UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA

Environmental drivers of amphibian communities in a coastal protected area of Portugal

Bruna Meirelles

Mestrado em Biologia da Conservação

Dissertação orientada por:
Doutor Neftalí Sillero
Professor Doutor Rui Rebelo

2025

“But man is a part of nature,

and his war against nature is inevitably a war against himself.”

Rachel Carson, in Silent Spring

Agradecimentos

A realização desta dissertação só foi possível graças à ajuda e ao apoio de muitas pessoas, às quais deixo aqui o meu mais sincero agradecimento.

Agradeço profundamente ao meu orientador, Neftalí, por todo o apoio e dedicação ao longo deste trabalho. A realização das amostragens com o drone, que sem você não teriam sido possíveis, foi essencial para o desenvolvimento desta pesquisa. A sua paciência, disponibilidade e confiança foram fundamentais para o meu aprendizado e para a concretização desta dissertação. Muito obrigada por tudo.

Agradeço também ao Rui Rebelo pela orientação, pelas contribuições e pela revisão cuidadosa desta dissertação.

Mesmo à distância, sou imensamente grata à Angélica Crottini pela oportunidade de integrar a sua equipa de pesquisa e por idealizar o projeto que deu origem a este trabalho.

À Marisa, agradeço por todos os ensinamentos transmitidos durante as amostragens. Sem ti, nada disso teria acontecido. Obrigada por estares sempre presente em campo, por me ensinares tanto sobre as espécies e sobre tudo o que envolve a Reserva. A tua dedicação é inspiradora e tenho certeza de que a tua própria dissertação irá muito longe!

As amostragens não teriam sido possíveis sem a ajuda de outros biólogos apaixonados por anfíbios, que aceitaram o desafio de ir à Reserva a noite, mesmo em dias de chuva. Muito obrigada, Miguel, e grazie mille, Luca.

Realizar o percurso de mais de 300 km mensalmente foi um desafio maior do que eu imaginava. Por isso, agradeço aos meus amigos Beatriz e Breno por disponibilizarem um colchão para dormir quando precisei.

Nada disso seria possível sem o apoio incondicional dos meus pais e da minha avó. Muito obrigada por me incentivarem constantemente, mesmo sem entenderem nada da tese e estando do outro lado do oceano. Amo vocês!

Agradeço também à minha psicóloga, Mauren, pelo suporte essencial durante todo este processo.

Por fim, mas não menos importante, deixo o meu agradecimento aos amigos que tornaram esta caminhada mais leve. Ao Bruno, por me apresentar a oportunidade que tornou esta experiência possível. Aos meus amigos do Oceanário, que mesmo sem acompanharem de perto o progresso do trabalho, sempre demonstraram interesse e carinho. À Maria, companheira nesta jornada intensa chamada “tese de mestrado”. A Brenda, Jade, Gustavo e Luís, que mesmo a 200 km ou a 6.300 km de distância, estiveram presentes todos os dias a me ouvir reclamar e a incentivar a não desistir. E ao Vasco, por me lembrar e fazer com que eu continuasse a viver nesse período.

Resumo

Os anfíbios constituem um dos grupos mais diversos e ecologicamente relevantes de vertebrados, desempenhando papéis essenciais em cadeias tróficas e servindo como bioindicadores altamente sensíveis às alterações ambientais. Com mais de 8.600 espécies descritas, eles ocupam uma ampla variedade de habitats terrestres e aquáticos, incluindo rios, lagoas, zonas húmidas temporárias, florestas, prados, rochas e camadas superficiais do solo, distribuindo-se por biomas tropicais, subtropicais e temperados e diversificando-se ao longo de gradientes altitudinais. A alternância entre fases aquáticas e terrestres permite a transferência de energia, nutrientes e minerais essenciais entre ecossistemas, contribuindo significativamente para a regulação de populações de presas e predadores. Apesar de sua relevância ecológica, os anfíbios enfrentam declínios globais sem precedentes, com cerca de 40% das espécies atualmente em risco de extinção. Esses declínios são impulsionados por múltiplos fatores, frequentemente atuando de forma sinérgica, incluindo perda e degradação de habitats, doenças emergentes como a quitridiomicose, mudanças climáticas, poluição ambiental e espécies exóticas invasoras.

A ecologia dos anfíbios é fortemente condicionada por fatores ambientais, particularmente a disponibilidade de habitats aquáticos e terrestres e a variabilidade climática. A água desempenha papel central na fisiologia e reprodução desses organismos, uma vez que sua pele fina e permeável é essencial para trocas gasosas e regulação osmótica, mas também os torna vulneráveis à desidratação e à degradação da qualidade hídrica. Espécies que depositam ovos em corpos de água dependem diretamente da presença destes habitats, enquanto espécies com desenvolvimento direto ou terrestre ainda necessitam de solos húmidos e microclimas favoráveis. Micro-habitats terrestres, como cavidades de solo, vegetação densa, troncos em decomposição e pedras soltas, fornecem abrigo contra predadores, proteção contra a dessecação e oportunidades de forrageamento. A conectividade entre habitats aquáticos e terrestres é crucial para a movimentação dos indivíduos e para a dispersão entre populações, especialmente em espécies de baixa capacidade de deslocamento. Além disso, variáveis climáticas como temperatura, precipitação e humidade relativa regulam a atividade e a fenologia reprodutiva, sendo que eventos extremos podem causar desacertos entre a reprodução e a disponibilidade de água. Isto implica que em certos ciclos anuais muito secos, não haja reprodução em alguns locais por falta de corpos de água.

