{ij}\right)$$

where p_{ij} is the detection probability in site i at survey j. To test candidate drivers of detection probability, and thus implicitly of activity rate, p_{ij} can be modeled as:

$$\text{logit}\left(p_{ij}\right) \sim \alpha_p + \beta_{p[1]} \times x_{1[ij]} + \beta_{p[2]} \times x_{2[ij]} \ldots + \beta_{p[n]} \times x_{n[ij]}$$

where α is the intercept and x_1, $x_2 \ldots x_n$ are variables potentially affecting activity rate.

Case 3: Abundance categories

Count data may be difficult to obtain in cave systems, as conditions may be suboptimal and multiple counting of the same individual might occur. Nonetheless, abundance data may provide more information compared to simple observation data (Falaschi et al., 2025). For these reasons, it might be useful to categorize observations in "abundance categories" when count data are difficult to obtain. When abundance categories are available, we propose to analyze changes in activity rates using multistate occupancy models, with three or more possible states for the species. For instance, given an abundance threshold k, observations can be categorized as: A (absent; 0 individuals), P (present; $<k$ individuals), M (abundant; $\geq k$ individuals). Modeling detection probabilities of the different states allows us to retrieve information on the effects of potential zeitgebers on the different abundance classes. In this case, it is possible to infer more insights on biological rhythms than using simple occupancy models: For species easily detectable, it might be relevant to discriminate between detecting a few individuals, which might be independent from any zeitgeber, and detecting many individuals, which may be a stronger indication for an underlying biological mechanism.

In the ecological part of the models, the true state of the species at site i can be defined as z:

$$z_i \sim \text{Categorical}(\Omega_i)$$

where Ω_i is the state vector, describing the probability of each state for site i. Ω_i is defined based on using the two parameters—ψ, occurrence probability; and m, the conditional probability to have $>k$ individuals in an occupied site:

$$\Omega_i = \left[1-\psi \;\; \psi(1-m) \;\; \psi m\right]_i$$

with ψ and m following a Bernoulli distribution. In the observation model, the observed state follows a categorical distribution conditional on the true state y of the site. The observed state of a site i in survey j is defined as:

$$y_{ij} \mid z_i \sim \text{Categorical}\left(\Theta_{zoij}\right)$$

where the indices of the four-dimensional observation array (Θ) are the true state z, the observed state o, site i, and replicate j. Hence, the observation array Θ shows the true state in the row and the observed state in the columns:

$$\Theta_{zoij} = \begin{bmatrix} 1 & 0 & 0 \\ 1-p_P & p_P & 0 \\ 1-p_{MP}-p_{MM} & p_{MP} & p_{MM} \end{bmatrix}_{ij}$$

To test candidate drivers of detection probability, we should separately model detection probability of state P when the true state is P; detection probability of state P when the true state is M, and detection probability of M as:

$$\text{logit}\left(p_{Pij}\right) \sim \alpha_{Pp} + \beta_{Pp[1]} \times x_{1[ij]} + \beta_{Pp[2]} \times x_{2[ij]} \ldots + \beta_{Pp[n]} \times x_{n[ij]}$$

$$\text{logit}\left(p_{MPij}\right) \sim \alpha_{MPp} + \beta_{MPp[1]} \times x_{1[ij]} + \beta_{MPp[2]} \times x_{2[ij]} \ldots + \beta_{MPp[n]} \times x_{n[ij]}$$

$$\text{logit}\left(p_{MMij}\right) \sim \alpha_{MMp} + \beta_{MMp[1]} \times x_{1[ij]} + \beta_{MMp[2]} \times x_{2[ij]} \ldots + \beta_{MMp[n]} \times x_{n[ij]}$$

where α is the intercept and x_1, $x_2 \ldots x_n$ are variables potentially affecting the activity rate.

Case study 1: *P. anguinus*

The results showed no circadian rhythmicity in cave populations of *P. anguinus* (mean = 0.271; 95% CrI = −0.106 to 0.641). This finding is in agreement with experimental studies that found no evidence of daily variation of activity or resting metabolic rate (Hervant et al., 2000, 2001; Schatz et al., 1997). However, detection probability

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exhibited a strong seasonal pattern, with higher detection during summer and winter (Figure 2), which, in the study area, are the seasons with the lowest precipitation amount. Previous assessments found seasonal peaks in activity levels during September, November, and February in the absence of light and temperature fluctuations (Schatz et al., 1997), representing a potential validation of the validity of our approach and of the robustness of our findings.

