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From laboratory validation to field ecology: biologging and
dead-reckoning reveal amphibians' movement

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Riassunto

Gli anfibi rappresentano uno dei gruppi di vertebrati più minacciati al mondo, e la comprensione della loro ecologia del movimento su scala fine è ancora limitata a causa di vincoli logistici e tecnici. Questa tesi integra una revisione globale, esperimenti comportamentali controllati e applicazioni di tecnologie di biologging miniaturizzate per migliorare l'affidabilità dei metodi di tracciamento negli anuri e valutare la rilevanza ecologica dei dati ottenuti.

Il Capitolo I fornisce la prima revisione completa (1970-2023) della telemetria elettronica in anfibi e rettili, mettendo in evidenza forti bias geografici, un uso predominante dei radiotrasmettitori, la quasi assenza di test pre-rilascio e una reportistica incoerente sugli effetti dei dispositivi. La revisione evidenzia lacune importanti nei metodi di fissaggio, nella registrazione di mortalità e nella perdita di dispositivi, proponendo la necessità di protocolli etici standardizzati e ulteriori progressi nella miniaturizzazione.

Il Capitolo II presenta una validazione sperimentale dell'uso di accelerometri in *Aquarana catesbeiana*. Solo un'imbracatura su misura si è rivelata efficace, evidenziando la necessità di metodi di fissaggio specifici per specie. Il carico del dispositivo ha avuto effetti limitati sulla locomozione: il salto è rimasto invariato, mentre il nuoto ha richiesto maggiore energia, soprattutto negli individui più leggeri. La classificazione comportamentale basata sull'accelerometria è risultata altamente accurata, confermando la validità dell'approccio anche sul campo.

Il Capitolo III valuta sperimentalmente gli effetti di dispositivi esterni su *Bombina variegata*, *Bufo viridis* e *Bufo bufo* in condizioni acquatiche e terrestri. I test con e senza *device* mostrano che locomozione e uso dello spazio risultano ampiamente conservati, anche con carichi superiori alla soglia del 10%, con variazioni principalmente

individuali e non specie-specifiche. Le analisi basate su PCA, *heatmap* e modelli misti lineari hanno rivelato solo effetti sottili a breve termine. Parallelamente, la classificazione comportamentale basata su accelerometro ha raggiunto una precisione del >96%, dimostrando la robustezza del *biologging* multi-sensore per dedurre il comportamento locomotore. Nel complesso, il capitolo conferma la fattibilità del *biologging* negli anuri e fornisce un flusso di lavoro validato per analisi comportamentali e spaziali, indicando che i vincoli tecnici tradizionali sono stati spesso sopravvalutati.

Il Capitolo IV testa la fattibilità di integrare la radiotelemetria VHF con dispositivi dotati di accelerometri e magnetometri per ricostruire percorsi di movimento su scala fine in *Bufo viridis*. Il *dead-reckoning* (DR) forniva traiettorie continue sostanzialmente più lunghe e dettagliate rispetto a percorsi basati su GPS, rivelando chiari schemi di attività notturna e una finestra di attività termica ristretta. Nonostante alcuni eventi di mortalità legati ai dispositivi, lo studio dimostra che la DR può catturare con successo comportamenti spaziali ad alta risoluzione in piccoli anfibi criptici, offrendo uno strumento potente per comprendere l'uso degli habitat e l'ecologia del movimento sul campo.

Il Capitolo V estende l'approccio di *dead-reckoning* sviluppato nel Capitolo IV per analizzare la variazione stagionale del movimento e dell'attività in *Bufo bufo*. Integrando i punti GPS ricavati tramite VHF con le ricostruzioni DR basate su accelerometri e magnetometri, lo studio ha rivelato traiettorie molto più lunghe e dettagliate rispetto ai soli fix. Sono emersi pattern stagionali netti: in estate l'attività era limitata alle ore notturne e concentrata in piccoli micro-habitat umidi, mentre in inverno i movimenti avvenivano a temperature più basse e su aree più ampie, nonostante una minore frequenza locomotoria complessiva. Nel complesso, i risultati evidenziano come il DR permetta di

individuare ritmi comportamentali fini e fattori ambientali che guidano il movimento, sottolineando l'importanza di considerare la componente stagionale nell'interpretazione dell'ecologia spaziale degli anfibi.

Il Capitolo VI estende ulteriormente l'approccio di *dead-reckoning* testandone la fattibilità in *Bombina variegata pachypus*, uno degli anuri più piccoli e minacciati mai studiati con questa tecnica. Nonostante alcune limitazioni tecniche, il DR ha permesso di ricostruire traiettorie continue e ad alta risoluzione, rivelando distanze di movimento e modalità di utilizzo dello spazio molto maggiori rispetto a quanto suggerito dalle sole osservazioni sul campo. Gli individui hanno mostrato schemi di attività termica e temporale altamente variabili e influenzati dai microhabitat occupati. Nel complesso, il capitolo dimostra che il DR può essere applicato con successo anche ad anfibi di piccole dimensioni, offrendo informazioni senza precedenti fondamentali per la conservazione di *B. v. pachypus* e di altre specie minacciate.

Nel complesso, questa tesi dimostra che il *biologging*, quando affiancato da una rigorosa validazione sperimentale, da un'attenta progettazione dei dispositivi e da approcci analitici avanzati come il *dead-reckoning*, può offrire una comprensione senza precedenti del movimento e del comportamento degli anfibi. Collegando esperimenti controllati in laboratorio ad applicazioni in campo su più specie, il lavoro definisce un quadro pratico ed etico per ampliare l'uso del *biologging* nei vertebrati di piccola taglia. Oltre all'innovazione metodologica, i risultati mostrano come i dati di movimento a scala fine possano contribuire direttamente alla pianificazione della conservazione, migliorando la capacità di individuare habitat critici, finestre temporali di attività e requisiti ecologici in specie esposte a rapidi cambiamenti ambientali.

Summary

Amphibians are among the most threatened vertebrate groups worldwide, yet our understanding of their fine-scale movement ecology remains limited by logistical and technical constraints. This thesis integrates a global review, controlled behavioural experiments, and applications of miniaturised biologging technologies to improve the reliability of tracking methods in anurans and to assess the ecological relevance of the movement data they generate.

Chapter I provides the first comprehensive review (1970-2023) of electronic telemetry applied to amphibians and reptiles. The review reveals pronounced geographical biases, a predominant reliance on radio transmitters, a near absence of pre-release testing, and inconsistent reporting of device effects. Major gaps are identified in attachment methodologies, mortality reporting, and device loss, emphasising the need for standardised ethical protocols and further technological miniaturisation.

Chapter II presents an experimental validation of accelerometer use in *Aquarana catesbeiana*. Among the attachment methods tested, only a custom-made harness proved reliable, underscoring the necessity of species-specific attachment strategies. Device load had limited and context-dependent effects on locomotion: jumping performance was unaffected, while swimming required greater energetic effort, particularly in lighter individuals. Accelerometer-based behavioural classification achieved high accuracy, confirming the suitability of this method for field applications in large-bodied amphibians.

Chapter III experimentally evaluates the effects of external devices on *Bombina variegata*, *Bufotes viridis*, and *Bufo bufo* under both aquatic and terrestrial conditions. Comparisons between trials with and without devices showed that locomotor

performance and space use were largely preserved, even when tag mass exceeded the conventional 10% threshold. Observed variation was primarily individual rather than species-specific. PCA, heatmaps, and mixed models revealed only subtle short-term effects, while accelerometer-based behavioural classification exceeded 96% accuracy. Together, these results demonstrate the feasibility of biologging in anurans and provide a validated workflow for behavioural and spatial analyses, indicating that traditional technical constraints have often been overstated.

Chapter IV tests the feasibility of integrating VHF radiotelemetry with accelerometer- and magnetometer-equipped devices to reconstruct fine-scale movement paths in *Bufo viridis*. Dead-reckoning (DR) produced continuous trajectories that were substantially longer and more detailed than those inferred from GPS fixes alone, revealing clear nocturnal activity patterns and a narrow thermal activity window. Despite some device-related mortality events, this chapter demonstrates that DR can successfully capture high-resolution spatial behaviour in small, cryptic amphibians, offering a powerful tool for field-based movement ecology.

Chapter V extends the DR framework to examine seasonal variation in movement and activity in *Bufo bufo*. By integrating VHF-located GPS points with DR reconstructions, the study revealed trajectories far longer and more complex than suggested from positional data alone. Clear seasonal differences emerged: summer activity was confined to nocturnal periods and small, humid microhabitats, whereas winter movements occurred at lower temperatures and across wider areas despite reduced locomotor frequency. These results demonstrate the value of DR for identifying fine-scale behavioural rhythms and environmental drivers of movement, highlighting the importance of accounting for seasonal dynamics when interpreting amphibian spatial ecology.

Chapter VI further extends the DR framework by testing its feasibility in *Bombina variegata pachypus*, one of the smallest and most threatened anurans studied with this technique. Despite technical limitations, DR successfully reconstructed continuous fine-scale trajectories, revealing movement distances and spatial use far greater than indicated by traditional field observations. Individuals showed highly variable space-use patterns and idiosyncratic thermal and temporal activity, strongly influenced by microhabitat features. These results confirm that DR can be effectively applied even to very small sedentary amphibians, providing unprecedented insights for the conservation of *B. v. pachypus* and similarly threatened species.

Overall, this thesis demonstrates that biologging, when paired with rigorous experimental validation, careful device design, and advanced analytical approaches such as dead-reckoning, can advance an unprecedented understanding of amphibian movement and behaviour. By bridging controlled laboratory tests with field applications across multiple species, this work establishes a practical and ethical framework for expanding biologging in small-bodied vertebrates. Beyond methodological innovation, the findings show how fine-scale movement data can directly inform conservation planning by identifying critical habitats, temporal activity windows, and ecological requirements in species facing rapid environmental change.

General Introduction

Movement ecology, the study of how organisms move, use space, and interact with their environment across spatial and temporal scales, provides a fundamental framework for understanding animal behaviour, population dynamics, and species persistence (Jeltsch et al., 2013; Nathan et al., 2008). By linking movement patterns to ecological processes such as resource selection, dispersal, reproduction, and survival, movement ecology allows researchers to quantify how individuals exploit heterogeneous landscapes and respond to environmental change. This discipline has become central to modern conservation biology, as spatial data are increasingly used to identify critical habitats, delineate home ranges, and inform the design of protected areas and ecological corridors (Hromada et al., 2020).

To investigate these spatial and behavioural processes, a wide range of tracking and biologging technologies has been developed, each tailored to different spatial scales, taxa, and research objectives:

- Very High Frequency (VHF) radio transmitters are among the earliest and most widespread tools, emitting low-power signals that can be manually tracked using directional antennas and receivers (Rueda-Urbe et al., 2024). Because their use requires the presence of a field operator to obtain each position fix, they typically provide a limited number of spatial locations per individual. Nevertheless, VHF telemetry remains invaluable for both small- and large-bodied terrestrial species thanks to the light weight of the transmitters, their long battery life, and relatively low costs.
- Harmonic Direction Finder (HDF) systems represent a more recent alternative. These passive transponders, which lack an internal power source, reflect an

external signal emitted by a handheld antenna, enabling short-range localisation (typically within a few metres). Their negligible mass and absence of batteries make them particularly suitable for fine-scale studies on small-bodied animals with limited home ranges (Leskovar & Sinsch, 2005). As with VHF telemetry, the presence of an operator is required to obtain each position fix, which inherently limits the number of spatial locations that can be recorded for each individual.

- Global Positioning System (GPS) loggers provide highly accurate geolocations via satellite triangulation, making them ideal for studying home range size, habitat selection, and long-distance movements (Smith et al., 2018). One of their major advantages is the ability to collect a large number of location fixes automatically, depending on the device settings, without requiring the continuous presence of a field operator. However, these benefits come at a relatively high cost, and the use of GPS technology remains mostly restricted to larger species or open environments due to constraints related to device size, energy consumption, and the need for unobstructed satellite visibility. In recent years, technological miniaturisation has significantly advanced, leading to the development of lightweight GPS tags that can even be deployed on small species (Wild et al., 2022). This progress suggests that the future application of GPS technology may extend to increasingly smaller taxa. Nevertheless, environmental factors such as dense vegetation or water bodies still represent major limitations, as they can prevent signal transmission to satellites (Forrest et al., 2022; Ironside et al., 2017).

In addition to positional devices, inertial sensors such as tri-axial accelerometers and magnetometers have revolutionised the study of animal movement and behaviour. Accelerometers measure the specific force acting on the sensor along three orthogonal

axes (X, Y, Z), typically expressed in units of gravitational acceleration (g; 1 g = 9.81 m/s²; Brown et al., 2013). In behavioural studies, tri-axial accelerometers are commonly used, with each axis describing a distinct type of body movement: surge (antero-posterior movements), heave (dorso-ventral movements), and sway (lateral movements). These correspond to the pitch, roll, and yaw known in aircraft dynamics (Whitford & Klimley, 2019). The static component of acceleration reflects the orientation of the body relative to the Earth's gravitational field, whereas the dynamic component arises from body motion. Static acceleration is relatively easy to interpret. For instance, an accelerometer mounted on the back of a standing quadruped will typically record 0 g on the surge axis, +1 g on the heave axis (gravity acting downward), and 0 g on the sway axis. If the animal lies on its side, the sway axis would instead register ± 1 g depending on which side it rests, while the other two axes would approach 0 g (Figure 1; Whitford & Klimley, 2019).

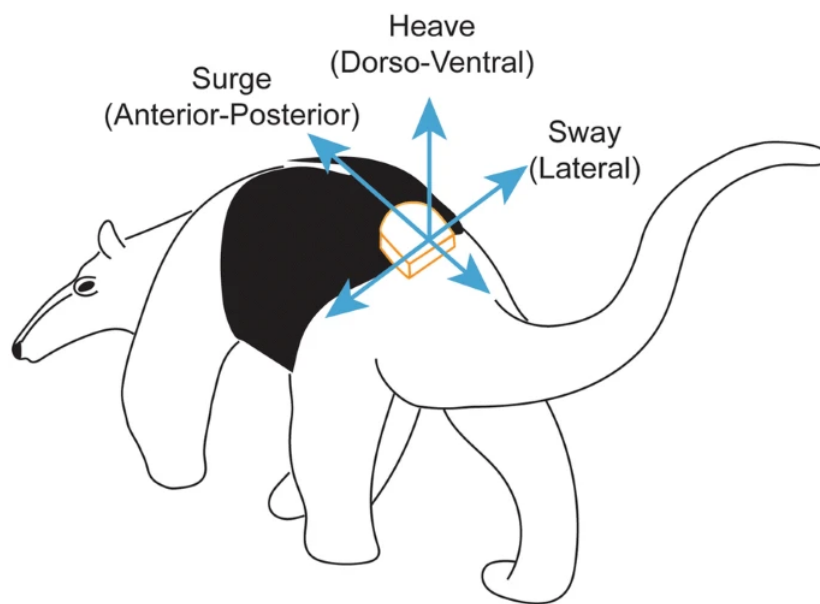


Figure 1. Schematic representation of accelerometer placement on a quadrupedal vertebrate (anteater example), showing the orientation of the three orthogonal axes used to record surge (antero-posterior), heave (dorso-ventral), and sway (lateral) movements (Whitford & Klimley, 2019).

By filtering out the static gravitational component, the remaining dynamic acceleration reflects changes in movement and locomotor intensity. From these values, researchers can derive the Overall Dynamic Body Acceleration (ODBA), the sum of the absolute

dynamic accelerations across the three axes, or its vectorial equivalent (VeDBA; Qasem et al., 2012). These metrics provide robust proxies for energy expenditure, activity levels, and behavioural states. Tri-axial accelerometers thus offer a precise and fine-scale quantification of body orientation and movement, enabling the reconstruction of detailed behavioural profiles and the estimation of energetic costs in free-ranging animals.

However, accelerometers alone have several limitations when used to infer animal behaviour. Most notably, they cannot determine the animal's heading, making it difficult to reconstruct the direction of movement (Williams et al., 2017). Moreover, acceleration signals can be influenced by external forces not generated by the animal itself, such as wave motion affecting seabirds resting on the surface or air-flow vibrations in flying species, thereby increasing the signal-to-noise ratio (Williams et al., 2015, 2017). Accelerometers also perform poorly when the animal moves at a constant velocity, as during gliding or slow, steady movements, where little or no dynamic acceleration is produced (Williams et al., 2017). Finally, although postural information can often be derived from the static component of acceleration, this approach fails during rapid turns or strong manoeuvres (e.g., banking or diving), when additional forces act on the body and distort gravitational reference (Williams et al., 2015, 2017).

To overcome this limitation, tri-axial magnetometers are commonly integrated within inertial measurement units (IMUs; Martín López et al., 2016; Noda et al., 2014). Magnetometers record the intensity and direction of the Earth's magnetic field along three orthogonal axes, typically expressed in microteslas (μT). These measurements provide a three-dimensional magnetic vector that can be used to infer the animal's heading relative to magnetic north. In an ideal, undisturbed magnetic field, the three components of the magnetometer (M_x , M_y , M_z) describe a sphere of constant radius corresponding to the

local magnetic field strength. Once calibrated, the magnetometer output can be combined with accelerometer data to compute pitch, roll, and yaw angles, thus resolving both the animal's body orientation and its directional heading in space (Williams et al., 2017).

Thus, when combined, accelerometers and magnetometers provide a comprehensive description of body dynamics and heading, forming the core of modern inertial navigation and dead-reckoning systems in animal biotelemetry. This integration enables the reconstruction of detailed movement paths even in environments where GPS signals are intermittent or unavailable, such as under dense vegetation or underwater.

While the development and miniaturisation of these biologging tools have revolutionised movement ecology across many taxa, most of our current understanding still comes from studies on large and highly mobile species, such as birds, mammals, and marine organisms, whose body size accommodates satellite-based telemetry and long-term monitoring devices (Joo et al., 2022). In contrast, amphibians, despite their key ecological roles and alarming global decline (Kiesecker, 2011; Stuart et al., 2004), remain largely understudied in this context. Their small body size, cryptic behaviour, and association with complex terrestrial and aquatic habitats pose logistical challenges that have long hindered the application of high-resolution spatial tracking techniques. This knowledge gap is particularly concerning given that amphibians are among the most threatened vertebrates, with more than 40% of species currently at risk of extinction according to the IUCN Red List (Luedtke et al., 2023).

Over the past decades, continued advances in biologging technologies, including miniaturised radio transmitters, GPS loggers, accelerometers, and magnetometers, have begun to provide unprecedented opportunities for fine-scale studies of increasingly smaller animals, enabling the collection of high-resolution movement and behavioural

data even in species that were previously too small for traditional tracking devices (Gould et al., 2024; Liechti et al., 2018).

However, the widespread application of biologging in small-bodied animals remains constrained by both practical and ethical considerations. Guidelines such as the commonly cited “10% rule” for the device-to-body-mass ratio are still widely referenced, yet their universal applicability across diverse morphologies and locomotor types is questionable and often not supported by rigorous pre-deployment testing (Heyer et al., 1994; see discussion in Chapter I). Moreover, the method of device attachment, whether harness, adhesive, or implantation, can strongly influence tag retention, injury risk, and mortality. These factors are frequently underreported in the literature, highlighting the need for standardised, ethically sound protocols.

From a methodological standpoint, several challenges persist. These include: (i) the ongoing miniaturisation of devices to allow deployment on increasingly smaller and more sensitive species; (ii) the development of attachment methods that ensure animal welfare while providing reliable recordings; (iii) the calibration and validation of behavioural classification algorithms to accurately distinguish between different activity states; (iv) the reconstruction of movement paths as close as possible to reality, in order to better understand the spatial and ecological requirements of species of conservation interest; and (v) the proper management and standardisation of data to facilitate meta-analyses and the sharing of datasets. The experimental chapters in this thesis address many of these issues, presenting high-accuracy behavioural classifiers, empirical tests of tagging effects, and comparative evaluations of dead-reckoning versus GPS performance.

Finally, from a conservation perspective, the outcomes of biologging studies must be translated into actionable management strategies: identifying key breeding and refuge

sites, delineating connective corridors, and optimising monitoring protocols to enhance detection probabilities during surveys. Standardised reporting, including metadata on device-to-body-mass ratio, tag loss, mortality, and monitoring duration, and the systematic inclusion of pre-release trials, should become prerequisites for future research aiming to effectively inform amphibian conservation planning.

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Chapter I - How to track herps? A review of electronic telemetry techniques for monitoring Amphibians and Reptiles and future perspectives.

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Abstract

This review brings together the literature relating to the biotelemetry of amphibians and reptiles, focusing on the use of technologies like radio transmitters, harmonic direction finders and GPSs to understand their movement ecology. The integration of these technologies has proven invaluable in revealing small-scale behaviour such as habitat selection along movement paths. We analysed critical aspects of biotelemetry studies, including the device attachment method, the transmitter type, device-to-body weight ratio, and associated rate of injury, mortality, and device loss.

This systematic analysis of studies from 1970 to 2023 indicates a continuous increase in scientific interest in herpetofauna tracking and the adoption of new methods. Geographically, North America dominates research while in the austral hemisphere, excepting Australia, tracking studies are almost neglected, underlining the need for a more equitable distribution of studies and resources globally. The increasing use of GPS transmitters suggests a transition to advanced tracking technologies, while traditional radio transmitters remain prevalent due to their accessibility and effectiveness.

The diversity in attachment methods for reptiles and amphibians reflects the anatomical and behavioural differences among groups, emphasising the need for species-specific approaches. High heterogeneity in sample sizes and duration among studies highlights the complexity of monitoring research through telemetry, which requires approaches adaptable to the specificities of the species and the different habitats.

The review underscores the importance of implementing standardised reporting practices to facilitate comparison and synthesis of results among studies, thus promoting a more comprehensive understanding of monitoring dynamics and their impact on species conservation. Addressing gaps in the reporting of injured animals and establishing standardised reporting protocols for injury and mortality is crucial to fully understanding the effectiveness of the method used and the implications of monitoring on populations. The development of new technologies and materials, as well as the degree of miniaturisation that can be achieved in the future, opens the door to new possibilities for tracking animals hitherto not contemplated in this type of study because of the complexity of attaching the devices to the body or the very small body size.

Introduction

Spatial ecology explores how species interact with and within their habitats, examining processes such as succession, adaptations, species diversity maintenance, and predator-prey dynamics (Legendre and Fortin, 1989; Semlitsch, 2008). These insights are essential for addressing applied ecological challenges, including the conservation of endangered species and the control of invasive species (Perry et al., 2002).

Despite its importance, spatial ecology research has historically focused on avian and mammalian species, leaving a critical gap in understanding the spatial dynamics of

amphibians and reptiles (herps) - a pressing concern in herpetology (Smith et al., 2018). Herps are often elusive and cryptic, making traditional long-term observational methods both labour-intensive and inefficient. To overcome these challenges, biotelemetry has become an invaluable tool for remotely tracking physiological, behavioural, and energetic parameters in free-living animals (Cooke et al., 2004).

Recent technological advances have overcome many limitations related to traditional approaches in movement monitoring, enabling data collection with unprecedented resolution and reliability, comparable to that of laboratory environments (Ropert-Coudert and Wilson, 2005; Rutz and Hays, 2009; Kays et al., 2015; Allan et al., 2018). Commonly used electronic devices include: (i) radio-transmitters, which emit radio signals detected by an operator's antenna; (ii) harmonic direction finders (HDFs), which are battery-free and reflect external signals emitted and received by an antenna; and (iii) GPS (Global Positioning System) units, which autonomously record location data via satellite connections. Each method has distinct strengths and limitations. For example, radio-transmitters and GPS units enable individual recognition through unique frequencies or internal memory, whereas HDFs are lightweight and suitable for small organisms (i.e., insects) due to their battery-free design. (O'Neal et al., 2004). GPS units provide continuous spatiotemporal data collection but are heavier and require greater power, while radio transmitters and HDFs depend on operators for real-time location data (Fancy et al., 1988).

Integrating these tracking methods can significantly enhance our understanding of spatial ecology. For instance, combining precise visual locations obtained via radio telemetry with frequent GPS recordings offers detailed insights into habitat selection, movement patterns, and time-activity budgets. The resulting dataset has the potential to unveil

detailed behavioural information of elusive animals that would be impossible to observe and follow otherwise (Thurfjell et al., 2014; Avgar et al., 2016). Such integrative approaches can also improve predictions of species movements, enabling more effective conservation, management, and removal efforts.

This review synthesises current applications of electronic tagging technologies in herpetology, critically evaluating their effectiveness for taxa with diverse sizes, morphology, skin properties, behaviours, and ecological roles. By identifying best practices and limitations, this work seeks to advance applied ecological research and inform biodiversity conservation strategies for amphibians and reptiles.

Methods

We conducted a systematic review using Google Scholar to identify studies on biotelemetry tracking in amphibians and reptiles. Searches included the first 10 pages of results for each of the following keywords: “tracking amphibian”, “tracking reptile”, “radio telemetry amphibian”, “radio telemetry reptile”, “GPS tracking amphibian”, “GPS tracking reptile”, “radio tracking reptile”, “radio tracking amphibian”, “harmonic tracking reptile” and “harmonic tracking amphibian”. Studies employing non-electronic methods, such as fluorescent powders or thread bobbins, were excluded.

To systematically extract comprehensive information, we designed a structured table to document key parameters from each selected study. 1) Paper metadata: title, authors, publication year and journal; 2) geographic information: study location (country and continent); 3) study subject details: species, taxonomic class (Amphibia or Reptilia), order (Anura, Crocodilia, Rhynchocephalia, Squamata, Testudines or Urodela), family, and IUCN Red List category (IUCN, 2024); 4) research specifics: study objective, type

of electronic transmitter (radio-transmitter, harmonic direction finder [HDF], or GPS), brand of the transmitter, unit name, and attachment method (“harness”, “surgical implants”, “glue”, “carapace-mounted”, “sewn” or “stomach-inserted”); 5) device and animal data: attachment method, transmitter weight, average animal mass, percentage transmitter-to-body weight ratio, and sample size; 6) monitoring parameters: duration of the tracking period, injury and mortality rates, causes of mortality, devices loss rate, and pre-release tests conducted under controlled conditions (e.g. behaviour, weight, or hormones tests).

The percentage transmitter-to-body weight ratio was evaluated against the guidelines stating that transmitters should weigh less than 10% of the animal's body weight (Heyer et al., 1994). As many studies only reported average weights, we used these values to ensure comparability across studies. Individual animals exceeding the 10% threshold could not have been considered, as individual weights were not consistently provided.

If a study analysed multiple species, transmitters, attachment methods, or pre-release tests, we recorded each combination as a separate entry in the table. This approach ensured comprehensive coverage of the methods used across studies. This systematic approach enabled us to extract and organise key information from diverse studies, providing a global perspective on biotelemetry practices for herps.

All analyses and data visualisation were performed using R 4.4.0 (R Core Team, 2024; see Data Availability).

Results

3.1 Trends in tracking research

Between 1970 and 2023, research on biotelemetry tracking in herps exhibited steady growth, peaking in the last decade (Figure 1). However, a significant decline occurred between 2010 and 2014, contrasting with the preceding upward trend. Despite this dip, GPS transmitter usage increased significantly compared to other transmitter types over time.

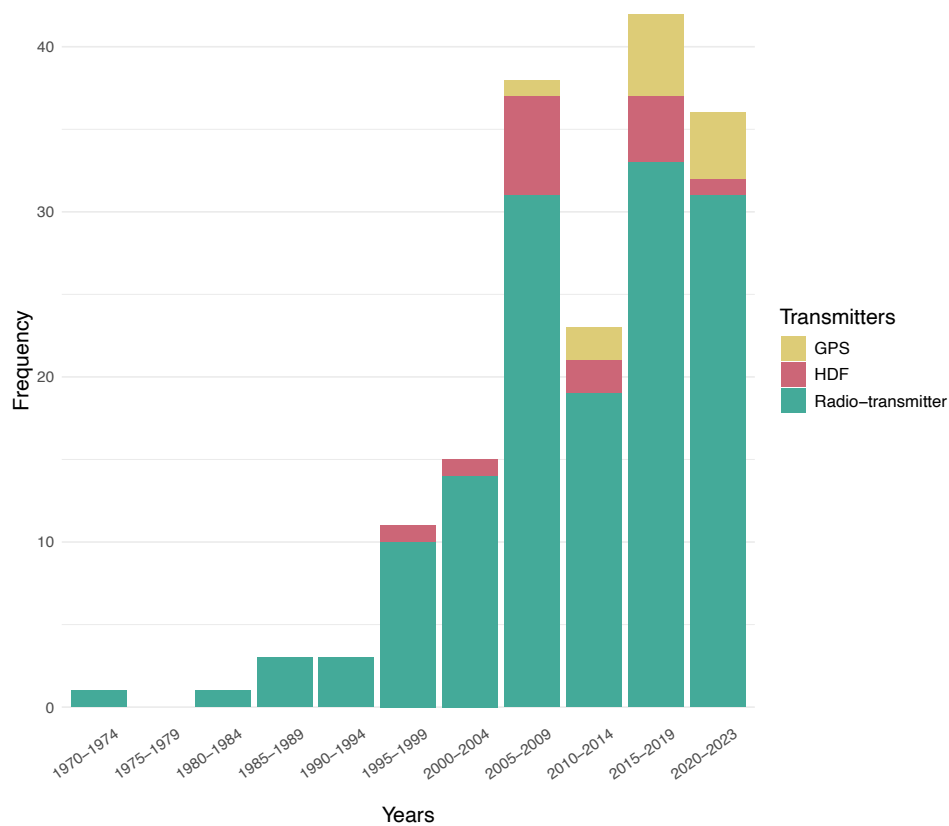


Figure 1. Trends in the number of papers on amphibian and reptile tracking over time. The histogram depicts the number of papers published on amphibian and reptile tracking from 1970 to 2023, segmented into five-year intervals. Different colors represent the types of transmitters used: GPS, HDF (Harmonic Direction Finder), and Radio-transmitters.

3.2 Geographical distribution

The geographical distribution of tracking studies reveals substantial regional variation (Figure 2). North America accounted for the highest number of studies (61 articles, 30 on reptiles, 31 on amphibians). Conversely, South America and Africa showed lower research activity, with 8 and 5 studies, respectively. South America included 4 studies each on amphibians and reptiles, while Africa comprised 2 studies on amphibians and 3 on reptiles.

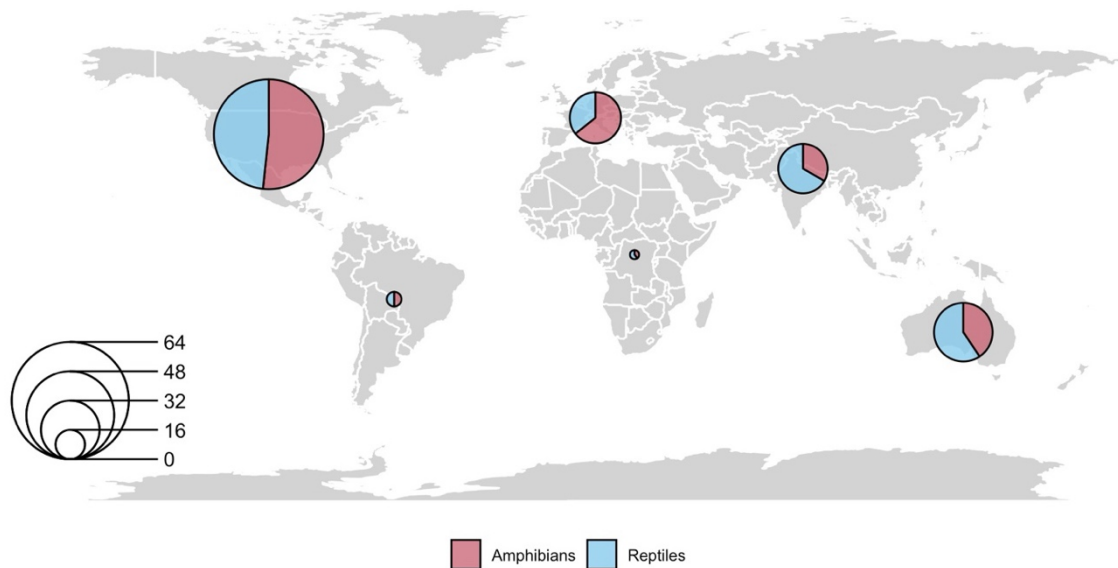


Figure 2. Geographic distribution of tracking research by continents. The world map illustrates the geographic distribution of tracking research on amphibians and reptiles. Each continent is represented by a pie chart divided into segments for amphibians and reptiles, with the size of each chart proportional to the number of articles published.

3.3 Species conservation status

Among the 160 reviewed articles, 123 species were studied, of which 26 belonged to threatened IUCN categories: 16 Vulnerable (VU), 6 Endangered (EN), and 4 Critically Endangered (CR). One was Data Deficient (DD), and 4 species had not been assessed (NA). The majority (92 species) were non-threatened, classified as Least Concern (LC, 86), Lower Risk (LR, 1), or Near Threatened (NT, 5).

3.4 Transmitter usage

Radio transmitters were the most commonly used tag type, employed in 83 studies on amphibians and 86 on reptiles (Figure 3). HDF transmitters showed a significant preference for amphibians (20 studies) over reptiles (3 studies; Chi-square: $\chi^2 = 12.565$, $df = 1$, $P < 0.001$). GPS transmitters, used exclusively for reptiles (14 studies), were primarily applied to Varanidae due to weight constraints.

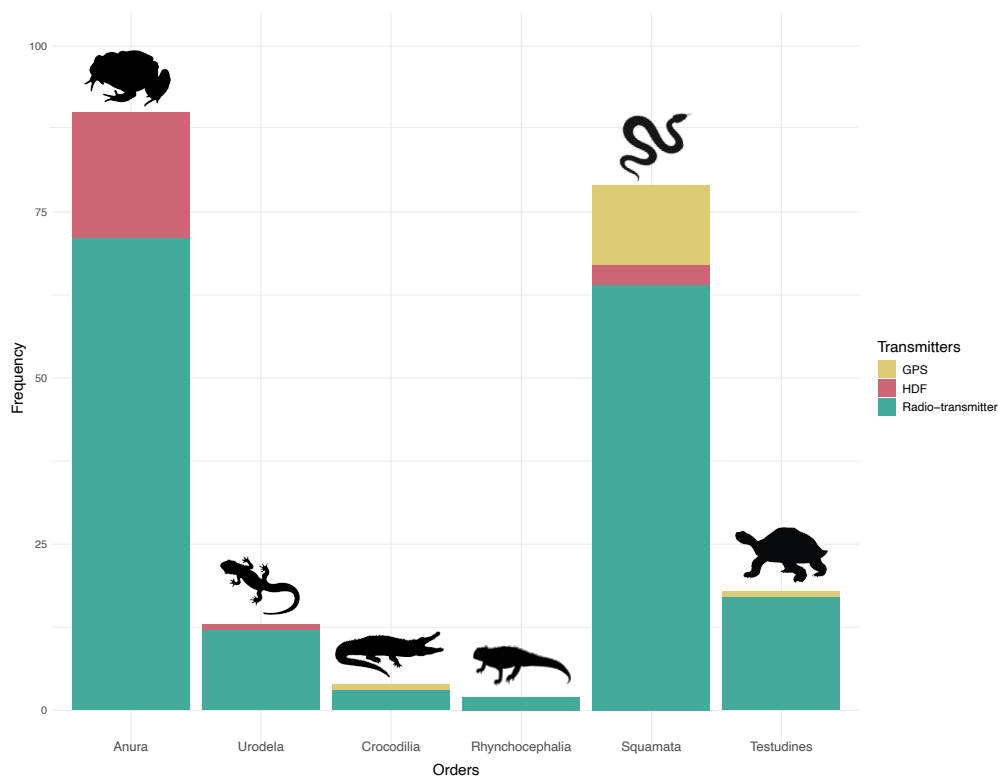


Figure 3. Distribution of tracking techniques by amphibian and reptile orders. The histogram illustrates the distribution of tracking techniques used across different orders of amphibians and reptiles. Different colours represent the types of transmitters used: GPS, HDF (Harmonic Direction Finder), and Radio-transmitters.

3.4.1 Transmitter brands and weight guidelines

One transmitter brand (Holohil Systems Ltd.) was the most frequently used, appearing in 55.6% of studies. Some studies used more than one brand. The 10% weight guideline was adhered to in 94.4% of studies, while 5.6% deviated from this standard. In 15% of studies, the transmitter-to-body weight ratio was either unreported or could not be calculated.

Notably, 33 studies failed to indicate compliance with the guideline; of these, 10 involved threatened species, and 2 featured unassessed species (1 DD, 1 NA).

3.5 Attachment methods

Attachment methods significantly varied between amphibians and reptiles (Figure 4). For reptiles, surgical implantation was the most common approach, used in 44 of 83 studies, predominantly on snakes. Exceptions included two lizards (Phrynosomatidae and Teiidae) and one skink (Scincidae). In amphibians, harnesses were the preferred method, employed in 56 of 77 studies, mainly on anurans (families: Hylidae, Bufonidae, Ranidae, Limnodynastidae, Leiopelmatidae, Rhacophoridae, Bombinatoridae, Aromobatidae) except for one study on *Triturus cristatus* (family: Salamandridae). The harness types varied, with most used designs including backpacks, belts with beads, and silicone belts.

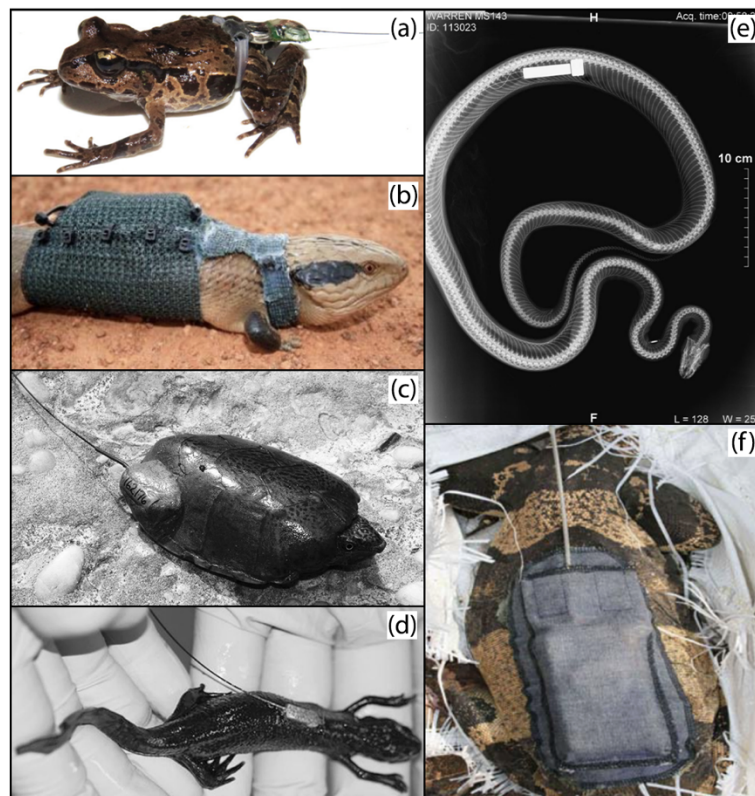


Figure 4. Six examples of electronic device attachment methods include: (a) a waistband created with cotton thread in a silicone tube on *Leiopelma archeyi* (Altobelli et al., 2023), (b) a wrap-around vest with a collar made of a layer of nylon flyscreen mesh and a layer of super-grip fabric on *Tiliqua multifasciata* (Price-Rees and Shine, 2011), (c)

Strenotherus depressus with transmitter attached by cement putty (Jenkins et al., 2022), (d) a transmitter sutured on the back of *Triturus cristatus* (Dervo et al., 2010), (e) a surgical implant in *Morelia spilota* (Bryant et al., 2010) and (f) a denim pouch attached with contact cement on *Varanus varius* (Flesch et al., 2009).

Other attachment methods include glueing, which was more prevalent in reptiles (16 studies) compared to amphibians (1 study). Glueing was employed in reptile families such as Alligatoridae, Varanidae, Phrynosomatidae, Agamidae, and Iguanidae and for Bufonidae among amphibians. Suturing devices directly onto the skin or using implants that extended from inside to outside the body were reported in 5 reptile studies (Alligatoridae, Leiocephalidae, Colubridae and Iguanidae) and 1 amphibian study (Rhacophoridae). For Testudines, transmitters were often mounted directly onto the carapace. Stomach-implanted transmitters were used in three studies, one involving reptiles (Colubridae) and two involving amphibians (Salamandridae).

3.5.1 Harnesses for amphibians

Harnesses for amphibians commonly combine tubing with cotton or nylon threads to ensure secure attachment or biodegradability (i.e., if the animal is not recovered at the end of the study). This approach has been used in species such as *Rhinella marina* (Shaykevich et al., 2022), *Litoria serrata* (Greenspan et al., 2023), and *L. lesueurii* (Rowley and Alford, 2007a, b, c). In *Rhinella marina*, the device loss rate was 14% with no reported mortality. For *L. lesueurii*, no losses or mortality were recorded. Elastic thread, often enhanced with glass beads to reduce abrasion, have also been employed, providing a lightweight and adjustable solution suitable for small species, particularly in *L. sylvaticus* (Muths, 2003; Blomquist and Hunter, 2007, 2010; Rittenhouse and Semlitsch, 2007; Bishir et al., 2018). Loss rates ranged from 0% to 45%, with mortality and injury rates varying between studies. For longer studies, more durable materials such as polyethylene or nylon fishing lines have been utilised, as seen in *Epidalea calamita*

(Miaud et al., 2000; Miaud & Sanuy, 2005; Sinsch et al., 2012) and *Anaxyrus terrestris* (Fritts et al., 2015). In *E. calamita*, device loss ranged from 0% to 8%, with no reported mortality. In *A. terrestris*, device loss and mortality were 3% and 5%, respectively. Recent trends emphasise biodegradable or natural materials (cotton threads and gauzes) to minimise environmental impact. These materials used on *Hyla japonica* showed no mortality or device loss, but injuries ranged 4-7% (Borzee et al., 2016; Borzee et al., 2018). In *Leiopelma hamiltoni*, there was a 30% device loss rate, but no mortality or injuries occurred (Altobelli et al., 2023). Some studies have experimented with more unconventional materials, such as aluminium chains, covered or not with spray plasticizer. In *Bufo viridis* no injuries or device loss occurred, but mortality reached 29% (Indermaur et al., 2008). Satin ribbons, as trialled in *Rana luteiventris* (Bull, 2000), resulted in 19% injuries, 45% device loss, and no mortality; using carpet thread on forelimbs increased injuries to 67%, with 4% device loss and no mortality. In *Triturus cristatus* (Dervo et al., 2010), backpack harnesses, the only reported use in urodeles, faced 100% device loss, underscoring retention challenges in this group.

3.5.2 Harnesses for reptiles

Harness designs for reptiles vary depending on species and morphology. For *Varanus panoptes*, a denim harness with epoxy resin led to 56% device loss but no injuries or mortality (Doody et al., 2014). In *Varanus olivaceus*, a butyl waist belt caused injuries in all individuals, with no loss or mortality (Bennett, 2014). For Scincidae, adhesive tapes were used at the tail base in *Oligosoma otagense* (Germano, 2007) and *Plestiodon fasciatus* (Brazeau & Hecnar, 2018), with this latter species experiencing 90% device loss and 10% mortality. In *Tiliqua multifasciata* and *T. scincoides* (Price-Rees et al., 2011), nylon mesh fabric and adhesive wrap-around vests led to 38% device loss rate but no

injuries or fatalities. Tail-mounted harnesses were tested in *Vipera berus* (Nash & Griffiths, 2018), where transmitters were attached to the tail base with surgical tape, resulting in 10% device loss but no mortality. In *Phrynosoma cornutum* (Richmond, 1998), a custom “U”-shaped harness made from a bicycle inner tube led to a 4% device loss rate with no mortality. For tortoises, materials such as latex belts (*Chelonoidis carbonarius*; Montaña et al., 2013) and polyester webbing and Velcro® harness (*Natator depressus*; Hounslow et al., 2022) were used, with minimal device loss (7%) and no recorded injuries or mortality. In *Sphenodon punctatus* (Jarvie et al., 2014; Ussher, 1999), elastic backpack harnesses showed varying injury rates (12-69%), low mortality (0-4%) and device loss (0-12%).

3.5.3 Surgical implants in reptiles

Surgical implants are commonly used in reptiles with variable outcomes. In *Salvator merianae*, no mortality, device loss, or injuries were observed (Pernas et al., 2012). In *Ophiophagus hannah*, the results were mixed: Rao et al. (2013) reported an 80% device loss rate, while Marshall et al. (2019) documented a 20% mortality rate and 7% device loss. Silva et al. (2018), however, observed no device loss or mortality in this species. *Python bivittatus* showed no mortality or device loss with intraperitoneal transmitters (Hart et al., 2015), while *Pseudonaja affinis* experienced a 70% mortality rate and 30% device loss from subcutaneous implants (Wolfe et al., 2018). In *Phrynosoma cornutum*, silicone adhesive with surgical implants resulted in 63% device loss rate and 7% mortality (Vesny et al., 2021). For *Crotalus adamanteus*, studies noted mixed outcomes, with 14% mortality and device loss rate (Hoss et al., 2010) and no adverse effects (Fill et al., 2015). *Trimeresurus albolabris* exhibited a high mortality rate of 56% but no device loss (Devan-Song et al., 2016). In *Crotalus horridus*, mortality rates from 20% to 28% (Reinert and

Rupert, 1999; Nordberg et al., 2021), with no device. *Agkistrodon contortrix* had a 7% loss rate without mortality (Carrasco-Harris et al., 2020). For *Sistrurus catenatus*, 8% loss and injuries were noted in one study (Weatherhead & Prior, 1992), while another reported no adverse effects (DeGregorio et al., 2018). In *Zamenis longissimus* (Major et al., 2023), the sole mortality was due to predation by another tracked individual. Lastly, *Vipera latastei* showed no loss or mortality (Brito, 2003).

3.5.4 Surgical implants in amphibians

Surgical implants for attaching tracking devices in amphibians have produced varied results across species. In *Triturus marmoratus* (Le Chevalier et al., 2017), an 8% mortality rate with no device loss was reported. In *T. cristatus*, predation and device loss ranged from 17% (Jehle and Arntzen, 2000) to 40% mortality and 20% injuries without device loss (Dervo et al., 2010). In *Paramesotriton hongkongensis*, 57% mortality and 24% device loss occurred; harnesses in the same study resulted in 90% loss and 10% mortality (Lau et al., 2017). *Pelobates fuscus* showed no mortality or device loss (Eggert, 2002), while *Ambystoma maculatum* experienced 44% mortality due to implant complications, with no device loss (Rittenhouse and Semlitsch, 2006). In *Hoplobatrachus occipitalis*, a 15% device loss but no mortality was observed (Spieler and Linsenmair, 1998), while *Ambystoma laterale* exhibited 5% mortality and a 59% device loss (Ryan and Calhoun, 2014). *Rhinella marina* showed a 40% device loss and no mortality (Seebacher and Alford, 1999). For *Ambystoma talpoideum*, 30% mortality and 50% device loss were reported (Moseley and Castleberry, 2005). *Cryptobranchus alleganiensis* suffered 24% mortality due to implant complications, with no device loss (Kraus et al., 2017). In *Dryophytes versicolor*, devices implanted in the coelomic cavity resulted in 9% mortality

from predation or post-removal complications and 27% device loss (Johnson, 2006). In *Epidalea calamita*, no mortality or device loss was reported (Sinsch et al., 2012).

3.5.5 Glue

Adhesive methods for attaching tracking devices have been applied primarily in reptiles, with mixed success. In *Caiman yacare* and *Melanosuchus niger*, glue combined with nylon fishing lines caused 10-25% mortality and 25-30% device loss (De la Quintana et al., 2020). In *Varanus varius*, superglue and linen tape at the tail base resulted in no mortality or device loss (Lei and Booth, 2018). Conversely, *Phrynosoma cornutum* experienced 7% mortality from predation and a 63% device loss when silicone adhesive was applied to the dorsum (Veszy et al., 2021). A denim pouch fixed with contact cement on *Varanus varius* led to a 0-27% device loss but no fatalities (Flesch et al., 2009; Pascoe et al., 2019). In *Varanus rosenbergi*, two glued pouches placed on the tail led to a 33% device loss rate but no deaths (Fletcher et al., 2023). In amphibians, glue usage is rare; for example, cyanoacrylate glue was applied to *Bufo viridis* and *Epidalea calamita*, resulting in a 33% device loss rate (Leskovar and Sinsch, 2005).