Portugal representa um hotspot de biodiversidade para anfíbios, abrigando várias espécies endêmicas, como o sapinho de verrugas verdes lusitânico (*Pelodytes atlanticus*) e o tritão-de-ventre-laranja-meridional (*Lissotriton maltzani*). Apesar da diversidade, muitas populações enfrentam declínios, e três espécies estão listadas como ameaçadas no Livro Vermelho dos Vertebrados. A conservação eficaz desses animais requer atenção especial aos habitats atlânticos e mediterrâneos, caracterizados por elevada heterogeneidade ecológica, combinando ambientes aquáticos e terrestres. Habitats como charcos temporários e lagoas costeiras desempenham papel crucial na reprodução, mas são altamente vulneráveis a pressões antrópicas e mudanças climáticas.

A Paisagem Protegida Regional do Litoral de Vila do Conde e Reserva Ornitológica de Mindelo (PPRLVC-ROM) constitui um caso de estudo relevante para a conservação de anfíbios em Portugal. Esta área, a primeira oficialmente protegida no país, apresenta uma diversidade de habitats, incluindo dunas, depressões húmidas, caniçais, sapais, cursos de água, matos costeiros e paisagens agroflorestais, abrangendo 379,57 hectares. Apesar do estatuto de proteção, a reserva enfrenta pressões significativas, como urbanização, fragmentação de habitats, exploração agroflorestal, turismo e espécies invasoras, que ameaçam a fauna local, incluindo a comunidade de anfíbios. A expansão de eucaliptos, em particular, pode impactar negativamente a comunidade de anfíbios pela alteração da composição do solo, da

vegetação subarbórea e da disponibilidade de corpos de água, afetando espécies sensíveis a alterações de micro-habitat.

Este estudo teve como objetivo avaliar a estrutura espacial da comunidade de anfíbios na reserva e identificar os principais fatores ambientais que influenciam sua distribuição. Levantamentos mensais, de julho de 2024 a junho de 2025, foram realizados por meio de transectos noturnos e imagens aéreas obtidas por Veículo Aéreo Não Tripulado (UAV) ou drone. As imagens áreas permitiram a criação de ortofotos e modelos digitais de elevação para mapear corpos de água permanentes e temporários, enquanto os transectos registaram espécie, fase da vida (juvenil ou adulto), presença dentro ou fora da água e localização georreferenciada. Variáveis climáticas — temperatura, humidade relativa e precipitação — foram fornecidas pelo Instituto Português do Mar e da Atmosfera (IPMA), permitindo análise sazonal e correlação com padrões de atividade e abundância.

Foram registadas 10 espécies de anfíbios: sete anuros (*Alytes obstetricans, Bufo spinosus, Discoglossus galganoi, Epidalea calamita, Pelobates cultripes, Pelodytes atlanticus* e *Pelophylax perezi*) e três caudados (*Lissotriton helveticus, Salamandra salamandra* e *Triturus marmoratus*). A riqueza e abundância apresentaram forte variação sazonal, com picos no outono e inverno e mínimos no verão, refletindo a influência da disponibilidade de água e das condições climáticas sobre a atividade. Espécies como *S. salamandra* mostraram períodos de atividade estendidos de setembro a abril, com picos em novembro e primavera, enquanto *P. cultripes* apresentou comportamento fossorial durante os meses mais secos, permanecendo enterrados e reduzindo a atividade superficial para evitar desidratação. Estes comportamentos demonstram adaptações ecológicas específicas a variações sazonais, evidenciando estratégias distintas de sobrevivência.

A comparação com o estudo de Velo-Antón (2020) evidenciou mudanças na comunidade: duas espécies anteriormente registadas (*Lissotriton boscai* e *Hyla molleri*) não foram detectadas, possivelmente devido a alterações no habitat ou diferenças no desenho amostral. Três espécies (*L. helveticus, A. obstetricans* e *P. atlanticus*) apresentaram menor frequência, enquanto *B. spinosus* e *P. perezi* foram mais abundantes, possivelmente refletindo adaptações a habitats alterados e maior tolerância a impactos antropogénicos. Essas variações reforçam o papel central da estrutura do habitat e da disponibilidade de água na determinação da abundância e da presença das espécies. Além disso, evidenciam a importância de monitorizações interanuais para distinguir alterações de curto prazo de mudanças ecológicas persistentes.

A análise de afinidades espécies–habitat mostrou gradientes claros: florestas suportam espécies como *S. salamandra* e *T. marmoratus*, enquanto dunas favorecem *P. cultripes* e *E. calamita*. Trilhos, embora antropogénicos, funcionam como corredores ou locais de movimento, favorecendo espécies generalistas como *B. spinosus* e *P. perezi*. A disponibilidade de água emergiu como fator crítico em todas as categorias de habitat, com espécies altamente aquáticas permanecendo próximas a corpos de água, enquanto espécies mais terrestres ocupam distâncias maiores. A diferenciação em estratégias ecológicas evidenciou três grupos: espécies fortemente aquáticas, espécies adaptadas a ambientes terrestres e intermediárias.