Case study 2: *Niphargus* sp.

We did not detect any relevant effect of any tested cycle on the detection probability of *Niphargus* sp. (Figure 3). The difference between day and night, in particular, had a weak effect with a large credible interval (mean = −0.062; 95% credible interval [CrI] = −0.663 to 0.566). This indicates that in completely dark caves, the species does not exhibit rhythmic behavior in relation to time of the day nor seasons.

Case study 3: *S. bottazzii*

The results confirmed an effect of daylight and seasons on detection probability, thus on activity rates of *S. bottazzii* (Figure 4). Specifically, we observed a lower level of activity during the daytime for small populations (mean = −4.434, 95% CrI = −8.427 to −1.804). Moreover, there was a higher level of activity during spring for large populations (probability of observing ≤20 individuals: mean = 2.445, 95% CrI = 0.190–5.282; probability of observing >20 individuals: mean = 2.790, 95% CrI = 0.045–5.897). Moreover, dip-netting was less efficient than visual surveys in large populations (probability of observing ≤20 individuals: mean = −1.703, 95% CrI = −3.782 to −0.086; probability of observing >20 individuals: mean = −4.555, 95% CrI = −8.330 to −1.425).

Large populations were more commonly found in deeper sections of the caves than smaller ones. Thus, the daily variation in activity rates observed in small populations may be linked to their exposure to zeitgebers, given their proximity to the photic zone. It has been suggested that females of *S. bottazzii* migrate toward the photic zone between reproductive events to exploit the greater availability of food sources and then return to deeper sections of the underground environment during the incubation phase (Ariani & Wittmann, 1997). It is possible that the "small populations" observed near the cave entrances were females in reproductive stasis. However, recent evidence indicates no sexual dimorphism