3.5.6 Other attaching methods

Some less common attachment methods include sewing transmitters directly onto the skin. In amphibians, in *Zhangixalus arboreus*, stitching with cotton thread caused 8% mortality from predation and a 33% device loss (Kusano, 1998). For reptiles, stitched attachments produced variable outcomes. In *Leiocephalus carinatus coryi*, transmitters encapsulated in epoxy and secured with tubes attached to the anterior and posterior ventral edges showed no mortality or device loss, but all individuals experienced injuries (Knapp and Owens, 2005). A similar technique used for *Cyclura cychlura* resulted in 10% injuries but 87% of individuals were lost due to snake predation (Knapp and Owens, 2005). In *Hierophis viridiflavus*, dorsal anchoring using rubber tubes inserted through

subcutaneous holes created with a needle across the snake's body below the spine resulted in no mortality, but injuries in all cases (Ciofi and Chelazzi, 1994).

Stomach-inserted implants were also tested, with varying success. In amphibians, *Paramesotriton hongkongensis* experienced a 90% device loss (Lau et al., 2017), and *Triturus carnifex* exhibited a 92% loss when transmitters were inserted via the oesophagus (Schabetsberger et al., 2004). In reptiles, stomach implants were more effective; in *Nerodia fasciata* and *N. taxispilota*, silicone-coated transmitters introduced by force-feeding resulted in no mortality or device loss (Osgood, 1970).

For *Testudines*, carapace-mounted devices are common. In *Mauremys sinensis*, epoxy-cemented caused 23% mortality, with no devices lost (Chen and Lue, 2008). *Apalone spinifera* experienced a 9% device loss without mortality when transmitters were attached using braided nylon fishing line or stainless-steel wire (Galois et al., 2002). In *Testudo hermanni*, synthetic resin resulted in a 13% device loss (Villardell-Bartino et al., 2015). Mechanical fastenings were also employed, such as stainless-steel bolts combined with non-toxic aquarium silicone to minimise snagging in *Graptemys geographica* (Carrière et al., 2009), or aluminum plates secured with bolts or ties in *Chelodina longicollis*; neither method caused mortality or device loss (Roe and Georges, 2007). Similarly, devices attached near the posterior carapace margin in *Clemmys guttata* and *Emydoidea blandingii* showed no adverse effects (Beaudry et al., 2009). In contrast, transmitters affixed to the posterior left marginal scutes with galvanised wire and epoxy in *Deirochelys reticularia* resulted in 14% mortality from predation and a 43% device loss (Buhlmann et al., 1995). In *Pelomedusa galeata* and *Pelusios sinuatus*, plastic cable ties were used to secure transmitters above a hind leg on the carapace, with no reported mortality or device loss (Price et al., 2022).

3.6 Pre-release testing

Only 12 studies conducted pre-release testing under controlled conditions to assess the effects of transmitters on performance. Among these, 3 evaluated behaviour and weight, 5 focused solely on behaviour, 3 solely on weight, and 1 assessed stress-related hormone levels.

3.7 Sample size and monitoring duration

The average sample size was 33.73 individuals (range: 1-417), and the average tracking duration was 197.18 days (range: 1-8300). Reptiles had a significantly longer average tracking duration (338.65 days) compared to amphibians (50.12 days; Wilcoxon: $W = 940.5$, $P < 0.001$). Conversely, on average, amphibians had larger sample sizes (46.97) than reptiles (21.84; Wilcoxon: $W = 4662.5$, $P < 0.001$).

3.8 Mortality and device loss

In 50.6% of the studies, the number of injured animals was not reported. Mortality was reported in 84.4% of studies, with at least one death recorded in 25.6% of cases, and it did not differ between amphibians and reptiles. Causes of mortality included predation, vehicle collisions, and complications from surgical implantation. Mortality showed no significant correlation with transmitter-to-body weight ratios (0.058) or species taxonomy (family) (ANOVA: $F_{31,125} = 0.92$, $P = 0.592$).

Device losses were documented in 133 studies (83.1%), with an average loss rate of 16.1%. At least one device was lost in 67 studies, with loss rates ranging from 2% to 100% (mean: 32.0%). Device loss was significantly influenced by attachment methods (ANOVA: $F_{5,131} = 2.398$, $P = 0.041$), with higher loss rates for stomach-implanted devices

compared to surgical implants ($P = 0.028$). Tracking duration did not significantly predict device loss ($F_{1,134} = 1.516$, $P = 0.220$).

Discussion

The results highlight the steady growth in the application of electronic telemetry for amphibians and reptiles, emphasising its increasing recognition as a valuable tool for ecological and conservation research. However, the decline observed between 2010 and 2014 raises questions about the factors influencing this trend, such as changes in research priorities, economic constraints, or the emergence of competing methodologies.

4.1 Geographical disparities in research effort

The significant concentration of research in North America reflects the availability of resources and infrastructure in the region, alongside a well-established tradition of herpetological studies. Conversely, regions such as South America and Africa exhibit notably lower research output. This disparity likely stems from economic and logistical challenges rather than reduced ecological urgency, given the high biodiversity and vulnerability of herpetofauna in these areas (Measey et al., 2019). Addressing this imbalance will require targeted efforts to enhance research capacity in underrepresented regions, such as increased international collaborations and funding opportunities.

4.2 Bias toward non-threatened species

The preponderance of studies on non-threatened species raises ethical and practical concerns. While logistical and ethical challenges (i.e., health concerns for the impact of tracking devices on animals) and regulatory constraints (i.e., difficulties in obtaining permits) may partially explain this trend, it risks leaving critical knowledge gaps about

endangered species. Understanding the spatial ecology and habitat preferences of threatened taxa is vital for designing effective conservation interventions, such as the identification of critical habitats and movement corridors. Researchers and funding agencies should prioritise studies on vulnerable species, incentivising efforts to overcome regulatory and logistical hurdles and fostering methodological innovations that minimise potential adverse effects on endangered populations.

4.3 Evolution of tracking technologies

The growing adoption of GPS transmitters, particularly during and after the observed decline in overall research, underscores a shift toward leveraging advanced technologies. This trend reflects broader technological progress in ecological research tools, enhancing data precision and collection frequency. Advances in miniaturisation and battery efficiency have broadened GPS usage (Kays et al., 2015), although primarily in reptiles due to weight constraints. Despite its high accuracy (Hulbert and French, 2001), GPS telemetry can be distorted in dense vegetation (Gamo et al., 2000), requiring study designs to account for signal interference. The continued reliance on radio transmitters highlights their affordability and reliability, making them accessible for diverse research contexts. Similarly, the preference for Harmonic Direction Finder (HDF) transmitters in amphibians underlines the importance of lightweight solutions tailored to the anatomical constraints of specific taxa.

The predominance of a single transmitter brand in reviewed studies suggests confidence in its performance but also reflects limited industry interest in developing novel devices. Such stagnation in innovation might restrict future technological progress, particularly for taxa with specialised ecological or anatomical requirements.

4.4 Attachment methods and animal welfare

The results underscore distinct preferences for attachment methods across taxa, driven by anatomical and behavioural differences. Surgical implantation dominates reptile studies, particularly for snakes, where harnesses are impractical due to their morphology and behaviour, leading to high loss rates. In contrast, harnesses are preferred for amphibians, given their smaller size and fragile skin, which complicates surgical implantation.

While the 10% transmitter weight guideline (Heyer et al., 1994) demonstrates a general commitment to animal welfare, it has been tested on a narrow range of taxa and remains more theoretical than empirically validated, particularly when applied to passive tracking devices. Each new species studied should undergo specific tests to assess the effects of tracking devices on their performance, behaviour, and health.

Device impacts depend not only on body weight but also on the attachment method used (Mizrahy-Rewald et al., 2023), which are crucial for secure and durable positioning, maintaining natural behaviour, and preventing harm. These considerations emphasise the need for careful selection of attachment methods tailored to the anatomical and ecological traits of the species under study.

The limited use of pre-release testing is concerning, as such evaluations are essential for assessing the effects of devices on animal health and behaviour. Expanding the practice of pre-release testing would ensure adherence to ethical standards, enhance data validity, and minimise the negative impacts of tracking studies. Incorporating these practices more broadly would significantly improve the reliability of telemetry research while safeguarding animal welfare, ultimately contributing to more responsible and effective

scientific inquiry. Expanding the practice of pre-release evaluations could significantly enhance the reliability of tracking studies while safeguarding animal welfare.

While some studies focus on the mechanical and physiological impacts of tagging, the potential behavioural consequences remain largely underexplored, representing a critical knowledge gap. In anurans, externally attached devices may influence mating dynamics, particularly in species where scramble competition is the dominant breeding strategy. During explosive breeding events, male-male interactions rely on rapid movements, agility, and the ability to compete physically for access to females; any impediment caused by harnesses or external tags could reduce mating success or alter natural courtship behaviour. Although the reviewed literature does not provide empirical assessments of these effects, acknowledging this limitation is essential. Future work should prioritise evaluating whether biologging devices reduce male competitive ability, interfere with acoustic signalling, or modify female choice in species with different mating systems.

Similarly, tagging may affect individuals' ability to enter or maintain access to daily refugia. Amphibians frequently rely on narrow crevices, underground cavities, or dense vegetation for daytime shelter and thermoregulation. External devices could hinder entry into these microhabitats or increase detectability to predators. Assessing whether transmitter size, shape, or attachment method compromises refuge use is therefore crucial for accurately interpreting movement and activity patterns derived from telemetry studies. Addressing these overlooked behavioural dimensions will be essential for improving device design, refining welfare standards, and ensuring that biologging studies accurately reflect natural behaviours. Integrating such evaluations into pre-release testing and post-deployment monitoring should represent a key priority for future research.

4.5 Study design variability and reporting standards

The variability in sample sizes and monitoring durations reflects the diverse research objectives and ecological contexts of tracking studies. The substantial range in monitoring periods, varying from 1 to 8300 days, is likely influenced by diverse research objectives, species behaviours, and environmental factors. This variability underscores the necessity of tailoring monitoring durations to the specific requisites of each study, reinforcing the importance of adaptability in research methodologies. Gaps in the reporting of injured animals may stem from various factors, including divergent research priorities and potential challenges in identifying injuries during monitoring periods. While this flexibility is necessary, the lack of standardised protocols for reporting injury rates and the limited documentation of device losses are critical gaps. The high mortality rates observed, attributed to factors such as predation, vehicular collisions, and surgical implantation, emphasise the need for rigorous monitoring and mitigation strategies. Notably, the absence of significant correlations between mortality rates and transmitter-to-body weight ratios or taxonomic group suggests that other factors, such as environmental conditions or attachment methods, play critical roles in the survival of tracked animals. Future research should aim to elucidate these influences and develop best practices to minimise mortality risks.

4.6 Device loss and methodological challenges

Device loss, while expected in field studies, highlights operational challenges. The significant variation in loss rates across attachment methods suggests that careful consideration of species-specific traits and study environments is crucial. Efforts to improve attachment reliability, such as refining harness designs or employing innovative materials, could reduce device loss rates. Furthermore, standardising reporting practices

for device loss and related factors would enhance the reproducibility and comparability of tracking studies.

4.7 Toward a holistic framework for herps telemetry

This review delineates the evolving landscape of amphibian and reptile tracking research, highlighting consistent growth in scientific interest over the years. The observed decline in research activity during the 2010-2014 interval raises questions about shifting priorities, funding dynamics, or other contextual influences. Addressing these fluctuations is crucial to maintaining momentum in this vital area of ecological and conservation research.

Geographical disparities in research activity underscore a pressing need for more equitable distribution of resources to better understand and conserve herpetofauna globally. While North America has dominated tracking studies, underrepresented regions such as South America and Africa, despite their rich biodiversity, require greater focus and investment.

The increasing adoption of GPS transmitters signals a commitment to advanced tracking methodologies, enabling more precise and detailed spatial data. However, the widespread use of radio transmitters and the preference for Harmonic Direction Finder (HDF) transmitters in amphibians reflect the necessity of tailoring technologies to meet the unique requirements of specific taxa. Encouragingly, technological advancements, such as the continued miniaturisation of tracking devices, promise improved opportunities to study smaller and less accessible species, thereby broadening the ecological insights that can be gained.

These findings emphasise the complex interplay of methodological considerations, ethical concerns, and practical challenges in telemetry studies. The predominance of ethical adherence, as evidenced by the consistent application of the 10% weight rule, is commendable. However, the limited implementation of pre-release testing highlights a critical gap that must be addressed to ensure minimal adverse effects on the welfare of study animals and to enhance the reliability of collected data.

The variability in sample sizes and monitoring durations across studies underscores the need for adaptable and species-specific research designs. Standardised reporting practices, particularly for injury rates, mortality, and device loss, are vital for robust data collection and for evaluating the implications of tracking studies on animal welfare.

Moving forward, efforts to promote equitable research representation, prioritise threatened species, and minimise negative impacts on studied populations will be essential. Establishing standardised protocols and leveraging technological advances will enable the field to address existing challenges and provide deeper insights into the spatial ecology of herpetofauna. Ultimately, these measures will enhance our capacity to implement effective conservation strategies and safeguard the ecological roles of these critical yet often overlooked taxa.

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Data Availability

Data will be made publicly available upon publication of the corresponding manuscript.

Currently, all datasets and scripts are stored in a private GitHub repository (URL: https://github.com/BombinaPN/review_tracking), accessible upon request.

Chapter II - Evaluation of attachment methods and short-term behavioural effects of biologgers in the American bullfrog (*Aquarana catesbeiana*).

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Abstract

Biotelemetry provides critical insights into animal movement and behaviour, but its application to amphibians remains challenging due to skin sensitivity, dual terrestrial-aquatic lifestyles and biomechanical and hydrodynamic constraints. Additionally, the behavioural effects of externally attached devices remain insufficiently quantified in many amphibian species. Here, we assessed the short-term effects of external telemetry devices on locomotor behaviour in the American bullfrog (*Aquarana catesbeiana*), aiming to improving data reliability in amphibian biologging studies. We tested different harness designs on nine individuals from a Central Italy population under controlled conditions. Among nine attachment methods evaluated, a custom-fit O-ring harness proved to be the only reliable solution, ensuring device retention while preserving

mobility. Tri-axial accelerometers deployed on the body enabled accurate classification of jumping (94.06%) and swimming (90.66%) behaviours, supporting the suitability of accelerometry for behavioural inference in large-bodied amphibians. To quantify device effects, we estimated vectorial dynamic body acceleration (VeDBA) in individuals fitted with a dummy tag identical in shape and mass to a prospective telemetry device, representing 2.14-7.90% of body mass. External loading affected locomotor energetics in a mode- and size-dependent manner: jumping VeDBA decreased in lighter individuals, whereas swimming VeDBA increased, consistent with biomechanical and hydrodynamic constraints imposed by the load. Movement frequency was unaffected, indicating no effect of movement initiation, while jump duration showed a body mass-dependent response, and swimming duration remained unchanged, with heavier individuals showing reduced sensitivity to external loading.

Overall, our results indicate that telemetry devices can be deployed on *A. catesbeiana* under short-term conditions with limited and context-dependent behavioural effects. However, caution is warranted when extrapolating these findings to smaller amphibian species and for prolonged periods, highlighting the need for species-specific attachment strategies and longer-term evaluations.

Introduction

Animal movement plays a central role in shaping ecological and evolutionary processes, as behavioural decisions are driven by complex interactions between intrinsic factors (e.g., sex, physiological state, motivational state) and extrinsic factors (e.g., environmental conditions, predation, competition) (Nathan et al., 2008; Shepard et al., 2008). Understanding these decisions is therefore essential for advancing eco-biological

knowledge. In recent decades, the integration of electronic devices into ecological research has catalysed a methodological revolution (Altobelli et al., 2022).

Among these tools, accelerometers and other biologgers have empowered researchers to infer fine-scale behaviours from movement data that were previously accessible only under controlled laboratory settings (Allan et al., 2018; Kays et al., 2015; Ropert-Coudert and Wilson, 2005; Rutz and Hays, 2009). These technological advancements have overcome many limitations in field studies (Kays et al., 2015; Nathan et al., 2008; Wassmer et al., 2020), generating data that allow researchers to address broad ecological questions and strengthen conservation and management strategies (Cooke et al., 2004; Klimley et al., 2013; Rutz and Hays, 2009).

Despite their wide use in mammals, birds and marine species, the application of biologging to small-bodied vertebrates such as amphibians remains uncommon. This is largely due to a combination of technical and biological limitations: amphibians are generally small, lack anchoring structures, possess permeable and fragile skin, and move between aquatic and terrestrial habitats (Altobelli et al., 2022; Cooke et al., 2013; Le Chevalier et al., 2017). Unlike other tetrapods, which often have anatomical features that facilitate harnesses or adhesive attachment (Bertassoni et al., 2022; Houstin et al., 2022; Lei and Booth, 2017), amphibians present additional challenges for secure device placement.

Recent advances in miniaturisation of electronic components, however, are beginning to overcome these limitations (Toledo, 2025), opening new opportunities to investigate movement ecology in these historically understudied taxa (Boncourt et al., 2023; Hammond et al., 2021; Licata et al., 2023). Yet, critical methodological issues remain unresolved, including the impact of the devices on behaviour and the most effective and

safe attachment techniques (Geen et al., 2019). Furthermore, although accelerometer-based methods have been widely validated across diverse taxa (Eisenring et al., 2022; Lush et al., 2016; Studd et al., 2019), only a handful of studies have explored their potential for identifying behaviours in anurans (Halsey and White, 2010; Spießberger et al., 2023), limiting broader adoption of these tools in amphibian research.

In this study, we evaluate the application of biologging tools in amphibians using the American bullfrog (*Aquarana catesbeiana*) as a model species. Through controlled behavioural experiments, we (i) compare different external attachment methods suitable for medium to large-bodied semi-aquatic anurans; and (ii) assess the short-term effects of external devices on locomotion and movement energetics across terrestrial and aquatic contexts.

Our results are intended to inform the development of safe and reliable biologging protocols for amphibians, while explicitly acknowledging the species- and context-specific nature of device effects. In particular, this study provides practical guidance for researchers seeking to deploy biologgers in free-ranging anurans for investigations of activity patterns, locomotor behaviour, and energy expenditure. By clarifying both the potential benefits and the limitations of external attachment approaches, our work contributes to the broader integration of movement ecology into amphibian research, while highlighting the need for cautious interpretation and further validation across species, body sizes, and ecological contexts.

Materials and methods

2.1 Study species and area

Aquarana catesbeiana (Shaw, 1802), commonly known as the American Bullfrog, is a large-bodied semi-aquatic anuran native to North America that has been widely introduced across the Americas, Asia and Europe. It is listed among the world's 100 most invasive species (Bury and Whelan, 1985; Lowe et al., 2000). In Italy, repeated introductions began in the early 20th century, with subsequent spread to central and southern regions (Cox et al., 2006; Razzetti et al., 2025). We selected *A. catesbeiana* as a focal species because its semi-aquatic lifestyle, reliance on both jumping and swimming locomotion, and relatively large body size make it suitable for evaluating attachment strategies and short-term locomotor responses to externally attached devices under controlled conditions. Importantly, the species' invasive status allows experimental manipulation without negative conservation implications for native populations. In the present study, tests were conducted using individuals from the population located in Fiano Romano (Rome, Italy; 42°11'32" N 12°36'35" E). In April 2023, nine *A. catesbeiana* specimens were collected using dip nets. Each individual was weighed using a precision scale (maximum capacity: 1 kg, accuracy: 0.01 g) prior to the beginning of behavioural trials.

2.2 Experimental setup

To assess the short-term effects of external loading on locomotion, we compared movement metrics between individuals carrying an accelerometer alone (control condition) and individuals carrying an accelerometer plus a dummy device (experimental condition, see section 2.3). This design was intended to isolate the effect of additional mass while maintaining a consistent attachment configuration across treatments. Body

mass of the individuals ranged widely (mean $\pm$ SD: 307.89 $\pm$ 153.93 g). The mass of the dummy-equipped harness represented between 2.14% and 7.90% of individual body mass and never exceeded the commonly adopted 10% threshold (Table S1).

Behavioural trials focused on the two most common locomotor modes of *A. catesbeiana*: jumping and swimming. Two experimental arenas were used to represent terrestrial and aquatic contexts. Terrestrial trials were conducted in a 6-meter-long linear tunnel assembled by connecting two greenhouses (295x100x80 cm each, Outsunny® 845-234V04CR) and covering them with a dark tarp, leaving only the tunnel ends illuminated by natural light to encourage forward movements. Aquatic trials were conducted in a pool (258x179x66 cm, Avenli® 17805) filled with 40 cm of water sourced from the collection site and filtered using a pump (Intex®). Each individual experienced both conditions within the same day: first the control trials (accelerometer only) and subsequently the experimental trials (accelerometer + dummy). This approach allowed us to minimise variation caused by environmental factors such as temperature, light, or the presence of potential prey, which could differ across days and influence movement behaviour. Trials were video recorded for subsequent behavioural analysis. Terrestrial trials (~4 min) were filmed using a smartphone mounted on a tripod at one end of the greenhouse, while aquatic trials (~90 s) were recorded from an overhead view using a drone (DJI Mini II).

2.3 Attachment methods and accelerometer configurations

Because biologging applications in amphibians remain relatively uncommon, we evaluated multiple external attachment methods for dummy devices before selecting a configuration suitable for dummy deployment. Attachment methods were assessed based on their ability to remain attached during locomotion, minimise visible skin abrasion

during short-term use, and maintain a stable device orientation relative to the animal's body. Dummy devices were designed to simulate the size and shape of a potential GPS-accelerometer unit suitable for large anurans (Gipsy 6, Technosmart Europe srl, Rome, Italy, 4 x 2 cm, 8 g). We tested nine attachment configurations: (a) 1mm diameter silicone wire (Jinsion Crystal Tec®) wrapped around the waist and front limbs, (b) a nylon stocking arranged in a figure-eight pattern around the body, (c) a silicone elastic band positioned in a figure-eight configuration, (d) a flat haemostatic lace with holes for the front legs, secured around the abdomen, (e) a garment made from 70% polyamide and 30% elastane, fitted with openings for both front and hind legs, (f) a silicone tube (Patkil®, inner diameter: 1.5 mm, outer diameter: 2.5 mm) containing a cotton thread, fastened around the waist, (g) silicone tube rings (Patkil®) with a fishing line attachment for all four limbs, connected to the device via silk thread, (h) O-rings (CCLife®) placed on the front limbs and waist, connected to the device via silk thread, (i) O-rings (CCLife®) placed on the front limbs and waist, connected to the dummy via braided fishing line (Croch®, diameter: 0.25 mm, strength: 35 lb).

Accelerometers (Axy 5, Technosmart Europe srl, Rome, Italy; 4 g) were attached as close as possible to the animal body mass centre, and were configured to record at 100 Hz with a 10-bit resolution. To determine the appropriate G scale setting for the accelerometers, we deployed a device on an individual using the highest available setting (16 G) and used the data to inform the setting of other devices. As we recorded a maximum acceleration of 16 G, we set all the devices at 16 G, ensuring that all recorded accelerations fell within the device's operational range. Before data analysis, accelerometers were calibrated following Garde et al. (2022). Each device was placed stationary and sequentially oriented on each of its six axes for approximately 10 seconds

per position. The recorded outputs were then used in two calibration steps: (1) ensuring that the maximum and minimum absolute values for each axis were equal, and (2) applying a gain correction to standardise values to 1.0 g (Garde et al., 2022). In the present study, the accelerometer alone represented 0.81-3.19% of individual body mass. Although this proportion is low relative to commonly applied thresholds, we note that even lightweight devices may influence amphibian behaviour due to skin sensitivity and attachment constraints; consequently, all behavioural comparisons were conducted within individuals and across experimental conditions.

2.4 Animal housing and welfare

All experimental procedures were approved by the Italian Ministry of the Environment and Energy Security (authorisation no. 2023.0043372). Following capture, individuals were housed individually in plastic fauna boxes ($79 \times 57 \times 43$ cm) containing water and a stone to provide perching opportunities. Fauna boxes were maintained outdoors in proximity to the study pond to minimise transport-related stress. Animals were fed every two days with five live adult migratory locusts, and general health, skin condition and feeding behaviour were monitored daily throughout the study. Behavioural trials were designed to minimise handling and disturbance: each individual was tested for a maximum of 5.5 minutes per day and was returned immediately to its fauna box following each testing session. Attachment sites were inspected daily for signs of skin abrasion, irritation, or adverse reactions. In accordance with the national management plan for *A. catesbeiana*, captured individuals of this invasive species cannot be released into the wild and must be euthanised at the conclusion of experimental procedures. Euthanasia was performed using an overdose of buffered tricaine methanesulfonate (MS-222),

administered in closed containers to prevent environmental contamination (Adriaens et al., 2013), following international guidelines (AVMA, 2020) and national management regulations, to ensure a rapid and painless death.

2.5 Data preparation

Due to the peculiar locomotion of *A. catesbeiana*, and the practical challenges associated with external attachment, occasional displacement or misalignment of the accelerometer occurred, rendering some data unusable. To address this issue, all video recordings were carefully reviewed to i) identify instances of device misalignment and ii) construct a behavioural database for each individual at 1-second resolution, which was subsequently used for behavioural validation (see below). For each trial, a continuous 1-minute segment of accelerometer data was extracted, ensuring that the device remained correctly positioned throughout the selected interval. If an uninterrupted minute could not be identified due to device misalignment, the entire trial was excluded from subsequent analysis. For the selected accelerometer data, static acceleration was calculated using a 1-second rolling mean and subtracted from the raw data to isolate dynamic acceleration. Vectorial dynamic body acceleration (VeDBA) was then calculated as the square root of the summed squares of the three dynamic acceleration axes (Gleiss et al., 2011). VeDBA represents overall body movement and is widely used as a proxy for energy expenditure across a range of taxa and locomotory modes (Qasem et al., 2012; Wilson et al., 2020). Behavioural states were classified into stationary vs locomotion behaviours (jumping or swimming) using a K-mean clustering approach applied to moving standard deviation of absolute acceleration values for each axis (X, Y, Z), calculated at the native sampling frequency (100 Hz). The k-means algorithm was set to identify two clusters: "stop" (low acceleration variability, the stationary behaviour) and "swim/jump" (high acceleration

variability). To validate behaviour classification accelerometer-derived data were down-sampled from 100 Hz to 1 Hz to match the temporal resolution of the video-based dataset. Each second was assigned the most frequent behavioural class occurring within its corresponding 100 data points. From this dataset, 10,000 data points were randomly selected, and the percentage of correctly identified behaviours was calculated by comparison with video observations. Behaviour classification was derived from accelerometer data rather than directly on video recordings to assess the applicability of this approach to future field studies, where simultaneous video recordings are typically unavailable. Finally, using video-derived behavioural dataset, movement (jumping or swimming) duration and frequency were quantified. Each movement event was assigned a continuous integer value, movement frequency was calculated as the number of distinct events per trial, while movement duration was computed as the total number of seconds during which the same integer value remained constant.

2.6 Statistical analysis

To assess the effects of the dummy tracking device on locomotion, we fitted three sets of generalised linear mixed models, using Bayesian hierarchical modelling (brms package, R; (Bürkner, 2015), evaluating differences in VeDBA, movement frequency and movement duration. Models were fitted separately for swimming and jumping behaviours, resulting in six models overall. First, we fitted Gaussian models with log-transformed VeDBA as the response variable to ensure normality (Figure S1, S3, S4). Fixed effects included dummy device presence (binary), scaled body mass, scaled test day, and their interactions. Periods classified as stationary behaviour were removed prior to model fitting. Continuous predictors were standardised using z-scores to facilitate

model convergence and interpretation. Individual identity was included as a random effect. To account for potential temporal autocorrelation within trials, a first-order autoregressive structure was incorporated, assuming that successive measurements within the same test could be correlated over time. Subsequently, analogous models were fitted using movement frequency and duration as response variables. Jump frequency was modelled using a negative binomial distribution (Figure S2, S5), while swim frequency was modelled using a Poisson distribution (Figure S2, S6). Movement duration for both jumping and swimming was modelled using a Poisson distribution (Figure S2, S7, S8). Bayesian modelling was chosen due to the limited sample size (nine individuals) and the presence of missing data for some individuals on certain days, which could bias frequentist approaches. Bayesian inference allows for robust parameter estimation in unbalanced datasets and provides full posterior distributions of model parameters. Models were run using the cmdstanr backend, with four Markov Chain Monte Carlo (MCMC) chains of 4000 iterations each (1000 warm-up). All analyses were performed with R 4.4.0 (R Core Team, 2024).

Results

3.1 Attachment methods

Among the tested attachment configurations, only method i) (Figure 1) remained consistently attached throughout the trials. All alternative attachment methods were actively removed by *A. catesbeiana* within a short time. Based on this outcome, custom-fitted harnesses were individually tailored for each of the nine individuals using appropriately sized O-rings. The final harness assembly consisted of the tracking device secured by two O-rings placed around the front limbs and one around the waist, connected via braided fishing line.

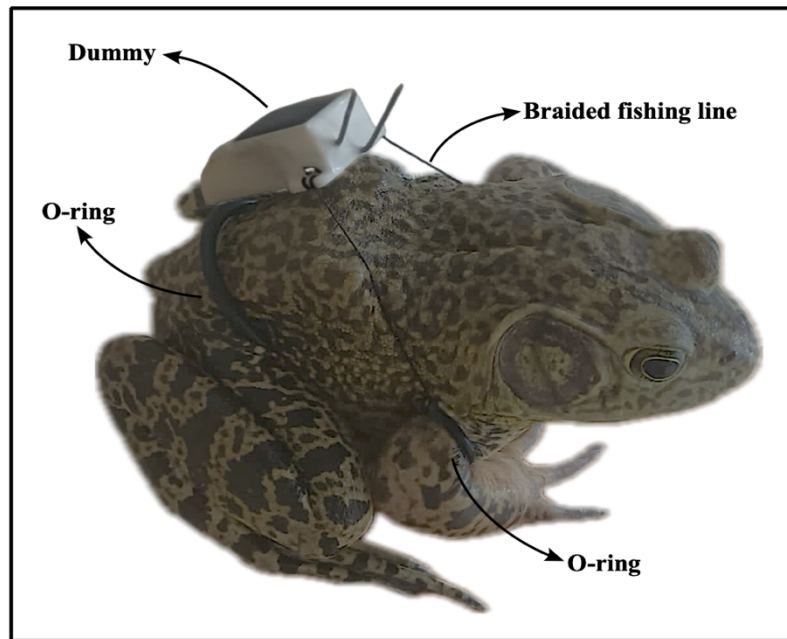


Figure 1. An American bullfrog equipped with a dummy of a potential tracking device. The dummy is secured using two O-rings on the front limbs and one around the waist, all connected to the dummy with braided fishing line.

3.2 Behavioural classification and data validation

The k-means clustering approach applied to accelerometer data successfully discriminated between stationary and locomotor behaviours. Classification accuracy

reached 94.06% for jumping and 90.66% for swimming when validated against video observations (Figure 2).

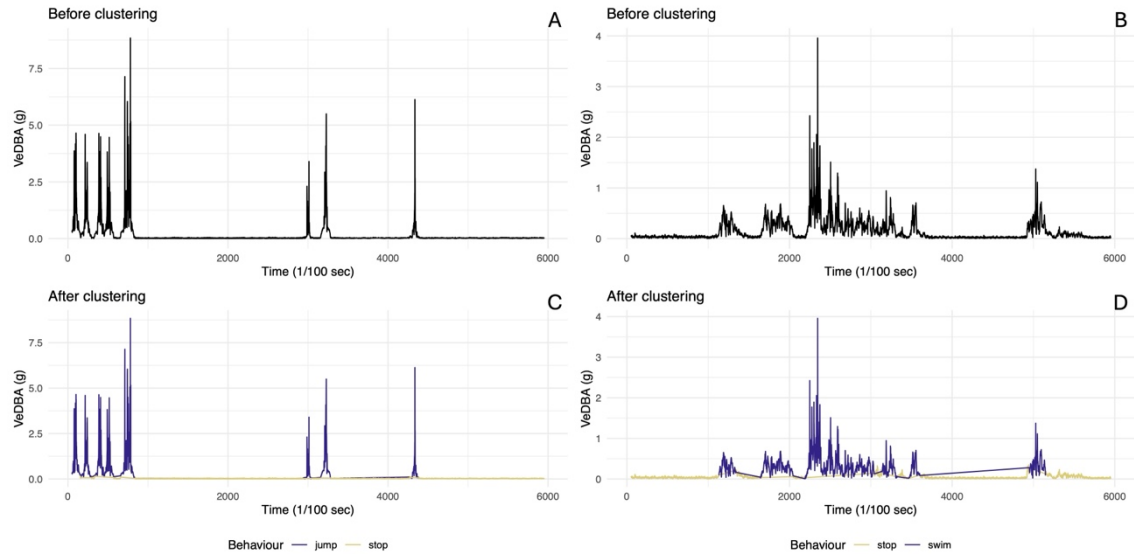


Figure 2. The four plots represent the performance of VeDBA for two individuals (LC07 and LC03) under the two test conditions. The plots on the left illustrate the data of LC07 during a land test with the dummy on day 4 (A and C), while the plots on the right show the data of LC03 during an in-water test with the dummy on day 1 (B and D). The upper plots show the VeDBA values before clustering the movements (A and B), while the lower plots represent the data after clustering (C and D).

3.3 Effects of increased load on locomotor energetics (VeDBA)

Bayesian analysis of locomotor energetics in *A. catesbeiana* revealed distinct effects of the dummy device on jumping and swimming behaviours. For jumping, VeDBA generally decreased with increasing body mass when no dummy was present, indicating lower relative energetic expenditure in heavier individuals. In contrast, when carrying the dummy, VeDBA increased with body mass, resulting in a positive interaction between dummy presence and body mass ($\beta = 0.15$, 95% CI: [0.05, 0.24]; Figure 3). The interaction between dummy and test day was not significant, nor was the three-way interaction between dummy, body mass, and test day (Figure 3).

In swimming, VeDBA increased with body mass in both conditions; however, the increase was steeper in the absence of the dummy, while the presence of the device

attenuated this slope, consistent with a significant negative interaction between dummy and body mass ($\beta = -0.07$, 95% CI: [-0.13, -0.01]; Figure 3). Furthermore, VeDBA during swimming decreased over successive test days when individuals carried the dummy ($\beta = -0.14$, 95% CI: [-0.20, -0.07]), suggesting a progressive reduction in locomotor effort across days. The three-way interaction between dummy, body mass, and test day was not supported (Figure 3).

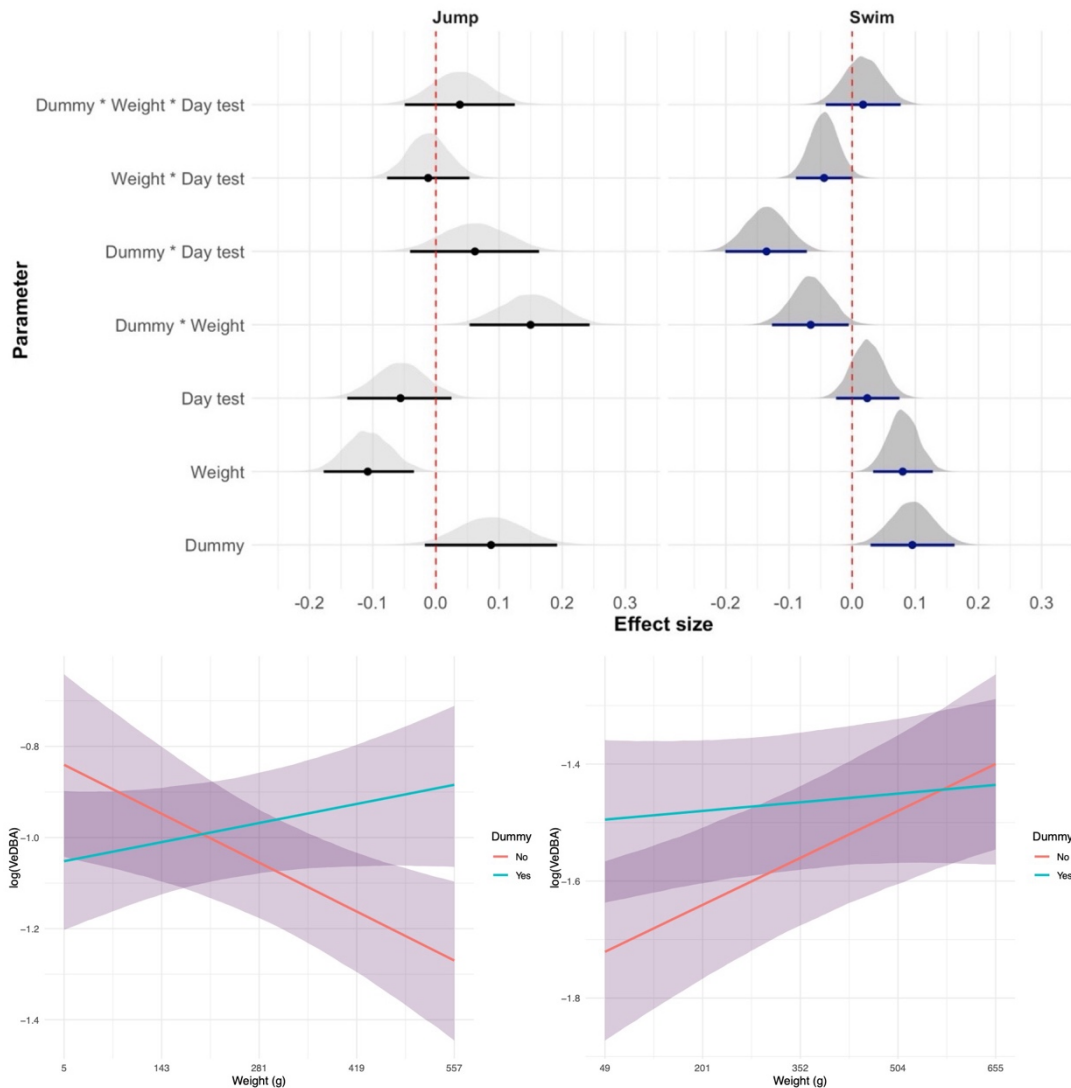


Figure 3. Top panel: posterior distributions of model parameters for Jump and Swim conditions. The plot displays the posterior distributions of the estimated effects from Bayesian models analysing the relationship between Dummy (device presence), Weight, and Day test on log-transformed VeDBA. The distributions represent the uncertainty around each parameter estimate, with the black (Jump) and dark blue (Swim) points and horizontal bars indicating the 95% credible intervals. Dashed red vertical lines denote a null effect (zero). The results suggest how different factors and their interactions influence VeDBA in each condition. Bottom panels: predicted log-transformed VeDBA as a function of body mass for jumping (left) and swimming (right). Red lines: absence of dummy; blue lines: presence of dummy;

grey shaded areas: 95% intervals. Body mass values correspond to the predicted range based on the nine individuals tested; scales differ between jump and swim panels due to model predictions.

3.4 Effects of increased load on movement frequency

Bayesian analyses of movement frequency revealed no clear effect of dummy presence on either jumping or swimming frequency. Posterior distributions for dummy presence, body mass, test day, and their interactions broadly overlapped zero for both locomotor modes (Figure 4).

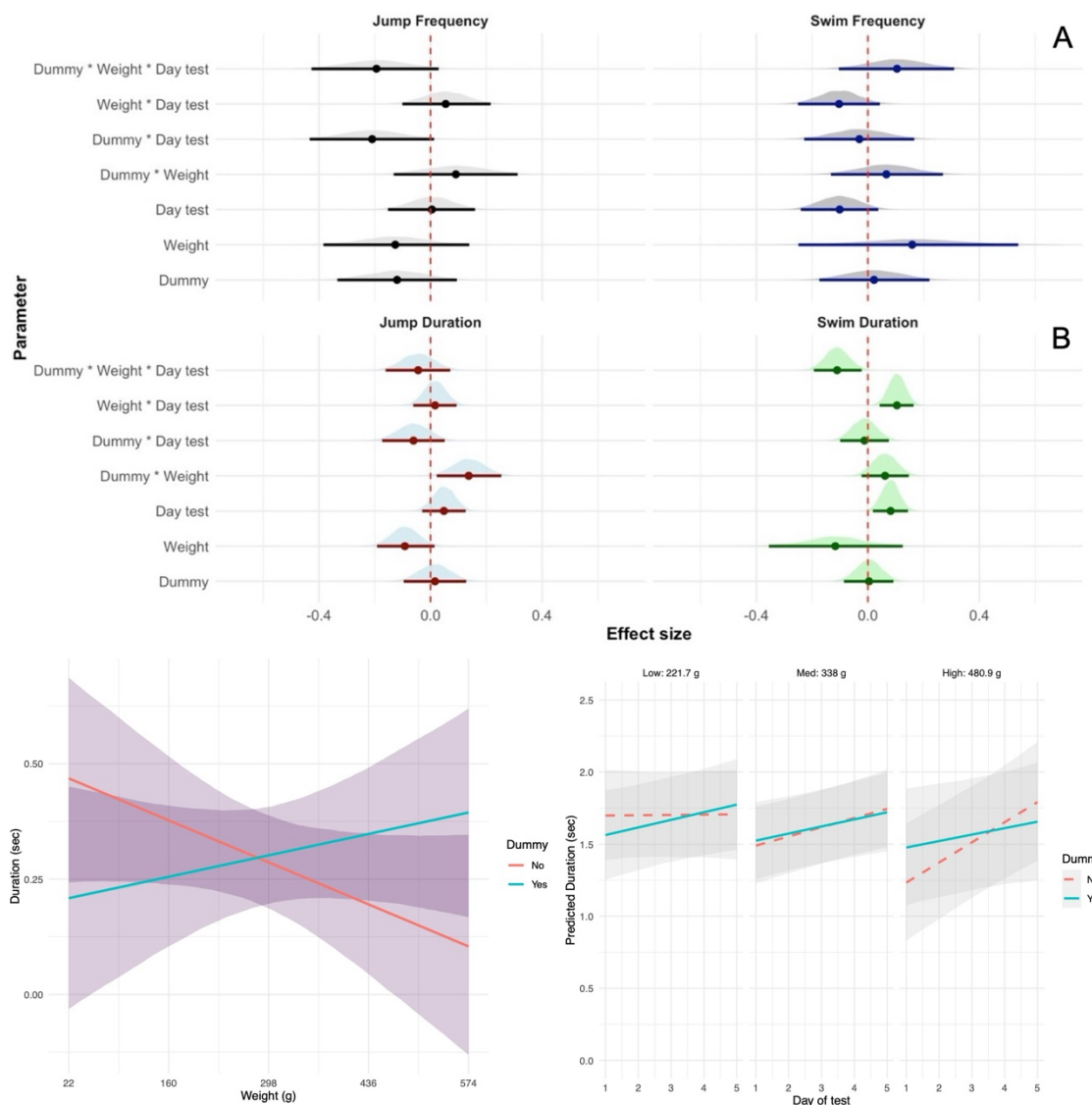


Figure 4. Top panel: Posterior distributions of the effect sizes for the frequency and duration of jumps and swims in animals tested with and without a dummy device. The four panels represent the results of Bayesian analyses for each locomotor mode: Jump Frequency (top left), Swim Frequency (top right), Jump Duration (bottom left), and Swim

Duration (bottom right). Each panel shows the estimated effect sizes with 95% credible intervals for the effects of the dummy device, body weight, and test day, as well as their interactions. Dashed red vertical lines denote a null effect (zero). Bottom left: interaction between dummy presence and body mass for jumping duration. Red line: absence of dummy; blue line: presence of dummy; grey shading: 95% intervals; x-axis shows individual body mass (not scaled). Bottom right: three-way interaction among body mass, dummy presence, and test day for swimming duration. Red line: absence of dummy; blue line: presence of dummy; grey shading: 95% intervals; x-axis shows test day (1–5), y-axis shows swim duration. Lines are shown for three representative body mass values (low = 25th percentile, medium = 50th percentile, high = 75th percentile).

3.5 Effects of increased load on movement duration

Movement duration analysis showed distinct responses to the tracking device across different locomotor modes. For jumping, a positive interaction between dummy presence and body mass was detected ($\beta = 0.14$, 95% CI: [0.02, 0.25]), indicating longer jumps in heavier individuals when carrying the dummy. The predicted values indicate that in the absence of the dummy, jump duration slightly decreased with increasing body mass, whereas in the presence of the dummy, jump duration increased with body mass, highlighting a compensatory effect in heavier individuals (Figure 4). No other main effects or interactions showed statistical significance. Swimming duration increased over successive test days ($\beta = 0.08$, 95% CI: [0.02, 0.14]). A positive interaction between body mass and test day ($\beta = 0.10$, 95% CI: [0.04, 0.16]) indicated that heavier individuals showed a stronger increase in swimming duration over time. Additionally, a significant three-way interaction among dummy presence, body mass, and test day ($\beta = -0.11$, 95% CI: [-0.19, -0.02]) revealed that the effect of the dummy varied with body mass over days: without the dummy, the increase in duration over time became progressively steeper with increasing body mass, whereas with the dummy, duration still increased across days, but the slope was largely independent of body mass and approximated the pattern of individuals with intermediate weight without the dummy (Figure 4).

All models showed good convergence ($R_{\text{hat}} = 1.00$) and high effective sample sizes ($\text{Bulk_ESS} > 4000$), confirming robust parameter estimation.

Discussion

4.1 Attachment reliability and behavioural classification

Our results indicate that, despite challenges related to attachment methods and measurable effects on some locomotor parameters, telemetry devices can be applied to *Aquarana catesbeiana* without causing pronounced short-term behavioural disruption. Among the attachment strategies tested, only a single configuration remained consistently secured, highlighting the inherent challenge of applying external devices to amphibians, particularly robust and agile species like *A. catesbeiana*. The effectiveness of the custom-fit harness underscores the necessity of species-specific attachment solutions in amphibian biologging and provides a practical reference for future studies targeting medium- to large-bodied anurans.

We successfully classified key locomotor behaviours using tri-axial accelerometer data, achieving high agreement with video observations. Classification accuracies were comparable to those reported in other biologging studies, where validation accuracies typically range from 80% to 100% (Bourke et al., 2016; Pavese et al., 2022). These results support the feasibility accelerometer-based behavioural inference in field studies, where direct observation or video validation is often impractical or impossible.

4.2 Mode- and size-dependent effects of external loading

External loading influenced locomotor energetics in a mode-specific manner. Jumping performance appeared largely resilient to the added load, whereas swimming showed a clearer energetic response. This contrast reflects fundamental biomechanical differences

between terrestrial and aquatic movement. During jumping, VeDBA decreased with increasing body mass in the absence of the dummy but increased in heavier individuals when the dummy was present. This suggests heavier individuals may better employ compensatory strategies to maintain jumping performance under external load, whereas lighter individuals experience a relatively higher burden (Azizi and Roberts, 2010).

In swimming, VeDBA increased with body mass in both experimental conditions; however, the energetic difference between frogs tested with and without the dummy was strongly mass dependent. Lighter individuals showed a pronounced increase in VeDBA when carrying the dummy compared to the control condition, likely reflecting a relatively greater hydrodynamic resistance imposed by the device, whereas heavier individuals exhibited similar VeDBA values irrespective of device presence, suggesting a higher tolerance to the added drag (Matley et al., 2024). Additionally, the progressive decline in VeDBA over time suggests adaptive responses to the external load, possibly involving behavioural compensation such as altered swimming kinematics to reduce drag or energy expenditure (Matley et al., 2024).

The absence of significant effects on jump and swim frequency suggests that the device did not significantly impair movement initiation. However, the interaction between dummy presence and body mass on jump duration indicates a clear mass dependent compensatory response. Predicted values show that, in the absence of the dummy, jump duration slightly decreased with increasing body mass, whereas when the dummy was present, heavier individuals performed longer jumps. This pattern suggests that larger frogs were able to adjust their jumping performance to offset the additional load, while smaller individuals showed limited capacity for such compensation. In swimming, our results revealed a progressive increase of over the test days across

individuals, suggesting a general habituation effect. This trend was further modulated by body mass: heavier individuals exhibited a steeper increase in movement duration over time, possibly due to better physical capacity. However, this mass dependent pattern differed markedly depending on dummy presence. The three-way interaction indicated that, without the dummy, the increase in swimming duration across days became progressively steeper with increasing body mass, indicating enhanced performance gains in larger individuals. In contrast, when frogs carried the dummy, swimming duration still increased over time, but the slope was largely independent of body mass and closely resembled the pattern observed in individuals of intermediate weight under control conditions. This suggests that external loading constrained the extent to which heavier individuals could capitalise on their greater body mass to enhance swimming performance over time, effectively homogenising behavioural responses across size classes.