O efeito de variáveis climáticas foi notável sobre abundância e atividade, mas menos sobre riqueza e composição comunitária. Temperaturas mais altas aumentaram a atividade de algumas espécies, enquanto espécies altamente aquáticas reduziram movimentos sob calor, possivelmente para evitar desidratação. Chuvas recentes estimularam a atividade, mas precipitação prolongada reduziu a deteção de indivíduos. A humidade relativa apresentou efeito limitado, exceto em *S. salamandra*, devido aos níveis noturnos geralmente altos no litoral português e à distância da Reserva ao centro de obtenção dos dados do IPMA.

Apesar da proteção formal, a PPRLVC-ROM enfrenta pressões significativas, incluindo expansão de eucaliptos, fragmentação de habitats, degradação de lagoas e impactos climáticos. A preservação de habitats aquáticos, incluindo criação, manutenção e restauração de charcos temporários e permanentes, é crucial, bem como a conectividade com habitats terrestres e o controlo de espécies exóticas. Recomenda-se ainda a gestão de trilhos para reduzir distúrbios durante a reprodução e a promoção de vegetação nativa para manter micro-habitats essenciais. A participação comunitária e programas de ciência cidadã podem reforçar a proteção e monitorização a longo prazo.

Limitações do estudo incluem a cobertura de apenas um ano e a não amostragem de todos os habitats, bem como dependência de variáveis climáticas obtidas a partir de uma estação distante e de métodos que podem reduzir a deteção de espécies pequenas ou semi-arbóreas. Futuras pesquisas devem incluir monitorização interanual, área de estudo maior, dados climáticos locais, métodos complementares (DNA ambiental, gravações acústicas) e a avaliação de interações bióticas e qualidade da água.

Em resumo, a estrutura da comunidade de anfíbios na PPRLVC-ROM é fortemente moldada pelo tipo de habitat, disponibilidade de água e variabilidade climática de curto prazo. A persistência dessas comunidades depende da proteção integrada de habitats aquáticos e terrestres, mitigação de pressões antropogénicas e monitorização contínua, garantindo a conservação de espécies endémicas e vulneráveis no contexto de paisagens costeiras Portuguesas.

Palavras-chave: Distribuição; Charcos temporários; Tipo de habitat; Disponibilidade de água; variáveis climáticas.

Abstract

Amphibians are recognised worldwide as key bioindicators due to their ecological roles, biphasic life cycles, and high sensitivity to environmental change. However, despite their importance, amphibian populations are experiencing unprecedented declines driven by habitat loss, pollution, climate change and emerging diseases. In Portugal, many species are under pressure, yet systematic studies remain scarce in several protected areas. This study assessed the spatial structure, dynamics and environmental drivers of the amphibian community in the Paisagem Protegida Regional do Litoral de Vila do Conde e Reserva Ornitológica de Mindelo (PPRLVC-ROM), Portugal's first officially protected area. Field surveys were conducted over the course of one year, combining visual encounter transect searches, Unmanned Aerial Vehicle (UAV) surveys, and GIS analysis to construct habitat maps. Climatic data were used to explore associations between environmental variables and amphibian activity. A total of ten species were recorded, confirming the Reserve as having one of the richest amphibian assemblages in Portugal. Community composition displayed seasonal variation, with activity concentrated near temporary ponds and humid depressions. Statistical analyses revealed that water availability, habitat type, and proximity to aquatic environments were key predictors of species distribution. Climatic variables, particularly temperature and rainfall, were found to significantly influence amphibian activity patterns. Comparisons with historical records indicated relative stability in overall richness but also suggested shifts in abundance and distribution linked to urban expansion, agroforestry practices, and habitat degradation. These findings highlight the ecological importance of small wetlands and habitat heterogeneity in sustaining amphibian populations in Portuguese coastal landscapes. Conserving the PPRLVC-ROM requires maintaining water quantity and quality, ensuring connectivity between terrestrial and aquatic habitats, and mitigating anthropogenic pressures. This study provides an updated ecological baseline for the PPRLVC-ROM and contributes to the broader understanding of amphibian conservation in Portuguese coastal ecosystems.

Keywords: Distribution; Temporary ponds; Habitat type; Water availability; Climatic variables.

Index

List of figures, tables and appendix

Figures

Tables

Appendix

1. Introduction

1.1 Amphibians: diversity, ecological roles and global decline

Amphibians are one of the most diverse groups of vertebrates, comprising over 8.600 described species that occupy a wide variety of terrestrial and freshwater environments (Luedtke *et al*., 2023). They are adapted to an impressive range of habitats, including aquatic systems such as streams and ponds, terrestrial ecosystems such as forests and grasslands, scansorial environments such as trees and rocky outcrops, and fossorial microhabitats such as leaf litter and the upper layers of organic soil. Amphibians are found in tropical, subtropical and temperate biomes, and they have diversified remarkably across different altitudinal gradients (West, 2018).

Beyond their diversity, amphibians play essential ecological roles and are widely recognised as key bioindicators due to their sensitivity to environmental change. They occupy multiple trophic niches, acting as both predators and prey and thereby contributing to the regulation of the abundance and biomass of other organisms (Hopkins, 2007). Their biphasic life cycle, which alternates between aquatic eggs and larval stages and terrestrial adult stages, enables them to transfer energy, nutrients, and essential minerals between ecosystems (Alagador, 2022). Amphibians can assimilate and concentrate contaminants in their tissues, including transient or diluted pollutants, enabling the early detection of ecological stressors that would otherwise go unnoticed, being considered as bioindicators (West, 2018).