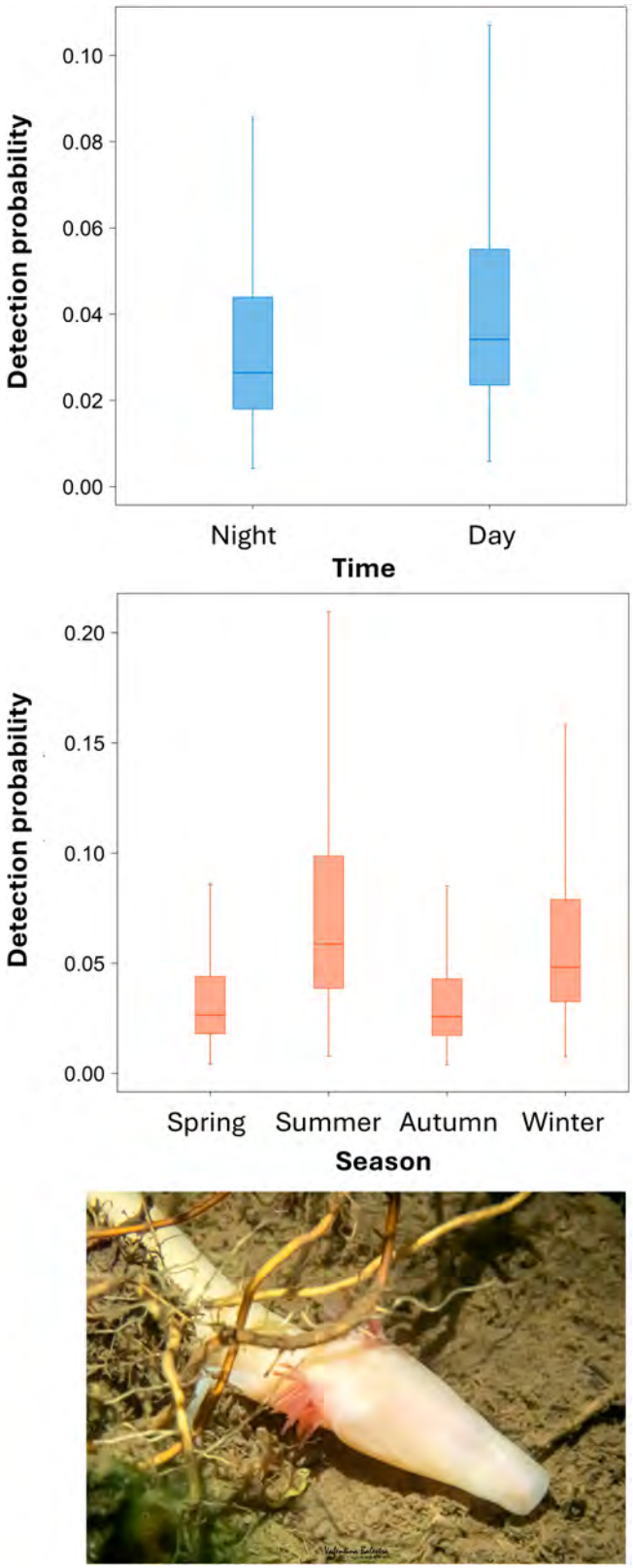

FIGURE 2 Daily and seasonal detection probability variation in *Proteus anguinus*. The midline represents the posterior median, boxes represent the interquartile range (25th–75th percentiles), and whiskers represent the 95% equal-tailed credible interval (2.5th–97.5th percentiles). Photo credit: Valentina Balestra.

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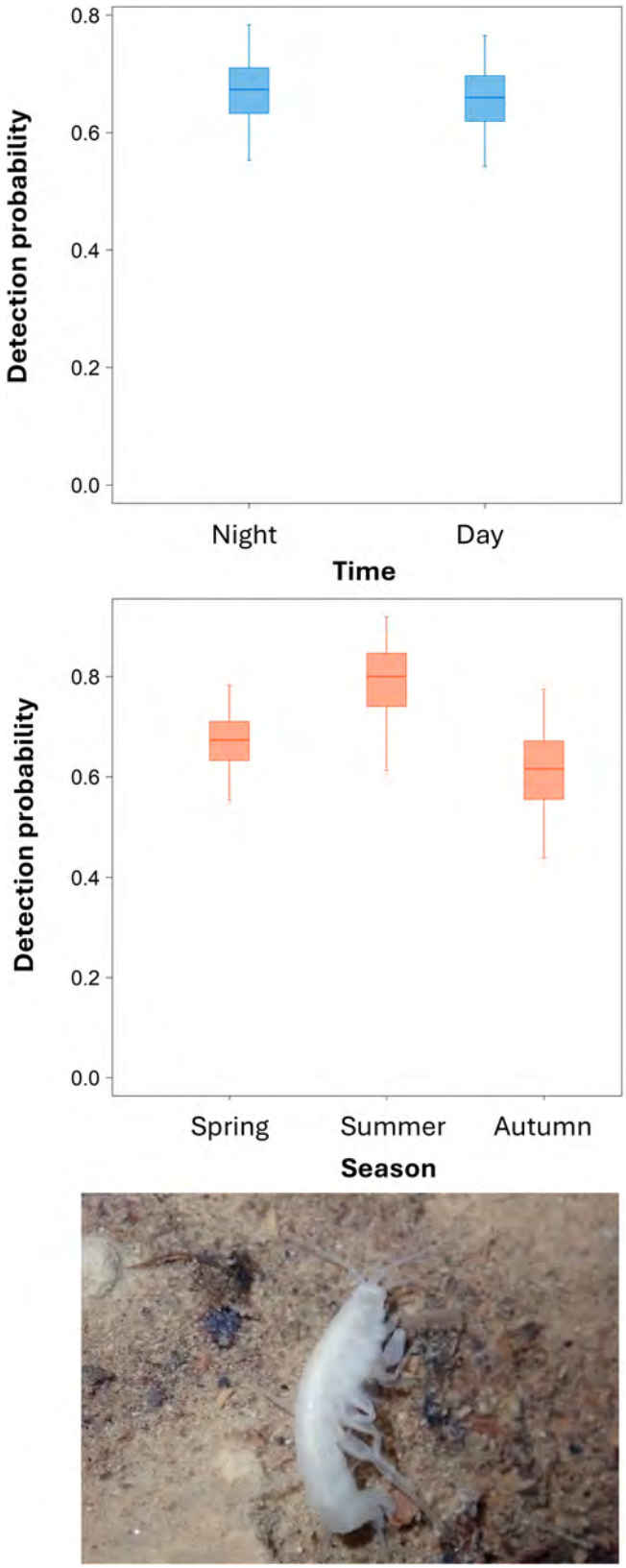