The analytical framework used here allowed for robust modelling of individual variation and hierarchical effects, providing a nuanced understanding of how body mass, device presence, and time interact to influence locomotor performance. One limitation of this study, however, is the assumption that the accelerometer alone, without the dummy load, has little to no influence on behaviour. While this allowed us to isolate the effects of additional external load, it does not account for subtler behavioural shifts caused by the attachment itself. Future studies should include control conditions to assess whether the presence of an accelerometer alone influences locomotor performance.

Long-term studies are also needed to evaluate whether animals fully compensate for external loading over time.

4.3 Implications for amphibian biologging

Overall, our findings demonstrate that external telemetry devices can be successfully deployed on large anurans using a species-specific attachment strategy, while inducing only moderate and context-dependent effects on locomotor performance. Among the tested attachment configurations, a custom-fit harness based on limb and waist fixation proved to be the only reliable solution, underscoring the importance of tailored attachment designs for amphibian biologging, particularly in robust and agile anuran species.

By integrating tri-axial accelerometer with video validation, we show that accelerometer data can reliably discriminate key locomotor behaviours in anurans, providing a robust framework for behavioural inference in situations where direct observation is not feasible. This represents an important methodological advance for amphibian movement ecology, a field that remains underrepresented in biologging research.

The effects of external load on locomotion were strongly mode- and size-dependent. Jumping performance was largely resilient to the added mass, with heavier individuals showing clear compensatory responses in both energetic output and movement duration when carrying the dummy. In contrast, swimming was more sensitive to external loading, particularly in smaller individuals, which exhibited higher energetic costs when equipped with the device. These results highlight the role of body mass in mediating the biomechanical and hydrodynamic consequences of external attachments, suggesting that smaller individuals experience proportionally greater constraints, while larger frogs are better able to buffer the effects of added drag and mass. Importantly, the progressive reduction in swimming energetic expenditure and the increase in movement duration across test days indicate partial behavioural adjustment to the external load over short

timescales. However, the presence of the dummy appeared to limit the extent to which larger individuals could further improve swimming performance over time, suggesting that external devices may constrain longer-term performance gains even in individuals with greater physical capacity.

In addition to locomotor effects, future biologging studies on anurans should also consider behavioural dimensions that were beyond the scope of our experimental design. In species characterised by scramble competition, externally attached devices could potentially interfere with mating performance by reducing agility during male-male interactions or affecting courtship dynamics. Although our controlled laboratory trials did not allow us to evaluate such effects, this remains an important ecological parameter to assess in future field-based validations. Likewise, external attachments may influence the ability of individuals to enter narrow refugia or use shelter microhabitats effectively, which could alter daily activity rhythms or predation risk. Incorporating these behavioural endpoints into future tests will be crucial for an evaluation of biologging impacts.

Taken together, these results emphasise that evaluating the impact of biologging devices in amphibians requires simultaneous consideration of locomotor mode, body size, and temporal dynamics. While short-term behavioural disruption appears limited under the conditions tested here, extrapolation to long-term deployments and field conditions should be approached cautiously. Continued efforts toward device miniaturisation, hydrodynamic optimisation, and long-term field-based validation will be essential to ensure both data quality and animal welfare in amphibian biologging studies.

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Supplementary

Statistical analysis

Data distribution

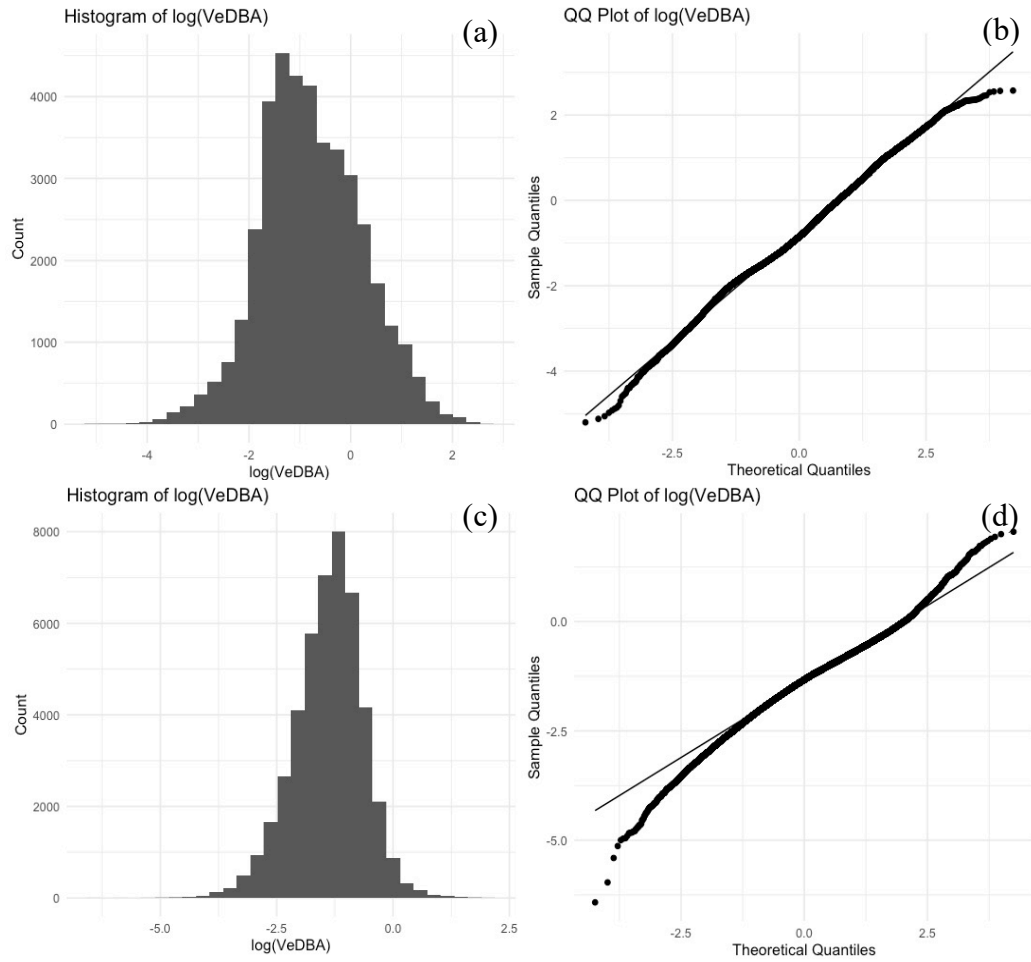


Figure S1. Distribution and normality assessment of log-transformed VeDBA values for jumping and swimming behaviours in *Aquarana catesbeiana*. (a) Histogram of $\log(\text{VeDBA})$ for jumping; (b) QQ plot of $\log(\text{VeDBA})$ for jumping; (c) Histogram of $\log(\text{VeDBA})$ for swimming; (d) QQ plot of $\log(\text{VeDBA})$ for swimming. Histograms illustrate the frequency distribution of log-transformation VeDBA values, while QQ plots assess their normality by comparing empirical quantiles to theoretical quantiles.

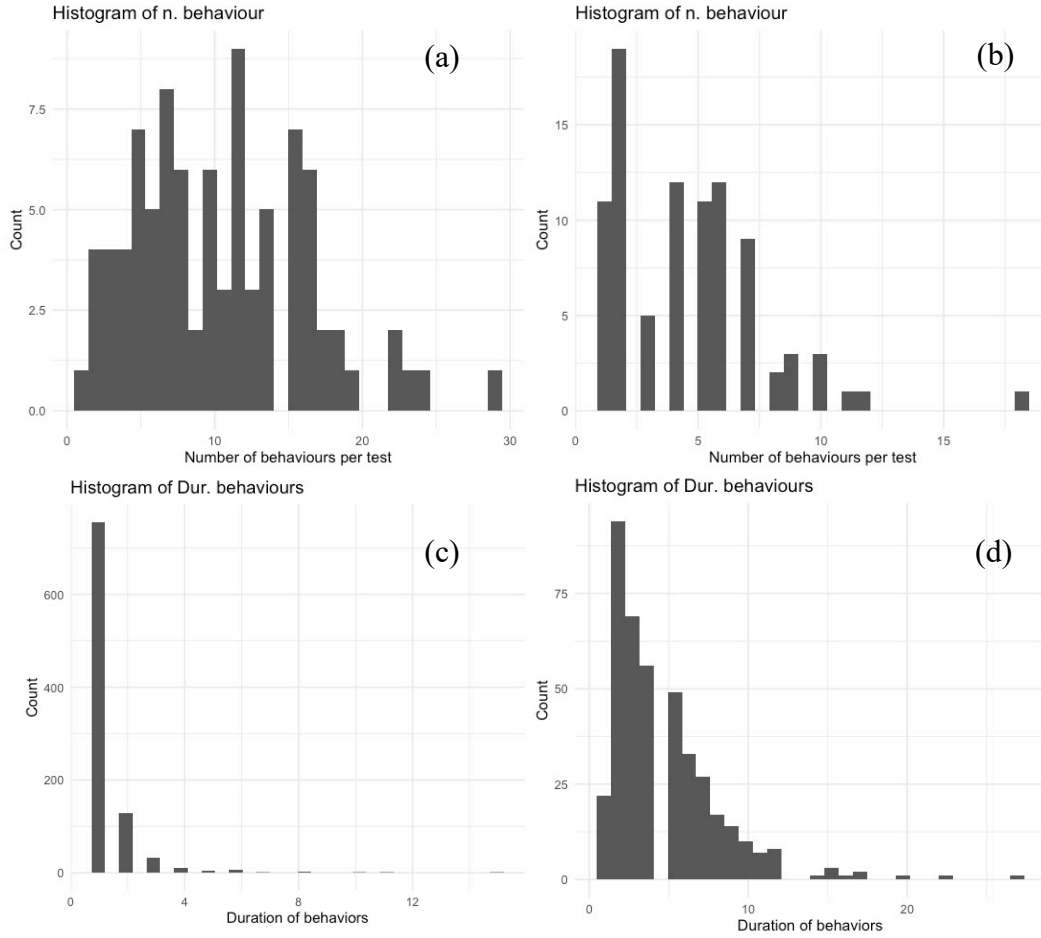


Figure S2. Frequency and duration distributions of jumping and swimming behaviours in *Aquarana catesbeiana*. (a) Histogram of the number of jumping events; (b) Histogram of the number of swimming events; (c) Histogram of the duration of jumping events; (d) Histogram of the duration of swimming events. Movement count distribution was assessed by comparing mean and variance; when variance exceeded the mean, a negative binomial distribution was used; otherwise, a Poisson distribution was applied. Specifically, jump frequency was modelled with a negative binomial distribution (mean = 10.49; var = 32.21), while swimming movement count followed a Poisson distribution (mean = 4.62; var = 9.09). For movement duration, given that duration is a continuous positive variable, a Gamma distribution with a log link function was chosen.

Model Assumptions

VeDBA jump

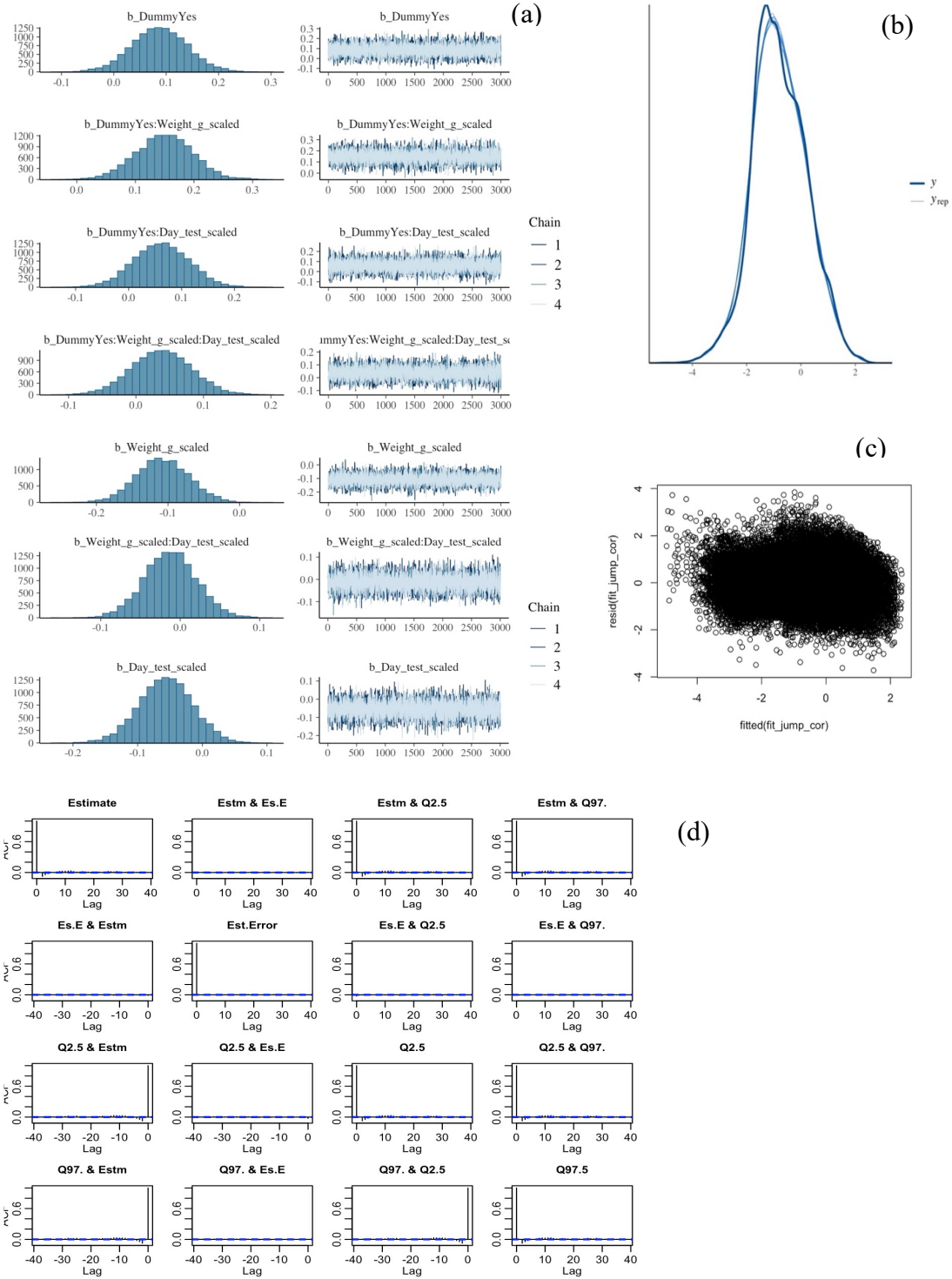


Figure S3. Diagnostic plots for the Bayesian model analysing the *VeDBA* of jumps performed by the animals. These plots assess key model assumptions and the adequacy of the Bayesian framework. (a) Posterior distributions and trace plots for the fixed effects of the Bayesian model. The posterior distributions appear approximately Gaussian, and the trace plots show good mixing across all four chains, with no evident signs of lack of convergence. These results suggest that the MCMC sampling was effective and that the model parameters are well estimated. (b) Posterior predictive check

based on overlapping density plots. The black curve represents the empirical distribution of the observed $\log(\text{VeDBA})$ values, while the blue curves show the distributions simulated from the model's posterior predictions. A strong overlap between the two indicates that the model adequately captures the observed data. (c) Residuals vs. fitted values plot for the Bayesian model ($\text{AR}(1)$, normal distribution on $\log(\text{VeDBA})$). The relatively homogeneous distribution of residuals suggests that the assumption of homoscedasticity is reasonably met, although some outliers are present at the margins. No clear systematic patterns are observed, indicating an adequate model specification. (d) Autocorrelation function (ACF) plots of the model residuals. The plots indicate low autocorrelation at all lags except for the first, which is expected given the $\text{AR}(1)$ structure specified in the model. These results suggest that the temporal correlation in the data has been effectively accounted for.

VeDBA swim

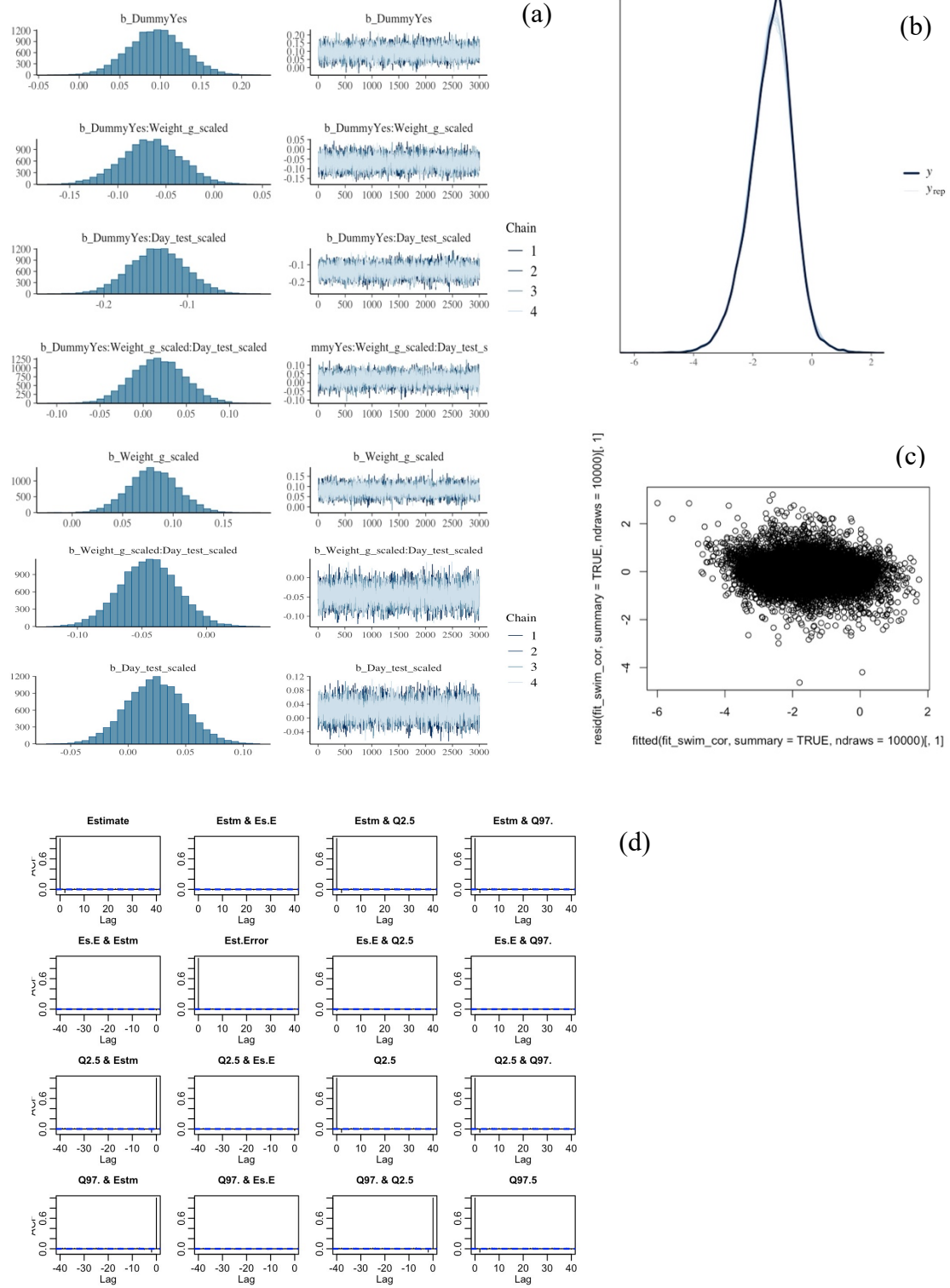


Figure S4. Diagnostic plots for the Bayesian model analysing the VeDBA of swimming performed by the animals. These plots assess key model assumptions and the adequacy of the Bayesian framework. The details of panels (a), (b), (c), and (d) are analogous to those in Figure S3, which refers to the jumping model.

Jump frequency

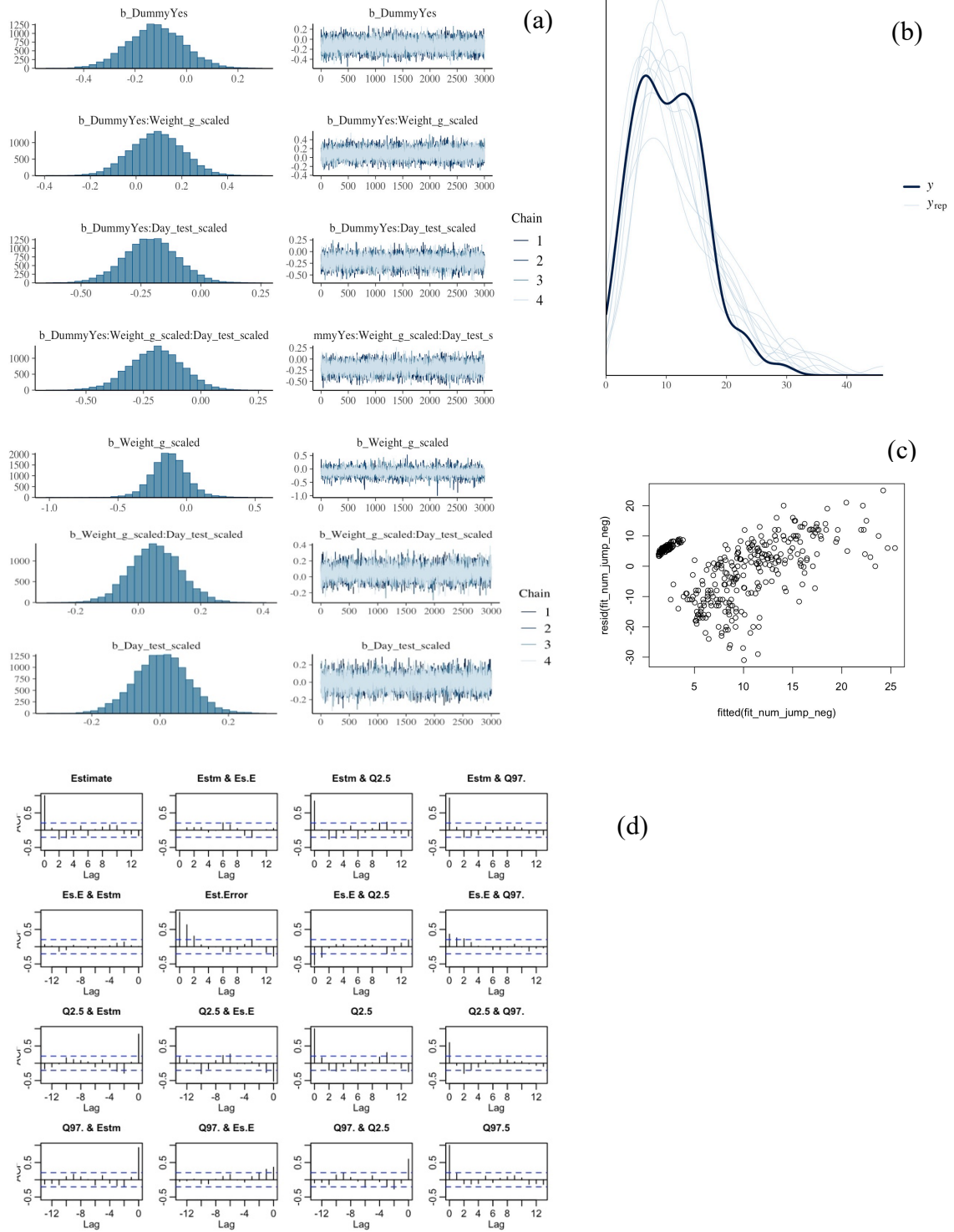


Figure S5. Diagnostic plots for the Bayesian model analysing the number of jumps performed by the animals. These plots assess key model assumptions and the adequacy of the Bayesian framework. The details of panels (a), (b), (c), and (d) are analogous to those in Figure S3.

Swim frequency

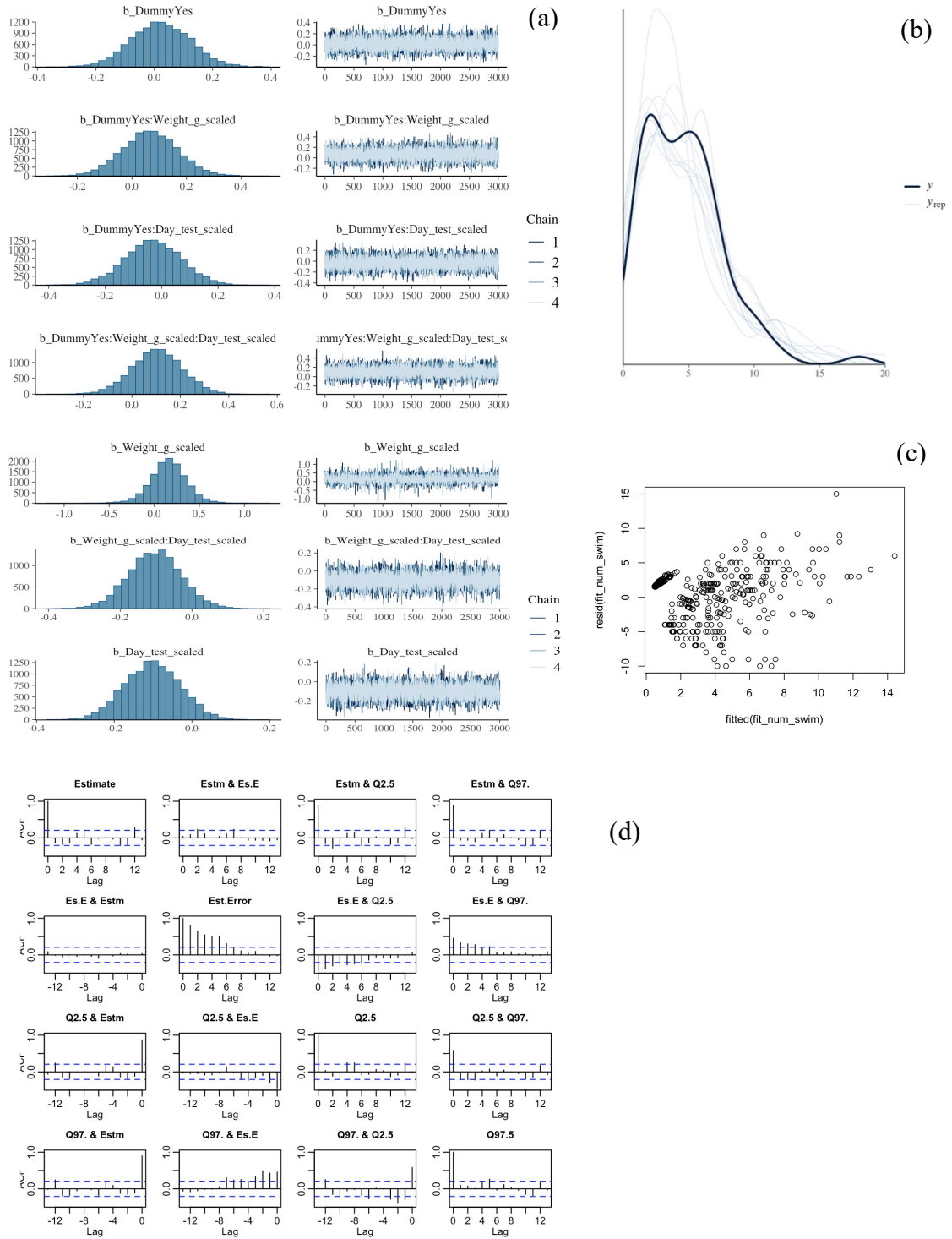


Figure S6. Diagnostic plots for the Bayesian model analysing the number of swimming performed by the animals. These plots assess key model assumptions and the adequacy of the Bayesian framework. The details of panels (a), (b), (c), and (d) are analogous to those in Figure S3.

Jump duration

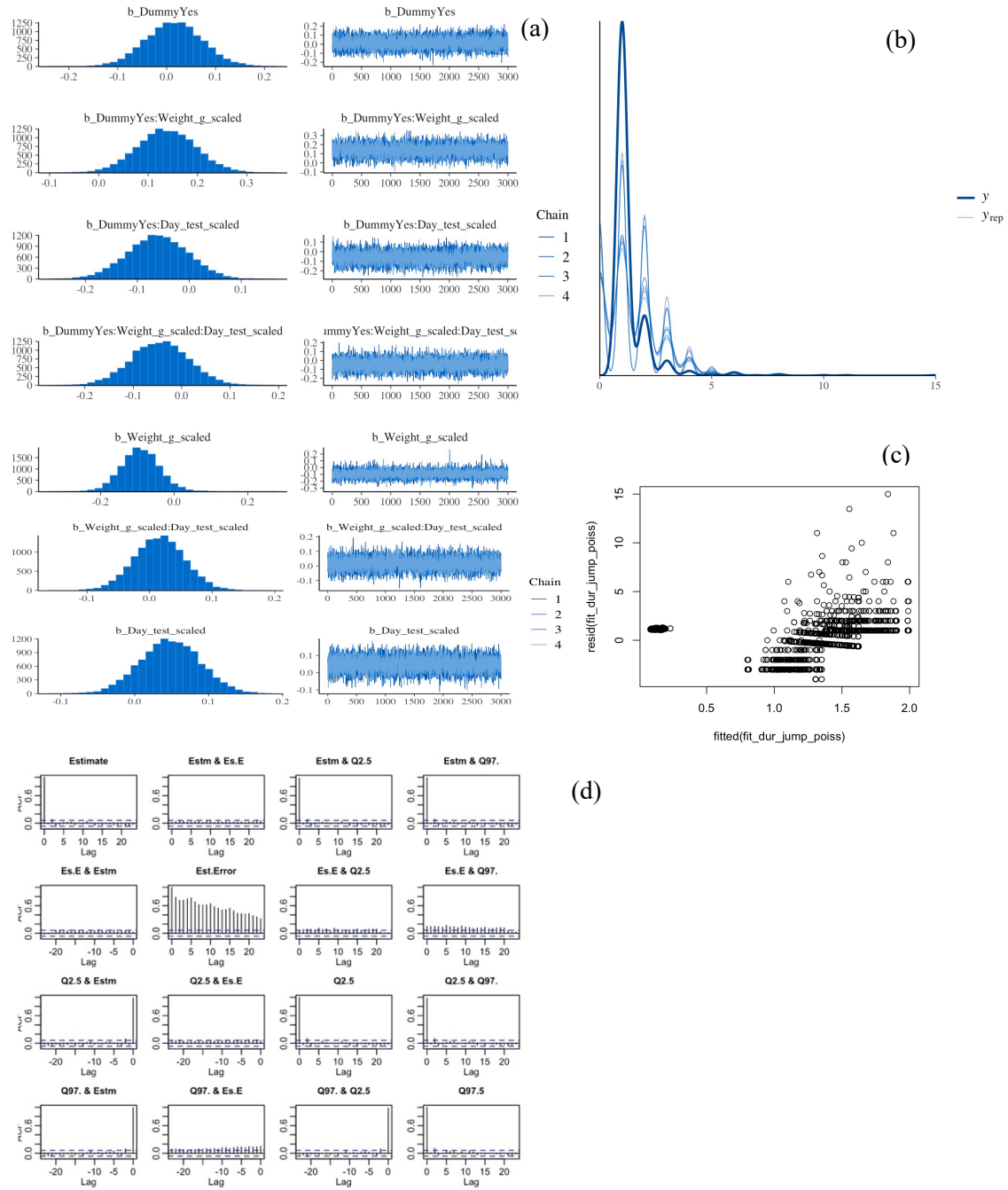


Figure S7. Diagnostic plots for the Bayesian model analysing the duration of jumps performed by the animals. These plots assess key model assumptions and the adequacy of the Bayesian framework. The details of panels (a), (b), (c), and (d) are analogous to those in Figure S3.

Swim duration

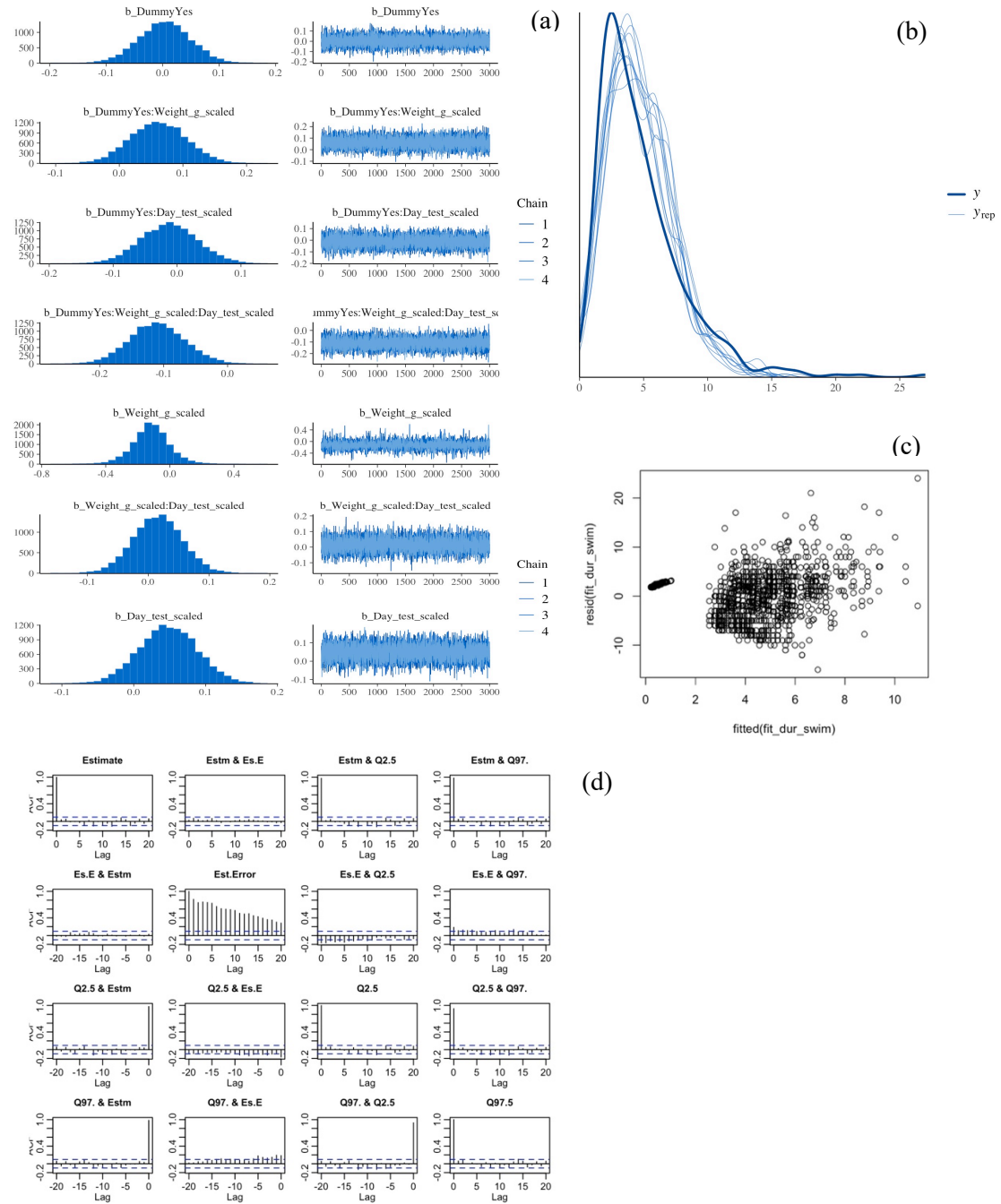


Figure S8. Diagnostic plots for the Bayesian model analysing the duration of swimming performed by the animals. These plots assess key model assumptions and the adequacy of the Bayesian framework. The details of panels (a), (b), (c), and (d) are analogous to those in Figure S3.

Weight information

Table S1. Information regarding the weights of the animals and their respective harnesses used in the study. The table includes the following columns: (i) Identifier assigned to each specimen, (ii) Sex of the specimen, (iii) Weight of the specimen in grams, (iv) Weight of the harness attached to each specimen in grams, (v) Percentage ratio of the harness weight to the animal weight.

ID	Sex	Animal weight (g)	Harness weight without accelerometer (g)	Harness-Animal weight ratio without accelerometer (%)	Harness weight with accelerometer (g)	Harness-Animal weight ratio with accelerometer (%)
LC01	F	299.22	10.55	3.53	14.55	4.86
LC02	F	221.73	10.12	4.56	14.12	6.37
LC03	F	496.21	10.64	2.14	14.64	2.95
LC04	M	472.67	10.59	2.24	14.59	3.09
LC05	F	480.93	10.64	2.21	14.64	3.04
LC06	M	376.70	10.56	2.80	14.56	3.86
LC07	M	171.45	10.05	5.86	14.05	8.19
LC08	F	125.39	9.91	7.90	13.91	11.09
LC09	M	126.70	9.91	7.82	13.91	10.98

Data Availability

Data will be made publicly available upon publication of the corresponding manuscript.

Currently, all datasets and scripts are stored in a private GitHub repository (URL: https://github.com/BombinaPN/bullfrog_test), accessible upon request.

Chapter III - To track or not to track? Evaluation of the influence of tracking devices on toads.

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Abstract

Understanding how biologging devices affect small vertebrates is crucial for balancing research needs with animal welfare, particularly in conservation-sensitive taxa such as amphibians. Despite their ecological importance and global declines, amphibians remain largely excluded from biologging studies, limiting the data available for effective management.

Here, we experimentally evaluated the behavioural and spatial effects of externally attached tracking devices in three anuran species (*Bombina variegata*, *Bufo viridis*, and *Bufo bufo*), under both aquatic and terrestrial conditions. Individuals were monitored with

and without devices across multiple days to test for short-term impacts on behaviour and potential habituation. We analysed movement metrics using heatmaps, PCA, and mixed models, while locomotor behaviours were classified from tri-axial accelerometer data using both unsupervised (K-means) and supervised (Random Forest) approaches.

Our results show that locomotor performance and space use remained largely unaffected by device presence, even when tag mass exceeded the conventional 10% threshold. Only minor inter-individual variability was observed, with no systematic behavioural disruptions at the species level. Random Forest classification achieved >96% accuracy, and species-specific VeDBA thresholds derived from clustering further confirmed the reliability of accelerometer-based behavioural inference.

By demonstrating both the feasibility and minimal short-term impacts of biologging in small- to medium-sized amphibians, our study challenges overly conservative assumptions about device constraints and provides a validated workflow for behavioural and movement analysis. Beyond methodological advancement, we highlight how biologging can deliver critical insights into movement ecology, home range, activity budgets, and phenology in amphibians—dimensions of their natural history that remain understudied but are vital for evidence-based conservation planning and management.

Introduction

Monitoring amphibians with tracking devices is a key approach for understanding their behaviour and habitat use, generating essential data for conservation. Amphibians are among the most threatened vertebrates worldwide, with 41% of species at risk of extinction, primarily due to habitat loss, emerging diseases, and climate change (Re:wild, Synchronicity Earth, 2023). To design effective conservation strategies, it is critical to

understand their movements and ecological requirements across the full range of environments they use during their life cycle, thereby avoiding the neglect of key habitats. Tracking methods such as radio-telemetry and harmonic radar have been successfully applied to amphibians to investigate movement patterns and thermal preferences (Borzée et al., 2016; Forget-Klein & Green, 2021). However, few studies have explicitly assessed the behavioural impacts of these tools (Blomquist & Hunter, 2007; Dervo et al., 2010; Gourevitch & Downie, 2018). Most rely on the widely adopted guideline that device mass should not exceed 10% of body mass (Heyer et al., 1994), assuming this threshold is sufficient to minimise negative effects. Yet, this “10% rule” may not be universally appropriate: in some cases, it may fail to prevent behavioural alterations, while in others it may be unnecessarily restrictive, not taking full advantage of the animal's ability to tolerate greater weight and limiting the deployment of more advanced devices.

Species differ in their morphology, ecology, and physiology, and these differences likely shape their tolerance to external tags. Identifying species-specific limits would allow researchers to exploit the benefits of modern biologging technologies such as accelerometers, thermometers, and magnetometers. These devices not only track position and movements, but also enable the study of behaviours, activity rhythms, and microclimatic conditions suitable for moving. Larger devices may also carry longer-lasting batteries, extending monitoring periods and improving our capacity to capture seasonal and long-term ecological processes.

The aim of this study is to evaluate whether tracking devices, specifically radio transmitters, harmonic radar tags, and tri-axial accelerometers, affect the behaviour of three anuran species of different body mass (*Bufo bufo*, *Bufotes viridis*, and *Bombina variegata*), under controlled conditions. By comparing behaviour with and without

devices, we assess the extent of any short-term changes and the potential to habituation. We further explore interspecific differences in tolerance, with the goal of identifying species-tailored solutions that maximise information gain without compromising animal welfare. This study addresses a significant gap in amphibian research, providing methodological guidance to improve monitoring approaches. The results will help ensure that biologging data accurately reflect natural behaviour and can be reliably used to inform conservation strategies that respond to the ecological needs of threatened amphibians.

Methods

2.1 Species and study areas

The experiments were conducted on three anuran species with contrasting ecological, morphological and locomotor traits: *Bufo bufo* (Linnaeus, 1758), *Bufotes viridis* (Laurenti, 1768), and *Bombina variegata pachypus* (Linnaeus, 1758). *Bufo bufo* is a large, mainly nocturnal toad (male SVL >10 cm, females up to 15 cm; Arnold & Ovenden, 2002), primarily terrestrial and moving by walking or short hops, inhabiting forests, wetlands, and anthropogenic areas (Trochet et al., 2019). *Bufotes viridis* is smaller (SVL 5-9 cm), adapted to dry habitats (Lanza et al., 2007), and alternates walking with moderate jumps. *Bombina variegata* is the smallest (SVL 3-5 cm; Plytycz & Bigaj, 1993), strongly tied to humid, montane or hilly habitats with temporary ponds or slow-flowing waters, and typically moves by short hops.

We selected these species to represent a gradient of body sizes, ecological adaptations, and locomotion styles, thereby enabling a comparative evaluation of device effects on movement in controlled conditions.

In addition to these broad ecological and locomotor differences, the three species also span functional trait gradients that are directly relevant to predicting tag impact. *Bufo bufo* exhibits relatively high fossorial tendencies and frequently uses burrows or tight subterranean refugia (e.g., rodent holes, root cavities), where external devices may increase friction or reduce the ability to fully enter narrow shelters. Its preferred habitats typically include dense leaf litter and understory vegetation, conditions in which protruding devices may snag or alter natural movement paths. *Bufotes viridis*, by contrast, occupies more open and sparsely vegetated habitats, with limited use of deep refugia and a predominantly surface-active lifestyle, suggesting a potentially lower mechanical interference from devices during routine movements. *Bombina variegata*, although semi-aquatic and less fossorial, relies on shallow water bodies, muddy substrates, and microrefugia such as crevices in rocks or vegetation mats; in such contexts, external tags could modify buoyancy, increase drag, or hinder access to tight aquatic shelters.

2.2 Field collection

A total of 18 adult individuals (six per species) from distinct populations were collected in 2024. *Bufo bufo* was sampled in June from the Natural Reserve of Insugherata (Rome, Italy; 41°57'01''N, 12°26'09''E), *Bufotes viridis* in April from Castel di Guido (Rome, Italy; 41°53'33''N, 12°16'57''E), and *Bombina variegata* in August from the Monti Lucretili hybrid population (42°05'41''N, 12°53'23''E), which includes genetic traits from both *B. v. variegata* and *B. v. pachypus* (Talarico et al., 2020).

Animals were transported to the Fondazione Bioparco di Roma and housed in species-specific terraria under controlled conditions. *Bufo bufo* and *Bufotes viridis* were kept in semi-dry enclosures with hiding spots and a water source, while *B. variegata* was

maintained in a humid setup with shallow water. All animals were acclimated for two days before experiments and were fed live invertebrates (crickets and mealworms). Body mass was measured with a digital scale (precision: 0.01 g).

All procedures complied with permits issued by the Italian Ministry of the Environment and Energy Security (authorisation no. 2023.0043372).

2.3 Experimental procedure

Each individual was tested for five consecutive days in two settings: a dry arena and an aquatic arena. Daily trials consisted of 5 minutes in a dry glass tank (100 × 60 × 60 cm), followed by 5 minutes in a water-filled tank. Water depth was adjusted for species-specific swimming style: 5 cm for *Bombina variegata*, 10 cm for *Bufo viridis*, and 20 cm for *Bufo bufo*. Arenas were substrate-free to standardise conditions and isolate locomotor behaviour, with a white panel placed beneath to improve contrast for video analysis.

Initially, trials were performed without devices for five days. Animals were then equipped with a miniature tracking device using a custom harness and allowed three days of acclimation in their terraria before repeating the same five-day protocol with the device attached.

All sessions were filmed from above using a ceiling-mounted camera for later locomotor and behavioural analyses.

2.4 Devices and attachment methods

Bufo bufo and *Bufo viridis* were equipped with identical custom-built devices (Axy 5, Technosmart Europe srl, Rome, Italy) integrating VHF transmitters with accelerometers, magnetometers, and temperature sensors (mass: 2.92 g). These devices allow for tracking

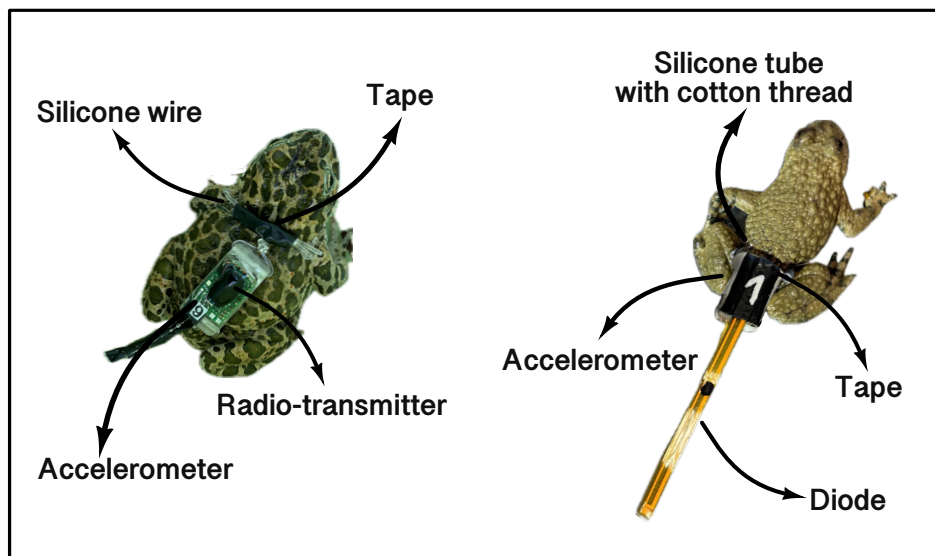
individuals via radiotelemetry while also recording behavioural data when animals are not actively tracked. The devices had two holes on opposite short sides for silicone cord attachment (1 mm, Jinsion Crystal Tec®). One cord looped around the waist; another looped around each forelimb, secured with reef knots reinforced by waterproof tape. This harness design placed the device dorsally and centrally, ensuring reliable acceleration data. The use of two independent cords allows the harness to detach through the degradation of the anterior tape, which loosens the front knot and enables the device to slide off, ultimately resulting in the complete release of the harness. Silicone minimises abrasion, while tape degrades over time, enabling eventual self-release in case of non-recapture (Figure 1).

For *Bombina variegata*, we used a lighter device (1.29 g, Technosmart Europe srl), including an accelerometer, magnetometer, and temperature sensor, but no VHF. Preliminary trials showed dorsal mounting impaired swimming, so we designed a waist-belt harness to position the device between the hind limbs. Silicone cord caused skin abrasions within 24 hours; therefore, we adopted a silicone tube (Patkil®, inner diameter: 1.5 mm, outer diameter: 2.5 mm) custom-fitted to each individual and secured with cotton thread. Each device also carried a RECCO diode (0.03 g) to facilitate field recovery (Figure 1).

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Figure 1. On the left, a *Bufo viridis* individual equipped with a device composed of a tri-axial accelerometer and a VHF radio-transmitter, attached dorsally using two 1-mm diameter silicone wires secured with waterproof tape. On the right, a *Bombina variegata* individual wearing a tri-axial accelerometer around the waist, fixed using a silicone tube with a cotton thread inside; a RECCO diode is attached to the accelerometer using waterproof tape.

2.5 Data analysis



Video recordings were processed with AnimalTA (Chiara & Kim, 2023), selecting for each video the optimal settings to enhance contrast between the animal and the background (see Data Availability for details). The generated trajectory was visually

inspected and manually corrected when necessary. From validated tracks, we obtained speed and the x-y coordinates.

Videos were also analysed in BORIS (Friard & Gamba, 2016) to mark movement and immobility periods, allowing calculation of discrete movement events.

Analyses were performed separately for dry and aquatic arenas. Two behavioural metrics were tested: mean speed and number of movement events. Speed (log-transformed) was modelled with linear mixed-effects models including device condition (presence/absence), body weight (standardised), test day (standardised), and species as fixed effects, and individual as a random effect. Movement events (count data) were analysed using negative-binomial GLMMs.