However, over recent decades, amphibians have undergone unprecedented global declines and are currently considered the most threatened class of vertebrates, with approximately 40% of species at risk of extinction (Luedtke *et al*., 2023). These declines are driven by multiple, often synergistic, threats. The most prevalent driver is habitat loss and degradation, primarily due to agriculture, deforestation, infrastructure development, and mining (West, 2018). Road construction and vehicle traffic further fragment habitats and cause significant mortality during seasonal migrations between aquatic and terrestrial environments (Ma *et al*., 2024). In recent years, infectious diseases, particularly chytridiomycosis caused by the fungi *Batrachochytrium dendrobatidis* and *B. salamandrivorans*, have emerged as a critical factor, contributing to the decline of at least 501 species and leading to 90 presumed extinctions (Fisher and Garner, 2020). Climate change has also become an increasingly important driver, altering thermal and hydrological conditions and contributing to 39% of status deteriorations recorded for amphibians since 2004 (Luedtke *et al*., 2023). Furthermore, persistent threats such as environmental pollution and the introduction of exotic and invasive species continue to increase the pressure on amphibian populations (West, 2018; Mathwin *et al*., 2020).

1.2 Habitat, water, and climate as drivers of amphibian ecology

Amphibian ecology is strongly influenced by environmental factors, particularly the availability of aquatic and terrestrial habitats, and climate variation. Their biphasic life cycle, spanning aquatic and terrestrial environments, makes them especially dependent on the integrity of both ecosystems (Hopkins, 2007).

Water plays a central role in amphibian physiology and reproduction. Their thin, permeable skin is essential for gas exchange and osmoregulation but also makes them highly sensitive to hydric conditions and desiccation (Hopkins, 2007; Çömden, Yenmiş and Çakır, 2023). Freshwater is indispensable for successful reproduction across all species; those that deposit eggs and larvae in aquatic habitats rely directly on bodies of water, while species with terrestrial or direct development remain dependent on soil moisture and microclimatic humidity (Walls, Barichivich and Brown, 2013). The physico-chemical quality of the water, the duration of the hydroperiod, and the presence of native and exotic predators can all strongly influence reproductive outcomes (Forio and Goethals, 2020). In Portuguese coastal regions, where water availability is highly seasonal and often unpredictable, the selection of breeding sites becomes particularly critical.

Terrestrial microhabitats are equally important for the persistence of amphibians. Features such as soil cavities, dense vegetation, decaying logs and loose stones provide essential refuge from predators and protection from desiccation, as well as foraging opportunities (Tong *et al*., 2023). Due to their generally low dispersal capacity, landscape connectivity is essential for movement between aquatic and terrestrial habitats throughout the life cycle, as well as for dispersal among populations (Hamer and McDonnell, 2008; Arntzen *et al*., 2017). The suitability of an area for amphibians is therefore closely linked to the availability and variety of suitable microhabitats.

Reproductive phenology and amphibian activity are tightly regulated by climatic conditions. Temperature and humidity strongly constrain behaviour and physiology. Extreme events may disrupt synchrony between breeding cycles and the availability of aquatic habitats. Such discrepancy can result in delayed or shortened breeding, extended aestivation, shifts in habitat selection or other adverse responses (Blaustein *et al*., 2010; Walls, Barichivich and Brown, 2013; Lertzman-Lepofsky *et al*., 2020). At the local scale, anthropogenic pressures such as urban development, pollution and habitat fragmentation increase these challenges, making amphibian populations more vulnerable to natural environmental fluctuations.

The global drivers of amphibian ecology and conservation are not uniform across the globe, and their effects vary depending on regional conditions. In Portuguese coastal landscapes, where hydrological cycles are highly seasonal and freshwater habitats are scarce and temporary (Gutiérrez-Rodríguez *et al*., 2022), amphibian populations are particularly vulnerable to habitat alteration and climatic extremes. These systems are valuable for studying how water availability, habitat structure, and climate shape amphibian communities.

1.3 Amphibian conservation in Portugal: a case study of the PPRLVC-ROM

The Iberian Peninsula is recognised as a biodiversity hotspot for amphibians, supporting a high proportion of endemic species due to its complex biogeographic history and diverse habitats (Gonçalves et al., 2009). Continental Portugal supports 17 native amphibian species, including several endemics such as the Lusitanian parsley frog (*Pelodytes atlanticus*) and the Portuguese Smooth Newt (*Lissotriton maltzani*). As in other world regions, several Portuguese amphibian species are experiencing population declines, with three listed as threatened in the national Red Book of Vertebrates (Cabral *et al*., 2005).

Habitats of particular importance for amphibians in Portugal include Mediterranean and Atlantic coastal ecosystems. These landscapes are characterised by a pronounced ecological heterogeneity, combining terrestrial and aquatic environments that sustain high levels of biodiversity (Gonçalves et al., 2009). Temporary wetlands and coastal ponds play a crucial role in providing essential breeding habitats for amphibians while simultaneously being among the most vulnerable ecosystems to human disturbance and climate change (Gutiérrez-Rodríguez *et al*., 2022).