FIGURE 3 Daily and seasonal detection probability variation in *Niphargus* sp. The midline represents the posterior median, boxes represent the interquartile range (25th–75th percentiles), and whiskers represent the 95% equal-tailed credible interval (2.5th–97.5th percentiles). Photo credit: Benedetta Barzaghi.

in energy use in *S. bottazzii*, which in turn suggests an absence of behavioral dimorphism as well (Boulamail, Fumarola, et al., 2025).

Regarding seasonal patterns, our results may be linked to piezometric variation. Rainfall is concentrated in autumn and winter in the study area; however, it is difficult to assess the direct effect of precipitation on groundwater level and resource availability in subterranean environments, as rainfall in the study area is concentrated in autumn and winter, and previous studies have reported aquifer response times of over 100 days for local aquifers (Balacco et al., 2022). The input of food sources from the surface environment via precipitation may therefore contribute to the increase in activity rates observed during spring.

DISCUSSION

This work provides a flexible and noninvasive approach to assess circadian and circannual patterns in animals by using detection probability as a proxy for activity levels. By adapting occupancy and abundance models to different types of observational data, our method overcomes several limitations that have long challenged chronobiological research in arhythmic environments. This approach proves especially valuable considering the ethical, logistical, and methodological constraints associated with both laboratory and field studies in cave environments. The flexibility of the framework makes it applicable across taxa, environmental settings, and data resolutions, helping to mitigate the effects of sampling heterogeneity and improving the comparability and repeatability of chronobiological research. This approach can be well-suited for estimating rhythmicity in arhythmic ecosystems because:

1. It is based on noninvasive data collection. Variations of detection probability are estimated from repeated surveys (Ficetola, Romano, et al., 2018; Guillera-Arroita et al., 2010). There are many different sampling strategies that can be used to collect observation and/or abundance data, such as visual surveys, dip-netting, pitfall traps, and baiting (Hunt & Millar, 2001; Wynne et al., 2019). Occupancy models and N-mixture models can be adapted to use data coming from most sampling strategies, particularly the less invasive ones. Given the sensitivity of arhythmic ecosystems, such as caves, to disturbance, being able to get robust estimates of circadian and circannual variations in activity levels from field data represents an advantage compared to laboratory experiments, which require animal collection and often the approval by an ethical committee (e.g., Manenti