Model assumptions were verified by inspecting residual distribution (histograms and QQ-plots) and by checking for influential outliers ($|z| > 3$). Multicollinearity among predictors was checked with Variance Inflation Factor (VIF; threshold = 5; Fox & Monette, 1992). Model selection followed an AIC-based dredging approach across all fixed effect combinations. Significant species interactions were explored with post hoc comparisons using estimated marginal means (EMMs), with Tukey-adjusted p-values. Finally, to assess whether the impact of the device depended on relative tag load, we ran species-specific generalised linear models (GLMs) with log-transformed speed as the response variable with device presence and relative device mass (% of body weight) including their interaction as predictors. These analyses allowed us to assess whether heavier relative loads produced measurable behavioural alterations within species.

To examine spatial behaviour, we generated heatmaps of activity and extracted six variables: (i-ii) weighted x-y means (activity centroids; i.e., where the animal spent most of its time); (iii-iv) x-y variance (spatial dispersion; i.e., how broadly individuals explored

the arena); (v) maximum density (site preference; i.e., lower values reflecting uniform movement across the arena and higher values indicating preferred areas or localised activity) and (vi) active area (number of cells accounting for 80% of activity; i.e., lower values suggesting limited activity to specific locations and higher values indicated widespread use of the arena). Representative examples of individual heatmaps are shown in Figure S1, using one *Bufo viridis* individual as an example in both dry and aquatic arenas, with and without the device. All other individual heatmaps are available in the Data Availability section. These variables were summarised by PCA, and PCs were analysed with mixed-effects models as above.

Finally, we combined annotated behaviour with tri-axial acceleration data (after calibration; Garde et al., 2022; Nesi et al., Under review) to validate accelerometry as a proxy for movement recognition in the field. Accelerometer validation involved two approaches. First, Vectorial Dynamic Body Acceleration (VeDBA) was calculated as the square root of the sum of the three components of dynamic acceleration (Gleiss et al., 2011). Then, (i) K-means clustering was used to derive species-specific thresholds of VeDBA discriminating movement vs. non-movement. This threshold provides a simple and interpretable criterion for detecting movement events based on sensor data alone; and (ii) Random Forest classification, using VeDBA, dynamic acceleration on three axes, and interactions as predictors. Data were randomly split into 80% training and 20% test sets, ensuring that the model could be evaluated on previously unseen data. Class imbalance (fewer movement events) was corrected by weighting categories (movement:non-movement = 5:0.5). This improved detection of true movement events and increased model reliability. Model performance was evaluated using accuracy, balanced accuracy, sensitivity, specificity, and the Area Under the Receiver Operating Characteristic Curve

(AUC), which quantifies the model's ability to discriminate between movement and non-movement events (values closer to 1 indicate better discrimination).

Results

3.1 Device burden across species

We tested 18 adult individuals, six per species. As expected, interspecific variation in body size strongly influenced the relative burden of the tracking device. *Bufo bufo* was the largest species (185.00-448.37 g; mean $\pm$ SD: 318.10 ± 92.07 g), for which the device represented only 0.65-1.58% of body mass (mean: $0.99 \pm 0.33\%$). *Bufo viridis* exhibited intermediate body sizes (21.72-42.17 g; mean $\pm$ SD: 31.74 ± 7.30 g), with relative device weights of 6.92-13.44% (mean: $9.64 \pm 2.33\%$). *Bombina variegata* was the smallest (5.75-8.53 g; mean $\pm$ SD: 6.92 ± 0.98 g), and even the lightest device available (1.32 g) imposed a substantial burden: 15.36-22.78% of body mass (mean: $19.25 \pm 2.64\%$; Table S1).

3.2 Locomotor speed

Model selection results are reported in Table S2. Here, we summarised the final models. In dry arena trials, mean locomotor speed (log-transformed) was significantly affected by species identity (*B. variegata* - *B. bufo*: Estimate = 0.618, $p < 0.001$; *B. bufo* - *B. viridis*: Estimate = - 0.638, $p < 0.001$) and by test day (Estimate = 0.107, $p = 0.007$; Figure S2). No effect of device or device-related interactions was detected.

In aquatic trials, speed was influenced by both species (*B. variegata* - *B. bufo*: Estimate = -0.679, $p = 0.009$; *B. bufo* - *B. viridis*: Estimate = 0.953, $p < 0.001$) and test day (Estimate = 0.114, $p = 0.026$; Figure S3). For all species, device presence alone had no main effect, but its interaction with test day was significant (Estimate = -0.202, $p = 0.005$;

Figure S3). Furthermore, a significant device x species interaction emerged: swimming speed decreased with the device in *B. bufo* (Estimate = 0.516, $p < 0.0001$) and *B. viridis* (Estimate = 0.405, $p = 0.001$), but not in *B. variegata* (Figure S3).

When analysing each species separately, we detected species-specific thresholds in tag-to-body mass ratio. In *B. bufo*, swimming speed was significantly reduced at low (0.65%: Estimate = 1.422, $p = 0.0001$) and intermediate ratios (1.01%: Estimate = 1.189, $p = 0.0007$), but not at higher ones. In *B. viridis*, swimming speed declined significantly at higher ratios (10.20%: Estimate = 0.684, $p = 0.017$; 13.44%: Estimate = 1.115, $p < 0.001$). In *B. variegata*, effects were inconsistent: reduced speed was detected at an intermediate ratio (18.4%: Estimate = 0.736, $p = 0.0005$), whereas other comparisons were non-significant or even reversed (e.g., faster swimming at 21.6%: Estimate = -0.453, $p = 0.026$; Figure 2).

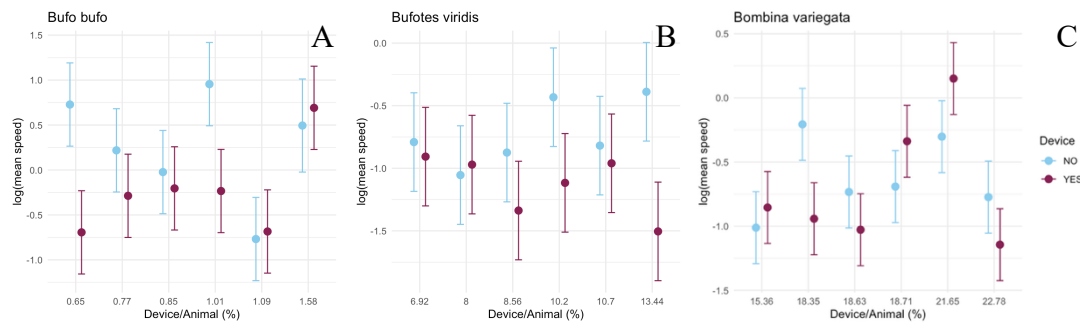


Figure 2. Effects of tag-to-body weight ratio on mean swimming speed in (A) *Bufo bufo*, (B) *Bufotes viridis*, and (C) *Bombina variegata*. Points represent estimated means $\pm$ 95% confidence intervals for individuals tested with (purple) and without (blue) a device, order by individual tag-to-body weight percentage.

3.3 Movement frequency

In dry arena trials, movement frequency was significantly influenced by test day (Estimate = 0.236, $p < 0.001$), species identity (*B. variegata* / *B. bufo*: Ratio = 3.723, $p < 0.001$; *B. bufo* / *B. viridis*: Ratio = 0.214, $p < 0.001$), and their interaction (Figure S4). Post hoc tests showed that *B. variegata* exhibited a steeper increase in movement

frequency across days compared to both *B. bufo* (Estimate = 0.182, $p = 0.030$) and *B. viridis* (Estimate = 0.170, $p = 0.018$). A three-way interaction between species, device, and test day indicated that *B. bufo* exhibited a marked decrease in movement frequency across days when wearing the device (Estimate = 0.376, $p < 0.001$), whereas no temporal effect of the device was detected in *B. viridis* or *B. variegata* or (Figure S4).

Device-to-body mass ratio further modulated these patterns. In *B. bufo*, individuals with intermediate ratios showed a significantly sharper decline compared to controls (0.85%: Estimate = 0.489, $p = 0.003$). In *B. viridis*, heavier animals showed more positive activity trends compared to control (8.56%: Estimate = -0.285, $p = 0.021$), while other ratios were non-significant. No ratio effects were detected in *B. variegata* (Figure 3).

In aquatic trials, both the device presence (Estimate = -0.420, $p < 0.001$) and species identity (*B. variegata* / *B. viridis*: Ratio = 1.929, $p = 0.007$; *B. bufo* / *B. viridis*: Ratio = 2.286, $p < 0.001$) significantly influenced movement frequency (Figure S5).

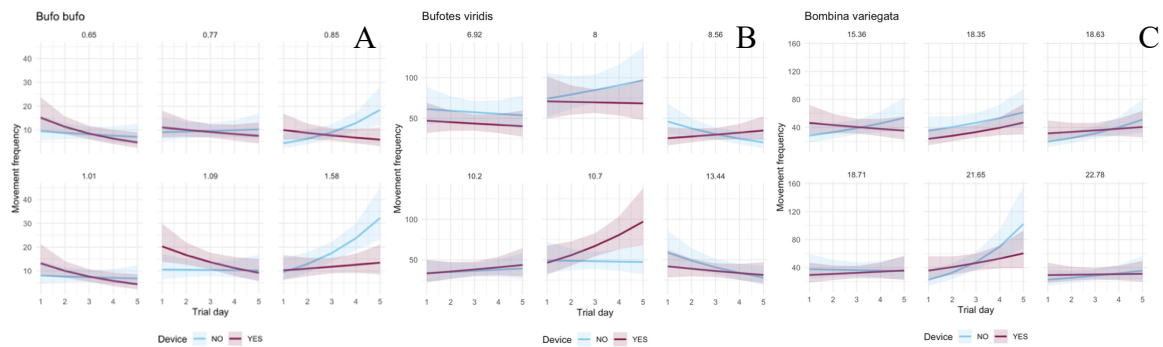


Figure 3. Daily trends in movement frequency during dry arena trials for individuals of (A) *Bufo bufo*, (B) *Bufotes viridis*, and (C) *Bombina variegata*, order by tag-to-body weight ratio. Each subplot shows changes in movement frequency across five consecutive trial days for animals with (purple) and without (blue) tracking devices.

3.4 PCA and analysis of heatmaps

In dry arena trials, the first three PCs explained 81% of the total variance (PC1 = 42.6%, PC2 = 23.6%, PC3 = 14.5%). PC1 was associated with low x-y variance and active area

(loadings: -0.71 to -0.77), PC2 with mean spatial position (x-mean: -0.78; y-mean: -0.64), and PC3 with maximum density (-0.87; Figure 4).

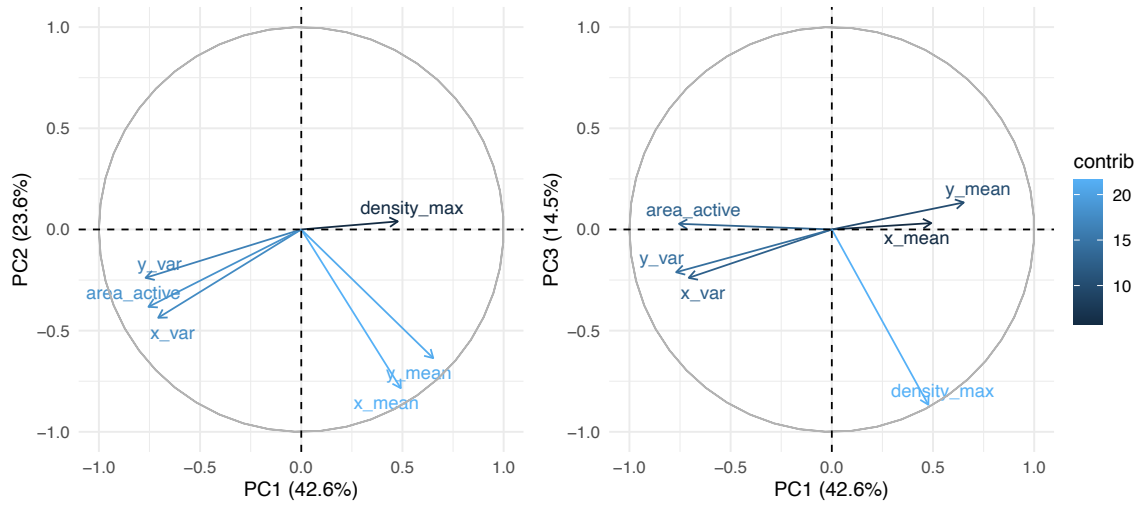


Figure 4. PCA of behavioural variables recorded in dry arena trials. The left plot represents the correlation circle for PC1 and PC2, while the right plot represents PC1 and PC3. Variable vectors indicate the contribution of each behavioural parameter to the principal components, with colour intensity proportional to the variable's contribution.

Linear mixed models revealed that PC1 decreased significantly over time (Estimate = -0.444, $p = 0.007$; Figure S6) and varied among species with *B. bufo* showing higher values than *B. variegata* and *B. viridis* (Estimates = -1.150 and 1.865, $p = 0.044$ and 0.002, respectively; Figure S6). A significant interaction between species and test day was found between *B. variegata* and *B. bufo* (Estimate = -0.647, $p = 0.016$; Figure S6). PC2 was significantly affected by device presence (Estimate = 0.836, $p = 0.005$) and by device x species interaction, with *B. variegata* showing higher PC2 scores when carrying the device (Estimate = -0.836, $p = 0.005$; Figure S6). PC3 did not show significant effects. Device-to-body mass ratio modulated PC2 in *B. variegata* (21.65%: Estimate = -1.694, p

= 0.037) and in *B. viridis* (8.0%: Estimate = -1.553, $p = 0.047$) but not in *B. bufo* (Figure 5).

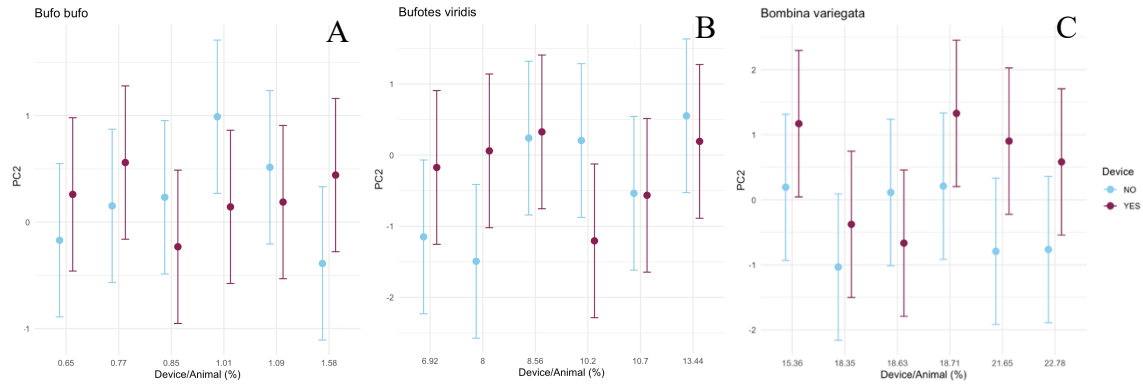


Figure 5. Effects of tag-to-body weight ratio on PC2 during dry arena trials in (A) *Bufo bufo*, (B) *Bufotes viridis*, and (C) *Bombina variegata*. Points represent estimated means $\pm$ 95% confidence intervals for individuals tested with (purple) and without (blue) a device, order by individual tag-to-body weight percentage.

In aquatic arena trials, the first three PCs explained 81% of variance (PC1 = 42.6%, PC2 = 22.4%, PC3 = 15.9%). PC1 was negatively associated with x-y variance and active area (-0.73 to -0.78), and positively with y-mean (0.70); PC2 was driven by x-mean (-0.76), and PC3 by maximum density (-0.95; Figure 6). Device presence significantly affected all three components (PC1: Estimate = 0.719, $p < 0.001$; PC2: -0.346, $p = 0.039$; PC3: 0.083, $p = 0.044$; Figure S7).

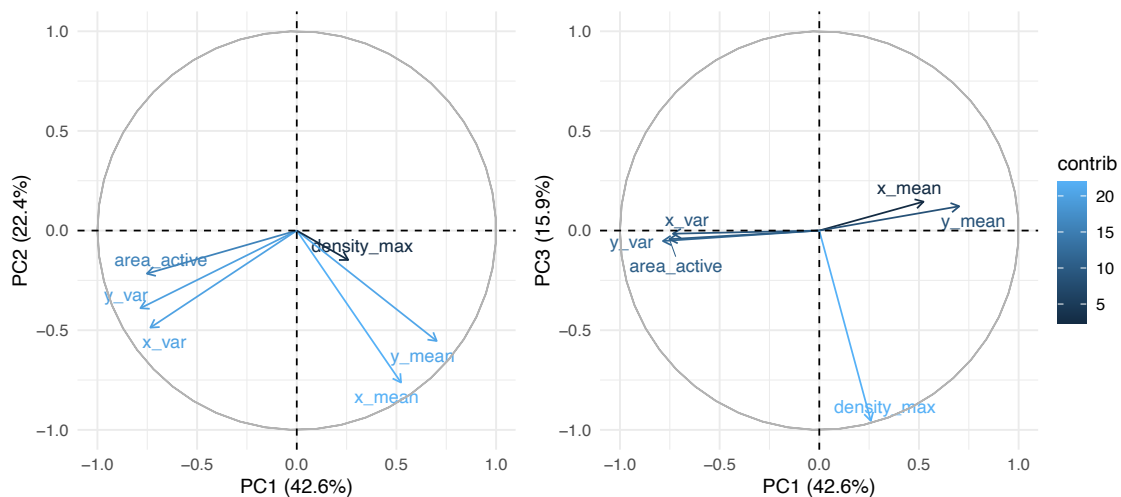


Figure 6. PCA of behavioural variables recorded in aquatic arena trials. The left plot represents the correlation circle for PC1 and PC2, while the right plot represents PC1 and PC3. Variable vectors indicate the contribution of each behavioural parameter to the principal components, with colour intensity proportional to the variable's contribution.

3.5 Validation

K-means clustering of VeDBA values distinguished active vs. inactive bouts with >85% accuracy across species (*B. variegata*: 88.0%, *B. bufo*: 88.2% and *B. viridis*: 85.6%). The VeDBA thresholds were 0.196 (*B. variegata*), 0.092 (*B. bufo*), and 0.109 (*B. viridis*).

Random Forest classification further improved accuracy, achieving >96% in all species (*B. viridis*: 96.2%; *B. bufo*: 97.6; *B. variegata*: 96.8%). Balanced accuracy ranged from 86.2% (*B. viridis*) to 92.3% (*B. bufo*). Sensitivity varied from 73.9% (*B. viridis*) to 85.9% (*B. bufo*), while specificity exceeded 98% in all cases, indicating robust performance despite class imbalance. The models also exhibited excellent discrimination ability, with AUC values of 0.986 for *B. viridis*, 0.993 for *B. bufo*, and 0.991 for *B. variegata*.

Discussion

4.1 Device burden across species

The three tested species differed substantially in body mass, which directly influenced the relative load imposed by tracking devices. This variation allowed us to assess device effects across a broad gradient of weight ratios, including values well above standard recommendations. Current guidelines advise that device mass in amphibians should not exceed 10% of body mass to minimise interference with natural behaviour and locomotion (Heyer et al., 1994). While this threshold was generally respected for *Bufo bufo* and *Bufo viridis*, in the smallest species, *Bombina variegata*, even the lightest device reached 22.8% of body mass. This was a deliberate choice, designed to test whether higher relative weights are tolerated, and to detect possible behavioural thresholds when the 10% rule is exceeded. Such a gradient-based approach provides new

insights into species-specific sensitivity to device burden and highlights the importance of tailoring tracking methodologies to the morphological constraints of each species.

4.2 Locomotor speed

In aquatic trials, swimming performance was impaired by devices in *B. bufo* and *B. viridis*, confirming that external tags can hinder locomotion in water (Bridger & Booth, 2003). In contrast, *B. variegata* showed no reduction in speed, possibly due to the waist-mounted attachment reducing drag and improving balance. These results highlight that not only tag mass, but also attachment position and shape, play a critical role in determining behavioural impact (Longarini et al., 2023; Mizrahy-Rewald et al., 2023; Tian et al., 2020). On land, locomotor speed remained unaffected in all species, suggesting that negative effects were specific to the aquatic environment. This aligns with ecological traits of the study species: *B. bufo* and *B. viridis* are primarily terrestrial outside the breeding season and less adapted to swimming (Ročková & Roček, 2005), making them more sensitive to hydrodynamic drag, whereas the more aquatic *B. variegata*, may compensate more effectively.

Weight-ratio analyses further reveal that in *B. bufo*, even light tags (<1% body weight) impaired swimming speed, suggesting that minimal burdens can affect aquatic performance in species poorly adapted to water. Conversely, *B. viridis* tolerated devices up to 10% before significant effects emerged. In *B. variegata*, responses were inconsistent across weight classes, indicating non-linear and context-dependent effects, likely reflecting the interplay of device shape, buoyancy, and behavioural plasticity. Similar complex patterns have been reported in other taxa, where tag design, position, and

locomotor mode interact in unpredictable ways (Mizrahy-Rewald et al., 2023; Tomotani et al., 2021).

4.3 Movement frequency

In dry arenas, movement frequency was influenced by the interaction between species, device, and test day. In *B. bufo*, tagged individuals showed progressive activity decline, in contrast to increase movement in untagged trials, suggesting persistent discomfort or energetic cost imposed by the carried device. Being terrestrial and slow-moving (Daversa et al., 2012; Sinsch, 1988), the device may have disrupted their natural energy allocation or motivation to explore. The other tested species did not show significant movement frequency alterations over time. These divergent trajectories highlight that tagging effects are species-specific.

Weight-ratio patterns revealed further nuances: in *B. bufo*, intermediate burdens (~0.85%) reduced activity over time; in *B. viridis*, higher ratios (8.6 %) stimulated increased activity; and in *B. variegata*, no ratios produced measurable effects. It should be noted, however, that each individual carried a unique ratio (since device burden scaled with body mass), so these patterns may also partially reflect individual behaviour (i.e. response to harness) rather than purely load-dependent responses. Notably, the waist-mounted harness in *B. variegata* may have mitigated interference, suggesting that placement matters as much as mass. This finding aligns with growing recognition in telemetry studies that tag impact depends not just on mass but on harness design and positioning (Kölzsch et al., 2016; Mizrahy-Rewald et al., 2023).

More broadly, these species-specific trajectories highlight contrasting strategies of behavioural adjustment: *B. bufo* showed persistent reductions in activity, suggesting

limited tolerance; *B. viridis*, in contrast, may initially respond cautiously but later resume normal activity once the device is integrated into its body schema, consistent with its more exploratory disposition (Spießberger et al., 2023); while *B. variegata* maintained stable movement patterns, likely aided by attachment position.

Overall, our results reinforce that device/body mass ratios alone are poor predictors of behavioural impacts. Instead, effects emerge from the interaction of morphology and life-history, harness design, and compensatory responses (Longarini et al., 2023; Mizrahy-Rewald et al., 2023; Tian et al., 2020).

4.4 PCA and analysis of heatmaps

Overall, in both terrestrial and aquatic trials, space use appeared broadly consistent across species, although some effects of device presence were detectable. In dry arenas, the attitude of animals to explore the space decreased over time, reflecting changes in activity distribution, with *B. bufo* showing higher values than the other species. In *B. variegata*, PC2 (representing the spatial position of individuals within the arena) was influenced by the presence of the device, with tagged individuals showing higher scores. This result is not easily interpretable, as the arena was homogeneous and the tendency of individuals to occupy specific positions may reflect idiosyncratic behavioural preferences rather than an effect of the device. The device-to-body mass ratio significantly modulated PC2 at specific ratios in two species (21.65% in *B. variegata* and 8% in *B. viridis*), whereas no such effect was detected in *B. bufo*. However, these effects do not appear to reflect a consistent species-level response: in each of the two species with a significant ratio effect, only one individual out of six showed a marked change in PC2, and no individuals in the third species exhibited such an effect. This pattern suggests that the observed modulation

of spatial position within the arena is more likely driven by individual-level variation in tolerance or sensitivity to the device (e.g. differences in harness fit, behavioural temperament, or prior experience) than by a general species-specific response. In other words, idiosyncratic factors affecting a few individuals, rather than a pervasive effect across conspecifics, probably underlie the significant results at those specific weight ratios.

These results suggest that, while the presence of a device can modulate movement metrics, the general structure of space use is not dramatically altered, even individuals carrying devices >10% of their body mass, indicating that the commonly cited 10% threshold may be overly conservative. These findings reinforce that behavioural tolerance is shaped by individual and species-level traits, as well as by device design and environmental context, rather than by fixed weight ratios alone.

4.5 Validation

K-means clustering on VeDBA values identified distinct species-specific thresholds, with the highest in *B. variegata* (0.196), followed by *B. viridis* (0.109) and *B. bufo* (0.092). Interestingly, while *B. bufo* and *B. viridis* shared similar thresholds (~0.1), *B. variegata* displayed a markedly higher value (~0.2). This discrepancy likely does not reflect true interspecific differences in VeDBA patterns but rather results from the attachment method: waist-mounted attachment in *B. variegata* not only move more compared to back-mounted devices in toads, but may also experience impact against the substrate during locomotion, leading to higher recorded accelerations. Random Forest classification further enhancing accuracy, achieving >96% correct assignment, with high balanced accuracy ($\geq 86\%$) and specificity (>98%) across species. Slightly lower

sensitivity in *B. viridis* (73.9%) compared to *B. bufo* (85.9%) may stem from subtle, fragmented movements that were harder to detect. Overall, the agreement between unsupervised and supervised methods validates our behavioural metrics and demonstrates the robustness of accelerometer-based classification across taxa, highlighting the potential of this protocol for broader use in movement ecology studies of small vertebrates.

4.6 Ecological behaviours: mating performance and refuge access

Beyond locomotor and spatial metrics, device effects should also be evaluated in relation to behaviours with direct ecological relevance, such as mating performance and access to refugia. Although our experimental design was not aimed at quantifying reproductive behaviour, opportunistic observations in *Bombina variegata* showed that amplexus and oviposition occurred even when individuals were equipped with the device. This suggests that, at least in this species, the carried tag did not preclude successful mating interactions nor interfere with the physical positioning required for egg deposition. Whether similar levels of tolerance occur in species with different body plans, mating systems, or habitat use remains an open question, particularly for taxa that rely on narrow shelter microhabitats, where external devices might hinder refuge entry or increase predation risk. Future studies should therefore incorporate these behavioural endpoints to more comprehensively assess the ecological consequences of tagging across species.

Conclusions

Together, these findings demonstrate that tagging effects in amphibians are not dictated by simple weight ratios but result from a complex interplay of morphology, locomotor

ecology, behavioural flexibility, and harness design. Individuals varied in their responses: some showed persistent reductions in activity, others initially responded cautiously but gradually resumed normal activity as they integrated the device, while more agile or behaviourally flexible individuals maintained stable movement patterns. This underscores that behavioural tolerance to devices is context-dependent and mediated by both intrinsic traits and external factors, rather than by fixed thresholds.

Our study demonstrates the strong potential of biologging for small- to medium-sized amphibians, showing that tracking devices, when carefully selected and deployed, can provide valuable insights into movement ecology and behaviour without causing major detrimental or ethically concerning effects, even at relatively high load ratios. Nevertheless, subtle behavioural alterations were detected in some species or individuals, including changes in activity patterns or space use, which may influence behavioural inferences in field studies. These effects were not solely dependent on device weight but were also influenced by attachment method, species-specific traits (morphology, ecology, and life-history), and environmental context. Some species were able to compensate for added load, while others exhibited temporary reductions in activity. These findings underscore the importance of including control groups and evaluating device design and attachment strategies in a species-specific and preferably short-term framework. Although short-term effects appear minimal, long-term consequences, such as energetic costs of behavioural compensation or responses under stressful conditions, cannot be excluded. Overall, our results support the cautious but effective use of biologging in amphibians, enabling robust behavioural and spatial data collection while emphasising the need for careful interpretation of results in light of possible device-induced effects.

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Supplementary

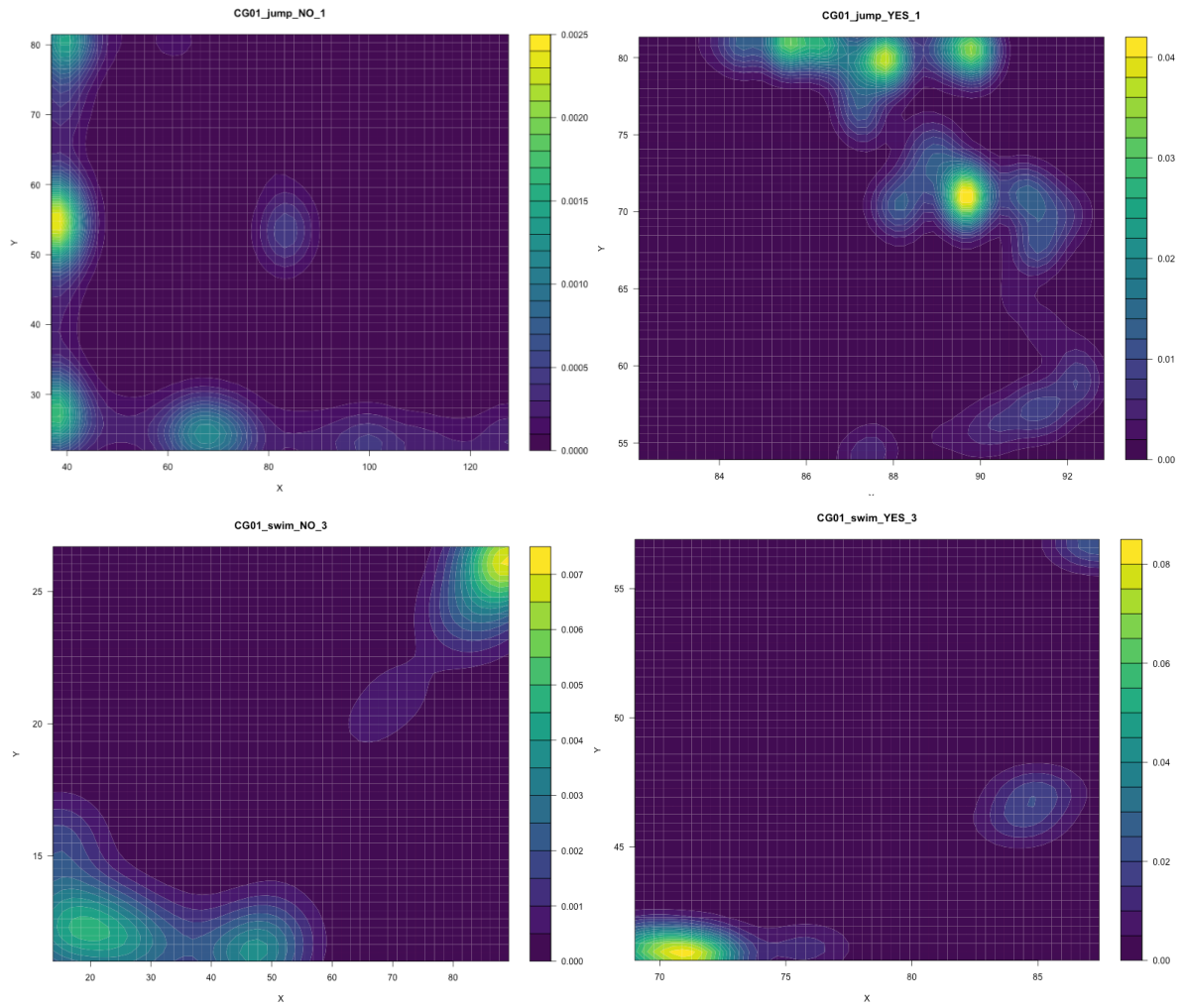


Figure S1. Representative activity heatmaps for individual CG01 (*Bufo viridis*) in dry (top row) and aquatic (bottom row) arenas, without (left) and with (right) the tracking device. Heatmaps illustrate spatial activity patterns across the arena. Dry arena tests were recorded on the first experimental day, and aquatic arena tests on the third day.

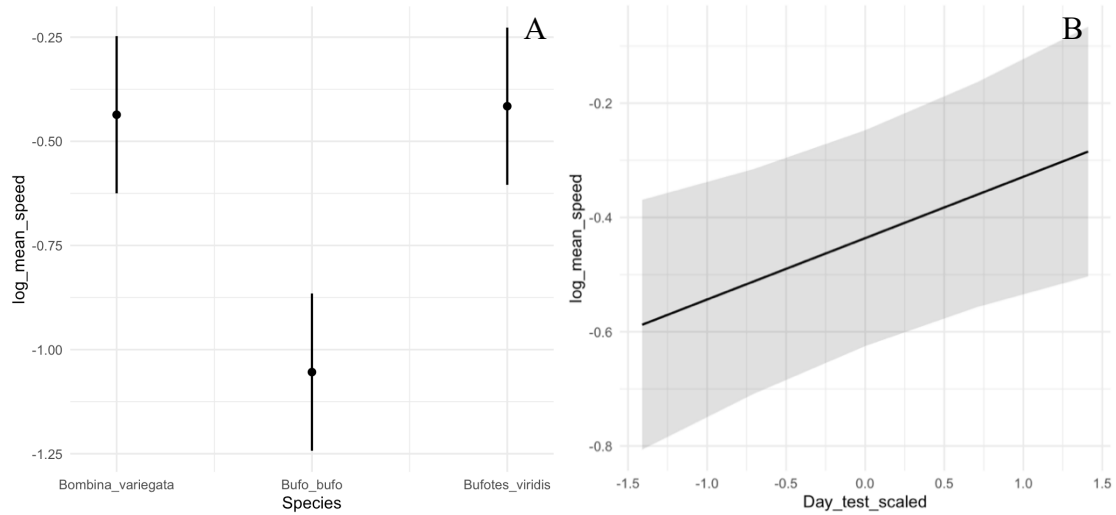


Figure S2. Differences in mean locomotor speed (log-transformed) among species during dry arena trials. (A) *Bufo bufo* exhibited significantly lower speed compared to both *Bombina variegata* and *Bufotes viridis* ($p < 0.001$). (B) Effect of test day on mean locomotor speed across individuals. A significant increase in speed was observed over time ($p = 0.007$).

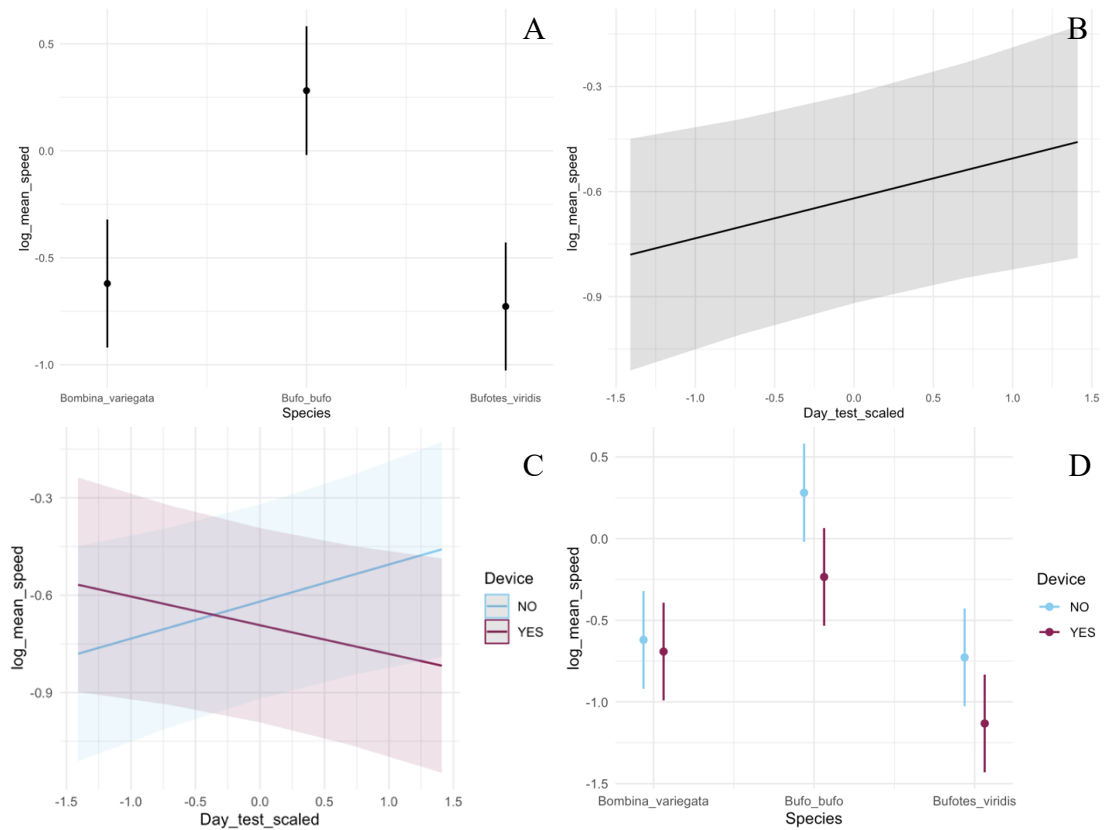


Figure S3. Differences in mean locomotor speed (log-transformed) among species during aquatic arena trials. (A) *Bufo bufo* exhibited significantly higher speed compared to both *Bombina variegata* and *Bufotes viridis* ($p < 0.01$). (B) Effect of test day on mean locomotor speed across individuals. A significant increase in speed was observed over time ($p = 0.026$). (C) Interaction between test day and device presence on mean swimming speed. Individuals tested without a device showed increased speed over time, while those with a device showed a slight decrease ($p = 0.005$). (D)

Interaction between species and device. Post hoc comparisons revealed a significant reduction in swimming speed in *Bufo bufo* ($p < 0.0001$) and *Bufo viridis* ($p = 0.001$) when wearing a device, while no significant effect was found in *Bombina variegata*.

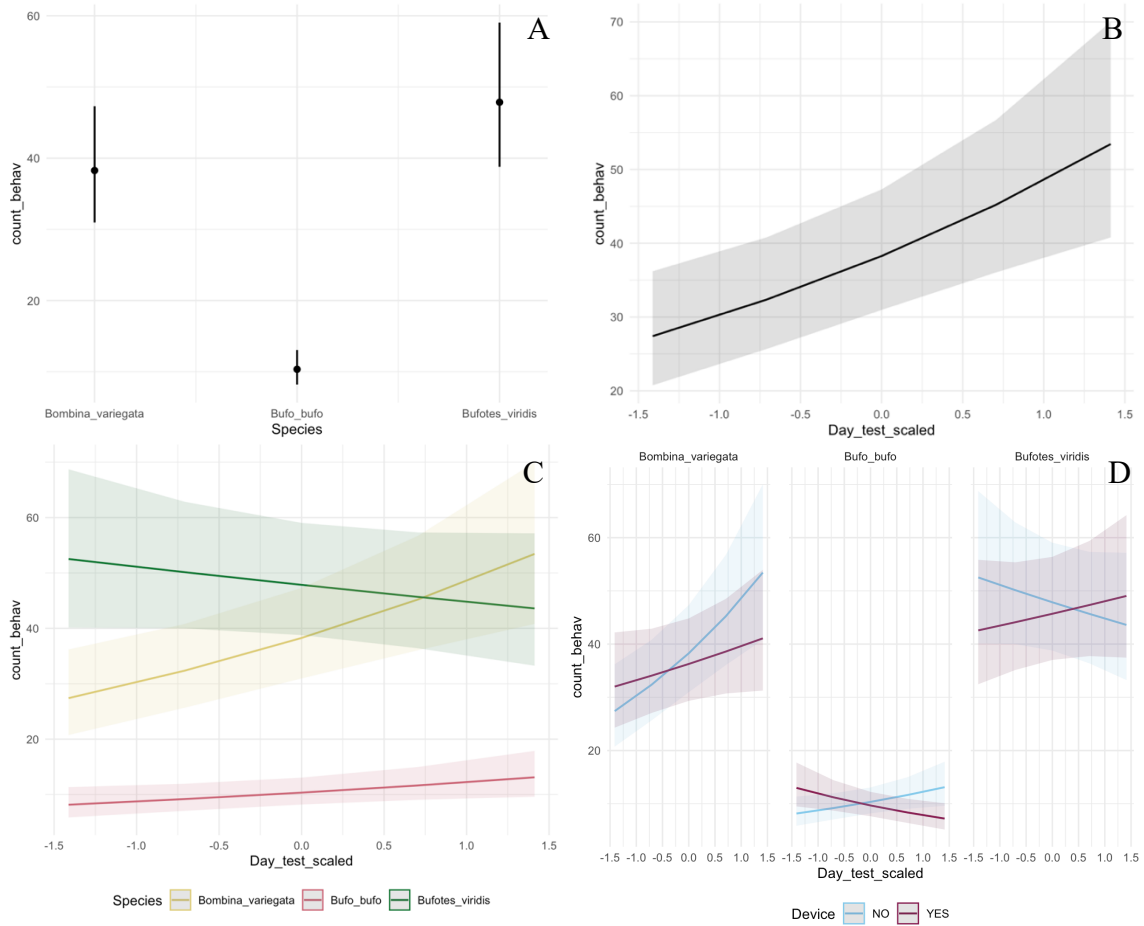


Figure S4. Differences in number of movements among species during dry arena trials. (A) *Bufo bufo* exhibited significantly lower movement frequency compared to both *Bombina variegata* and *Bufo viridis* ($p < 0.0001$). (B) Effect of test day: a significant increase in movement frequency was observed over time ($p < 0.001$). (C) Interaction between test day and species: *B. variegata* showed a significantly steeper increase in movement frequency over time compared to both *B. bufo* and *B. viridis* ($p < 0.03$). (D) Interaction between species, day test and device: *B. bufo*

exhibited a significant reduction in movement frequency over time when wearing the device compared to when untagged ($p < 0.001$).

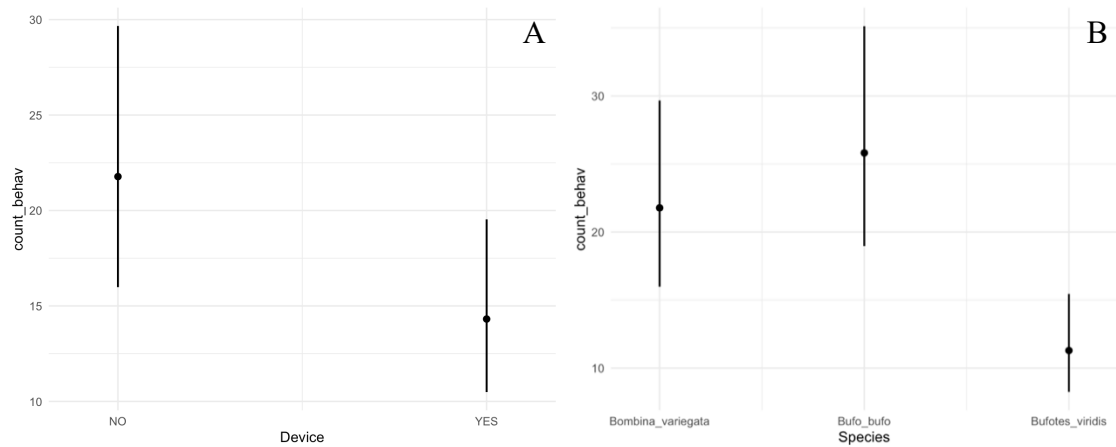


Figure S5. Differences in number of movements among species during aquatic arena trials. (A) Individuals equipped with a device exhibited significantly fewer movements than untagged individuals ($p < 0.001$). (B) *Bufotes viridis* exhibited significantly lower movement frequency compared to both *Bombina variegata* and *Bufo viridis* ($p < 0.01$).

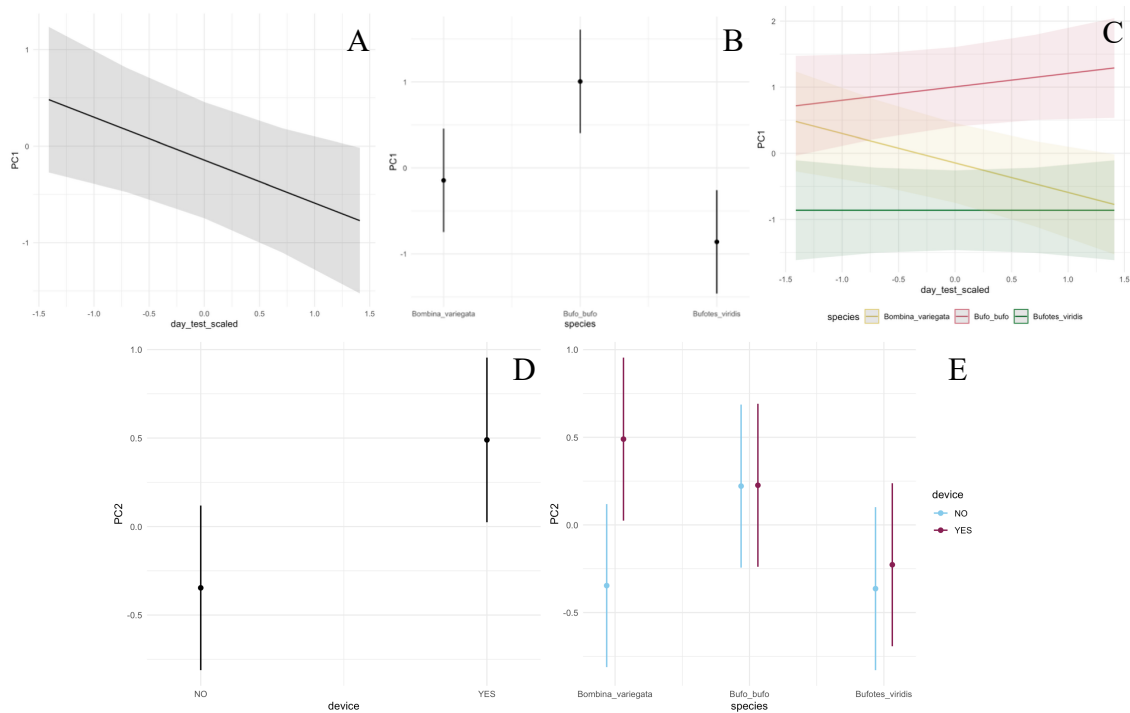


Figure S6. Differences in PCA of spatial behaviour in dry arena trials. (A) PC1 scores significantly decreased over test days ($p = 0.007$). (B) PC1 varied among species, with *Bufo bufo* showing higher scores than *Bombina variegata* and *Bufotes viridis* ($p = 0.044$ and 0.002). (C) Interaction between species and test day significantly affected PC1 trends (notably between *B. variegata* and *B. bufo*: $p = 0.016$). (D) PC2 scores were significantly higher in individuals ($p = 0.001$). (E) PC2 scores were significantly higher in individuals ($p = 0.001$).

carrying a device ($p = 0.005$). (E) A significant interaction between device and species was found for PC2, with *B. variegata* showing increased scores when equipped with a device ($p = 0.005$).

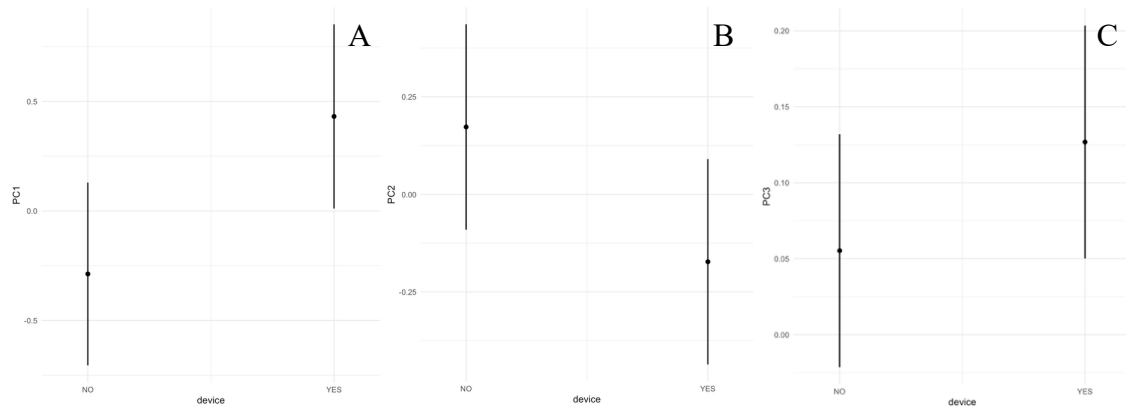


Figure S7. Differences in PCA of spatial behaviour in aquatic arena trials. (A) PC1 scores were significantly higher in individuals carrying a device ($p < 0.001$). (B) PC2 scores were significantly lower in individuals carrying a device ($p = 0.039$). (C) P31 scores were significantly higher in individuals carrying a device ($p = 0.044$).