Within this ecological and conservation framework, the Paisagem Protegida Regional do Litoral de Vila do Conde e Reserva Ornitológica de Mindelo (PPRLVC-ROM) is a notable case study. Located on the north-western Portuguese coast within the Porto metropolitan area, it has a diverse mosaic of habitats including dune ridges, humid depressions, reed beds, salt marshes, streams, coastal scrub and agroforestry landscapes (Silva, 2017). Despite its modest size (379.57 hectares), the PPRLVC-ROM supports an impressive variety of amphibians, with twelve species having been recorded to date, including caudates and anurans (Velo-Antón, 2020).

This area was the first in Portugal to be protected, receiving official designation as Reserva Ornitológica de Mindelo (ROM) in Government Gazette No. 204, Series II, on 2 September 1957. The initiative was led by several ornithologists visiting the region, notably Professor Joaquim Santos Júnior, who promoted the establishment of the reserve to provide conditions for ornithological and botanical research (Macedo, 2002). The Reserve was initially created under the forestry regime and functionally supervised by the Dr Augusto Nobre Institute of Zoology, whose director was Santos Júnior. However, the ROM was not included in the first National Network of Protected Areas established in the 1970s. Together with pressure from the region's industrial, tourism, and urban development sectors, this exclusion resulted in the area's natural conditions gradually deteriorating (Honrado *et al*., 2005).

In 2009, the Assembleia Metropolitana do Porto granted the site Regional Protected Landscape status, officially creating the Paisagem Protegida Regional do Litoral de Vila do Conde e Reserva Ornitológica de Mindelo (Notice No. 17821/2009, Diário da República, 2nd Series, 12 October 2009). Within the protected landscape, three protection categories are established: Partial Protection Area Type I, Partial Protection Area Type II, and Complementary Protection Area (Notice No. 13081/2020, Assembleia Metropolitana do Porto, 4 September 2020). The regulation prohibits, among other activities, the circulation of unauthorised motor vehicles, the discharge of any waste including wastewater, alteration of vegetation cover and soil disturbance, the introduction of non-native or invasive species, hunting, and any action that may disturb wildlife. The regulation also promotes ecotourism, environmental education, and habitat restoration, reflecting a model of regional landscape protection that seeks to balance conservation and sustainable local use.

Even though it is protected by law, the PPRLVC-ROM faces pressures that threaten the local fauna and flora. The construction of buildings within and around the protected area is driven by significant urban pressure in the region (Macedo, 2002). The habitats are currently being fragmented by buildings, paved and unpaved roads, and pedestrian trails, which is increasing roadkill (Velo-Antón, 2020). Another significant pressure is the increased use of the protected area for commercial purposes, such as agricultural and eucalyptus plantations. The PPRLVC-ROM is well known for its tourist attractions, such as recreational activities and birdwatching, as well as ecotourism opportunities and environmental observation and education. However, environmental degradation resulting from human activity is also evident. This can be seen through trampling in the dune area, as well as the presence of solid waste and non-native species (Honrado *et al*., 2005), particularly ice plants (*Carpobrotus edulis*), which are found throughout the area. The contamination and degradation of water bodies have a substantial impact on amphibians, as these water bodies flow from urbanised areas (Honrado *et al*., 2005).

Many studies conducted in the PPRLVC-ROM have focused on geographical characterisation (Silva, 2007), land use (Macedo, 2002), sediment composition (Ribeiro *et al*., 2014) and genetics and environmental DNA focused on *S. salamandra* (Alarcón-Ríos *et al*., 2019; Mulder *et al*., 2016; Peixoto *et al*., 2020). Despite the area's ecological importance and the presence of a diverse amphibian community, research and species identification within this taxonomic group are scarce compared to ornithological or botanical studies (Honrado *et al*., 2005). Much of the existing information is fragmented and is often derived from incidental observations (Velo-Antón, 2020) or unpublished reports. This results in limited and outdated knowledge of the amphibian community. Together, these factors make the PPRLVC-ROM a promising study site for amphibian conservation in Portugal.

1.4 Objectives

The general objective of this study was to assess the spatial structure of the amphibian community in the Reserve, identifying the main environmental factors influencing its distribution, abundance, and aboveground activity. The specific objectives were:

1) To describe the composition and seasonal dynamics of the amphibian community over one year.

2) To compare current results with historical data, identifying potential changes in the community over time.

3) To analyse the associations between each amphibian species and different habitats.

4) To investigate the influence of water availability and proximity to water bodies on the spatial distributions of species.

5) To evaluate the effects of climatic variables on amphibian aboveground activity, abundance and habitat use.

6) To suggest conservation implications focusing on the amphibian community.

2. Methods

2.1 Study area

The research was conducted in the Paisagem Protegida Regional do Litoral de Vila do Conde e Reserva Ornitológica de Mindelo (PPRLVC-ROM). The PPRLVC-ROM is located on Portugal's north-west coast, specifically within the Porto metropolitan area (Figure 1). It covers five municipalities: Azurara, Árvore, Mindelo, Vila Chã and Labruge. The Reserve covers a total area of 379.57 hectares and extends 8.5 km along the coast. The region has a temperate climate, characterised by rainy winters and dry, hot summers. The protected area holds significant biological and landscape value, including a variety of habitats such as sandbanks, dune ridges, humid intradune depressions, reed beds, salt marshes and streams (Silva, 2017). Despite the heterogeneity of the biotopes, these habitats represent approximately 10% of the PPRLVC-ROM. The predominant biotopes consist of agricultural land (33.8%), pine and deciduous forests (23.4%), sand dunes and coastal areas (17%) and interdune scrub (15.4%) (Silva, 2017). Consequently, the Protected Area is dominated by coastal agroforestry biotopes (Silva, 2017).