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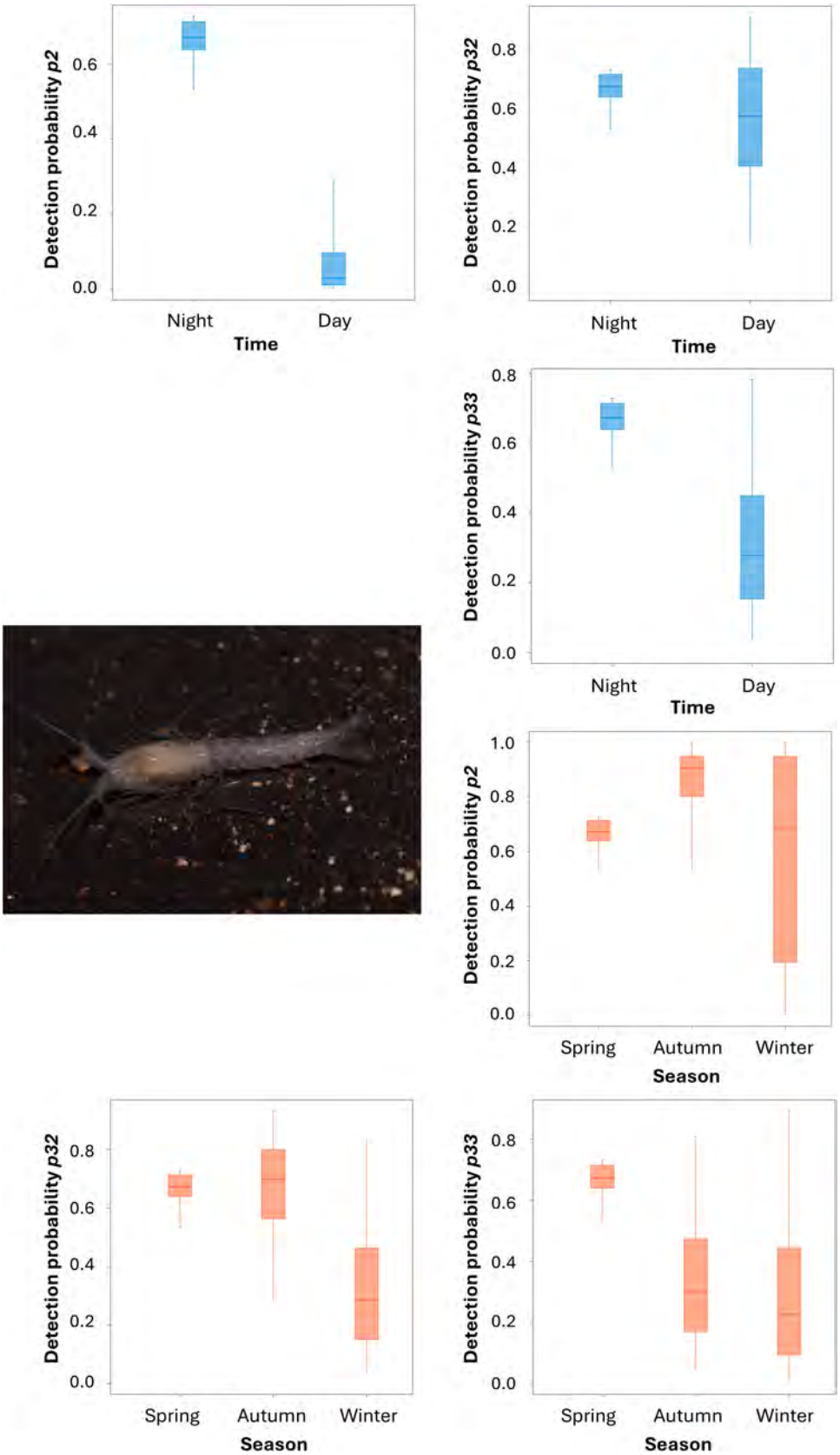

FIGURE 4 Daily and seasonal detection probability variation in *Spelaeomysis bottazzii*. *p2*, probability of observing <20 individuals in small populations; *p32*, probability of observing <20 individuals in large populations; *p33*, probability of observing ≥20 individuals in large populations. The midline represents the posterior median, boxes represent the interquartile range (25th–75th percentiles), and whiskers represent the 95% equal-tailed credible interval (2.5th–97.5th percentiles). Photo credit: Benedetta Barzaghi.

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et al., 2023). Moreover, the required sampling effort is relatively inexpensive, as it requires just visual assessment surveys and information about date and time. However, it is important to ensure a constant protocol throughout surveys, as well as ensuring that all observers have been trained in the same way, as this can directly impact on imperfect detection, thus complicating the biological interpretation of the models.

2. It is extremely flexible. The possibility of using different monitoring strategies means that this framework can be easily adapted to different species, to the specific characteristics of the surveyed environments, and to the type of data available (qualitative, quantitative, or semiquantitative). Habitat features can be integrated in this framework to account for a variety of cases. Sampling in caves and other arhythmic environments can be extremely challenging, as they require a big sampling effort and advanced techniques (Christman et al., 2016; Mammola et al., 2021). Thus, the possibility of using different types of data, tailored to the specific situation of the sampling, represents an important characteristic for any modeling approach.
3. It is easily reproducible. A common malpractice in biospeleological research is the lack of repeatability (Wynne et al., 2019). This framework can be easily reproducible, as long as key information is reported. Specifically, information that should be available to the readers are: (1) number and location of the surveyed sites; (2) number of surveys in each site; (3) the survey method; (4) the script used to model detection probability, or at least a detailed description of the model.