Table S1. Information on the species, body weights, and corresponding harnesses of the specimens used in the study. The table includes the following columns: (i) Species to which each individual belongs, (ii) Identifier assigned to each specimen, (iii) Sex of the specimen, (iv) Body weight (g), and (v) Percentage ratio of harness weight to body weight.

Species	ID	Sex	Animal weight (g)	Device-Animal weight ratio (%)
<i>Bufo bufo</i>	IN01	F	377.95	0.77
	IN02	F	342.43	0.85
	IN03	F	267.11	1.09
	IN04	F	287.72	1.01
	IN05	F	448.37	0.65
	IN06	F	185	1.58
<i>Bufo viridis</i>	CG01	F	34.12	8.56
	CG02	M	42.17	6.92
	CG03	M	36.48	8
	CG04	M	27.3	10.7
	CG05	M	28.62	10.2
	CG06	M	21.72	13.44
<i>Bombina variegata</i>	CP07	F	7.14	18.35
	CP09	M	7	18.71
	CP10	M	5.75	22.78
	CP11	M	7.03	18.63
	CP013	F	8.53	15.36
	CP14	F	6.05	21.65

Table S2. Summary of the models tested for each response variable under jump and swim behavioural contexts. The table reports the full model structures considered during model selection, including fixed effects, random effects, and relevant interaction terms.

	<i>Jump</i>	<i>Swim</i>
Speed	$\log_mean_speed \sim Day_test_scaled + Device + Species + (1 ID) + Day_test_scaled:Device$	$\log_mean_speed \sim Day_test_scaled + Device + Species + (1 ID) + Day_test_scaled:Device + Device:Species$
Movement frequency	$count_behav \sim Day_test_scaled + Device + Species + (1 ID) + Day_test_scaled:Device + Day_test_scaled:Species + Device:Species + Day_test_scaled:Device:Species$	$count_behav \sim Device + Species + (1 ID)$
Heatmap: PC1	$PC1 \sim day_test_scaled + species + (1 id) + day_test_scaled:species$	$PC1 \sim device + (1 id)$
Heatmap: PC2	$PC2 \sim device + species + (1 id) + device:species$	$PC2 \sim device + (1 id)$
Heatmap: PC3	$PC3 \sim (1 id)$	$PC3 \sim device + (1 id)$

Data Availability

Data will be made publicly available upon publication of the corresponding manuscript. Currently, all datasets and scripts are stored in a private GitHub repository (URL: https://github.com/BombinaPN/arena_test), accessible upon request.

Chapter IV - Where do green toads go when no one is looking? Reconstructing movement patterns using a dead-reckoning approach.

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Abstract

Amphibians are among the most threatened vertebrates globally, yet fine-scale movement data remain scarce due to their small size, cryptic behaviour, and challenging habitats. In this study, we tested the feasibility of combining VHF radiotelemetry with miniaturised accelerometer- and magnetometer-equipped devices to reconstruct continuous movement paths in the European green toad (*Bufo viridis*). Six adult individuals were tracked during the post-reproduction phase for one week in their natural habitat, with devices recording tri-axial acceleration, magnetic heading, and temperature. Continuous trajectories were reconstructed using dead-reckoning (DR) and validated against GPS fixes.

Data were successfully retrieved for five of the six individuals. Mortality events were caused by a toad being crushed by a horse, entanglement in vegetation due to the VHF antenna leading to overheating, and predation by a snake (*Natrix helvetica*), the latter providing a unique opportunity to capture both predator activity and digestive tract dynamics. As expected, DR reconstruction revealed substantially longer distances travelled compared to GPS-based paths (632 ± 175 m vs. 289 ± 84 m), highlighting systematic underestimation by traditional GPS methods. Activity analyses indicated strong nocturnal patterns, with movement commencing around 21:00-22:00 and ceasing between 05:00-07:00, and a clear thermal window of 10-15°C for peak activity.

These findings demonstrate that DR provides high-resolution, continuous movement data, capturing fine-scale spatial behaviour that is undetectable with conventional GPS fixes. The integration of VHF telemetry and accelerometer/magnetometer data is thus a feasible and powerful approach for studying the movement ecology of small, cryptic amphibians. Our study highlights the importance of device design, attachment, and monitoring protocols to minimise animal risk. This methodology offers a promising avenue for ecological and conservation research, enabling precise characterisation of habitat use, activity patterns, and fine-scale spatial ecology in species threatened by habitat loss and climate change.

Introduction

Amphibians are currently considered the most threatened vertebrate group globally, with over 41% of species at risk of extinction (Re:wild, Synchronicity Earth, 2023). Habitat loss, climate change, emerging diseases, and pollution are among the main drivers of this ongoing and pervasive decline (Re:wild, Synchronicity Earth, 2023). Understanding

amphibian spatial ecology and behavioural patterns is therefore crucial to inform effective conservation strategies and mitigate further population loss.

Despite this urgency, fine-scale movement data remain scarce for most amphibian species (Rowley and Alford, 2007). Existing knowledge derived from short-term mark-recapture studies or low-resolution tracking approaches, which can reveal broad-scale patterns such as seasonal migrations but often fail to capture detailed, fine-scale behaviours. The small size, cryptic habits, and the complex habitats of amphibians often constrain the use of traditional tracking methods. For instance, GPS loggers, valuable for larger vertebrates, are often unsuitable for amphibians due to their small size, difficulty of attaching devices to permeable skin, and frequent signal loss under dense canopy or within burrows (Gamo et al., 2000).

Radio-telemetry, although widely used in amphibian research and successful in mapping movements over days to months (Ocock et al., 2014; Sinsch et al., 2012), also presents significant limitations. The method requires the continuous presence of an operator in the field to manually record positional fixes, thereby constraining the temporal resolution of data collection and preventing the reconstruction of continuous movement paths. Consequently, resulting datasets often underestimate path tortuosity and total distance travelled, overlook short-term excursions, and fail to capture activity patterns and habitat use occurring outside periods.

In this study, we used the European green toad (*Bufo viridis*), a small-bodied anuran, as a model species to test the feasibility and potential of applying dead-reckoning (DR) for amphibian tracking. This technique combines data from tri-axial accelerometers and magnetometers to reconstruct fine-scale movement paths, representing the first application of DR in an amphibian. Dead-reckoning has been successfully implemented

across a variety of taxa, from large mammals to birds (Bidder et al., 2015; Gunner et al., 2021; Magowan et al., 2022), providing detailed and continuous trajectories in environments where GPS signals are unreliable or unavailable.

The adoption of this approach for amphibians is particularly promising, as it may overcome the size and habitat constraints that limit other tracking methods. However, its applicability has never been tested in anurans or other amphibians, likely due to the difficulty of deploying sufficiently miniaturised devices without compromising animal welfare or behaviour. By applying dead-reckoning to *B. viridis* under controlled conditions, we aimed to (i) reconstruct continuous and accurate movement paths, and (ii) characterise temporal activity patterns in relation to temperature and time of day. Our study provides a methodological first in amphibian research and highlights the potential of DR to advance the study of the fine-scale movement ecology in small, cryptic vertebrates in natural environments.

Methods

2.1 Study Animals and Device Attachment

Six adults *Bufotes viridis* (Laurenti, 1768) individuals were collected in April 2024 from the Castel di Guido population (Rome, Italy; 41°53'33''N, 12°16'57''E; Figure S1). Following capture, the animals were temporarily housed at the Fondazione Bioparco di Roma, where behavioural experiments were conducted to verify that the chosen tracking devices did not impair their performance (Nesi et al., Under review). On 23 June 2024, all individuals were released at the original capture site, marking the start of a one-week tracking period. The study group consisted of one female (34.12 g; device-to-body mass

ratio = 8.56%) and five males (42.17, 36.48, 27.3, 28.62, and 21.72 g; device-to-body mass ratios ranged 6.92-13.44%).

Custom-built biologging devices (Axy 5, Technosmart Europe srl, Rome, Italy) combined VHF transmitters, tri-axial accelerometers, magnetometers, and temperature sensors, with a total mass of 2.92 g. The accelerometer was set to record at 25 Hz, while the magnetometer sampled at 2 Hz, providing sufficiently fine resolution for activity classification and heading estimation. Each device featured two holes on opposite short sides to allow attachment using 1 mm diameter silicone cords (Jinsion Crystal Tec[®]). One cord was passed through the lower holes and secured around the waist, while another passed through the upper holes and was tied around each forelimb with reef knots, reinforced with an additional knot and sealed with water-resistant tape (Nesi et al., Under review). This harness positioned the device centrally on the body, ensuring reliable acceleration measurements while minimising interference with natural movement.

All procedures were approved by the Italian Ministry of the Environment and Energy Security (authorisation no. 2023.0043372).

2.2 Field Tracking

Immediately before release, magnetometers were calibrated at the release site by rotating each device along its y-axis (calibration of x and z axes) while the operator simultaneously rotated on their own axis, ensuring proper calibration also for the device's y-axis (Williams et al., 2017). After calibration, animals were released at the capture site at 19:30, and the first radiotracking session was conducted at 21:00. Individuals were then located every 12 hours using radiotelemetry, with searches starting at 09:00 and 21:00 and continuing until all toads were found, and location coordinates were recorded using

a smartphone equipped with the Commander Compass GO application. Homing was used to reach each individual's exact GPS position. At every relocation, observers recorded whether the animal was visible and whether it was active or inactive. These GPS fixes were essential reference points for reconstructing continuous movement paths through DR.

2.3 Track reconstruction

Track reconstruction was performed in R v. 4.5.1 (R Core Team, 2025) separately for each individual using the *Gundog.Tracks* function (Gunner et al., 2021). GPS and accelerometer files were first imported and calibrated (Garde et al., 2022). Time intervals corresponding to magnetometer calibration were annotated in the column *Marked.event* with the label "M". Magnetometer calibration was then conducted using the function *Gundog.Compass* (Gunner et al., 2021), with the parameter `method = 1`, which assumes that the tri-axial sphere is offset. This option was chosen because it provides a robust correction when only a simple translation of the magnetic field is required, as recommended by the authors.

To discriminate between active and inactive phases, we applied a Random Forest model previously developed for the same species, which classified the animal's state (movement vs. stop) based on accelerometer axes and VeDBA (Vector of the Dynamic Body Acceleration = $\sqrt{Ax^2 + Ay^2 + Az^2}$; Gleiss et al., 2011) values. Prior to VeDBA calculation, the static component of acceleration was removed from each axis to isolate the dynamic signal associated with movement. The predicted output integrated into *Marked.event*, assigning the value 0 when the animal was stationary and 1 when in

motion. Elevation data were interpolated between GPS fixes obtained during radiotracking sessions.

Finally, movement paths were reconstructed using the function *Gundog.Tracks* (Gunner et al., 2021). As starting coordinates, we set the release location of each individual (lo and la, corresponding to longitude and latitude). Heading was provided by the yaw angle (h), and speed was estimated from VeDBA values (v) with a linear calibration factor ($m = 1$, $c = 0$). Interpolated elevation was included (elv), together with pitch (p) to account for three-dimensional movement. Activity state was incorporated from the *Marked.event* column (ME), restricting track advancement to periods classified as movement by the Random Forest model. Verified Positions (VPs), obtained from GPS fixes during radiotracking, were supplied (VP.lon and VP.lat). To maximise correction accuracy, all available VPs were retained by setting VP.ME = FALSE, ensuring that positional drift was corrected even during stationary phases. The method = "All" option was selected, meaning that every VP was used in the dead-reckoning correction procedure without under-sampling. This approach was chosen to fully exploit the high-resolution GPS data available and to minimise drift throughout the entire reconstructed track. The track was bounded between the first and last VP (bound = TRUE) and reconstructed in the forward direction (Outgoing = TRUE). Diagnostic plots were generated (plot = TRUE) to visually compare dead-reckoning trajectories with VP-corrected tracks.

2.4 Activity patterns analysis

Activity patterns were derived from the Random Forest outputs (Nesi et al., Under review), which classified each time point based on accelerometer data as either movement or stationary. To investigate temporal activity, time stamps were grouped into hourly bins:

each hour represents the period from HH:00:00 to HH:59:59. For each individual, the number of movement events within each hour was divided by the total number of events (movement plus stationary) occurring in that hour, yielding the proportion of time spent moving per hour.

A similar approach was applied to temperature. Recorded temperatures were rounded to the nearest integer (e.g., 18.6°C rounded to 19°C), and for each individual, the proportion of movement events relative to total events was calculated for each temperature bin.

Finally, temporal and thermal data were jointly binned to examine how the proportion of movement varied across combinations of time and temperature. For each individual, we calculated the percentage of accelerometer records classified as movement within each hour-temperature cell, producing a two-dimensional activity surface. This approach made it possible to identify the specific thermal and temporal windows in which individuals were most likely to be active.

Results

3.1 Fate of tracked individuals

All six individuals were released at the original capture site and tracked over the course of several days (23-29 June 2024), although the duration of successful tracking and the recovery of data varied among animals. Individual CG01 was monitored until June 29 but was not observed after the first tracking point on the evening of release (21:32), and the device could not be recovered, precluding data retrieval. CG02 was tracked until the morning of June 28, when it was found dead, apparently submerged in horse faeces, which likely caused its death. Data from this individual were successfully downloaded on the nights of June 25 and 27. Observations indicated that CG02 was active during the first,

second, and fourth nights. CG03's device was downloaded on the night of June 27, and the animal was observed active during the first, fourth, and fifth nights. On the morning of June 29, radiotelemetry indicated that the signal was coming from a snake (*Natrix helvetica*) occupying the crevice normally used by CG03 as a resting site. This suggested that the toad had been predated by the snake. The device was later recovered following its natural expulsion from the predator on July 2, allowing retrieval of the remaining movement data. CG04's device was downloaded on the night of June 25, and the animal was observed active during the first three nights. From the morning of June 28, CG04 was no longer moving, and the device was recovered on the morning of June 29, though the animal itself had apparently removed the harness between the night of June 27 and the morning of June 28. CG05 was tracked until the evening of June 29, with device downloads occurring on June 25 (evening), June 27 (morning), and after replacement of the device on June 26 due to battery issues during the initial download. Observations indicated activity during the first, third, fourth, fifth, and seventh nights, as well as during the second morning. Finally, CG06 was tracked until the morning of June 29, when it was found dead due to overheating after entangling the VHF antenna around a plant and being unable to free itself. Device downloads for CG06 occurred on June 25 and 27, and the animal was observed active on the first, third, fourth, and fifth nights.

3.2 Comparison between GPS and DR tracks

Reconstructed movement tracks were successfully obtained for five of the six monitored individuals, with the exception of CG01, whose device was not recovered. Both VHF-based tracks (validated positions, VP) and dead-reckoning (DR) trajectories were generated, highlighting clear differences in the distances travelled estimated by the two

methods (Figure 1; S2). The DR paths indicate that individuals primarily used dry grasslands during the tracking period, whereas CG05 was the only individual to also exploit a moister area formed by the overflow from the spring near the release site.

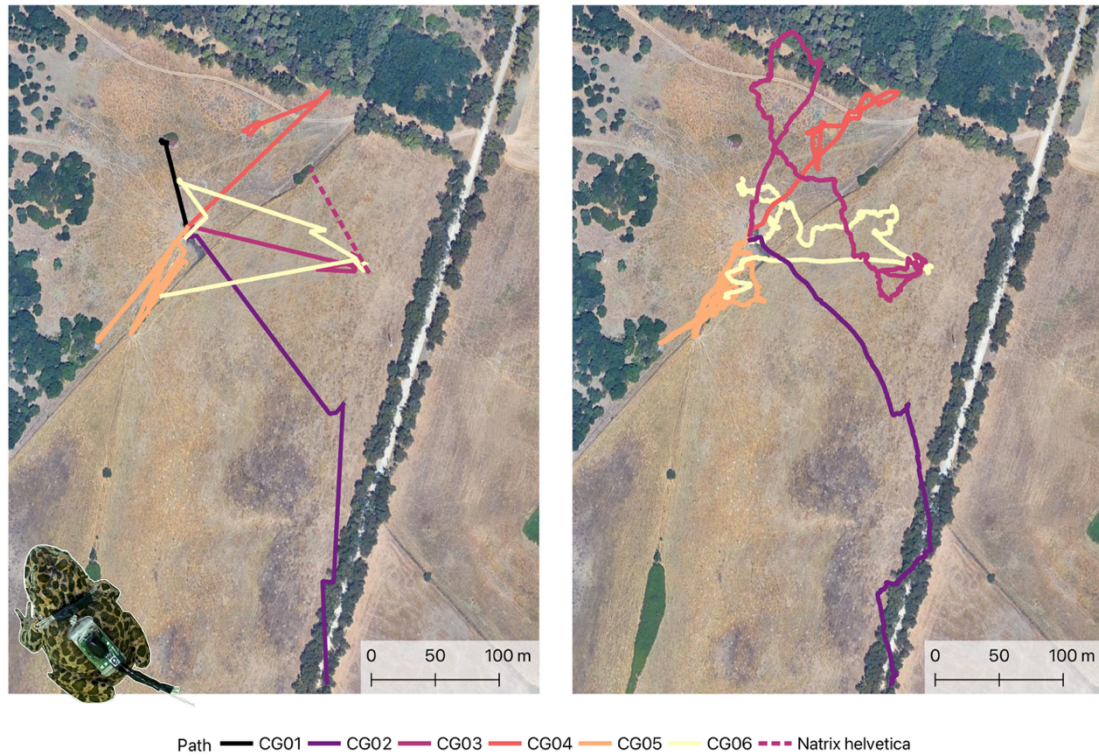


Figure 1. Reconstructed movement tracks of *Bufotes viridis* individuals obtained with different methods. Left panel: VHF-based tracks interpolated from validated positions (VP). The dashed line represents the likely trajectory of a *Natrix helvetica* during the predation event of individual CG03. Right panel: Dead-reckoning (DR) trajectories, showing more detailed reconstructions of the animals' paths. Colours correspond to individuals.

On average, VHF estimates yielded a path length of 367.8 ± 97.9 m (range: 251-465 m), whereas DR reconstructions produced longer distances both in 2D (755.8 ± 243.4 m; range: 454-997 m) and in 3D (776.8 ± 247.1 m; range: 472-1021 m; Table 1). These differences illustrate how radiotracking systematically underestimates travelled distances compared to DR.

Table 1. Distances (m) estimated from validated VHF positions (VP), 2D dead-reckoning (DR) and 3D dead-reckoning (DR) for the five successfully reconstructed individuals.

ID	VP 2D (m)	DR 2D (m)	DR 3D (m)
CG02	427	538	553
CG03	251	997	1021
CG04	274	454	472
CG05	465	922	944
CG06	422	868	894

3.3 Predation event

During the tracking period, individual CG03 experienced a predation event by a snake (*Natrix helvetica*). Radiotelemetry indicated that the signal originated from the predator occupying the toad's habitual resting crevice on the morning of 29 June, indicating that the toad had been captured. Concurrent movement-sensor data provided additional detail on the event: the VeDBA profile indicated an abrupt cessation of normal toad activity at 05:30 on 29 June. Thereafter, the signal displayed irregular patterns consistent with both the snake's locomotion and the normal peristaltic movements of its digestive tract. At 20:10 on 1 July, the device was naturally regurgitated by the snake, after which all remaining data were successfully recovered (Figure 2).

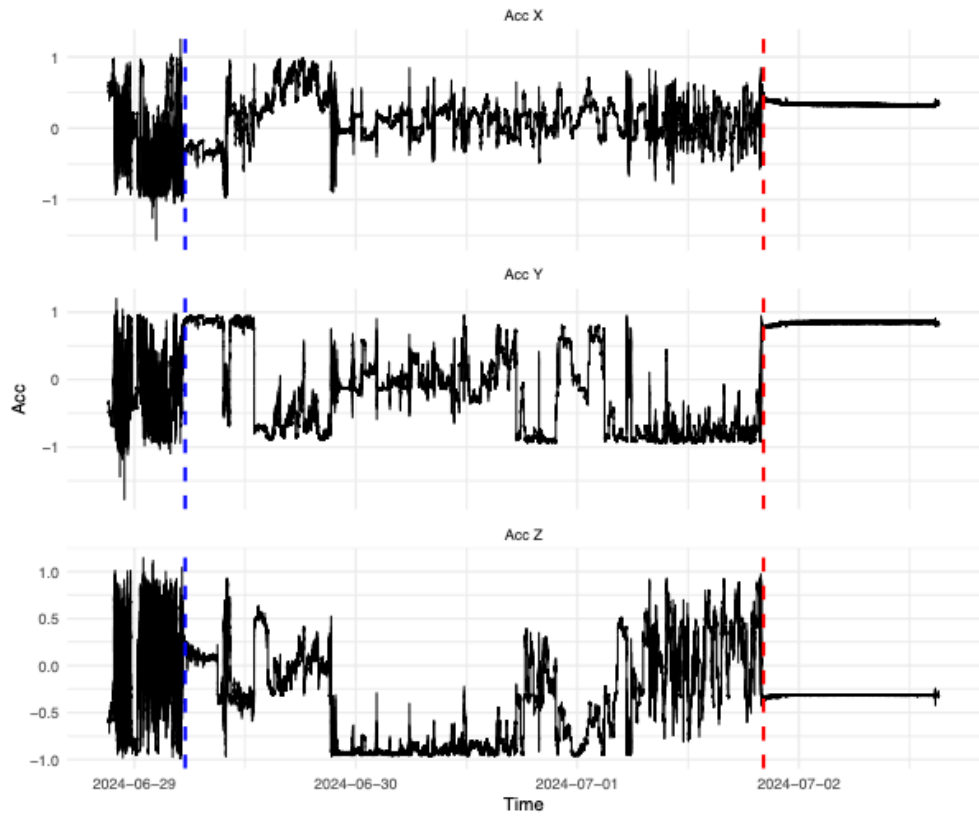


Figure 2. Acceleration recorded by the device along the three movement axes (x , y , z) during a predation event of *Bufo viridis* by *Natrix helvetica*. The blue line indicates the predation phase, during which the device was still attached to the toad's body; the intermediate section shows irregular movements generated by the snake and its digestive process; the red line marks the moment of device expulsion.

3.4 Activity patterns

The percentage of movement recorded by accelerometers varied consistently across individuals and time of day (Figure 3). *Bufo viridis* predominantly primarily nocturnal activity pattern: movement typically began between 21:00 and 22:00 and ceased between 05:00 and 07:00. Daytime hours were characterised by very low activity, with only sporadic movements recorded in a few individuals.

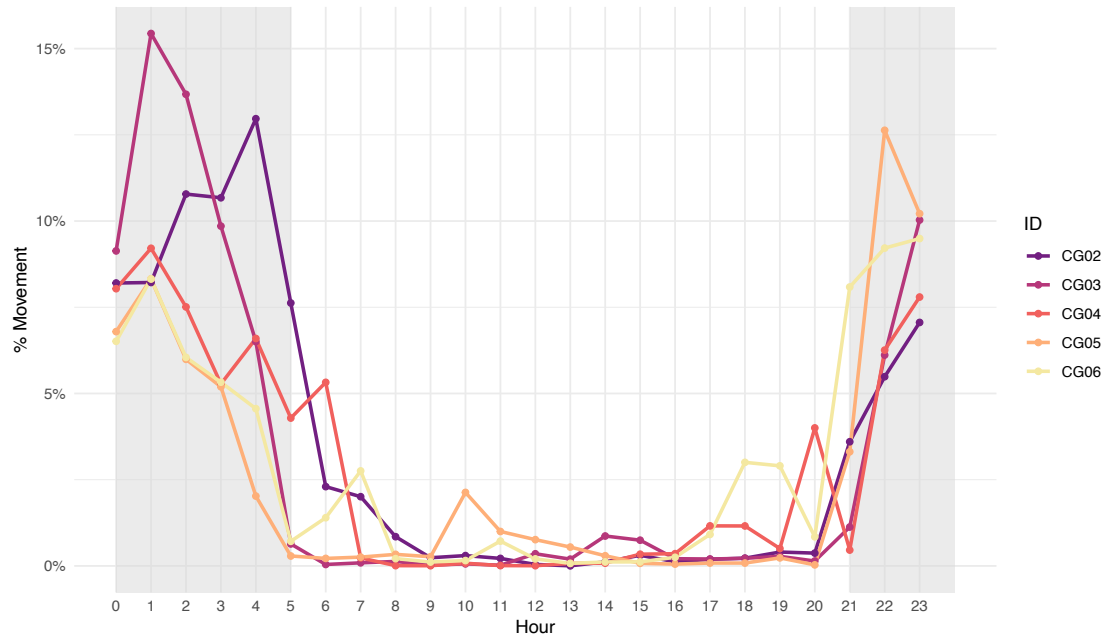


Figure 3. Hourly movement activity of *Bufotes viridis* individuals as recorded by accelerometers. Movement was expressed as the percentage of activity events relative to the total number of events recorded within each hour. The grey background from 21:00 to 05:00 indicates the nocturnal period during the study.

Activity also varied with temperature (Figure 4). Individuals were most active between 10 and 15°C, with movement gradually decreasing at higher temperatures and ceasing almost entirely around 20°C. Occasional movements were detected above this threshold, but these were isolated events. One individual (CG05) displayed atypical activity, with elevated movement detected at temperatures exceeding 40°C and around 10:00 in the morning. These abnormal patterns likely correspond to the moment when the animal became entangled in vegetation due to the VHF antenna, an event that led to heat stress and ultimately its death.

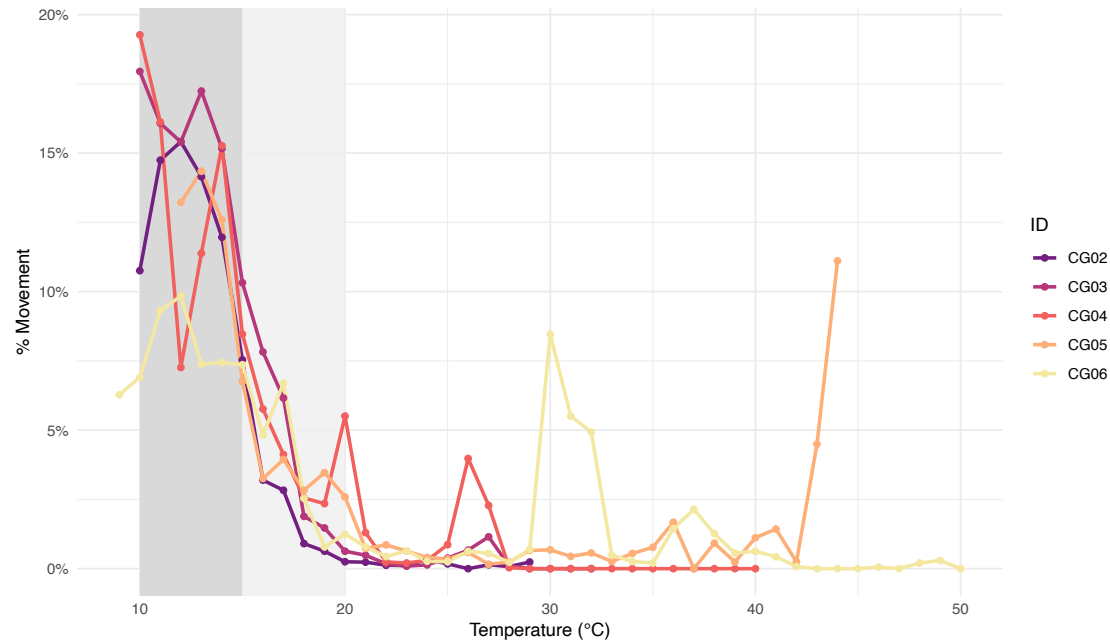


Figure 4. Movement activity of *Bufo viridis* individuals as recorded by accelerometers in relation to ambient temperature. Movement was expressed as the percentage of activity events relative to the total number of events across all temperatures. The shaded grey band between 10 and 15 °C highlights the temperature range associated with peak activity, while the lighter band between 15 and 20 °C represents the range in which movement progressively decreased.

Across all monitored toads, movements were concentrated between 21:00 and 05:00, corresponding to the coldest hours of the day during late June and early July, when temperatures ranged approximately between 10 and 23 °C. Individuals CG02, CG03, and CG04 predominantly occupied cooler microhabitats and seldom experienced temperatures above 25 °C, whereas CG05 and CG06 were often exposed to warmer conditions (frequently > 30 °C), despite remaining largely inactive at those times. The only exceptions were the brief, atypical activity peaks in CG05 (> 40 °C) and in CG06 (~35 °C; Figure 5).

Overall, the heatmaps illustrate that *B. viridis* activity is tightly restricted to nocturnal, cooler periods, while higher daytime temperatures largely coincided with inactivity, even though microhabitat exposure sometimes forced individuals into warmer conditions.

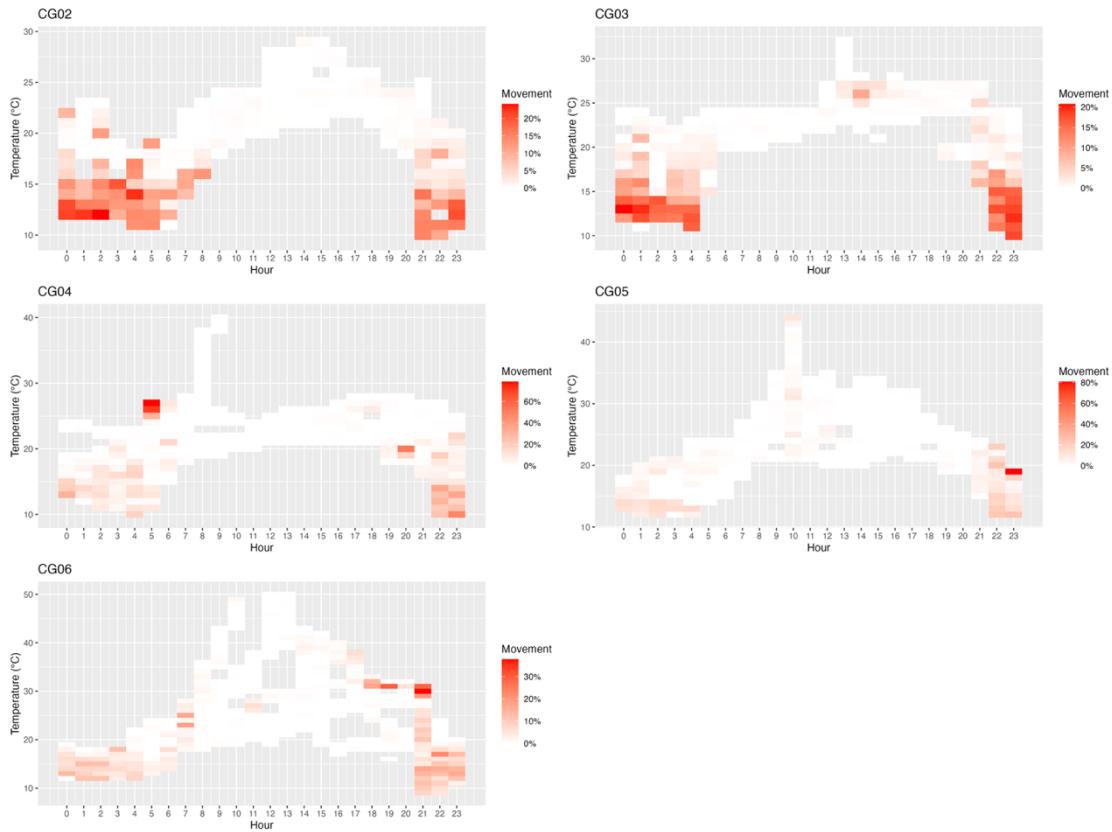


Figure 5. Heatmaps showing the proportion of time spent moving as a function of hour of day (x-axis) and ambient temperature (y-axis) for each individual *Bufotes viridis* (CG02-CG06). Activity is expressed as the percentage of events classified as “Movement” per bin, with warmer colours indicating higher activity levels.

Discussion

Our study demonstrates, for the first time in an amphibian, that dead-reckoning can be successfully applied using miniaturised multi-sensor devices to reconstruct fine-scale movement and activity patterns. Although dead-reckoning has been widely used in other vertebrate groups (Bidder et al., 2015), its application to anurans has been limited by body size, behaviour, and welfare constraints. By deploying lightweight custom-built tags on *Bufotes viridis* in semi-natural conditions, we show that the technique can generate

continuous movement trajectories and high-resolution activity metrics, overcoming the temporal gaps and coarse spatial resolution inherent to traditional radiotelemetry. The method also revealed behavioural processes, such as short-term movements, fine-scale nocturnal activity rhythms, and responses to thermal conditions, that are often missed by conventional tracking. The detection of a predation event further illustrates the potential of multi-sensor data to capture rare or cryptic behaviours. Together, these results indicate that dead-reckoning offers a promising and feasible tool for studying the movement ecology of small amphibians, with potential to complement or refine existing methodologies in field contexts.

Tracking of *Bufo viridis* revealed both the strengths and the limitations of combining VHF radiotelemetry with accelerometer-equipped devices in small amphibians. Successful data collection was achieved for five of six individuals, confirming the feasibility of the approach, while the loss of one device underscores that tag detachment and technical failure remain common challenges when working on small, elusive taxa (Altobelli et al., 2023; Pellet et al., 2006; Roznik and Alford, 2015). Mortality events, including the likely trampling of CG02 by horses and the entanglement and subsequent overheating of CG06, underscore the role of environmental hazards in determining the fate of tagged animals. These incidents emphasise the need for careful evaluation of device placement, mass, and attachment method, particularly for small terrestrial amphibians inhabiting structurally complex habitats.

In our field observations, toads equipped with the device were consistently able to enter and use natural refugia, including shallow burrows, vegetation clumps, and soil crevices. This indicates that the tag did not hinder access to microhabitats that are ecologically essential for thermoregulation, predator avoidance, and moisture balance in *Bufo*

viridis. The ability to successfully exploit these shelters aligns with the tolerance observed in captivity-based experiments (Nesi et al., Under review) and suggests that, at least for this species, the external device does not impose substantial mechanical constraints during refuge use.

The predation of CG03 by *Natrix helvetica* provides an additional ecological insight. Beyond confirming predator identity, the sensor data captured locomotor and digestive movements within the predator's body, highlighting the potential of multi-sensor systems to document rarely observed predator-prey interactions (Łaciak et al., 2023; Roznik and Johnson, 2009). Conversely, the case of CG04, apparently able to remove its harness, raises questions about individual behavioural responses to tagging and the need to refine attachment systems to minimise interference.

Despite these challenges, the observed activity patterns suggest that the devices were generally well tolerated and provided reliable behavioural data. Activity typically started shortly after 20:00, peaked between 22:00 and 03:00, and declined around 07:00, with occasional short resting phases at night or brief daytime movements. This pattern closely mirrors the nocturnal activity rhythm described by Spießberger et al. (2023) for post-breeding *B. viridis* under warm, dry summer conditions, supporting the ecological validity of our accelerometer-based inferences.

Comparisons between validated VHF trajectory and dead-reckoning (DR) reconstructions revealed systematic differences in estimated distances travelled. VHF-based paths consistently underestimated movement, whereas DR reconstructions yielded substantially longer distances (Bidder et al., 2015; Magowan et al., 2022). These results confirm that high-resolution DR can capture fine-scale displacements missed by coarser,

intermittently sampled VHF fixes, providing a more accurate and complete estimate of movement and space use in small amphibians.

Activity heatmaps further suggest potential differences in microhabitat use among individuals. While toads were largely inactive under the environmental conditions tested, some displayed prolonged exposure to warmer microhabitats, which may reflect variation in refuge availability, accidental exposure, or differences in microhabitat selection.

The marked reduction in activity above 25-30 °C demonstrates the strong thermal constraints limiting *B. viridis* movements during the post reproductive phase, restricting activity to narrow nocturnal windows when temperatures fall within physiological tolerance. This pattern is particularly relevant in the context of ongoing climate change: increases in night-time temperatures could reduce the duration of suitable activity periods, with potential consequences for foraging, reproduction, and dispersal.

Conclusions

This study shows that integrating VHF radiotelemetry with miniaturised accelerometer–magnetometer loggers and dead-reckoning provide a powerful approach for capturing the fine-scale movements of small amphibians. This method reveals spatial and behavioural patterns that conventional low-resolution tracking cannot detect, offering new insights into microhabitat use, thermal constraints, and activity rhythms. Despite occasional tag loss and predation, the results highlight the strong potential of dead-reckoning to support amphibian conservation by improving our understanding of how individuals navigate and respond to their environments.

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Supplementary

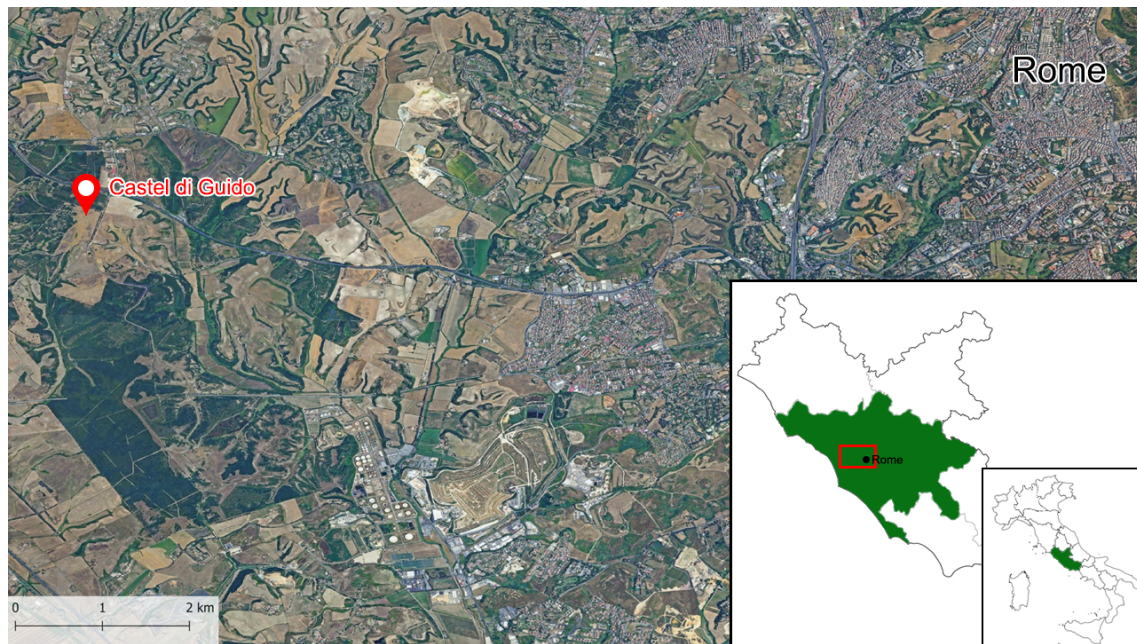
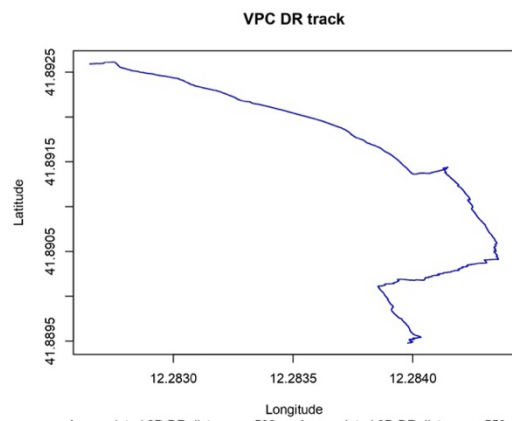
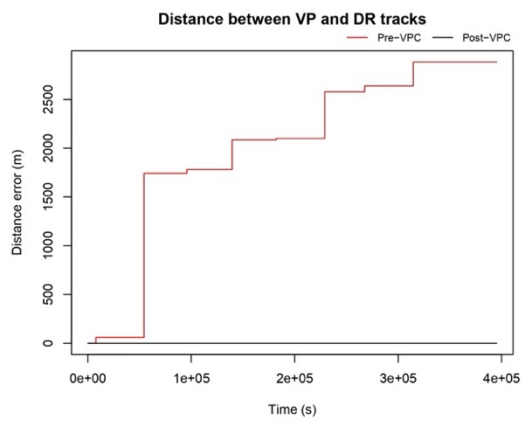
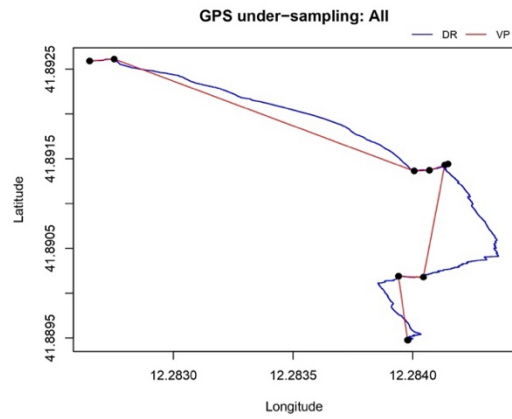
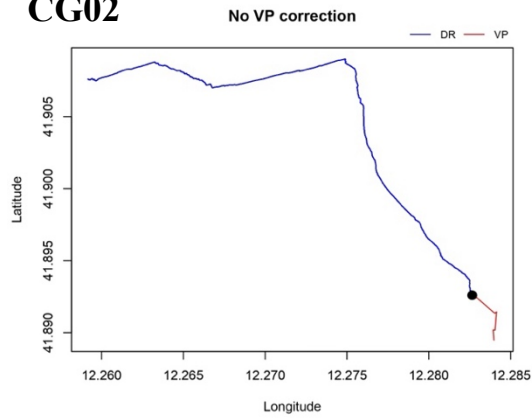
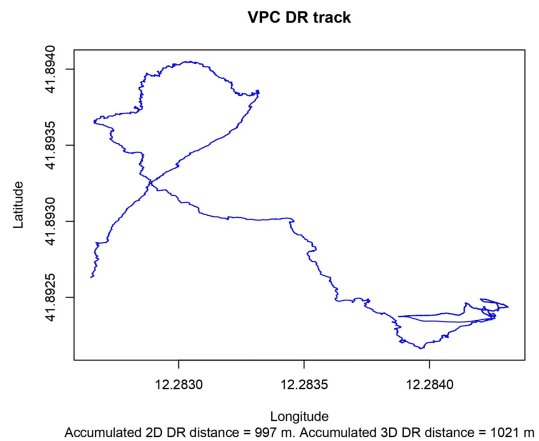
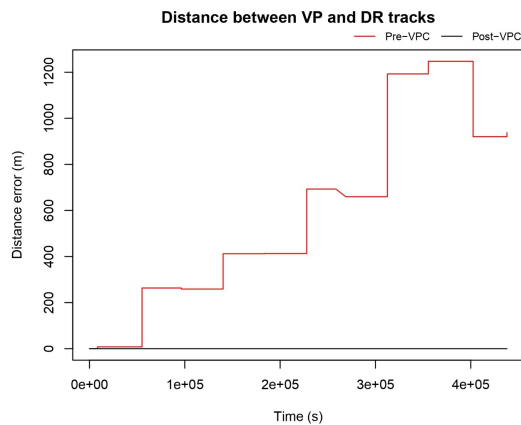
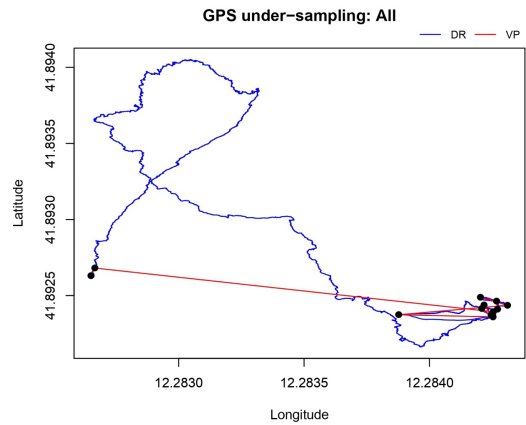
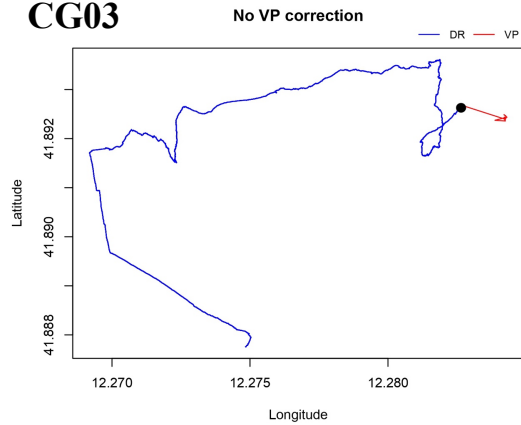


Figure S1. Sampling area of the *Bufotes viridis* population at Castel di Guido (Rome, Italy). The map shows the location of Castel di Guido highlighted in red, indicating its position within the central Italian peninsula.