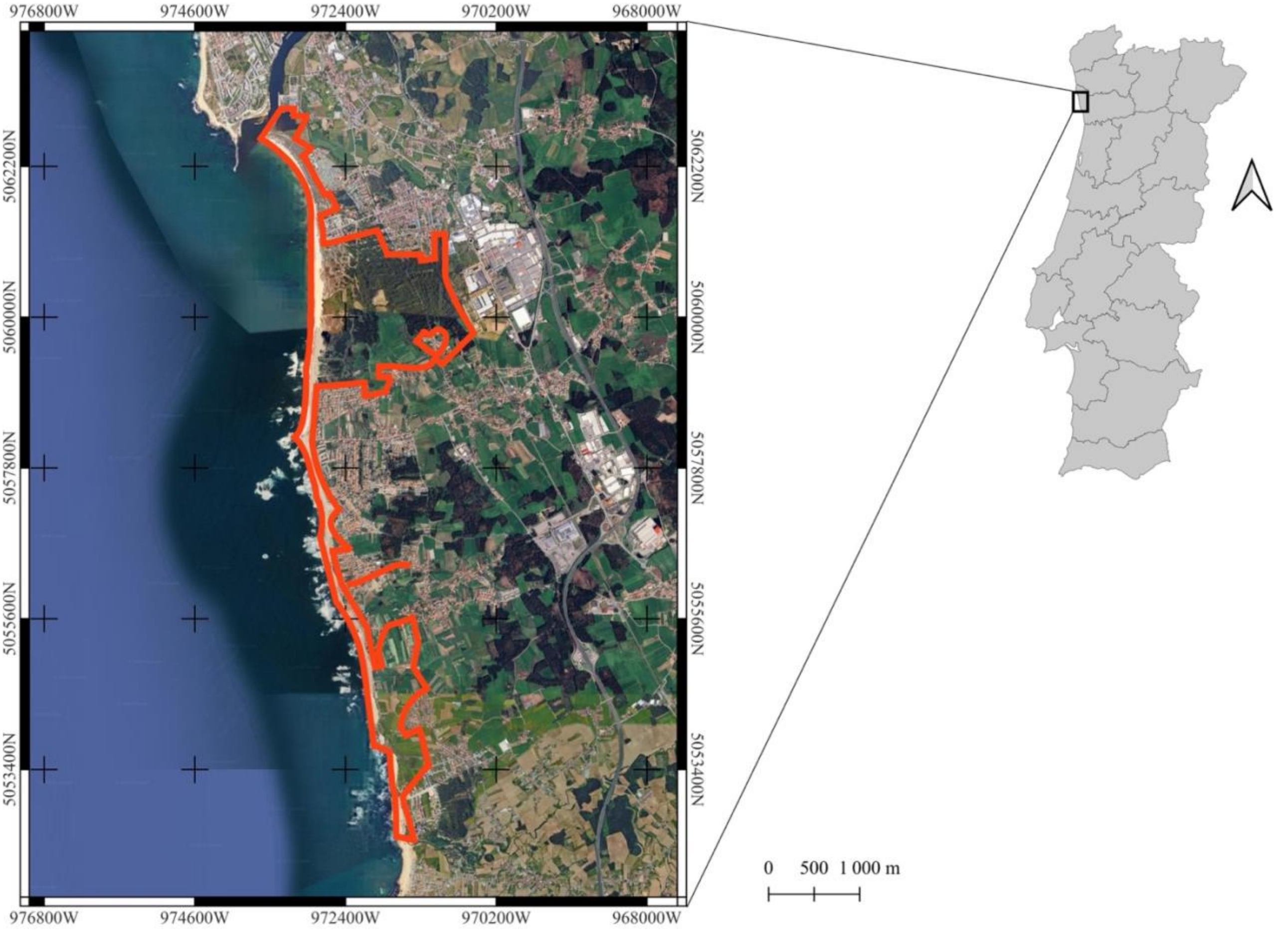


Figure 1 Map of Paisagem Protegida Regional do Litoral de Vila do Conde e Reserva Ornitológica de Mindelo, showing the limitations in orange. The image was created using the QGIS program (version 3.44.0) based on the work of Silva (2017).

The largest published study of amphibians in the protected area was conducted by Guillermo Velo-Antón (2020), who identified twelve species during nine years of sporadic visits. Four caudates were found:

the Common Fire Salamander (*Salamandra salamandra*), the Palmate Newt (*Lissotriton helveticus*), the Marbled Newt (*Triturus marmoratus*) and Bosca's Newt (*Lissotriton boscai*). Eight species from the order Anura were found: the Natterjack Toad (*Epidalea calamita*), the Iberian Painted Frog (*Discoglossus galganoi*), the Western Spadefoot (*Pelobates cultripes*), the Lusitanian Parsley frog (*Pelodytes atlanticus*), the Common Midwife Toad (*Alytes obstetricans*), the Spiny Toad (*Bufo spinosus*), Perez's Frog (*Pelophylax perezi*) and the Iberian Tree Frog (*Hyla molleri*).