Our findings confirm that even in the apparent constancy of subterranean ecosystems, detectable temporal variation in species activity can occur. Such variation could be linked to endogenous rhythms entrained by subtle or indirect environmental cues, or to behavioral plasticity and partial exploitation of surface habitats. In this context, our framework may also support future investigations into the evolutionary maintenance or loss of biological clocks in isolated cave lineages. Given the current biases in chronobiological research, we argue that future efforts should prioritize expanding the taxonomic breadth of studied species, especially freshwater and non-model taxa, and increasing field-based data collection in poorly studied regions. Integrating our modeling framework into broader monitoring programs could also contribute to long-term conservation strategies by identifying periods of maximum activity or vulnerability of species living in these extremely sensitive environments.

AUTHOR CONTRIBUTIONS

Conceptualization: Elia Lo Parrino, Stefano Lapadula, and Raoul Manenti. *Data curation*: Elia Lo Parrino, Stefano Lapadula, Damiano Brognoli, Giorgia Terraneo, Benedetta Barzaghi, Valeria Messina, and Raoul Manenti. *Formal analysis*: Elia Lo Parrino, Stefano Lapadula, and Mattia Falaschi. *Funding acquisition*: Francesco Cozzoli and Raoul Manenti. *Investigation*: Elia Lo Parrino, Stefano Lapadula, Damiano Brognoli, Giorgia Terraneo, Benedetta Barzaghi, Sarah Boulamail, Valentina Balestra, Matteo Galbiati, Jacopo Luciano Gandini, Giorgio Grassi, Mattia Falaschi, Gentile Francesco Ficetola, Salvatore Inguscio, Valeria Messina, Edgardo Mauri, Francesco Passoni, Veronica Zampieri, Francesco Cozzoli, and Raoul Manenti. *Methodology*: Elia Lo Parrino, Stefano Lapadula, Mattia Falaschi, and Gentile Francesco Ficetola. *Supervision*: Mattia Falaschi, Gentile Francesco Ficetola, Francesco Cozzoli, and Raoul Manenti. *Validation*: Mattia Falaschi, Gentile Francesco Ficetola, Francesco Cozzoli, and Raoul Manenti. *Visualization*: Elia Lo Parrino, Stefano Lapadula, and Mattia Falaschi. *Writing—original draft*: Elia Lo Parrino and Stefano Lapadula. *Writing—review and editing*: Elia Lo Parrino, Stefano Lapadula, Damiano Brognoli, Giorgia Terraneo, Benedetta Barzaghi, Sarah Boulamail, Valentina Balestra, Matteo Galbiati, Jacopo Luciano Gandini, Giorgio Grassi, Mattia Falaschi, Gentile Francesco Ficetola, Salvatore Inguscio, Valeria Messina, Edgardo Mauri, Francesco Passoni, Veronica Zampieri, Francesco Cozzoli, and Raoul Manenti.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and code (Lo Parrino et al., 2026) are available from Figshare: https://doi.org/10.6084/m9.figshare.30217528.

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ETHICS STATEMENT

The authors declare that no animal was mistreated for this work and no environment was damaged.

ORCID

Elia Lo Parrino https://orcid.org/0000-0001-9619-9568
Stefano Lapadula https://orcid.org/0000-0001-8578-960X
Giorgia Terraneo https://orcid.org/0009-0004-2548-0546
Benedetta Barzaghi https://orcid.org/0000-0003-0414-3693
Sarah Boulamail https://orcid.org/0000-0001-7105-6704
Valentina Balestra https://orcid.org/0000-0002-8529-468X
Matteo Galbiati https://orcid.org/0000-0003-1345-2893
Mattia Falaschi https://orcid.org/0000-0002-4511-4816
Gentile Francesco Ficetola https://orcid.org/0000-0003-3414-5155
Valeria Messina https://orcid.org/0009-0004-8545-6118
Veronica Zampieri https://orcid.org/0009-0000-8362-2558
Francesco Cozzoli https://orcid.org/0000-0002-6413-0977
Raoul Manenti https://orcid.org/0000-0001-6071-8194

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