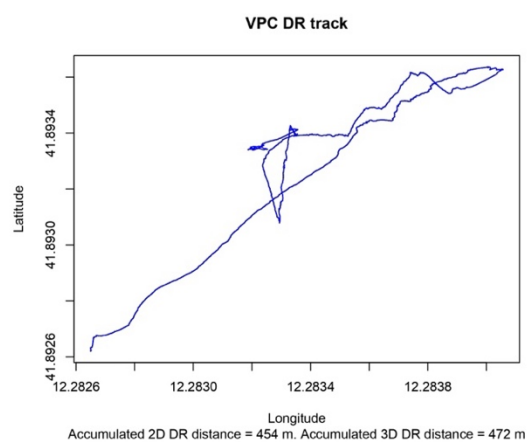
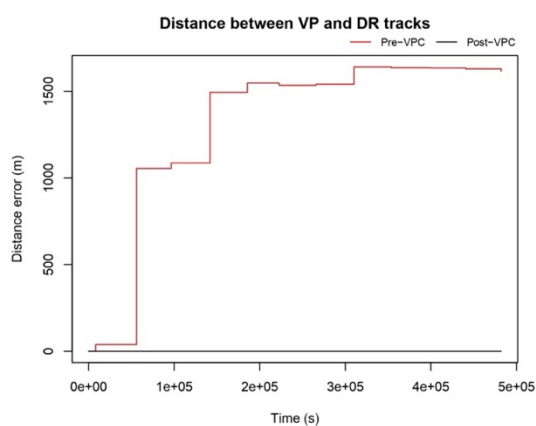
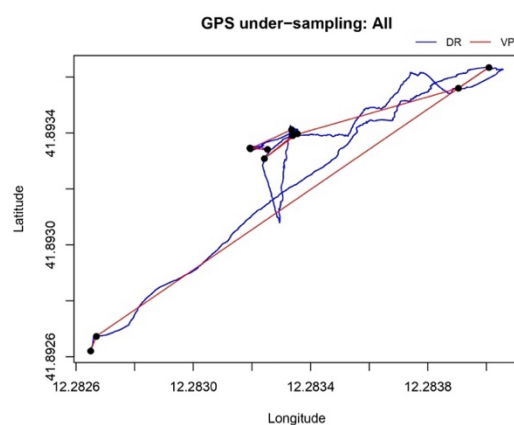
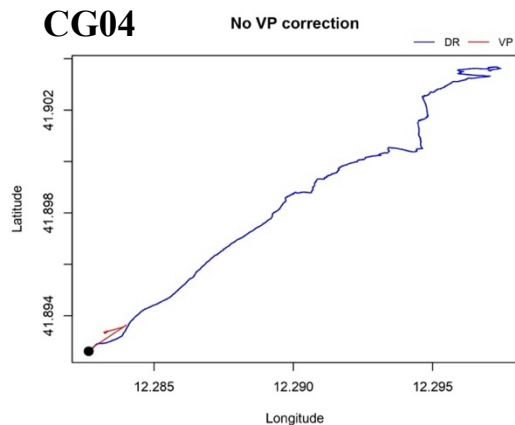
CG02



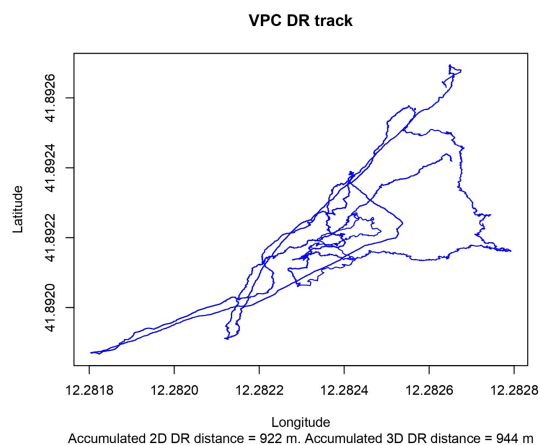
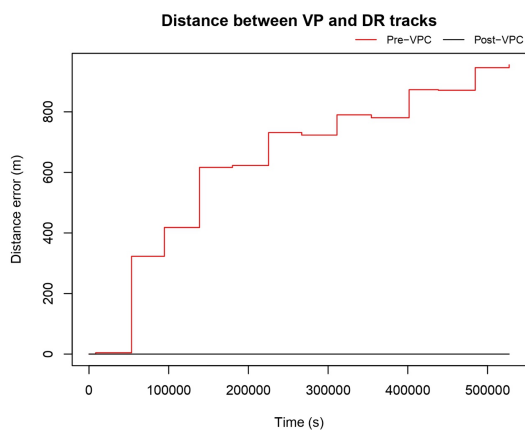
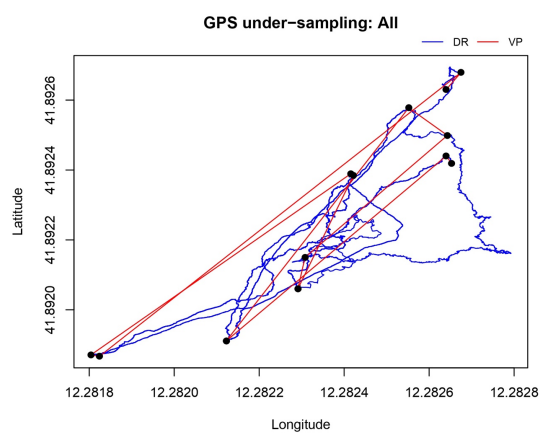
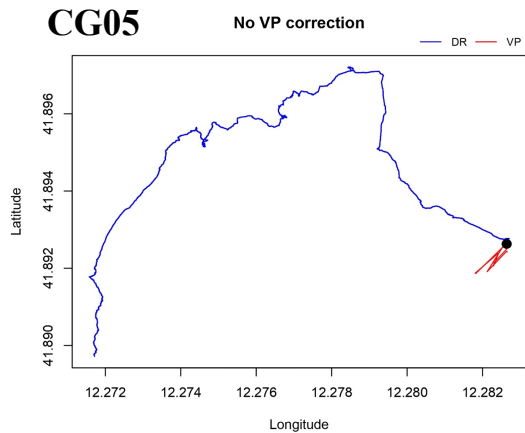
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CG04



CG05



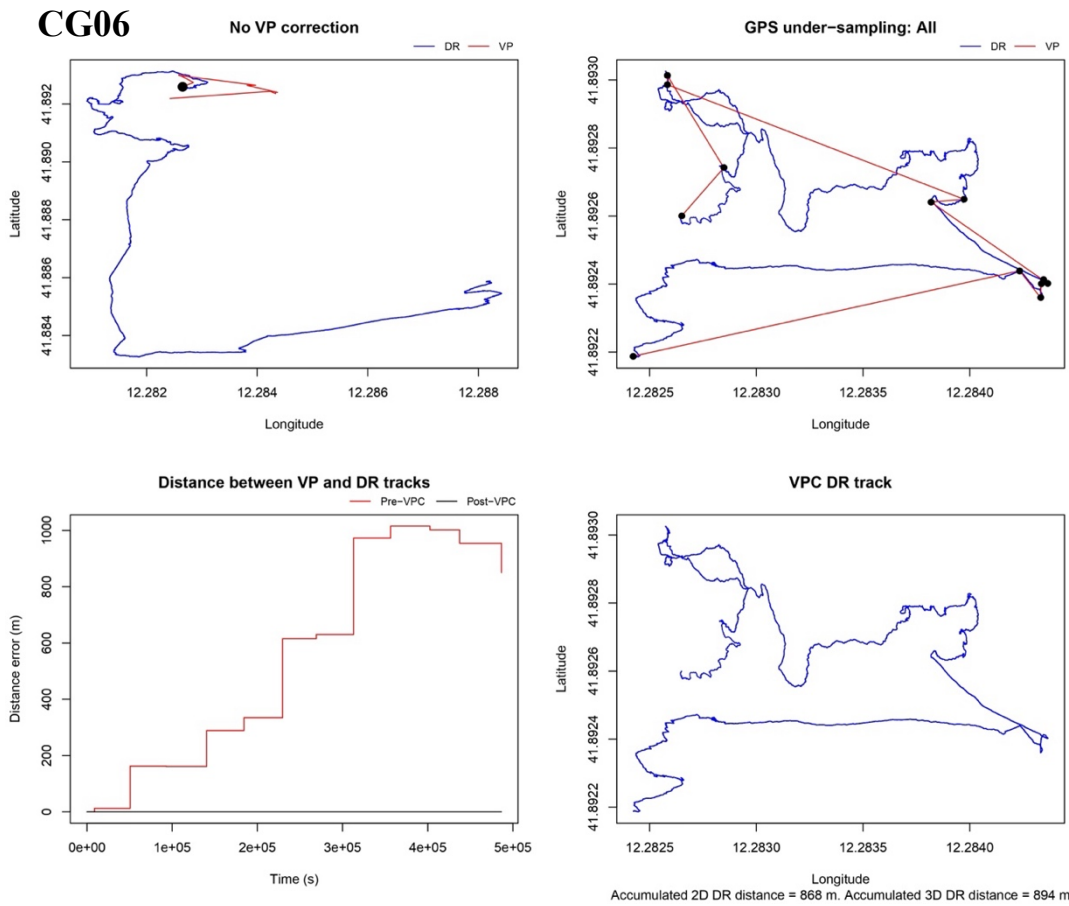


Figure S2. Reconstruction of movement tracks for individuals CG02-CG06 using the *Gundog.Track* function. The output plot is divided into four panels: (top left) red line represents the track reconstructed from validated positions (VP), while the blue line shows the uncorrected dead-reckoning (DR) track derived from accelerometer data; the black dot indicates the starting point of the track. (top right) red points represent the VP-based track, while the blue line shows the DR track corrected using VP data. (bottom left) distances between VP and the VP-corrected DR track. (bottom right) the final DR track reconstruction.

Data Availability

Data will be made publicly available upon publication of the corresponding manuscript. Currently, all datasets and scripts are stored in a private GitHub repository (URL: https://github.com/BombinaPN/DR_Bufotes_viridis), accessible upon request.

Chapter V - Following the footsteps of *Bufo bufo*: seasonal insights from dead-reckoning tracking.

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Manuscript in preparation.

Abstract

Understanding how environmental conditions shape the movement and activity of amphibians is essential for effective conservation planning. Using multi-sensor loggers combining VHF, accelerometers, and magnetometers, we investigated the fine-scale spatial and behavioural ecology of *Bufo bufo* across contrasting seasonal contexts in a Mediterranean protected area. Dead-reckoning (DR) reconstructions successfully complemented GPS data, revealing longer and more tortuous trajectories than positional fixes alone, and providing unprecedented temporal resolution of individual movements. Accelerometer-derived activity patterns showed marked seasonal differences. In summer, toads were mainly active at night within a thermal range of 13-24 °C, often restricting movements to small, moisture-rich forested patches. In contrast, during winter, activity

occurred at much lower temperatures (4-10 °C) and over wider areas, with individuals exhibiting larger home ranges despite lower overall locomotor frequency. This seasonal variation in home range size likely reflects both the capacity to exploit more suitable conditions for movement and the need to locate suitable refuges and thermally stable microhabitats under colder, more variable conditions.

Together, GPS and DR data revealed a strong influence of temperature and humidity on both the extent and timing of movement. From a conservation perspective, the seasonal shift in microhabitat use underscores the importance of protecting both summer and winter habitats and maintaining ecological connectivity between them. Our study demonstrates the feasibility and potential of lightweight multi-sensor biologging in amphibians, offering new opportunities to link fine-scale movement, behaviour, and environmental drivers in species of conservation concern.

Introduction

Amphibians are among the most threatened vertebrate groups worldwide, with nearly half of all species experiencing population declines (Re:wild, Synchronicity Earth, 2023). The causes of this global decline are multifaceted, including habitat loss and fragmentation, pollution, disease, climate change, and the spread of invasive species (Re:wild, Synchronicity Earth, 2023). Because amphibians play key ecological roles as both predators and prey, their decline can have cascading effects on ecosystem dynamics (Alonso et al., 2024; Hocking & Babbitt, 2014).

Understanding the spatial ecology of amphibians is crucial to developing effective conservation strategies. Movement data provide insights into habitat use, dispersal potential, and the connectivity of populations across landscapes (Joly, 2019; Reyes-Moya

et al., 2022). Fine-scale tracking allows researchers to identify environmental conditions that promote activity and movement, as well as those that constrain it.

The common toad (*Bufo bufo*), despite its name, is undergoing population declines in many parts of Europe (Petrovan et al., 2025). This widespread species is considered ecologically generalist and often used as a model in ecological and behavioural studies (Bókonyi et al., 2016; Mikó et al., 2017). However, detailed information on its movement patterns, especially across different seasons, is still limited. Studying how *Bufo bufo* moves and behaves in contrasting environmental conditions can provide valuable insights into its ecological requirements and resilience.

Traditional tracking techniques such as VHF telemetry, while informative, are limited in their ability to capture continuous, fine-scale movement data in small terrestrial animals. Dead-reckoning, which integrates data from accelerometers and magnetometers to reconstruct trajectories between GPS fixes, overcomes many of these limitations and offers unprecedented temporal resolution (Bidder et al., 2015; Magowan et al., 2022; Redcliffe et al., 2025).

In this study, we applied dead-reckoning to track the movements of *B. bufo* individuals during both summer and winter. Our aim was to investigate how toads move and behave under different seasonal conditions, providing new insights into their activity patterns and habitat use. In addition, we examined the environmental and temporal factors influencing their movements, specifically identifying the temperature ranges and time periods at which individuals were most active. This allowed us to infer when toads are more likely to disperse across the landscape, along which path they move in patchy habitats, and when they seek shelter underground. Ultimately, our findings contribute to a more

comprehensive understanding of amphibian spatial ecology and to the development of more effective conservation strategies.

Methods

2.1 Study Animals and Device Attachment

In June 2024, six individuals of *Bufo bufo* were captured in the Natural Reserve of Insugherata (Figure S1). After capture, the animals were temporarily housed at the Bioparco di Roma, where behavioural trials were conducted to ensure that the selected tracking devices did not negatively affect their performance (Nesi et al., Under review) . On 7 July 2024, all individuals were released back at their original capture site, initiating a one-week monitoring period during the summer season. For the winter season, six additional individuals were captured on the night of 5 March 2025 and released the following afternoon for an additional one-week tracking period. Although this period is referred to as “winter season” throughout the manuscript, early March represents a transitional phase in the annual cycle of *Bufo bufo*. In this area, breeding migrations typically occur between January and March, but their exact timing is highly variable among years. During our sampling period, egg clutches were already present in nearby water bodies, suggesting that at least part of the local population had already bred, while others may still have been moving toward or away from breeding sites. Therefore, individuals tracked in March may have been in different phases of the reproductive cycle, ranging from late-winter foraging to pre- or post-breeding movements.

We used custom-made tracking devices (Axy 5, Technosmart Europe srl, Rome, Italy) that integrated VHF transmitters, tri-axial accelerometers, magnetometers, and temperature sensors, with a total weight of 2.92 g per device. Accelerometer data were

collected at a frequency of 25 Hz, while magnetometer readings were sampled at 2 Hz, providing sufficiently high resolution for both activity classification and heading estimation. Each device featured two holes on the short opposing sides to facilitate attachment using 1 mm diameter silicone cords (Jinsion Crystal Tec®). One cord was threaded through the lower holes and secured around the waist, while the second cord passed through the upper holes and was tied around the forelimbs using reef knots, reinforced with an additional knot and sealed with water-resistant tape (Nesi et al., Under review). This harness design positioned the device centrally on the toad's body, ensuring accurate accelerometer measurements while minimising any interference with natural movements.

All experimental procedures were performed in compliance with the guidelines of the Italian Ministry of the Environment and Energy Security (authorisation no. 2023.0043372).

2.2 Field Tracking

Prior to release, each device was calibrated on-site by rotating it along its y-axis while the operator also turned in place, ensuring accurate magnetometer calibration along all axes, including the y-axis (Williams et al., 2017). Following calibration, the toads were released at their original capture locations at 19:50 during the summer season and at 19:00 in winter. The first tracking sessions began at 21:00 on both seasons. After release, individuals were located at 12-hour intervals using radiotelemetry, with search sessions conducted at 09:00 and 21:00, continuing until every toad had been relocated. Homing procedures were employed to determine the precise GPS position of each individual. At each relocation, observers noted whether the animal was visible and whether it was

actively moving or stationary. These GPS reference points served as critical anchors for reconstructing continuous movement trajectories through dead-reckoning.

2.3 Track reconstruction

Reconstruction of individual movement tracks was carried out in R v. 4.5.1 (R Core Team, 2025) using the *Gundog.Tracks* function (Gunner et al., 2021). For each toad, GPS and accelerometer datasets were imported and calibrated following the procedure described by Garde et al. (2022). Time segments corresponding to magnetometer calibration were annotated in the *Marked.event* column using the label “M”. Magnetometer correction was then performed with the *Gundog.Compass* function (Gunner et al., 2021), specifying *method = 1*, which assumes an offset tri-axial sphere. This option was selected because it provides reliable correction when a simple translational shift of the magnetic field is present, as recommended by the package authors.

To distinguish active from inactive phases, we employed a Random Forest model previously developed for *B. bufo* (Nesi et al., Under review). The model classified behavioural states (movement vs. rest) based on accelerometric axes and Vectorial Dynamic Body Acceleration (VeDBA ; Gleiss et al., 2011) values. The predicted output was incorporated into the *Marked.event* column, assigning a value of 0 when the individual was stationary and 1 when active. Elevation data were linearly interpolated between GPS positions collected during radiotracking sessions.

Dead-reckoning trajectories were reconstructed with the *Gundog.Tracks* function (Gunner et al., 2021). Each track began from the individual’s release coordinates (longitude and latitude), with heading information derived from the smoothed yaw angle

(h) and movement speed estimated from VeDBA values (v) using a linear calibration factor ($m = 1$, $c = 0$). Interpolated elevation (elv) and pitch (p) were included to account for three-dimensional movement, while the activity state (ME) restricted track progression to periods classified as active by the Random Forest output. Verified Positions (VPs), obtained from GPS fixes during radiotracking, were supplied as VP.lon and VP.lat.

All available VPs were retained (VP.ME = FALSE) to correct potential drift even during inactive periods, and the parameter method = "All" was applied to ensure that every VP contributed to the dead-reckoning correction process. This setting allowed the use of the full set of high-resolution GPS data and minimised positional drift across the reconstructed path. Tracks were bounded between the first and last VP (bound = TRUE) and reconstructed in the forward direction (Outgoing = TRUE). Finally, diagnostic plots were generated (plot = TRUE) to visually compare the dead-reckoning and the VP-corrected trajectories.

2.4 Activity patterns analysis

Activity profiles were generated using the output of the Random Forest model (Nesi et al., Under review), which categorised each accelerometer record as indicating either movement or rest. To analyse how activity changed over the course of the day, timestamp data were organised into one-hour intervals (from HH:00:00 to HH:59:59). For each individual, we calculated an hourly activity index by dividing the number of records classified as movement by the total number of records (movement + rest) within that hour. The relationship between temperature and activity was evaluated using a comparable approach. Recorded temperature values were rounded to the nearest degree Celsius (e.g.,

15.4°C assigned to 15°C), and within each temperature class we computed the proportion of active records relative to all observations. Subsequently, the temporal and thermal datasets were integrated to investigate how the probability of movement varied jointly across different hours of the day and temperature conditions. This analysis made it possible to identify the periods and thermal ranges during which *B. bufo* was most likely to be active, offering insights into its preferred environmental and climatic conditions for movement.

2.5 Home range

To quantify the spatial extent of movements, individual home ranges of *B. bufo* were estimated seasonally using the Minimum Convex Polygon (MCP) method. Dead-reckoned GPS-corrected positions were first resampled at 1 Hz and projected into the Universal Transverse Mercator coordinate system (UTM zone 33N, WGS84 datum) to ensure accurate distance and area computations. Individual MCPs were then calculated using 100% of the available locations with the *adehabitatHR* package (Calenge, 2024). The resulting polygons represent the total area encompassed by each toad during the tracking period, providing an estimate of space use. To explore seasonal differences, individuals were grouped according to tracking period (summer: IN01-IN06; winter: IN07-IN12). For each season, all location points were pooled to compute a single seasonal MCP polygon, representing the overall area used collectively by all tracked individuals in that period.

Results

3.1 Fate of tracked individuals

In the summer season, six *B. bufo* females were tracked, with an average body weight of 318.1 ± 92.1 g (185-448.4 g) and a device-to-body weight ratio of $0.99 \pm 0.33\%$ (0.65-1.58 %; Table S1). For the winter season, six individuals were tracked, including four females and two males, with a mean weight of 173.7 ± 104.0 g (89.6-349.7 g) and an average device-to-body weight ratio of $2.13 \pm 0.93\%$ (0.85-3.26 %; Table S1).

In both seasons, all individuals were released within a single area, close to the location where they had been captured. The duration of tracking and the recovery of devices varied considerably between individuals. During the summer, individuals IN01, IN02, IN03, and IN06 were successfully tracked for the full one-week period (7-13 July), with devices recovered and complete datasets gathered. Individual IN04 lost signal after just one day, providing location points only from the first evening and the following morning; the VHF signal could not be detected from the second evening onward. This loss was likely caused either by damage to the device or to the toad itself, probably resulting from machineries operated by private people managing the trails in the reserve, which were observed in action on the morning of 8 July, coinciding with the last moment the VHF signal was detected. IN05 was last visually observed on the evening of 8 July (the second night of tracking), and its tracking continued until the device battery depleted. The sudden disappearance of the signal in the final days was likely due to predation by an adult *Natrix helvetica*, observed in the area where the VHF signal was strongest. The subsequent movements of the signal appeared unusually rapid and inconsistent with normal toad behaviour, suggesting that the device was carried by a predator rather than the toad itself.

During the winter season, individuals IN08, IN10, IN11, and IN12 were successfully tracked for the full one-week period (6-13 March), with their devices recovered and complete datasets obtained. Individual IN07 was tracked until the VHF battery was depleted. Data from the device were collected until the evening of 9 March; thereafter, attempts to locate the toad were complicated by challenging terrain, dense vegetation, and adverse weather conditions. According to the VHF signal, the individual had moved 388.0 m in ellipsoidal distance (496.9 m in Cartesian projection) from the location where it had been last found, preventing recovery of the remaining data for the second half of the week. For individual IN09, it was not possible to reconstruct any movement paths. The toad was last visually observed on the morning of 8 March (the final confirmed sighting), and data stored on the device were never downloaded. After this date, the animal likely retreated into a small stream densely overgrown with brambles, which could not be accessed before the VHF device battery was fully depleted, making further signal reception and tracking impossible.

3.2 Comparison between GPS and DR tracks

Dead-reckoning trajectories were successfully reconstructed for eight individuals out of the twelve tracked, yielding complete movement paths for four toads in summer and four in winter, plus one partial track covering half of the winter monitoring period. Both GPS-based tracks (validated positions, VP) and dead-reckoning (DR) trajectories were obtained, showing notable differences in the total distances travelled (Figure 1; S2). For all toads, including those for which DR tracks could not be obtained, the GPS-based positions are available in the Supplementary Materials (Figure S3).

Winter individuals frequently moved along existing human trails when available, using them as corridors that allowed faster and more linear movements by minimising physical

obstacles. This behaviour was rarely observed in summer, when movements occurred mainly through vegetated areas.

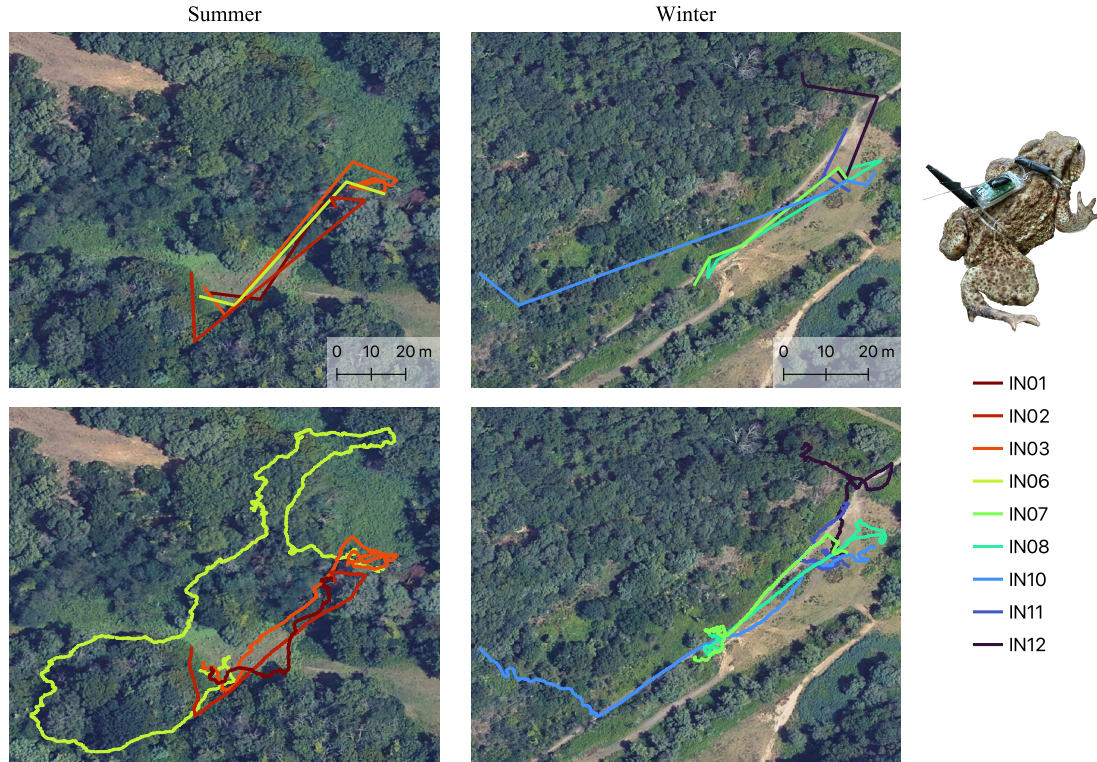


Figure 1. Reconstructed movement tracks of *Bufo bufo* individuals obtained using different methods. Panels on the left show individuals tracked during the summer (IN01, IN02, IN03, and IN06), while panels on the right show those successfully tracked during the winter (IN07, IN08, IN10, IN11, and IN12). Upper panels display trajectories reconstructed using validated GPS positions (VP), whereas lower panels show those obtained through dead-reckoning (DR).

Across all individuals, the mean distance estimated from GPS data (VP) was 114.4 ± 49.1 m (range: 58-220 m). In contrast, DR-based reconstructions produced substantially higher estimates: the average 2D DR distance was 239.7 ± 146.6 m (range: 99-577 m), and when elevation was incorporated, the 3D DR distance reached 251.9 ± 146.6 m (range: 117-596 m; Table 1). When examined by season, summer individuals (IN01-IN06) travelled on average 98.3 ± 35.9 m based on VP data (range: 58-139 m), while DR reconstructions increased these estimates to 245.8 ± 223.9 m in 2D (range: 99-577 m) and 273.3 ± 219.9 m in 3D (range: 117-596 m). In winter (IN07-IN12), the mean VP distance was $127.4 \pm$

59.3 m (range: 61-220 m), whereas DR-derived estimates reached 214.8 ± 62.9 m in 2D (range: 134-307 m) and 232.8 ± 69.8 m in 3D (range: 143-333 m). These results confirm that dead-reckoning provides a finer-scale representation of the animals' trajectories, capturing tortuous and small-scale movements that are not detectable through GPS fixes alone.

Table 1. Distances (m) estimated from validated GPS positions (VP), 2D dead-reckoning (DR) and 3D dead-reckoning (DR) for the five successfully reconstructed individuals.

ID	Season	VP 2D (m)	DR 2D (m)	DR 3D (m)
IN01	Summer	58	99	117
IN02	Summer	115	121	154
IN03	Summer	139	186	226
IN06	Summer	81	577	596
IN07	Winter	110	190	202
IN08	Winter	142	229	253
IN10	Winter	220	307	333
IN11	Winter	61	134	143
IN12	Winter	104	214	233

3.3 Activity patterns

During the summer tracking period, the four monitored individuals (IN01, IN02, IN03, and IN06) displayed marked temporal and thermal patterns of activity (Figure 2). The recorded temperatures throughout the monitoring sessions ranged 11-28 °C, but movements occurred predominantly within a narrower range, typically between 13 °C and 24 °C. Activity was concentrated mainly during crepuscular and nocturnal hours, although the specific timing varied among individuals. IN01 showed two main peaks of movement, at early morning (around 04:00-06:00) at approximately 19 °C, and during the afternoon (around 16:00) at 16-20 °C, with sporadic movements at cooler (12 °C, 07:00) and warmer (25 °C, 14:00) conditions. IN02 exhibited a predominant activity period between 19:00 and 21:00 at 23-24 °C, with minor movement detected near dawn

(05:00, 12-15 °C). IN03 was mostly active during the late evening and early night, particularly around 01:00 at 19-20 °C and 21:00 at 14-15 °C, with occasional activity near 19:00 at 23-24 °C. Interestingly, this individual also showed unusually low temperature values (11-14 °C) during the warmest daylight hours, a pattern inconsistent with the expected diel thermal trend. This pattern likely reflects periods spent in the water, as IN03 was observed in a small stream during the tracking period. IN06 showed two activity phases: one at early night (20:00-23:00, 20-23 °C) and another before dawn (02:00-03:00, 13-15 °C). The maximum proportion of movement within a given temperature–time bin reached 15 % in IN01, 23 % in IN02, 32 % in IN03, and 21 % in IN06, suggesting individual variation in both temporal and thermal preferences.

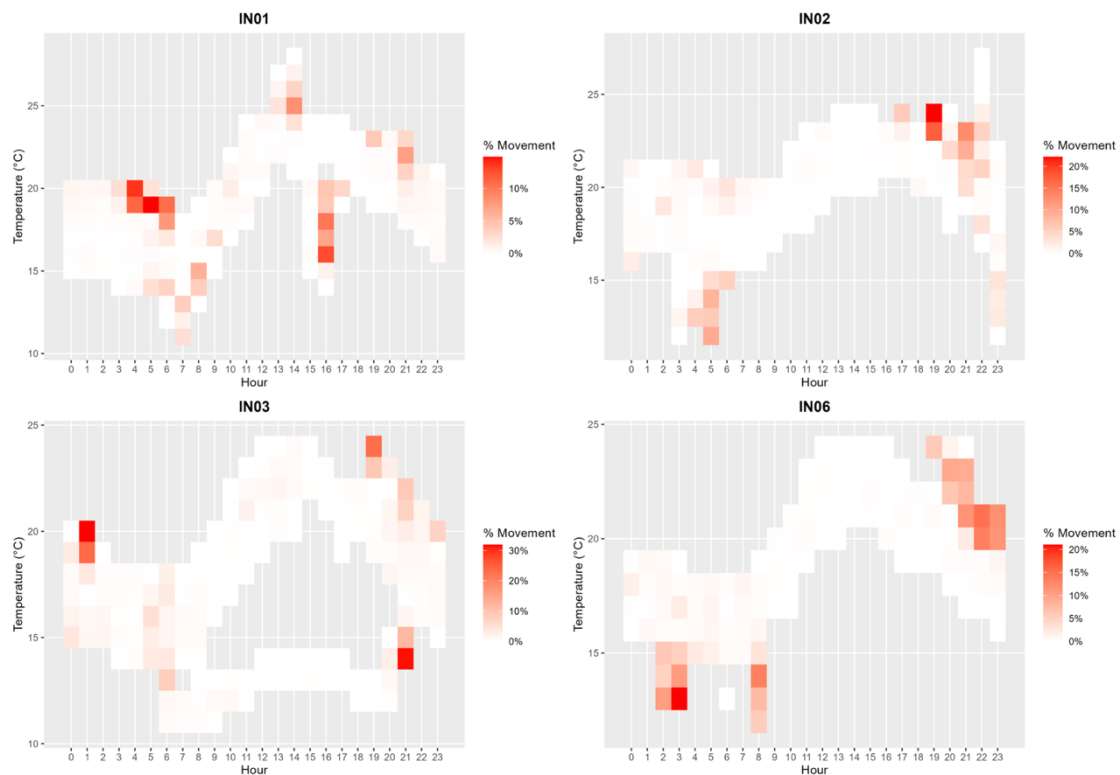


Figure 2. Heatmaps showing the proportion of time spent moving as a function of hour of day (x-axis) and ambient temperature (y-axis) for each individual *Bufo bufo* (IN01, IN02, IN03 and IN06). Activity is expressed as the percentage of events classified as “Movement” per bin, with warmer colours indicating higher activity levels.

During the winter tracking sessions, five out six individuals (IN07-IN12) were successfully monitored, showing distinct patterns of movement across a broad range of

recorded temperatures, from -1 °C to 22 °C (Figure 3). Overall, activity occurred mainly during evening and early night hours, between 18:00 and 22:00, and was generally associated with low ambient temperatures, typically between 4 °C and 10 °C. IN07 exhibited movements primarily around 21:00 at 3-6 °C, with occasional activity at 20:00 and 11 °C. IN08, which experienced the widest thermal range (-1-20 °C), was mostly active between 19:00 and 21:00 at 4-7 °C, but also showed sporadic movements between 00:00 and 05:00 at 10-12 °C. A short burst of movement was recorded around 13:00 at 20 °C, likely caused by researcher disturbance, as the individual was observed emerging from vegetation during fieldwork. IN10 moved mainly during the early morning (01:00-06:00) at 8-12 °C, and again around 19:00 at 5-6 °C. IN11 was active primarily in the evening (18:00-20:00) at 6-11 °C, with minor movements between 01:00 and 05:00 at 5-10 °C, while IN12 displayed its highest movement frequencies between 18:00 and 22:00 at 7-9 °C. The maximum proportion of movement observed within any temperature-hour

bin reached 17 % for IN07, 19 % for IN08, 42 % for IN10, 9 % for IN11, and 21 % for IN12.

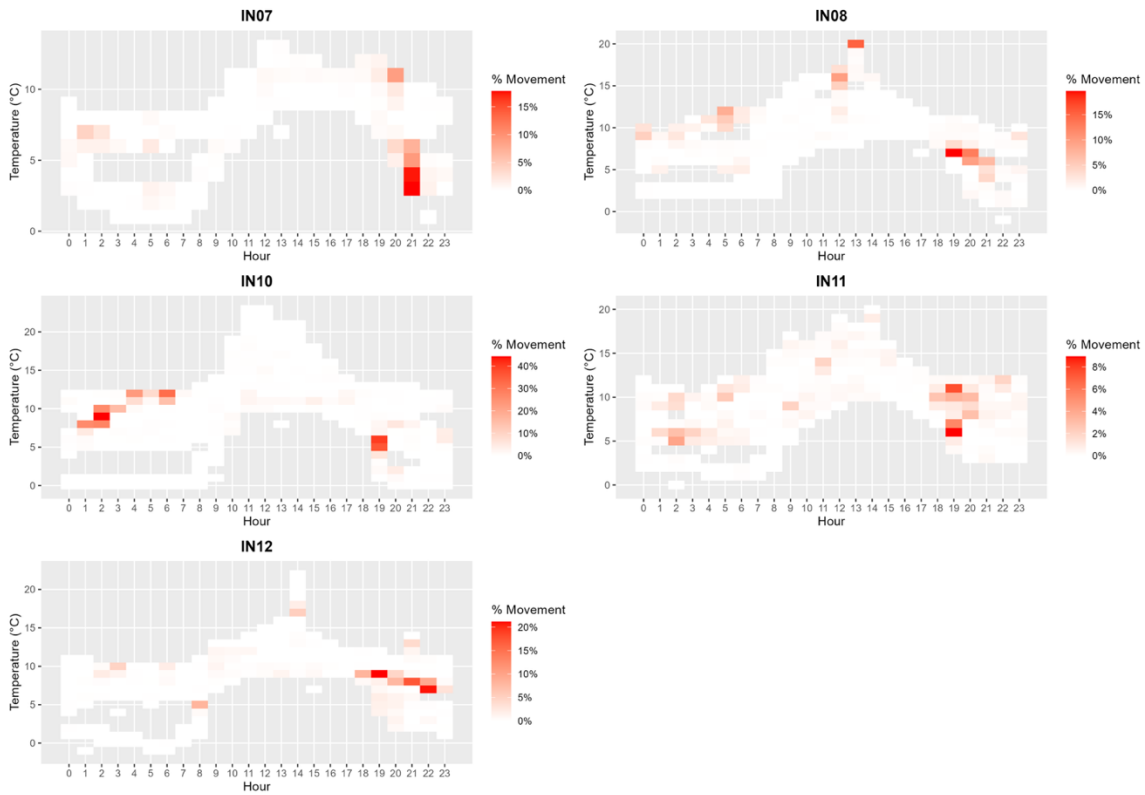


Figure 3. Heatmaps showing the proportion of time spent moving as a function of hour of day (x-axis) and ambient temperature (y-axis) for each individual *Bufo bufo* (IN07, IN08, IN10-IN12). Activity is expressed as the percentage of events classified as “Movement” per bin, with warmer colours indicating higher activity levels.

3.4 Home range

Minimum Convex Polygon (MCP) estimates revealed clear differences in the spatial extent of movements across individuals and seasons. During summer, individual MCP areas (Figure S4) ranged from 579 m² (IN01) to 6565.9 m² (IN06), with intermediate values for IN02 (1003.3 m²) and IN03 (988.4 m²). In winter, MCP areas (Figure S5) showed comparable variability, spanning from 593.1 m² (IN11) to 6521.4 m² (IN10), with IN07, IN08, and IN12 covering 1382 m², 1574.1 m², and 1751.3 m², respectively. When pooling all individuals tracked within each season, the total MCP area, representing the

collective home range of all monitored toads, was 7013 m² in summer and 15101.4 m² in winter (Figure 4).

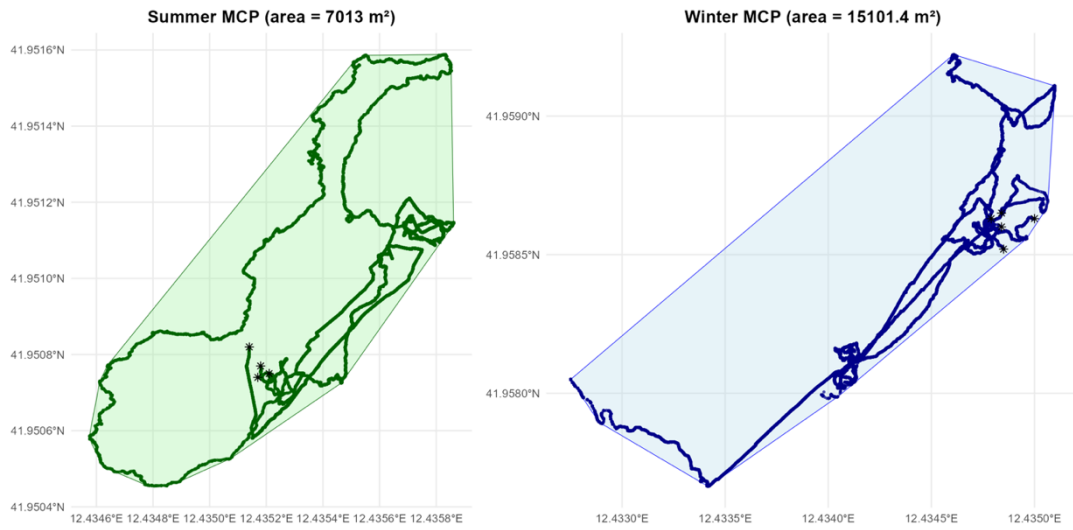


Figure 4. Comparison of the total home range areas (Minimum Convex Polygon, MCP) across seasons. The green map (left) represents the combined summer home range, while the blue map (right) shows the corresponding winter home range. Each polygon encompasses all movement locations recorded for individuals tracked during the respective season.

Discussion

The use of multi-sensor loggers combining GPS, accelerometers, and magnetometers has increasingly enhanced our understanding of animal movement and behaviour across a variety of taxa (Gunner et al., 2021; White & Lauder, 2024). In this study, we successfully applied this approach for the first time to *Bufo bufo*, providing new insights into the fine-scale movement ecology of the species across contrasting seasonal contexts. Despite the inevitable challenges associated with field deployment in free-ranging amphibians, data recovery was successful for most individuals, confirming the feasibility of applying lightweight dead-reckoning (DR) devices to this species in both summer and winter conditions. The variation in tracking duration and data completeness among individuals primarily reflected logistical constraints, environmental conditions, and, in a few cases, likely predation or interference events, rather than technical failure of the devices

themselves. These observations underscore the importance of integrating behavioural and ecological factors, such as microhabitat use and predation risk, when designing telemetry studies on small terrestrial vertebrates.

The comparison between GPS and DR trajectories highlighted the substantial underestimation of movement distances when relying solely on positional fixes. Dead-reckoning reconstructions consistently revealed longer and more tortuous paths than GPS-based estimates. This outcome aligns with previous findings in small or slow-moving animals, where the temporal gaps between GPS fixes can easily mask short, localised displacements (Bidder et al., 2015; Magowan et al., 2022). In this study, the incorporation of inertial data allowed the reconstruction of continuous trajectories, revealing complex fine-scale movements that would otherwise remain undetected. The similarity of results between 2D and 3D DR distances suggests that vertical displacements were relatively modest, consistent with the species' ground-dwelling habit, yet the modest elevation changes detected still contributed to a more realistic estimation of total movement effort. Moreover, the fine-scale reconstruction of paths clearly showed that toads frequently used trails, particularly when these were available within their home areas. This tendency suggests that such anthropogenic alteration of the natural habitats may represent energetically efficient movement corridors, offering plain and obstacle-free surfaces that facilitate terrestrial locomotion. These findings confirm that DR represents a powerful complement to conventional GPS tracking in amphibians, providing unprecedented temporal and spatial resolution of their movements.

The thermal and temporal activity patterns derived from accelerometer data further revealed marked seasonal differences, consistent with patterns already described for other bufonid species (Anderson et al., 2018). During the summer, activity was concentrated at

night and during crepuscular hours, mostly within a temperature range of 13-24 °C. Such conditions likely represent the optimal thermal window balancing locomotor performance with desiccation risk. Individual variation in timing and intensity of activity suggested behavioural flexibility, possibly reflecting differences in local microclimate, individual condition, or proximity to shelters and breeding sites. Notably, one individual (IN03) displayed temperature profiles inconsistent with the expected diel cycle, recording unusually cool values even during daylight hours. Field observations confirmed that this toad frequently occupied a small stream, indicating the use of aquatic refuges during the hottest periods of the day. This behavioural strategy highlights the capacity of *B. bufo* to exploit microhabitats with distinct thermal properties to buffer against excessive heat and maintain physiological performance under summer conditions.

During the winter season, activity occurred predominantly at much lower temperatures, generally between 4 and 10 °C, and was restricted to evening and early night hours. This pattern reflects the strong influence of ambient temperature on locomotor activity and confirms that *B. bufo* can remain active under cold conditions typical of Mediterranean winters. Nevertheless, individual differences in movement intensity and timing suggest that not all toads follow the same overwintering strategy. For instance, IN10, the only male successfully tracked during the winter period, displayed the highest activity level among all individuals, possibly reflecting sex-specific behaviour associated with pre-breeding movements or different energetic demands. The ecological context of the March tracking period requires careful consideration. In central Italian populations of *Bufo bufo*, the shift from overwintering to reproduction occurs over several weeks and does not follow a fixed calendar. Field evidence collected during our study, specifically the presence of newly laid egg masses, indicated that part of the population had already

completed spawning, while other individuals may still have been engaged in movements connected to breeding sites. As a result, the trajectories recorded during this period likely encompassed a mixture of late-winter activity and breeding-related displacements. This phenological heterogeneity offers one plausible explanation for the comparatively long and directional movements observed in some individuals, underscoring the need for cautious interpretation when assigning behavioural motivation to movements.

Home range analyses provided additional insights into the spatial ecology of *B. bufo* across seasons. Despite the reduced frequency of fine-scale activity in winter, individuals exhibited markedly larger total MCP areas compared to summer toads. This apparent expansion likely reflects the broader distribution of suitable microhabitats during the colder months, when increased humidity and frequent rainfall enhance surface moisture and reduce desiccation risk, allowing toads to move safely across otherwise inhospitable terrain. Moreover, suitable overwintering shelters may be more scattered in space, requiring longer displacements to locate optimal refuges. Conversely, in summer, drier conditions and higher evaporation rates likely constrain movement to smaller, moisture-rich patches such as shaded zones or areas near water bodies, resulting in smaller but more behaviourally active home ranges. These contrasting spatial patterns emphasise how seasonal variation in hydric and thermal conditions shapes both the extent and the structure of space use in this species. Field observations further confirmed that tagged individuals were able to access natural refugia without apparent difficulty. Toads were repeatedly found inside small burrows, partially buried in soil depressions, or sheltered beneath logs and root structures, indicating that the device did not hinder entry into narrow or structurally complex refuges. This is consistent with their known reliance on subterranean and covered microhabitats for thermoregulation and predator avoidance, and

suggests that the harnessed tags did not impose substantial mechanical constraints during refuge use.

Overall, these results provide a fine-scale description of the behavioural and thermal ecology of *B. bufo*, emphasising how activity and movement patterns are modulated by environmental temperature, humidity, and time of day. The combination of GPS and dead-reckoning data allowed the quantification of small-scale displacements otherwise missed by conventional telemetry, while accelerometer-derived activity metrics linked these movements to their underlying temporal and thermal context. Together, these findings highlight the potential of multi-sensor tracking to elucidate behavioural adaptations in amphibians, providing valuable information for understanding their spatial ecology and for improving habitat management and conservation strategies in both temperate and Mediterranean environments.

Conclusions

Seasonal comparisons revealed that individuals tracked during winter occupied markedly larger home ranges than those monitored in summer. This pattern suggests that, under colder and more variable environmental conditions, animals may increase their movement range and frequency to locate suitable microhabitats that ensure thermal and moisture stability and foraging opportunities. Conversely, during summer, individuals appear to restrict their movements within smaller areas, likely reflecting the local availability of optimal environmental conditions and resources.

From a conservation standpoint, these findings highlight the importance of considering seasonal variation in habitat use when designing management strategies. Protecting both summer and winter habitats, and ensuring ecological connectivity between them, is

crucial to support the full range of behavioural and physiological requirements of these amphibian populations.