All of the amphibians recorded in the study area are dependent on water and make use of the temporary ponds formed within the PPRLVC-ROM during the rainy season to breed (Velo-Antón, 2020). Currently, there is only one study on flood flows in this area. Hugo Miguel Oliveira da Silva (Silva, 2017), in his dissertation, used Geographic Information Systems to conclude that approximately 43% of the PPRLVC-ROM area would flood if the water level rose by one metre, with both estuarine and freshwater. This information is important because it enables the identification of areas susceptible to flooding, the determination of locations with the greatest potential for water accumulation and the prediction of flood zones where the salinity would be higher, affecting its suitability for use by amphibians.

2.2 Sampling

Two complementary sampling methods were employed. The first method involved using aerial imagery collected by an unmanned aerial vehicle (UAV) to produce a high-resolution orthophoto of the study area. The second method consisted of night-time transect-based visual surveys to record species observations. Sampling was conducted once per month for twelve months, from July 2024 to June 2025. To minimise variation in the presence of temporary ponds and vegetation, in each month, the two sampling methods were conducted as close together in time as possible.

2.2.1 UAV-based survey

Aerial images of the sampling area were taken using a DJI Mavic Air 2 drone. The drone's flight parameters were configured using Drone Harmony software (version 2.3.0). The perimeter of the sampling area was outlined manually for each flight. Images were captured at an altitude of 100 metres with 60% lateral and longitudinal overlap, at a flight speed of 5 metres per second. The aerial images from the September sampling were lost due to a memory card failure.

2.2.2 Nocturnal transect survey

A monthly transect survey was conducted to identify the amphibian distribution. The area under analysis, shown in Figure 2, covers approximately 32 hectares and includes a 2.1-kilometre circular route. This route encompasses various habitats, including dunes, pedestrian trails and forests. The study area contains two artificial ponds, the only permanent bodies of water in the area near the transect.

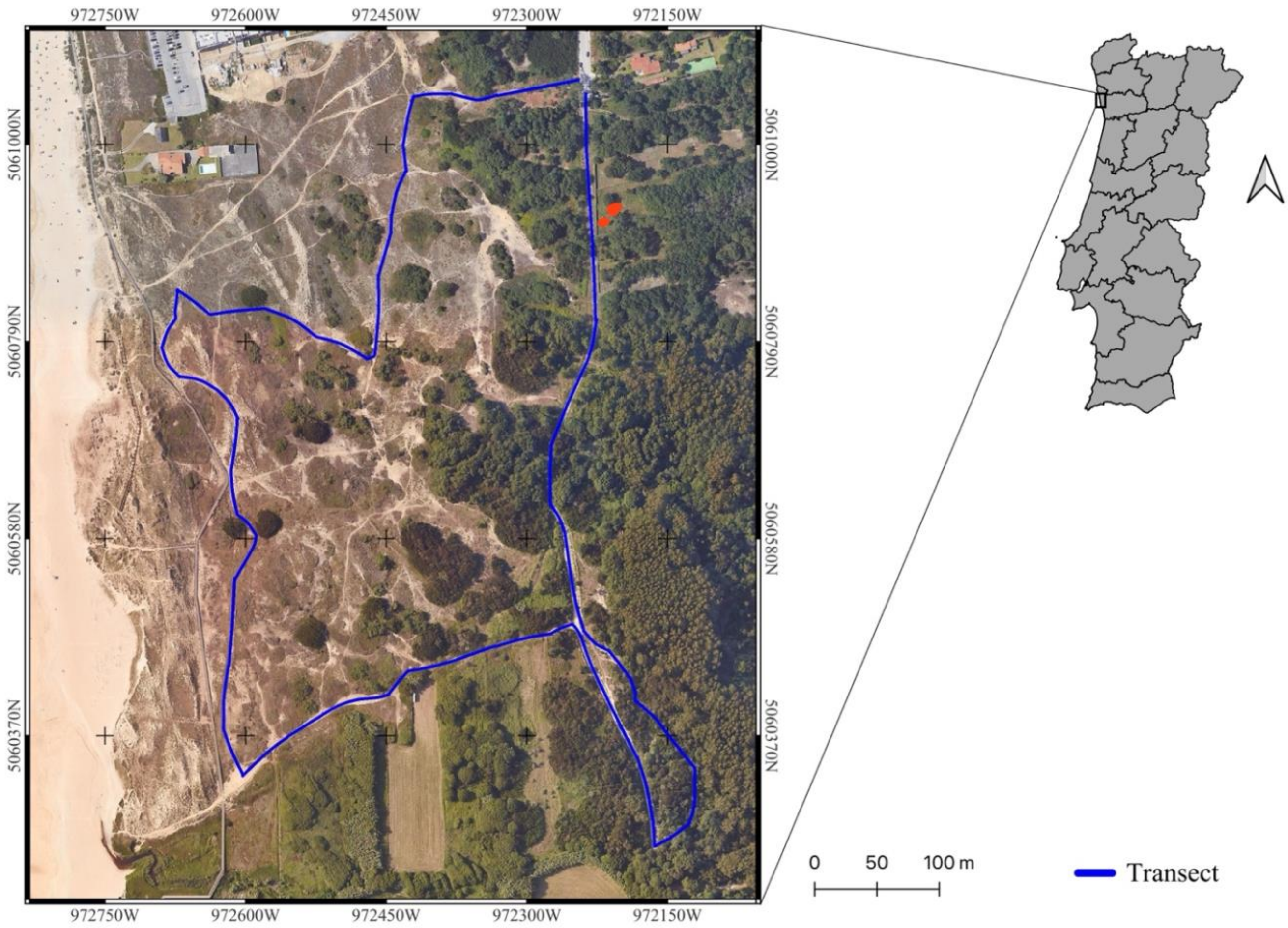


Figure 2 Study area. Blue line is the night sampling transect. Permanent artificial ponds are highlighted in orange. Image created in the QGIS program (version 3.44.0).