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Supplementary

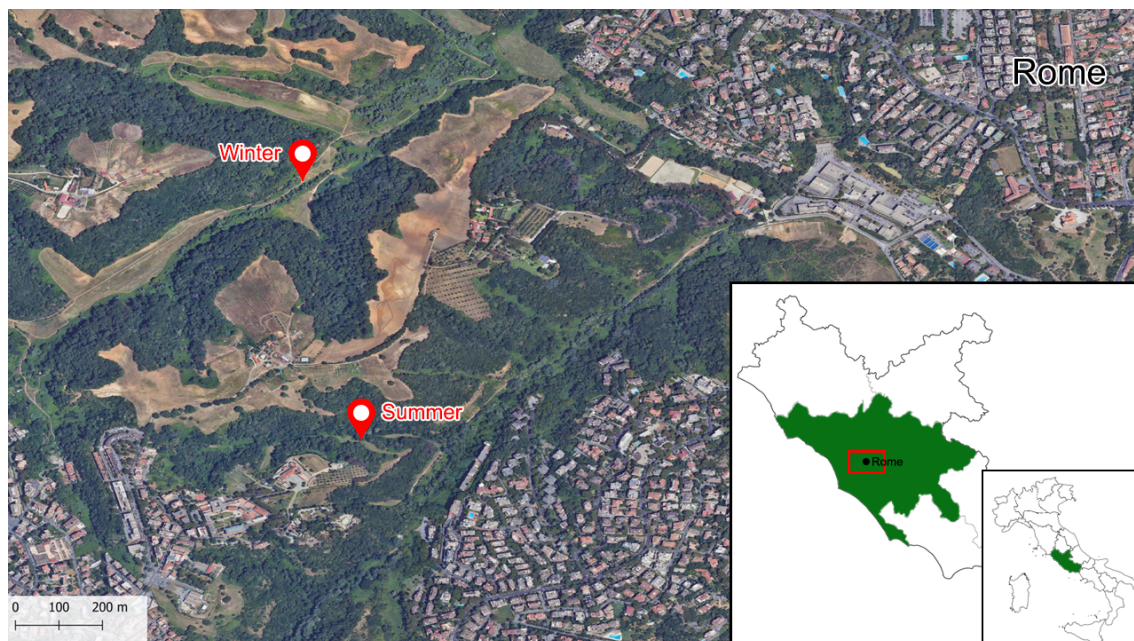
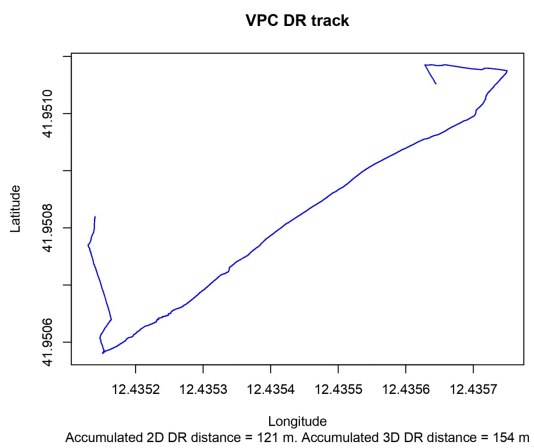
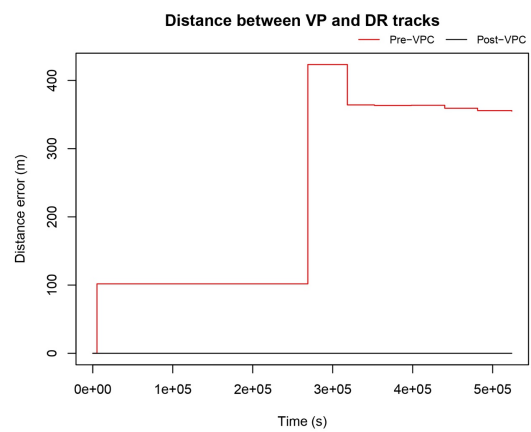
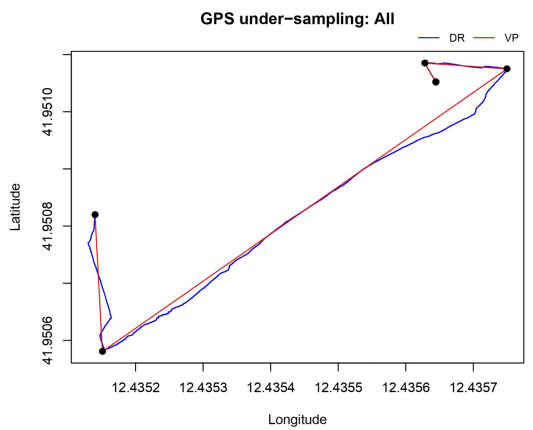
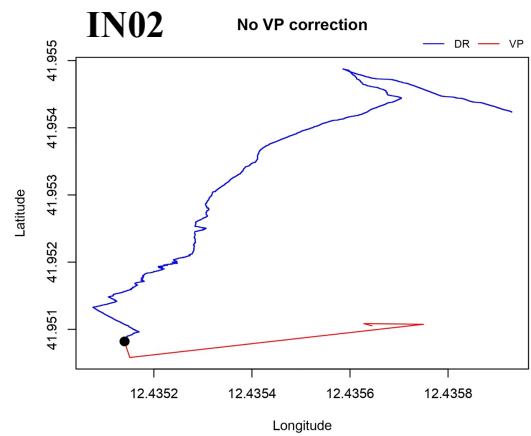
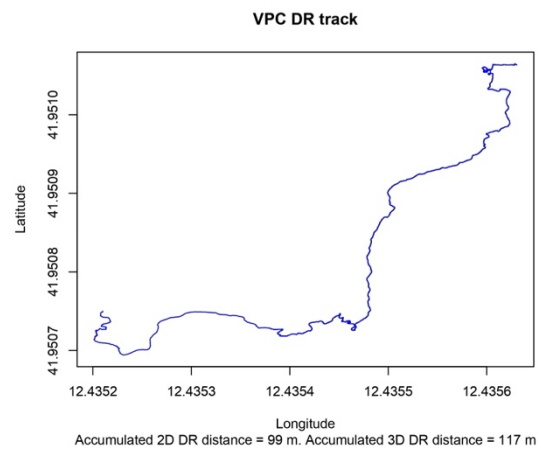
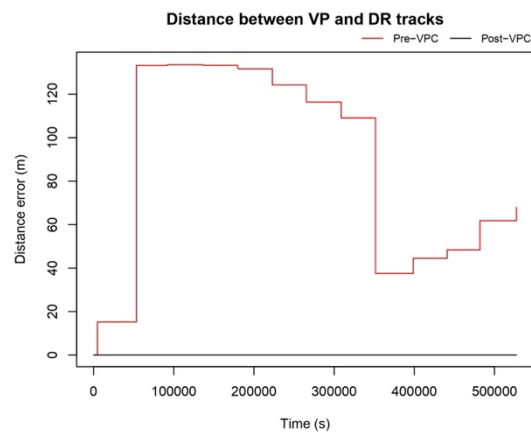
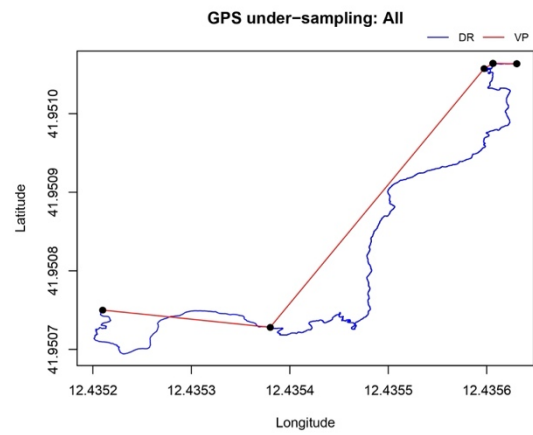
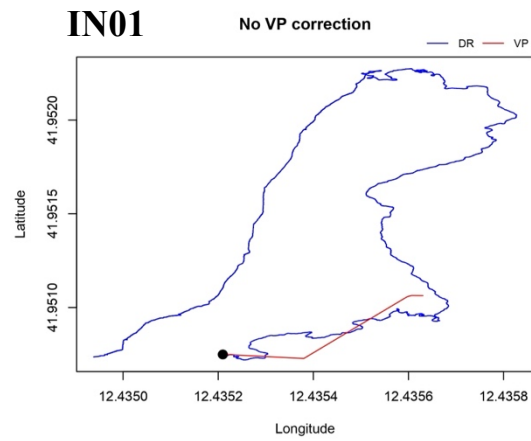
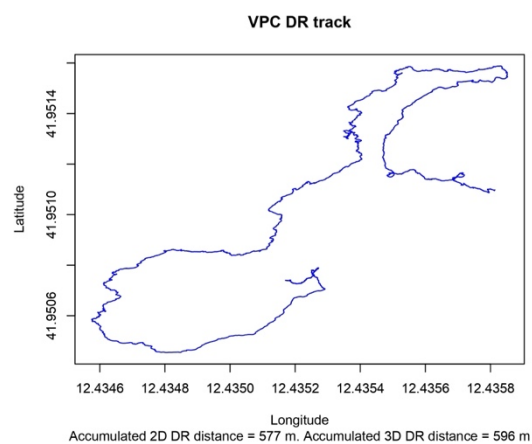
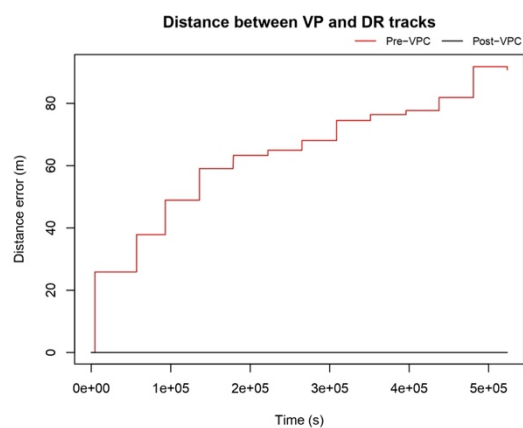
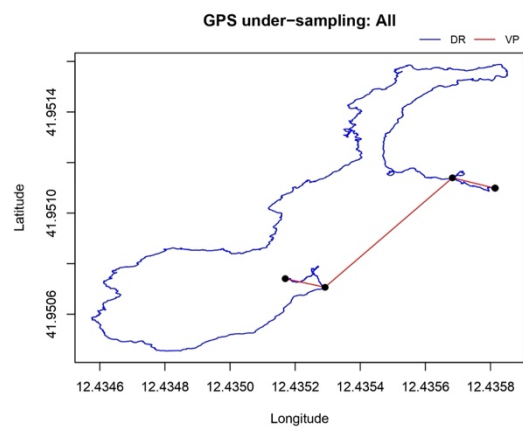
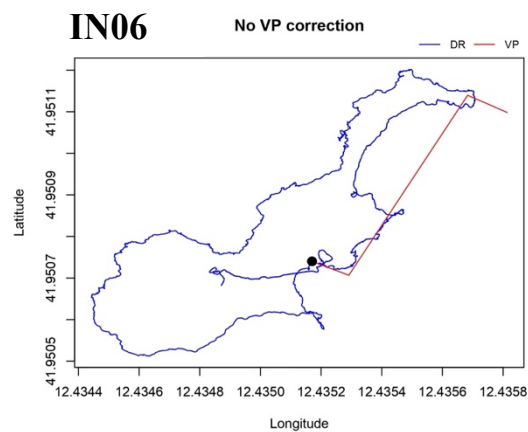
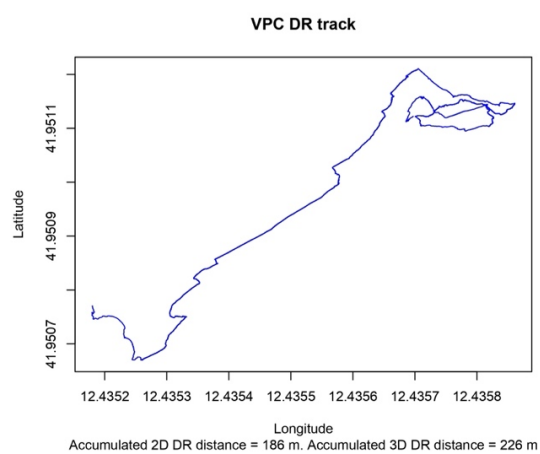
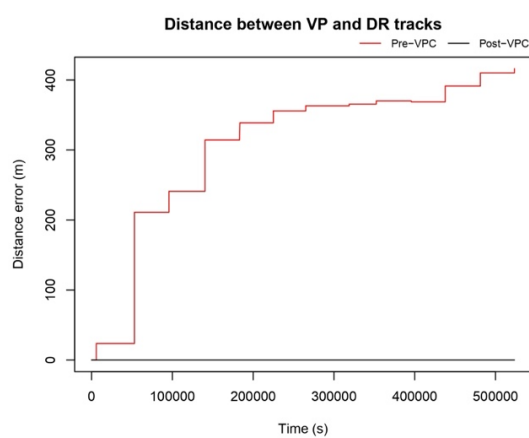
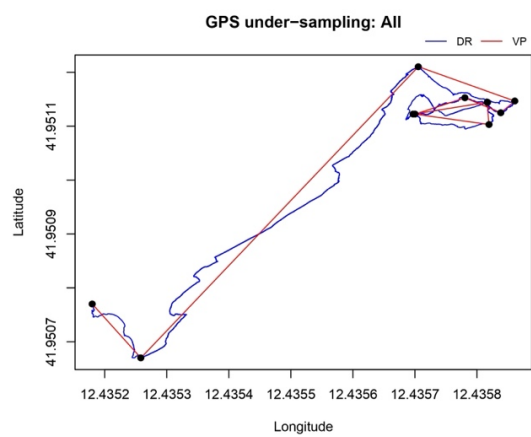
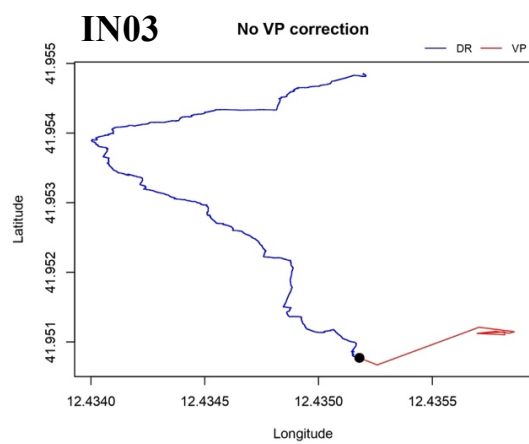
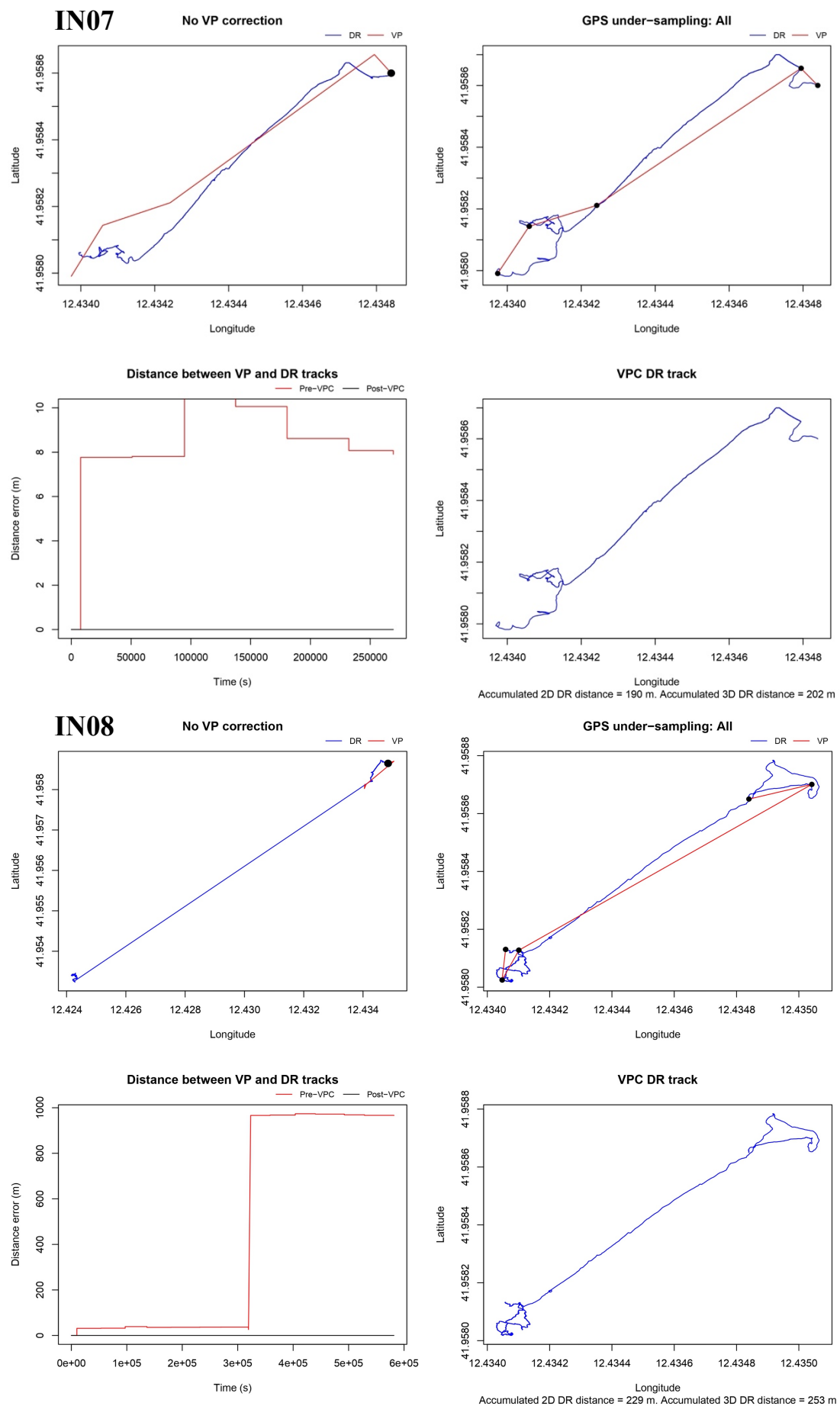
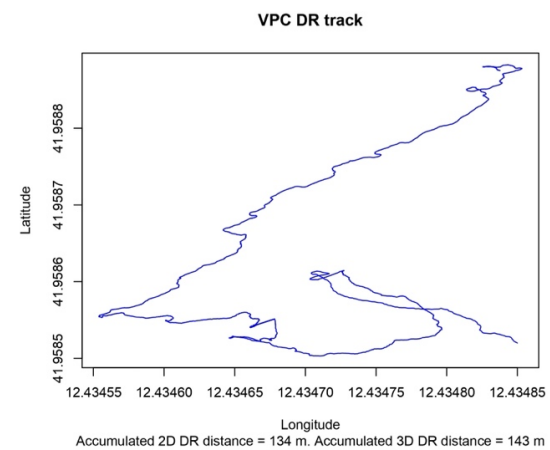
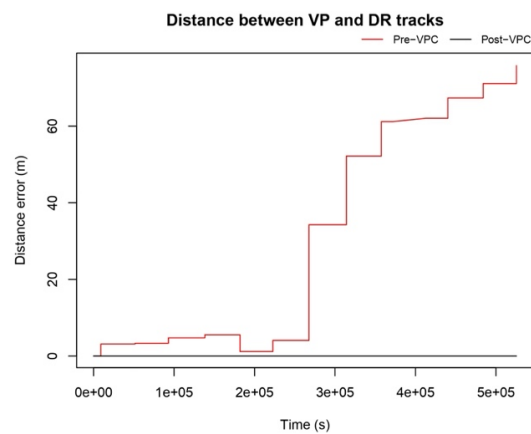
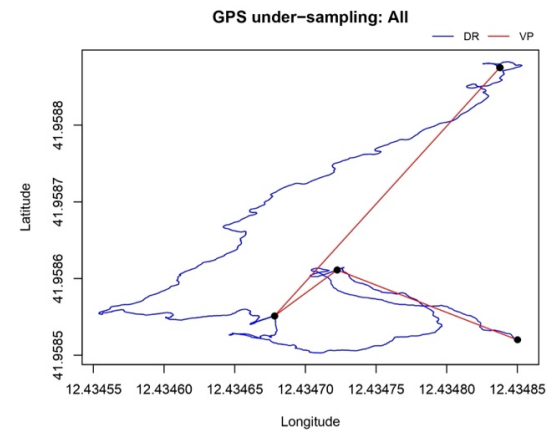
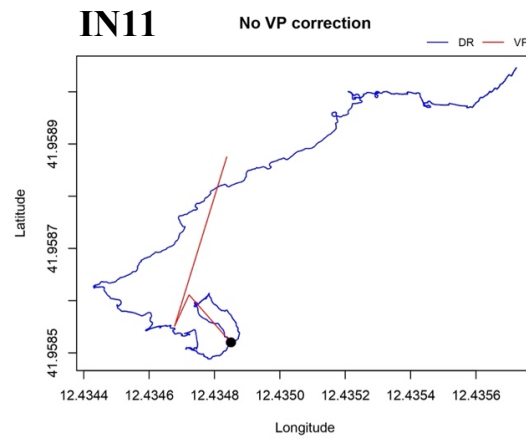
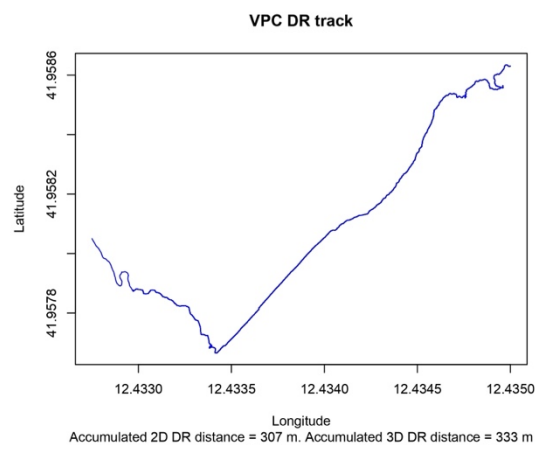
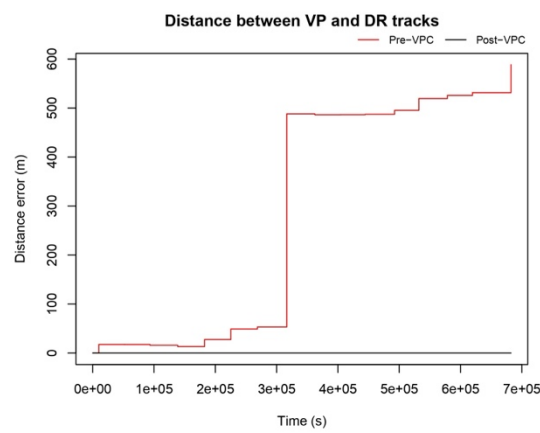
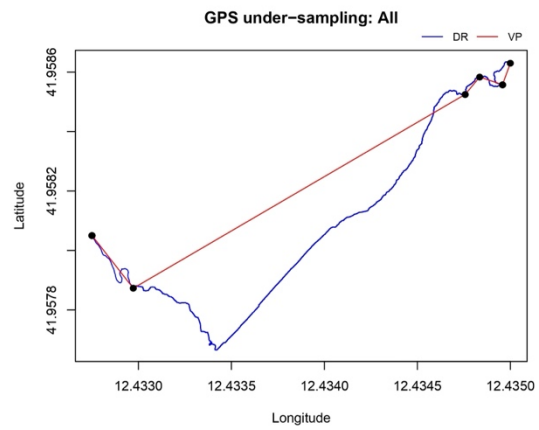
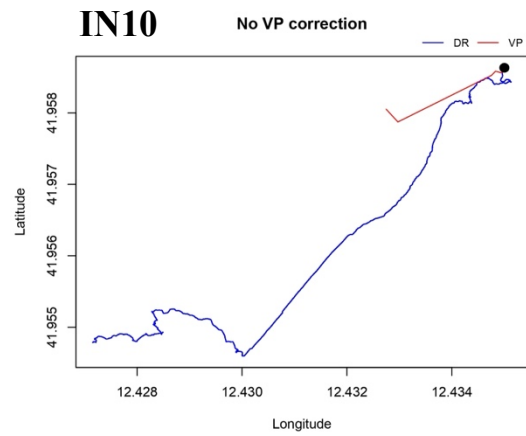


Figure S1. Map of the Insugherata Natural Reserve (Rome, Italy) showing the locations where *Bufo bufo* individuals were tracked during the winter and summer sessions. Coloured markers indicate the seasonal tracking sites, while the map inset highlights the position of the reserve within central Italy.









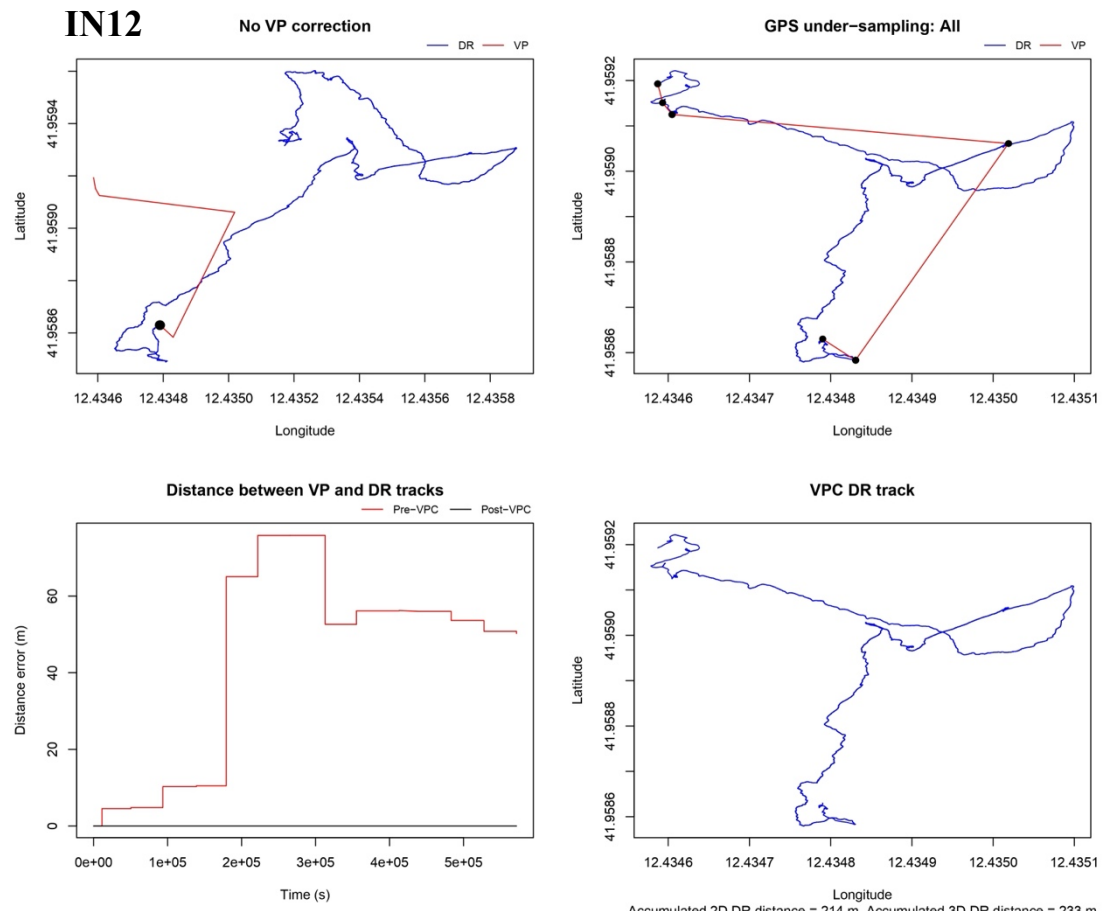


Figure S2. Reconstruction of movement tracks for individuals IN01-IN12 using the *Gundog.Track* function. The output plot is divided into four panels: (top left) red line represents the track reconstructed from validated positions (VP), while the blue line shows the uncorrected dead-reckoning (DR) track derived from accelerometer data; the black dot indicates the starting point of the track. (top right) red points represent the VP-based track, while the blue line shows the DR track corrected using VP data. (bottom left) distances between VP and the VP-corrected DR track. (bottom right) the final DR track reconstruction.

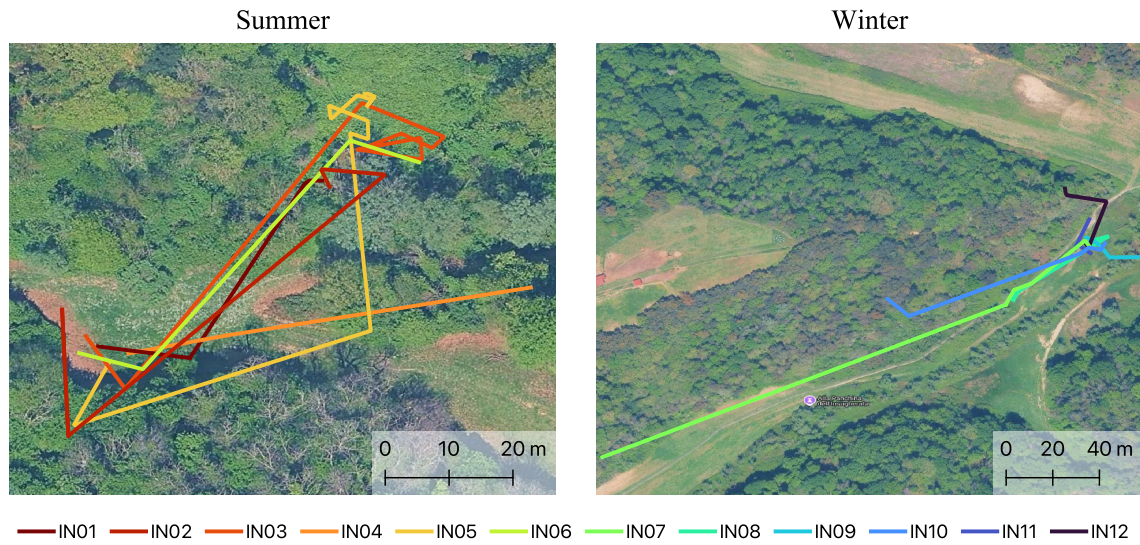


Figure S3. GPS locations of all *Bufo bufo* individuals tracked during the study, including those for which Dead-Reckoned (DR) tracks could not be reconstructed. Each point represents a GPS fix obtained during the one-week tracking period. Individuals tracked in summer (IN01-IN06) are shown on the left, while those tracked in winter (IN07-IN12) are shown on the right.

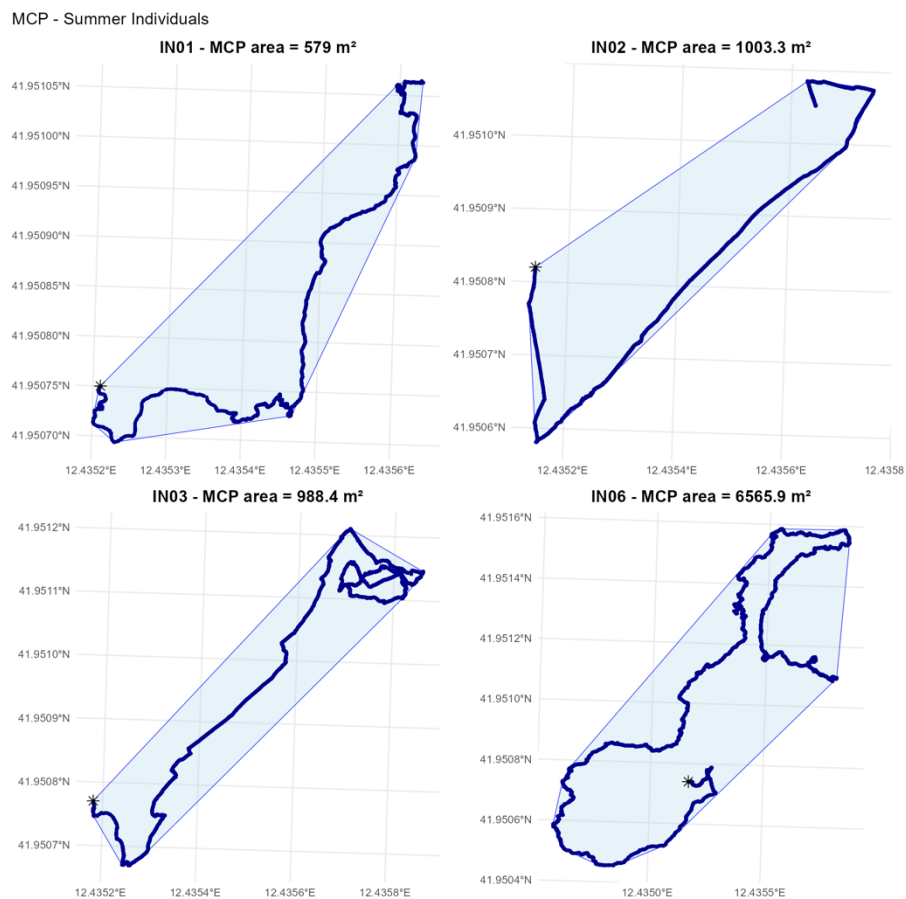


Figure S4. Spatial representation of individual movement tracks and home range areas estimated with the Minimum Convex Polygon (MCP) method for *Bufo bufo* individuals tracked during the summer season. The black asterisk indicates the animal's release location.

MCP - Winter Individuals

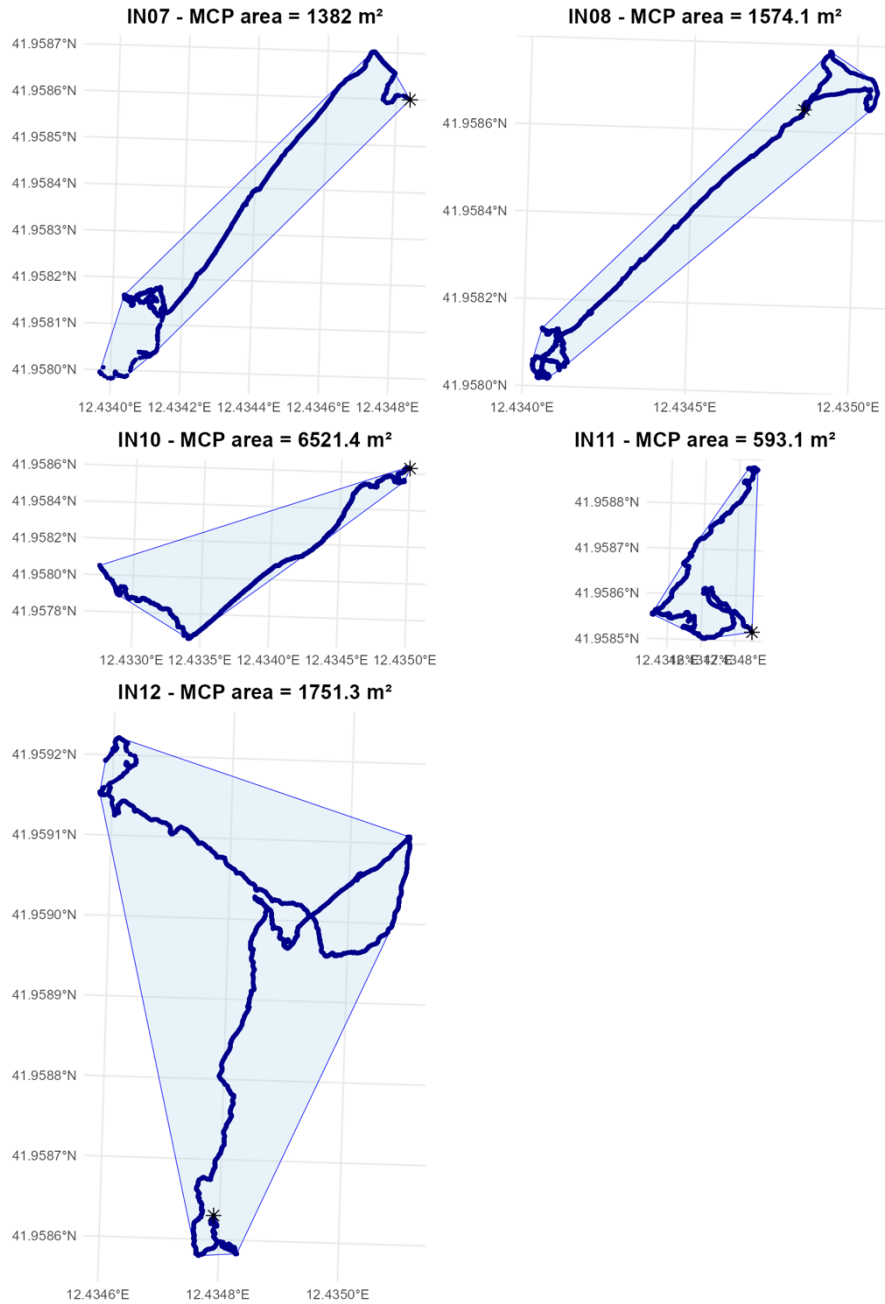


Figure S5. Spatial representation of individual movement tracks and home range areas estimated with the Minimum Convex Polygon (MCP) method for *Bufo bufo* individuals tracked during the winter season. The black asterisk indicates the animal's release location.

Table S1. Information on *Bufo bufo* specimens tracked during summer and winter. The table includes the following columns: (i) Season in which each individual was tested, (ii) Identifier assigned to each specimen, (iii) Sex of the specimen, (iv) Body weight (g), and (v) Percentage ratio of harness weight to body weight.

Season	ID	Sex	Animal weight (g)	Device-Animal weight ratio (%)
<i>Summer</i>	IN01	F	377.95	0.77
	IN02	F	342.43	0.85
	IN03	F	267.11	1.09
	IN04	F	287.72	1.01
	IN05	F	448.37	0.65
	IN06	F	185.00	1.58
<i>Winter</i>	IN07	F	252.49	1.16
	IN08	F	349.70	0.84
	IN09	M	124.53	2.35
	IN10	M	110.58	2.64
	IN11	F	115.50	2.53
	IN12	F	89.62	3.26

Data Availability

Data will be made publicly available upon publication of the corresponding manuscript.

Currently, all datasets and scripts are stored in a private GitHub repository (URL: https://github.com/BombinaPN/DR_Bufo_bufo), accessible upon request.

Chapter VI - Silent steps of a vanishing species: reconstructing fine-scale movement of *Bombina variegata pachypus* using a dead-reckoning approach.

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Manuscript in preparation.

Abstract

Biologging technologies are increasingly used to investigate fine-scale movements of small vertebrates, yet their application to amphibians remains limited. Here, we tested the feasibility of using miniaturised multi-sensor loggers for *Bombina variegata pachypus*, a small sedentary endangered anuran endemic to Italy. Six individuals were equipped with biologging devices combining accelerometers, magnetometers, and temperature sensors, and tracked for up to one week in ponds and natural streams. GPS reference points were manually recorded during field surveys to calibrate and validate dead-reckoning (DR) reconstructions. Despite technical issues, including premature battery depletion, data loss,

and occasional unrealistic straight-line paths, DR successfully reconstructed continuous, high-resolution trajectories. Estimated path lengths from DR averaged 59.9 ± 81.9 m in 2D (range: 1.2-213 m), revealing substantially greater movement than would have been inferred from sparse field observations alone. Individual Minimum Convex Polygon (MCP) areas ranged from 0.09 to 155.9 m², reflecting striking variation in space use even within small wetland networks. Activity analyses showed no consistent daily rhythm; instead, each toad exhibited idiosyncratic temporal and thermal activity patterns (5-28 °C), influenced by microhabitat characteristics such as shading, vegetation, and water depth. Notably, individuals occupying clusters of fenced ponds restored through conservation programs displayed the largest movement areas, highlighting the importance of protected and well-connected habitats. Overall, our findings demonstrate that dead-reckoning can be effectively applied to small amphibians, providing unprecedented insights into individual behaviour, habitat use, and activity patterns, with direct implications for conservation planning of *Bombina v. pachypus* and similar threatened species.

Introduction

The yellow-bellied toads (family Bombinatoridae) include three European taxa: *Bombina bombina*, distributed across eastern Europe; *B. variegata*, found in central and southeastern Europe; and the Apennine yellow-bellied toad, historically described as *B. pachypus* and now regarded as a subspecies of *B. variegata*, namely *B. v. pachypus* (Angelini et al., 2018; Fijarczyk et al., 2011; Pabijan et al., 2013; Pröhl et al., 2021). This taxon is endemic to the Italian peninsula, where it mainly occupies hilly and mid-mountain habitats, generally between 300-1000 m a.s.l., with occasional records up to

1900 m (Razzetti et al., 2025). The species occupies a variety of small lentic water bodies, including temporary ponds, oxbows of streams, drinking troughs, irrigation channels, and other artificial sites in agricultural and pastoral landscapes (Lanza et al., 2007).

Bombina v. pachypus has suffered a dramatic decline across its range, with over half of the known breeding sites lost between 1996 and 2004 and many populations now extinct or severely fragmented (Barbieri et al., 2004; Bologna et al., 2000, 2007; Stoch & Gringnetti, 2021). Although the IUCN does not treat *B. v. pachypus* separately from *B. variegata* (risk category: Least Concern), it is listed as Endangered in the Italian Red List (Rondinini et al., 2022), and is protected under the Bern Convention (Appendix II) and the EU Habitats Directive (Annexes II and IV). Major threats include the loss and degradation of small wetlands, prolonged droughts intensified by climate change, water abstraction, and stochastic demographic fluctuations typical of small populations (Razzetti et al., 2025).

In this context, a better understanding of the fine-scale movement ecology of *B. v. pachypus* can provide crucial information for planning conservation strategies at local and global scale (Pittman et al. 2014). Indeed, identifying when and under which thermal conditions individuals are active may inform both ecological knowledge and conservation practice (Bailey et al. 2019). For example, determining activity windows could improve the effectiveness of field surveys by helping operators focus on the times and conditions in which toads are more likely to be detected. Yet, detailed data on the daily movements and activity of this subspecies remain scarce.

Traditional approaches to studying amphibian movement, such as visual encounter surveys, capture–recapture, or radio-tracking, have clear limitations when applied to small and elusive species. These methods often yield sparse or biased data, as they depend

on observer presence and are constrained by the small body size and reduced displacements (Bower et al., 2014; Lardner et al., 2019). In this context, novel biologging tools such as accelerometers, temperature sensors, and dead-reckoning approaches are emerging as promising techniques to overcome such limitations (Bidder et al., 2015; Chakravarty et al., 2019). Dead-reckoning (DR), in particular, has been successfully applied to reconstruct animal trajectories from fine-scale sensor data (Bidder et al., 2015; Gunner et al., 2021; Magowan et al., 2022), but its application to small amphibians remains largely unexplored.

Developing and testing new tracking techniques on species of conservation concern is essential for both ecological research and applied management. In fact, biologging can provide continuous data on animal behaviour and movement even in the absence of an observer, greatly increasing the amount of information collected compared to traditional methods (Bidder et al., 2015; Magowan et al., 2022). Moreover, identifying the specific time windows and thermal conditions under which individuals are active can optimise monitoring efforts and inform habitat restoration strategies, reducing costs and improving the efficiency of field surveys and conservation management.

In this study, we explored the feasibility of applying dead-reckoning to *Bombina v. pachypus*. Specifically, we aimed to: (i) assess whether this technique can capture the fine-scale movements of a species characterised by extremely limited displacement; and (ii) investigate the temporal and thermal windows of activity. By combining ecological and methodological perspectives, our work contributes to both the conservation of a highly endangered amphibian and the development of innovative tools for amphibian monitoring.

Methods

2.1 Study Animals and Device Attachment

On 26 May 2025, we captured six adult *Bombina v. pachypus* in the Monte Navegna and Cervia Nature Reserve (Lazio, Italy; Figure S1). Individuals were selected based on a minimum body mass threshold of 5.75 g, established through preliminary laboratory tests (Nesi et al., Under review). The sampled toads belonged to two nearby but distinct subpopulations within the reserve. The three individuals from subpopulation NA inhabit a network of puddles created/restored in 2014 and fenced to prevent the use by cattle and wild boars for a restocking project of *B. v. pachypus*, while the three individuals from subpopulation FR inhabit a temporary brook nearby an artificial pond created in 2021 for a reintroduction project (Vignoli et al. 2021).

Immediately after capture, each individual was equipped with a custom-made biologging device (Technosmart Europe srl, Rome, Italy) incorporating a tri-axial accelerometer, a tri-axial magnetometer, and a temperature sensor. Devices weighed 1.29 g, and an additional 0.03 g Recco® diode was affixed to the device to facilitate animal relocation and recapture in the field.

Tags were attached around the waist of each toad using a silicone tubing belt (Patkil®, inner diameter: 1.5 mm; outer diameter: 2.5 mm). A cotton thread was threaded inside the tubing to allow for controlled degradation: in the event of animal loss, the device could be shed naturally over time, thereby minimising welfare risks (Nesi et al., Under review). The accelerometer was configured to record at 25 Hz, while the magnetometer sampled at 2 Hz, providing adequate resolution for activity classification and heading estimation. To maintain continuous recording at this resolution, devices were replaced

halfway through the tracking week, meaning that each individual carried two successive devices over the monitoring period.

All procedures were conducted under permits issued by the Italian Ministry of the Environment and Energy Security (authorisation no. 2023.0043372).

2.2 Field Tracking

Prior to release, magnetometer calibration was performed directly at the capture sites. Each tag was rotated along its y-axis while the operator simultaneously rotated around their own vertical axis, ensuring proper alignment and reducing potential distortions in heading estimation (Williams et al., 2017).

Following calibration, toads were released at their original point of capture at approximately 21:00. After a settling period of 30 minutes, the first tracking session was conducted to obtain initial GPS reference points. Subsequent relocations were carried out at 12-hour intervals, starting at 09:00 and 21:00, until all individuals had been detected. At each relocation event, observers recorded whether the toad was visible and whether it was active or inactive. These GPS fixes served as ground-truth reference points to anchor and validate the continuous movement trajectories later reconstructed via DR.

In addition, all individuals were weighed both prior to the start of tracking and again upon device recovery after one week using a precision balance with 0.01 g accuracy. This allowed monitoring of potential weight.

2.3 Track reconstruction

Track reconstruction was carried out in R v. 4.5.1 (R Core Team, 2025) each individual separately using the *Gundog.Tracks* function (Gunner et al., 2021). Raw GPS and accelerometer data were first imported and calibrated (Garde et al., 2022). Time intervals

corresponding to magnetometer calibration were annotated in the *Marked.event* column with the label “M”. Magnetometer calibration was then performed using the *Gundog.Compass* function (Gunner et al., 2021), setting method = 1 to correct for offsets in the tri-axial magnetic sphere. This approach provides a robust adjustment when only a simple translation of the magnetic field is needed, as recommended by the developers.

To differentiate active from inactive phases, a Random Forest model previously trained on the same species (Nesi et al., Under review) was applied. This model classified periods as movement or stationary based on accelerometer axes and VeDBA (Vector of the Dynamic Body Acceleration = $\sqrt{(Ax^2 + Ay^2 + Az^2)}$; Gleiss et al., 2011) values. The resulting activity predictions were integrated into the *Marked.event* column, with “1” denoting movement and “0” denoting inactivity. Elevation values were interpolated between GPS fixes obtained during field tracking sessions.

Movement paths were reconstructed using the *Gundog.Tracks* function, with the initial coordinates set at each individual’s release location (lo and la). Heading was derived from the smoothed yaw angle (h), and speed was estimated from VeDBA values (v) using a linear calibration factor (m = 1, c = 0). Interpolated elevation (elv) and pitch (p) were included to account for three-dimensional movement. Only periods classified as active by the Random Forest model (ME) were allowed to advance the track. Verified Positions (VPs) from GPS fixes were supplied (VP.lon and VP.lat) to correct for positional drift, and all VPs were retained by setting VP.ME = FALSE, ensuring that even stationary phases were corrected. The track was bounded between the first and last VP (bound = TRUE) and reconstructed in the forward direction (Outgoing = TRUE). Diagnostic plots were generated (plot = TRUE) to visually compare dead-reckoned trajectories against VP-corrected paths. To account for intrinsic GPS error and avoid over-representing

consecutive points separated by minimal distances, VP under-sampling was applied using the method = "Time_Dist" option with a distance threshold of $\text{thresh.d} = 5$ meters. This procedure ensures that consecutive GPS fixes less than 5 m apart are treated as a single point for correction purposes, improving the robustness of the reconstructed tracks while retaining the main spatial information.

Finally, the spatial extent of each individual's movement was quantified by calculating the Minimum Convex Polygon (MCP) based on the dead-reckoned coordinates. MCPs were computed in R (R Core Team, 2025) using the *adehabitatHR* package (Calenge, 2024) after projecting tracks to the appropriate UTM zone. Individual MCPs were generated with a 100% inclusion threshold, providing a first estimation of each toad's area of use during the tracking period.

2.4 Activity patterns analysis

Patterns of activity were quantified starting from the Random Forest classification (Nesi et al., Under review), which identified each accelerometer record as either active (movement) or inactive (stop). To examine daily rhythms, accelerometer time stamps were grouped into hourly intervals, with each bin covering the period from HH:00:00 to HH:59:59. For every hour and individual, we calculated the proportion of movements by dividing the number of active events by the total number of events (active + inactive) recorded in that interval.

Temperature effects on activity were assessed in a similar way. Raw temperature values were rounded to the closest integer (for instance, 15.7°C was assigned to 16°C), and within each temperature class the fraction of active versus total events was computed.

We then combined the temporal and thermal datasets to jointly evaluate how the likelihood of movement varied across hours of the day and temperature conditions. This procedure allowed us to highlight both the time windows and the thermal ranges in which *Bombina v. pachypus* showed a higher tendency to move compared to remaining inactive.

Results

3.1 Device Recovery and Failures

All six tagged toads were initially relocated, and all devices were retrieved between the night of 1 June and the morning of 2 June, with the exception of individual FR02, which could not be recovered despite repeated searches.

Several technical issues occurred with the biologging devices. For FR01, no data could be retrieved from the first tag. FR03 was not sighted at the mid-week check, preventing device replacement; consequently, the first tag eventually discharged in the field. For FR02 and NA04, the first device was replaced as scheduled, but the second unit failed to record any data. Notably, these two tags were the only ones that had been previously deployed, recharged, and reused. For NA05 and NA06, device replacement was successful, but the second units discharged prematurely after only two days (Table 1).

Table 1. Summary of tracking sessions conducted from May 26 to June 2 (first column). The letters M and N indicate morning (9:00) and night (21:00) tracking sessions, respectively. The columns show the IDs of the tracked individuals (FR01-FR03, NA04-NA06). A ✓ indicates that the animal was sighted and a GPS location was recorded, while ✕ indicates that the animal was not detected. Cells underlined in red represent cases where accelerometer data were not available.

	FR01	FR02	FR03	NA04	NA05	NA06
26 N	✕	✕	✓	✓	✓	✓
27 M	<u>✓</u>	✓	✓	✓	✓	✓
27 N	<u>✕</u>	✕	✕	✓	✕	✓
28 M	<u>✓</u>	✕	✕	✓	✓	✓
28 N	<u>✕</u>	✕	✕	✓	✓	✓

29 M	✓	x	x	✓	✓	✓
29 N	✓	x	x	✓	✓	✓
30 M	✓	x	x	✓	✓	✓
30 N	✓	✓	x	✓	✓	✓
31 M	✓	x	x	✓	✓	✓
31 N	x	x	x	✓	✓	✓
1 M	✓	x	x	✓	✓	✓
1 N	✓	x	✓	✓	✓	✓
2 M	✓	x		✓	✓	✓

Recco® reflectors failed to aid relocation as expected, despite specifications indicating detection capacity up to 20 cm underwater. All relocations were achieved via visual searches.

No injuries were observed in any toads, except for a minor superficial abrasion in one individual. Body mass changes ranged from -5% to +18% between release and retrieval (Table 2).

Table 2. Initial and final body mass of *Bombina v. pachypus* individuals equipped with biologging devices. Values indicate the mass (g) measured before deployment (W. Before) and after device retrieval (W. After). The percentage change in body mass (W. Variation, %) is calculated relative to the initial measurement. Missing values correspond to individuals whose devices could not be retrieved.

ID	Sex	W. Before (g)	W. After (g)	W. Variation (%)
FR01	M	12.09	12.53	+4
FR02	F	9.36	/	/
FR03	M	11.21	10.62	-5
NA04	M	7.45	8.82	+18
NA05	M	9.52	9.99	+5
NA06	M	9.18	8.85	-4

3.2 Comparison between GPS and DR tracks

Dead-reckoning reconstructions were generated between the first and last GPS fix for each individual. Track duration varied from approximately half a day to 3.5 days. No individual produced a full week of continuous data (Table 1).

For each toad, trajectories were reconstructed using both the GPS (VP) and the DR data (Figure 1, S2). FR01 and NA05 remained in the same site (artificial pond and roadside puddle, respectively) throughout the monitoring period. Others (e.g., FR02, FR03, NA04, NA06) were associated with the nearby stream or moved across the network of shallow puddles. Some reconstructed DR paths displayed straight-line anomalies.

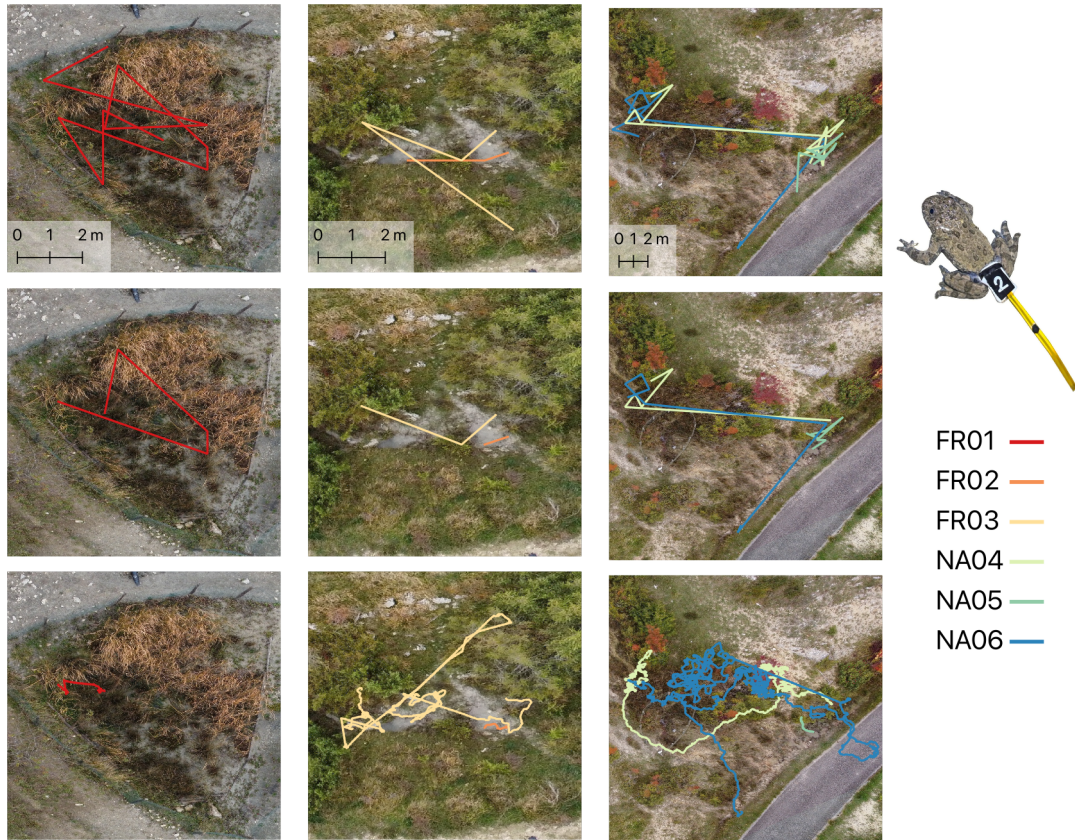


Figure 1. Tracking of *Bombina v. pachypus* individuals monitored for one week. The first row shows the reconstructed trajectories based on all GPS locations collected during the tracking period. The second row shows trajectories derived only from the GPS points suitable for dead-reckoning reconstruction (excluding those affected by intrinsic GPS error). The third row displays the trajectories reconstructed using the dead-reckoning method. The first column corresponds to the habitat used by individual FR01, the second to that used by FR02 and FR03, and the third to that used by NA04, NA05, and NA06.

VP-based path lengths averaged 14.2 ± 12.1 m (range: 0.6-32.0 m). DR paths were longer: 59.9 ± 81.9 m in 2D (range: 1.2-213.0 m) and 114.8 ± 162.7 m in 3D (range: 6.2-421.0 m; Table 3). In two cases, the difference in path length between 2D and 3D was unexpectedly large: 3D path length of FR01 exceeded 2D reconstruction of 60 m, despite inhabiting a shallow pool. Similarly, FR02's path increased from ~ 1 m (2D) to >6 m (3D), as this individual was located in a narrow stream pool characterised by limited horizontal extent but relatively high-water depth (~ 1 m).

Table 3. Distances (m) estimated from validated GPS positions (VP), 2D dead-reckoning (DR) and 3D dead-reckoning (DR) for each individual.

ID	VP 2D (m)	DR 2D (m)	DR 3D (m)
FR01	12.8	3.8	12.4
FR02	0.6	1.2	6.2
FR03	5.1	38.5	43.0
NA04	25.3	89.8	177.0
NA05	9.6	5.9	21.7
NA06	32.0	213.0	42.0

Minimum Convex Polygon (MCP) values based on DR trajectories ranged from 0.09 m² to 155.90 m² (mean $\pm$ SD = 44.58 $\pm$ 64.97 m²). FR01-FR03 had smaller MCPs (0.09-19.06 m²), whereas NA04-NA06 exhibited broader areas of use (0.44-155.90 m²).

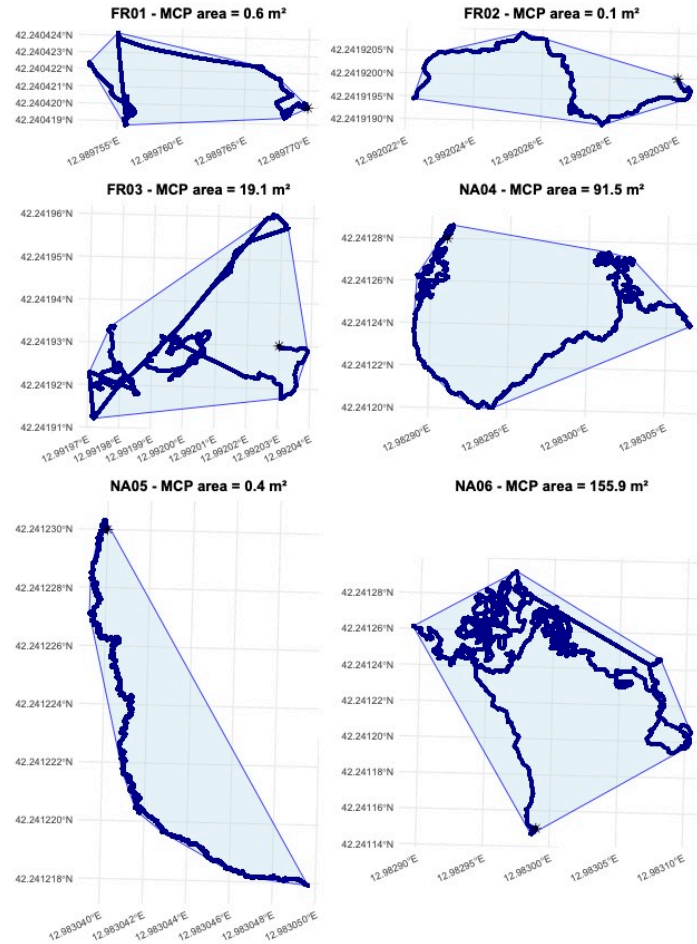


Figure 2. Spatial representation of individual movement tracks and home range areas estimated with the Minimum Convex Polygon (MCP) method. The black asterisk indicates the animal's release location.

3.3 Activity patterns

Hourly activity patterns did not reveal a consistent daily rhythm across toads (Figure 3). Instead, movement events appeared to be distributed idiosyncratically, with each animal showing distinct peaks of activity at different times of the day. NA06 concentrated movements between 15:00-21:00, whereas FR01 was more active in the early morning, with peaks between 04:00-12:00. NA04 showed reduced activity during the intervals 03:00-07:00 and 13:00-18:00, while NA05 increased movements in the morning (08:00-

12:00) and late afternoon to evening (16:00-23:00). FR02 and FR03, both located in the stream habitat, exhibited higher activity between 10:00-16:00, in contrast with the more variable patterns observed in the other individuals.

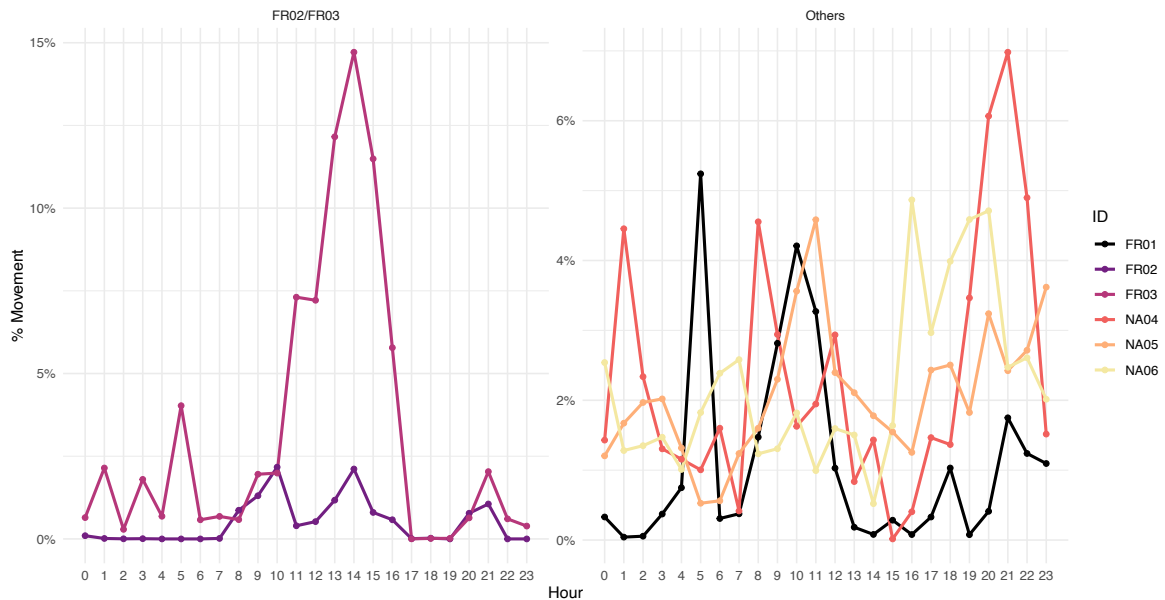


Figure 3. Hourly movement activity of *Bombina v. pachypus* individuals as recorded by accelerometers. Movement was expressed as the percentage of activity events relative to the total number of events recorded within each hour.

Temperature-activity relationships were highly individual. (Figure 4). FR01 was active primarily between 10-20 °C, with activity decreasing at higher temperatures, although it experienced conditions up to 28 °C. FR02 occupied a temperature range of 8-22 °C but displayed movement only at the upper end (21-22 °C). FR03, recorded between 5-18 °C, showed movement mainly at 5-7 °C and 14-18 °C. NA04 was most active between 7-16 °C, although temperatures ranged 5-23 °C. NA05 exhibited peak movements between 10-20 °C, with activity decreasing at higher temperatures (range 10-28 °C). NA06

showed increased activity between 11-14 °C and 17-20 °C, despite being recorded in temperatures spanning 7-24 °C.

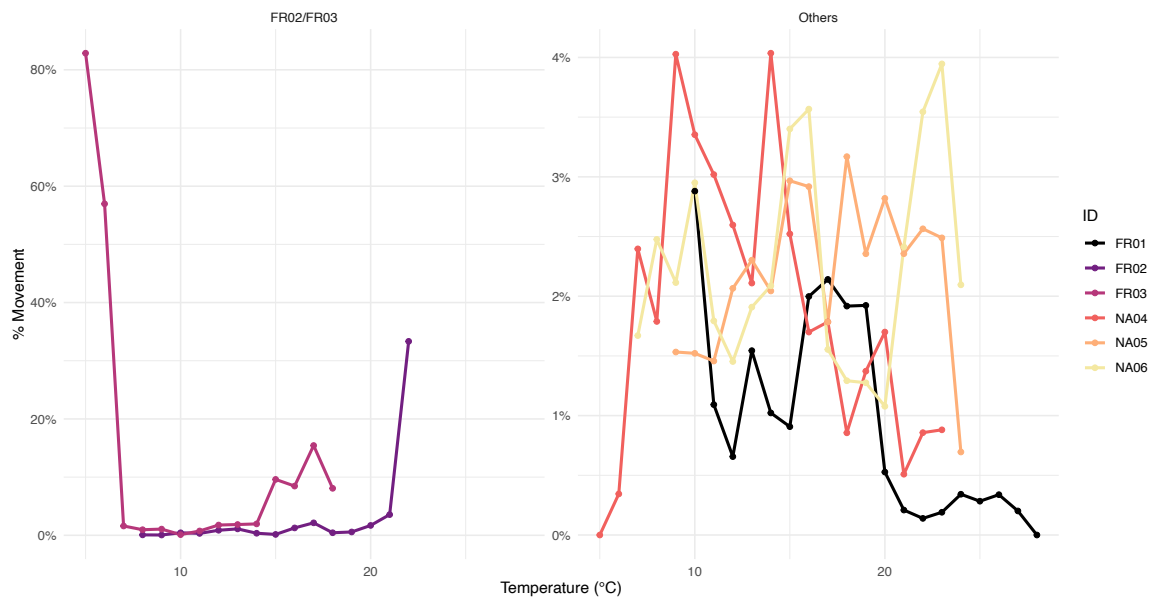


Figure 4. Movement activity of *B. v. pachypus* individuals as recorded by accelerometers in relation to ambient temperature. Movement was expressed as the percentage of activity events relative to the total number of events across all temperatures.

The movement activity of *B. v. pachypus* varied across individuals and habitats (Figure 5). NA04, NA05, and NA06, located in shallow pools with herbaceous and shrubby vegetation, showed movements distributed throughout most of the day. FR02 and FR03, which inhabited a shaded stream surrounded by shrubs and trees, concentrated their activity during midday and late afternoon, with clear movement peaks at relatively lower temperatures (10-15 °C) compared to individuals in the pools. Finally, FR01, found in a highly sun-exposed pool, exhibited pronounced activity in the late morning, coinciding with the temperature increase (up to 20 °C), whereas fewer movements were recorded during the hottest afternoon hours.

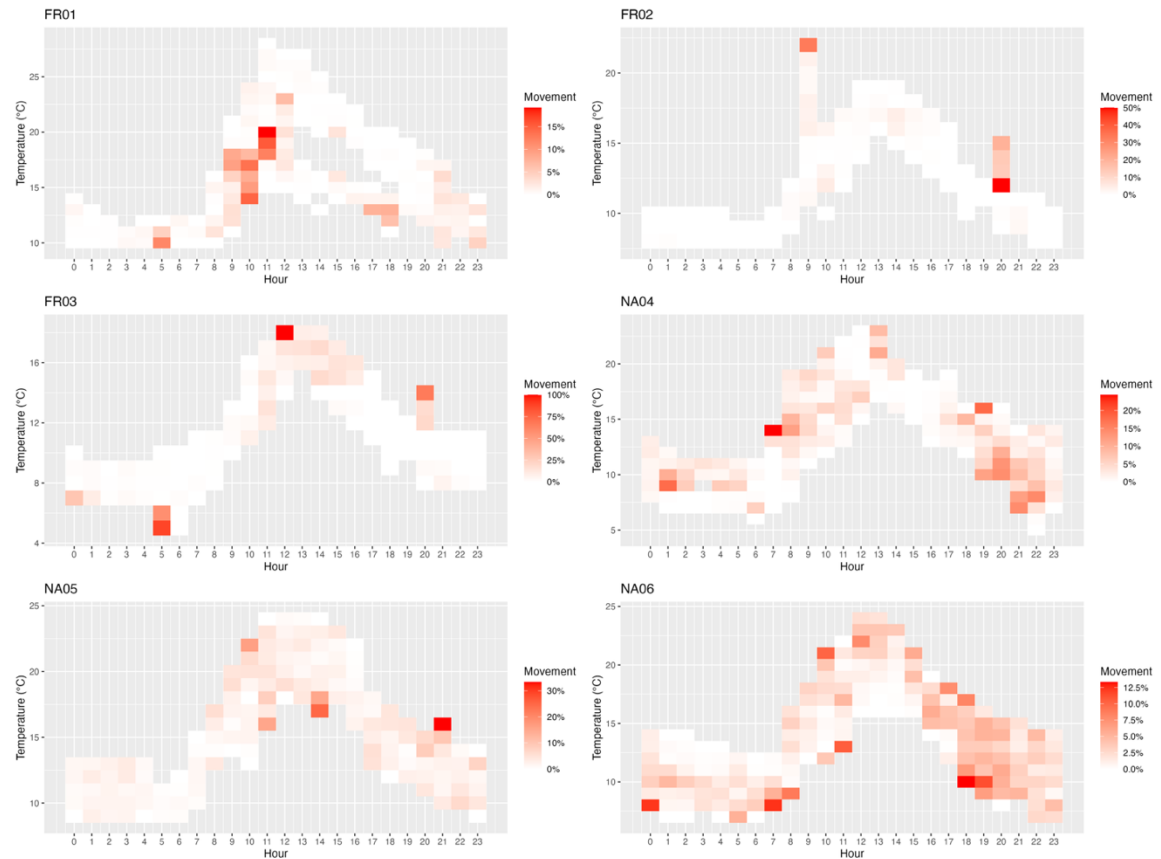


Figure 5. Heatmaps showing the proportion of time spent moving as a function of hour of day (x-axis) and ambient temperature (y-axis) for each individual *Bombina v. pachypus*. Activity is expressed as the percentage of events classified as “Movement” per bin, with warmer colours indicating higher activity levels.

Discussion

This study demonstrates the feasibility of applying dead-reckoning (DR) to track the movement small amphibians such as *Bombina variegata pachypus*. The DR approach consistently provided more detailed and continuous movement trajectories compared to GPS-only data, which systematically underestimated distances travelled, a pattern observed in similar DR applications in other taxa (Barbour et al., 2024; Dewhirst et al., 2016).

However, several technical issues emerged. Battery failures, data loss, and the generation of unrealistic straight-line paths (particularly in flowing water) suggest a need for improved device calibration, especially in aquatic environments. The reuse of biologging

tags appeared linked to increased malfunction rates, and the premature discharge of second units further highlights current battery limitations. Adjustments such as reduced sampling frequency (e.g., 2 Hz vs. 25 Hz) may help optimise recording duration in future applications without significantly compromising track resolution for such short-ranging species. Both 2D and 3D reconstructions revealed longer paths compared to GPS, but 3D distances were in some cases, possibly artificially inflated, likely due to accelerometer noise and habitat-specific conditions, such as pond depth or stream current.

Our findings also emphasise the high degree of individual variation in movement patterns, activity timing, and thermal preferences. No uniform diel activity rhythm was observed; instead, each toad displayed distinct temporal peaks, likely shaped by microhabitat characteristics such as sun exposure, vegetation, and water depth. For example, stream-inhabiting individuals exhibited narrower thermal activity windows than those in more variable, vegetated pool environments, suggesting that local environmental factors strongly shape behavioural strategies (Carilo Filho et al., 2021; Hubáček & Gvoždík, 2024).

Field observations also indicated that the devices did not prevent individuals from accessing the full range of microhabitats typically used by the species. Tagged toads were frequently observed sheltering under vegetation, burrowing into soft substrates, occupying shallow muddy depressions, or resting within submerged or partially submerged refuges, suggesting that the harness did not impair refuge entry or microhabitat selection. Moreover, several individuals were seen engaging in reproductive behaviours despite carrying the devices. This mirrors the tolerance observed in captive *Bombina variegata* and provides further support that the tag design does not interfere with key ecological functions in the field.

The spatial extent of movements, measured via MCPs, ranged from highly restricted ($<0.1 \text{ m}^2$) to relatively expansive ($>150 \text{ m}^2$). Although these values should be interpreted cautiously, given the limited monitoring time, suggesting flexible habitat use strategies even within small wetland mosaics. Notably, the most mobile individuals inhabited fenced ponds created through past conservation efforts, supporting the idea that habitat structure and connectivity influence movement behaviour and dispersal potential (Vignoli et al., 2021).

These findings highlight how biologging tools can uncover previously inaccessible dimensions of amphibian spatial ecology. By linking fine-scale movements with environmental variables, we can better understand how microhabitat features and thermal regimes influence behaviour, insights that are essential for conservation planning in fragmented and thermally variable landscapes.

Although our sample size was limited and the monitoring period short, the study provides a first demonstration that biologging tools can have direct applications for conservation. Combining DR-derived movement paths with spatial metrics (MCPs) could offer valuable insights into habitat connectivity and the functional role of small aquatic patches in supporting *Bombina* populations. By identifying the specific temporal and thermal windows of activity, such approaches may improve survey efficiency for endangered populations of *B. v. pachypus*, supporting more effective monitoring and management strategies (Sequeira et al., 2021; P. J. Williams et al., 2018).

Conclusions

This study represents a significant advance in the study of fine-scale movement ecology in small amphibians, a field historically limited by technological constraints. By

demonstrating the practical application of miniaturised biologging tools in *Bombina variegata pachypus*, we provide the first evidence that dead-reckoning methods can generate continuous, high-resolution movement data in small-bodied, semi-aquatic amphibians.

Our findings highlight how integrating movement data with environmental parameters, such as temperature and habitat structure, can reveal cryptic activity patterns and uncover spatial behaviours not detectable through conventional methods. This is especially critical for amphibians, whose behavioural ecology is strongly influenced by thermal constraints and whose populations are increasingly threatened by climate change and habitat fragmentation (Todd et al., 2009; Kearney & Porter, 2009).

Importantly, the ability to identify periods and habitats of peak activity offers direct applications for conservation. More efficient and targeted monitoring can improve detection rates in field surveys, while identifying the functional role of specific aquatic microhabitats (e.g., fenced ponds, stream fragments) can inform habitat restoration and protection strategies. These applications join with recent calls to integrate behavioural ecology into conservation frameworks (Caro & Sherman, 2012; Sequeira et al., 2021).

While the sample size and monitoring duration in this pilot study were limited, the methodology sets the stage for broader applications across small amphibians and other ectotherms. In doing so, it opens new avenues to explore how individual variation, microclimatic niches, and habitat connectivity jointly shape movement strategies and long-term population viability.

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Supplementary

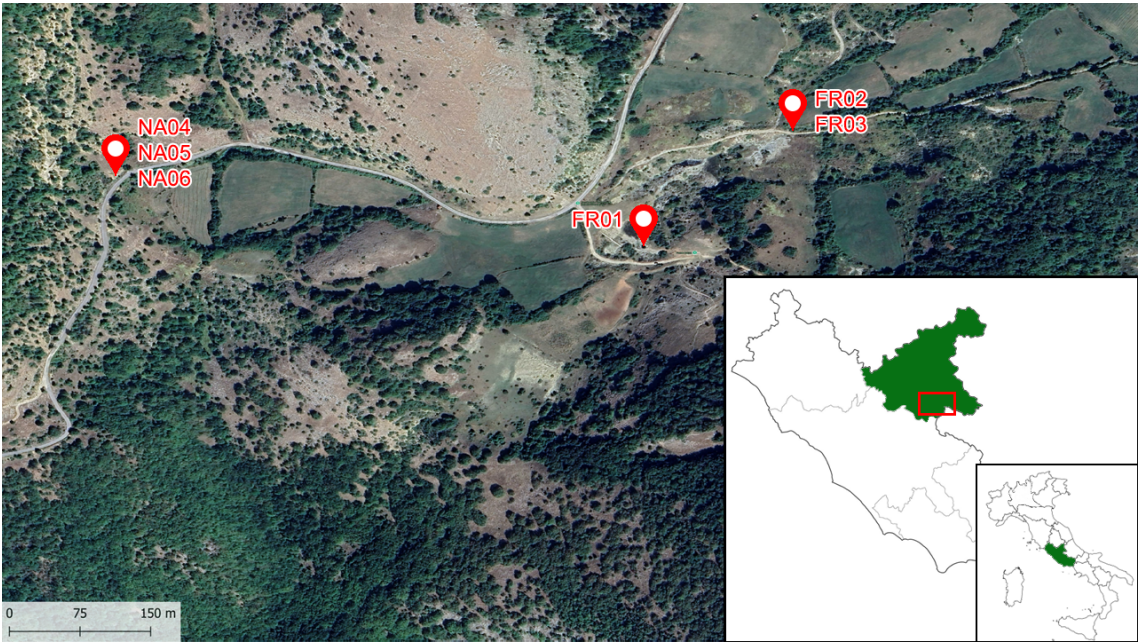
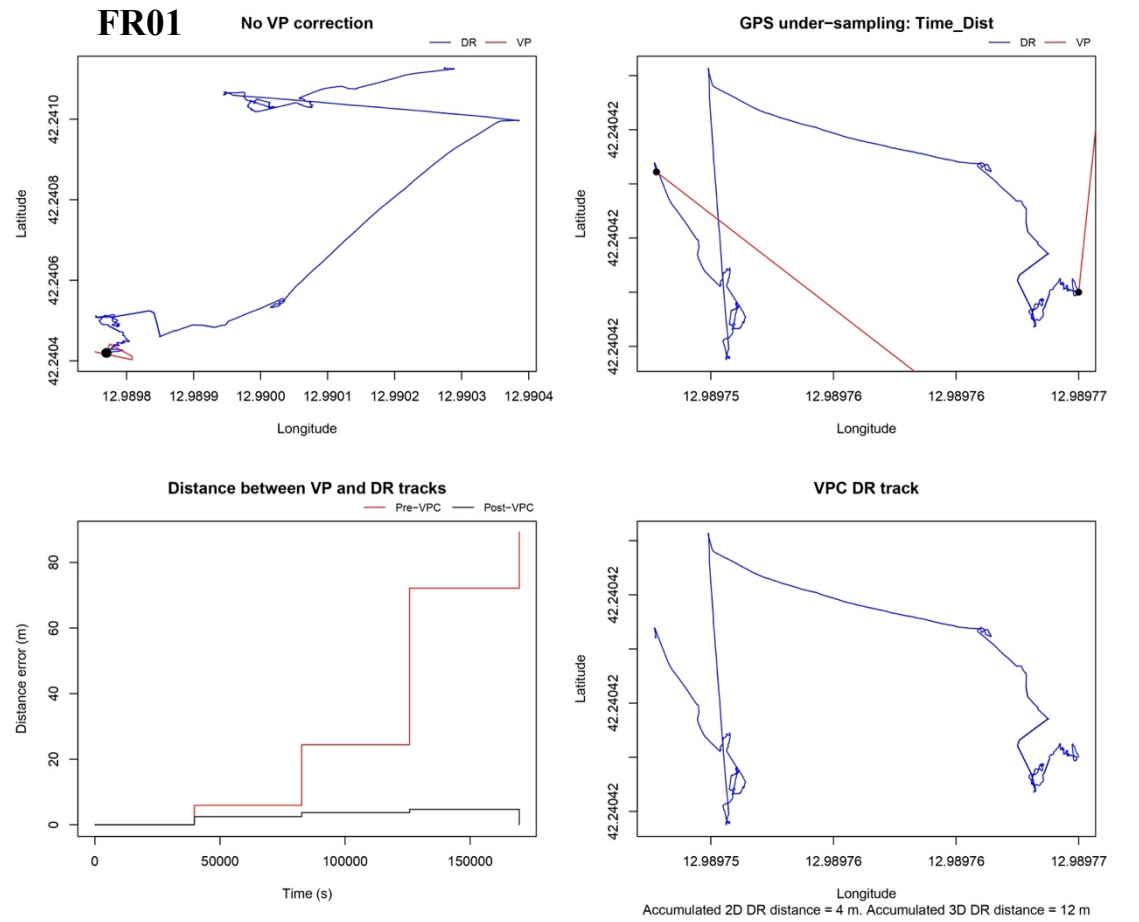
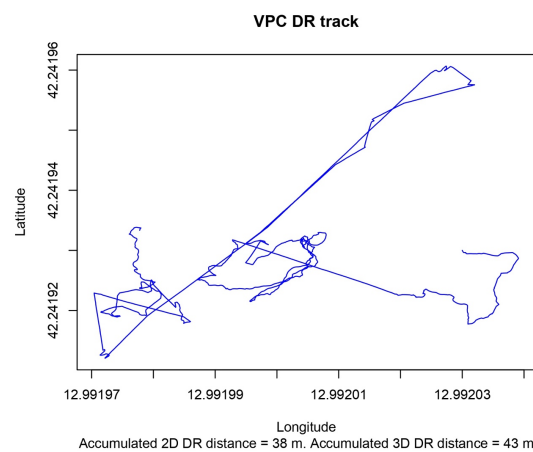
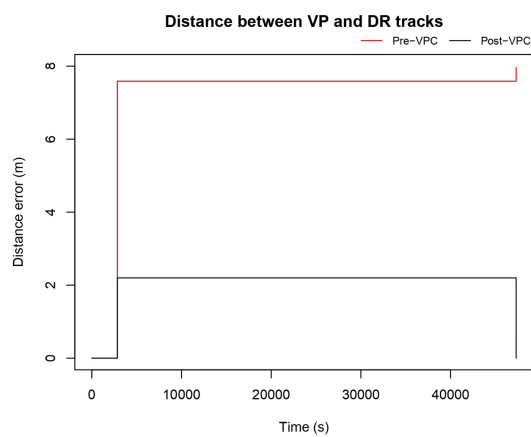
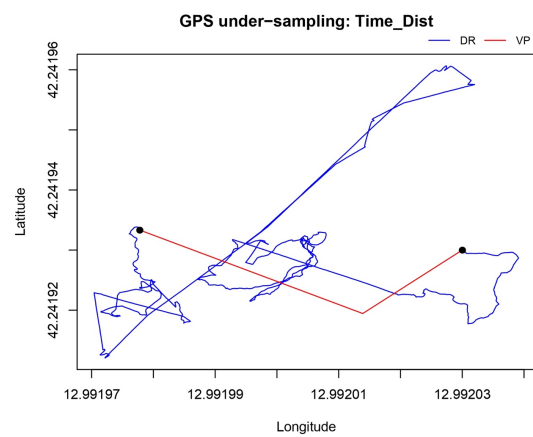
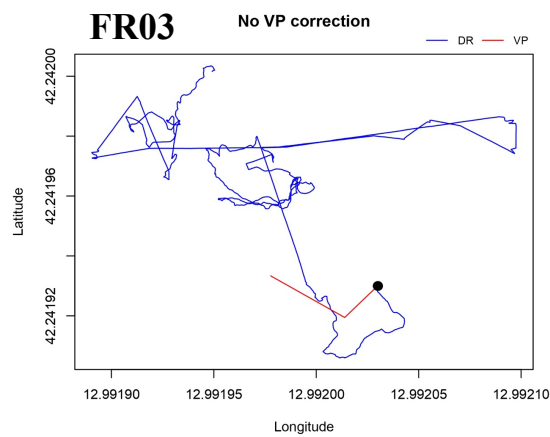
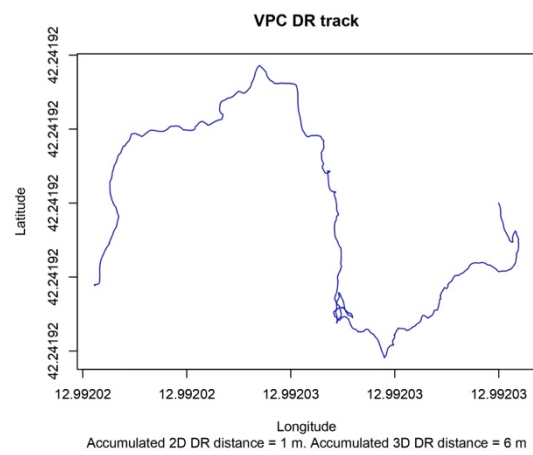
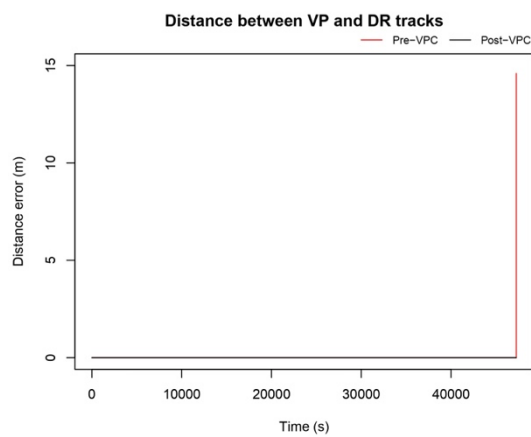
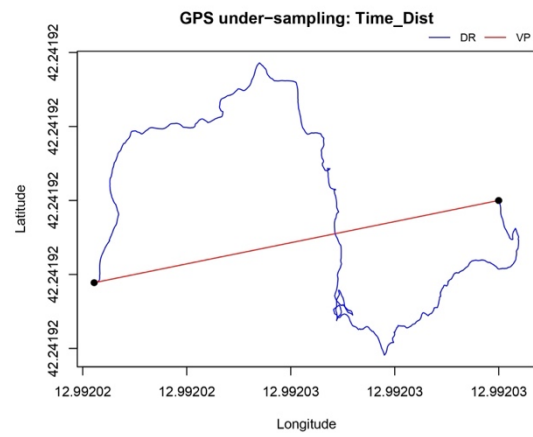
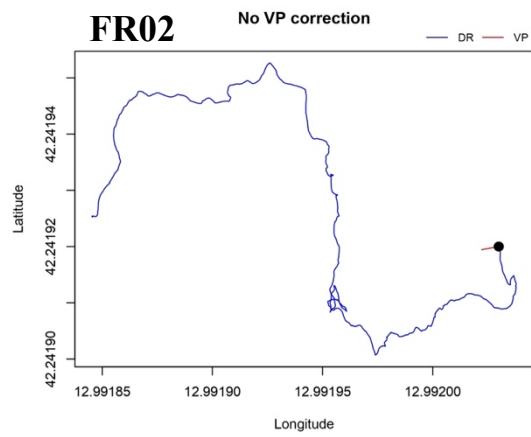
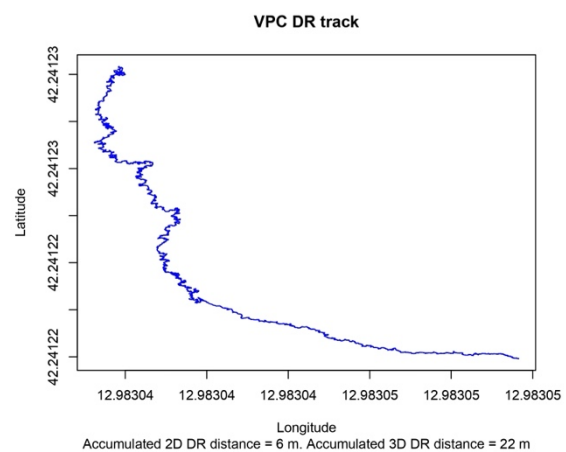
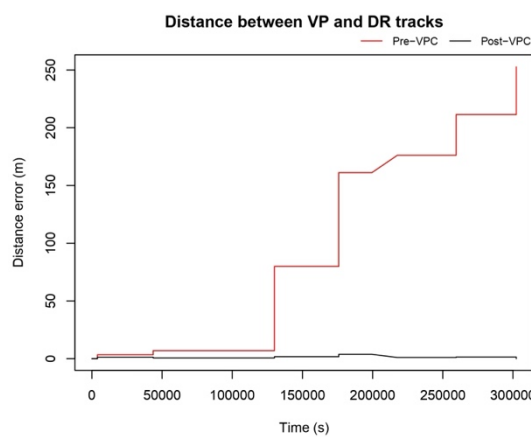
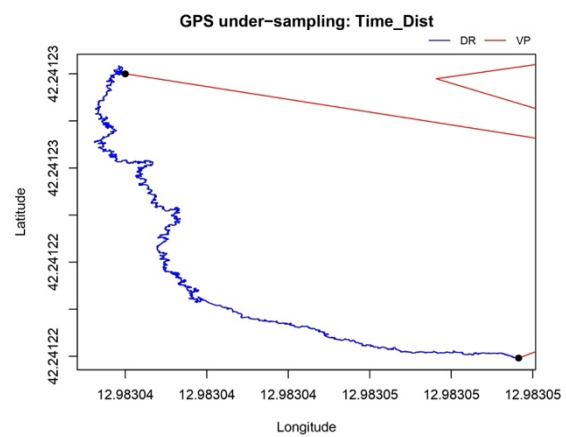
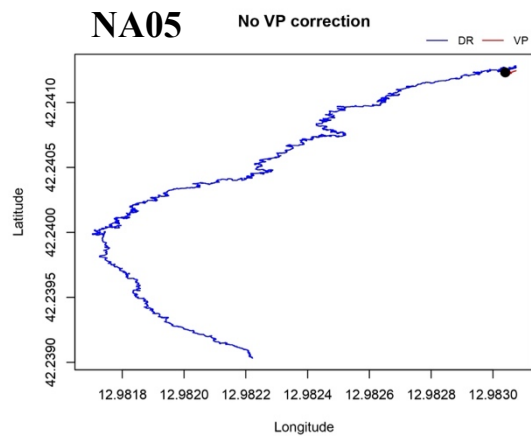
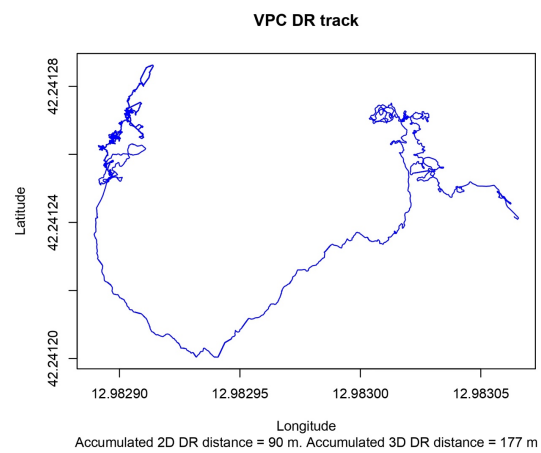
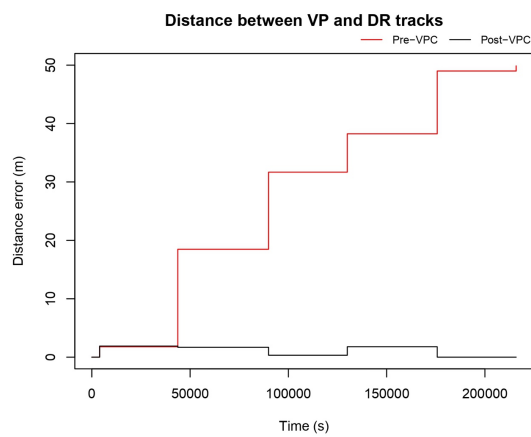
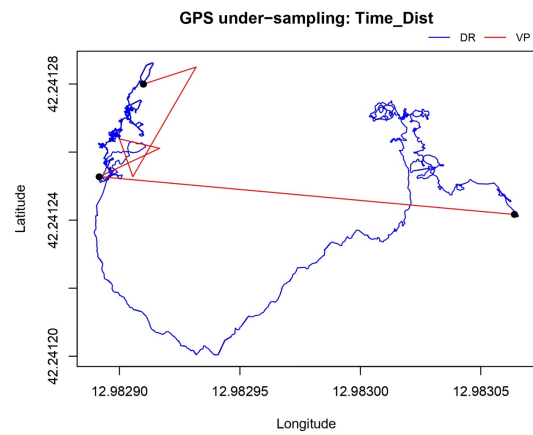
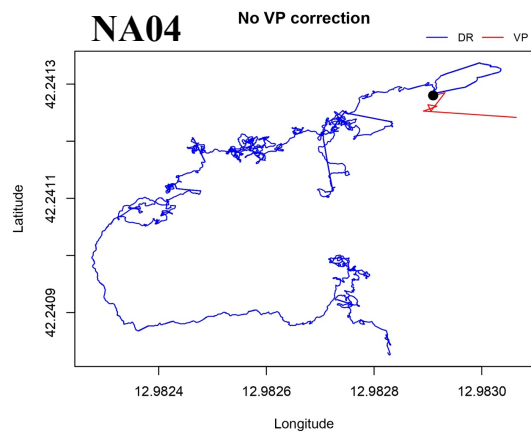


Figure S1. Map of the Monte Navegna Natural Reserve (Lazio, Italy) showing the tracking locations of *Bombina variegata pachypus* individuals. Markers indicate the positions of individuals NA04, NA05 and NA06, the location of FR01, and the area where FR02 and FR03 were recorded. The map inset highlights the position of the reserve within central Italy.







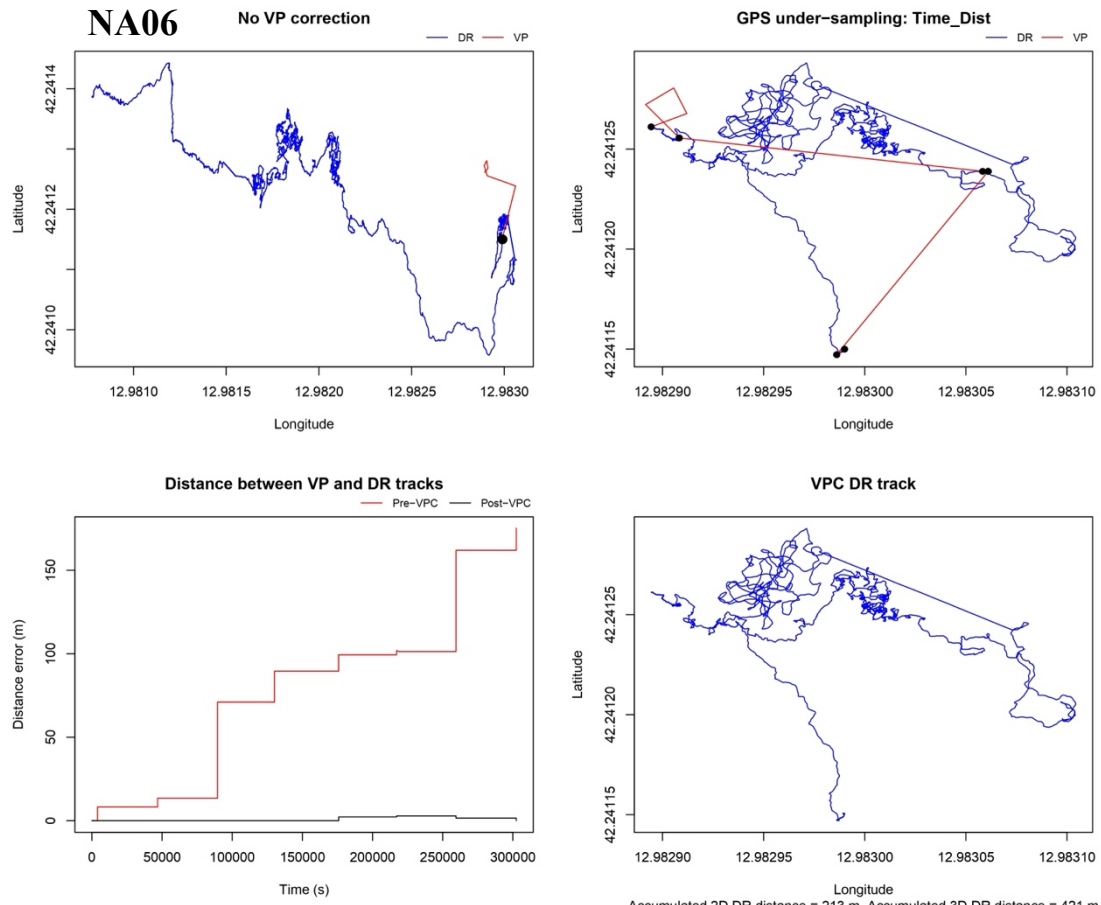


Figure S2. Reconstruction of movement tracks for *Bombina variegata pachypus* individuals FR01, FR02, FR03, NA04, NA05 and NA06 using the Gundog.Track function. The output plot is divided into four panels: (top left) the red line represents the track reconstructed from validated positions (VP), while the blue line shows the uncorrected dead-reckoning (DR) track derived from accelerometer data; the black dot indicates the starting point of the track. (top right) red points represent the VP-based track, while the blue line shows the DR track corrected using VP data. (bottom left) distances between VP and the VP-corrected DR track. (bottom right) the final DR track reconstruction.

Data Availability

Data will be made publicly available upon publication of the corresponding manuscript.

Currently, all datasets and scripts are stored in a private GitHub repository (URL:

https://github.com/BombinaPN/DR_Bombina), accessible upon request.

General synthesis and conclusions

Understanding how animals move through and interact with their environment is central to ecology and conservation. This thesis explored these processes in amphibians, a group that, despite being among the most threatened vertebrates (Kiesecker, 2011; Luedtke et al., 2023; Stuart et al., 2004), remains underrepresented in movement ecology research. By integrating technological, experimental, and analytical approaches, the chapters of this work collectively demonstrate that biologging and inertial sensor technologies can provide unprecedented insights into the spatial ecology and behaviour of small-bodied species, while also highlighting the methodological, ethical, and practical challenges that continue to limit their broader application.

Integrating technology and methodology in amphibian spatial ecology

Chapter I provided the first comprehensive review of tracking and biologging applications in amphibians and reptiles, revealing both the remarkable expansion of these methods and the persistent lack of standardisation in reporting tag effects, losses, and animal welfare outcomes. The synthesis demonstrated a clear temporal trend toward multi-sensor systems, especially GPS- and accelerometer-based loggers, while emphasised that VHF and harmonic direction-finding are essential tools for small-bodied, fossorial, or cryptic species. Importantly, the review demonstrated that the widely cited “10% rule” for device-to-body-mass ratio (Heyer et al., 1994) lacks robust empirical validation across taxa and locomotor modes, calling for systematic and more rigorous pre-deployment testing and transparent reporting practices.

Building on these insights, Chapter II experimentally tested the biomechanical and behavioural effects of external load in *Aquarana catesbeiana* using tri-axial

accelerometers. Beyond assessing tag effects, this chapter provided a key methodological contribution by demonstrating that, despite substantial morphological and hydrodynamic constraints, biologging can be successfully applied to amphibians. It also delivered the first empirical evidence that accelerometers can reliably capture and discriminate locomotor behaviours in anurans, establishing a foundation for future behavioural inference studies.

Chapter III extended this experimental framework to three European anuran species: *Bufo bufo*, *Bufotes viridis*, and *Bombina variegata*. The results revealed that tagging effects in amphibians are not governed by simple weight ratios but arise from a complex interplay among morphology, locomotor ecology, behavioural flexibility, and harness design. The integration of accelerometer data with Random Forest classification achieved behavioural prediction accuracy exceeding 96%, demonstrating that fine-scale behaviour can be inferred even when individuals are not directly observable. This capability substantially expands the scope of amphibian spatial ecology research in natural environments.

From controlled experiments to field applications

Chapters IV-VI translated laboratory insights into field applications by deploying multi-sensor devices that combine VHF telemetry or harmonic-reflective diodes with accelerometers and magnetometers across three target species. The implementation of dead-reckoning (DR) algorithms enabled the reconstruction of continuous trajectories for *Bufotes viridis*, *Bufo bufo*, and *Bombina variegata pachypus*, achieving a level of spatial and temporal resolution unattainable through GPS fixes alone (Bidder et al., 2015; Magowan et al., 2022). By integrating DR and GPS locations, drift could be corrected

and fine-scale movement paths validated, revealing microhabitat use, nocturnal activity windows, and movement tortuosity that are invisible to lower-resolution tracking.

These results highlight that high-resolution tracking approaches such as DR are particularly critical for sedentary species or those moving over very short distances, for which coarse positional data fail to capture biologically meaningful spatial use. At the same time, field deployments exposed persistent technological and logistical limitations, including battery failure, data loss, and challenges associated with device retrieval in complex habitats, which remain key barriers for long-term monitoring in small-bodied amphibians.

Towards standardised and ethical biologging practices

Across all chapters, a consistent message emerges: successful biologging in amphibians depends not only on technological miniaturisation but equally on methodological rigour and ethical responsibility. Transparent reporting of tag-to-body weight ratios, attachment methods, retention times, and welfare outcomes should become standard practice (Lameris & Kleyheeg, 2017; Meierhofer et al., 2025). The use of dummy trials and controlled laboratory tests prior to field deployment, as implemented throughout this thesis, represents a robust framework to ensure data reliability while minimising animal stress.

Ethical responsibility in biologging also extends to data sharing and analytical transparency. Making behavioural datasets and validated classification algorithms openly available would allow researchers to reuse and adapt existing validated models for species with similar morphologies or locomotor strategies, reducing the need for repetitive calibration trials and limiting animal handling. Such collaborative practices enhance

scientific reproducibility, and contribute to more ethical and efficient research (Bakker & Traniello, 2020).

Moreover, the integration of multiple sensors with environmental measurements offers powerful analytical opportunities. When combined with variables such as temperature, humidity, or substrate type, biologging data can reveal fine-scale environmental preferences and behavioural rhythms. These insights are essential for identifying critical habitats and temporal activity and can directly inform conservation planning, habitat restoration, and connectivity design (Eikelboom et al., 2020; Ellis-Soto et al., 2025).

Future research perspectives

The outcomes of this thesis open several promising avenues for future research:

- Miniaturisation and autonomy: continued advances in sensor design, energy efficiency, and data compression will further expand biologging applications to the smallest amphibian (and other vertebrate) species.
- Automated behavioural classification: machine-learning approaches, including deep neural networks, offer opportunities to refine behavioural inference from multi-axis inertial data.
- Integration with environmental modelling: combining biologging data with high-resolution environmental layers (e.g., microclimate, vegetation structure, hydrology) will enable predictive models of habitat selection and thermoregulation, which are critical under climate change scenarios (Chmura et al., 2018; Eikelboom et al., 2020).
- Ethical and welfare standardisation: collaborative networks should develop open-access databases of tag effects, attachment methods, and welfare outcomes to

support evidence-based guidelines for amphibian tagging (Arrondo & Pérez-García, 2025; Payne et al., 2024).

- Conservation applications: fine-scale movement data can inform the identification of functional corridors, breeding microhabitats, and refugia critical to population persistence, bridging behavioural ecology and applied conservation (Eikelboom et al., 2020; Ellis-Soto et al., 2025).

Final remarks

In summary, this thesis demonstrates that the integration of biologging technologies and inertial sensing provides a transformative framework for studying amphibian movement ecology. By combining conceptual synthesis (Chapter I), controlled experimentation (Chapters II-III), and field applications (Chapters IV-VI), it establishes a methodological and ethical foundation for future research on small-bodied organisms, especially vertebrates. More broadly, this work shows that technological innovation, when coupled with ecological insight and ethical responsibility, can unlock a new generation of movement ecology studies that not only deepen our understanding of animal behaviour but also strengthen the scientific basis for amphibian conservation in an era of rapid global change.

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