As amphibians are more active at night, the surveys began one hour after sunset. Two observers conducted the study. When there were more people present along the route during sampling, they did not participate in the search for amphibians. Walking speed was kept constant throughout the transect, and more time was allocated for observations at the temporary ponds. One data entry was made for each visual sighting. Acoustic detections were not considered. Information on location, date, time and species was recorded for each individual observed.

Initially, data was recorded using the Epicollect5 application (v. 86.2.1), which relied on the mobile device’s GPS. However, this method proved imprecise, with positional accuracy ranging from 3 to 17 metres, averaging 10 metres. In October 2024, the QField application (v. 3.6.2) was adopted, which enabled the use of the orthophoto created from the July drone imagery (explanation in section 2.3.2). This allowed observers to manually pinpoint the location of each sighting on the map, significantly reducing positional error.

Due to considerable errors in determining the geographical coordinates of the specimens' observations using the initial method, and due to the loss of drone images from September, analyses were conducted using orthophotography data from the nine samples collected in October 2024 onwards. Data collected between July and September 2024 were considered for analyses of amphibian richness and abundance.

2.3 Data organisation

2.3.1 Transect data

A database was created to compile observations collected during nocturnal transect sampling. All recorded locations were georeferenced using the coordinate system EPSG:3763 (ETRS89/Portugal TM06), consistent with the spatial reference standard, with all locations expressed in metres (Sillero *et al*., 2021). The type of habitat where the observations occurred was added to the database manually. The supplementary table Appendix 1 provides detailed metadata for each sampling event, including the date and time of sampling, sampling duration, rainfall, the name of active observers, the number of individuals and species detected, and additional field notes. Meteorological data were integrated, including hourly records of precipitation, relative humidity and temperature. These data were provided by the Instituto Português do Mar e da Atmosfera (IPMA) from the São Gerês meteorological station (41.1844° N, 8.6445° W) located in Porto, approximately 18 kilometres from the sampling site.

2.3.2 Orthophoto creation

To analyse the aerial images captured by the UAV, it was necessary to generate digital orthophotos. An orthophoto is an image that brings together the geometric precision of a map with the visual accuracy of a photograph (Shoab, Singh and Ravibabu, 2021). The images were processed using Agisoft PhotoScan software (v. 1.1.0). The workflow included image alignment, dense point cloud generation, mesh construction, and texture mapping.

During image alignment, the high accuracy setting was used with preselection disabled to maximise the number of tie points and ensure precise camera calibration. The dense point cloud was generated with high quality to obtain detailed surface information. The mesh was then built as a height field from the dense cloud, using the high polygon count and interpolation enabled to maintain surface continuity. Finally, the texture was created using the orthophoto mapping mode, producing a seamless mosaic optimised for planar surfaces.

The resulting orthophotos were exported in GeoTIFF format using the EPSG:3763 (ETRS89 / Portugal TM06) coordinate reference system. A digital elevation model (DEM) was also generated for each sampling area.

2.3.3 Spectral and topographic variables extraction

Several RGB-based spectral indices were calculated from the orthophotos to assist in the identification of water bodies. These indices enhance the contrast between vegetation, soil, and water based on differences in reflectance within the visible spectrum. The following indices were extracted:

The Excess Green Index (ExG) emphasises the green component of vegetation in relation to the red and blue bands (Woebbecke *et al*., 1995):

$$ExG = 2G - R - B \tag{2.1}$$

The Green Leaf Index (GLI) normalises the difference between the green and the average of the red and blue bands, improving robustness under variable illumination conditions (Louhaichi, Borman and Johnson, 2001):

$$GLI = \frac{2G - R - B}{2G + R + B} \tag{2.2}$$

The Normalised Green Red Difference Index (NGRDI) adapts the concept of the traditional NDVI to RGB imagery, using the green and red bands to highlight vegetation vigour (Gitelson *et al.*, 2002):

$$\text{NGRDI} = \frac{G - R}{G + R} \tag{2.3}$$

The Normalised Green Index (NGI) quantifies the green band's relative contribution to total brightness, making it useful for distinguishing vegetation from bare soil (Richardson *et al.*, 2007):

$$NGI = \frac{G}{R + G + B} \tag{2.4}$$

The Normalised Blue Index (NBI) measures the proportion of blue reflectance, which can assist in highlighting water or shaded areas (Richardson *et al.*, 2007):

$$NBI = \frac{B}{R + G + B} \tag{2.5}$$

The Normalised Green-Blue Difference Index (NGBDI) enhances the difference between green and blue reflectance, which makes it useful for distinguishing vegetation from water or shadows (Du and Noguchi, 2017):

$$NGBDI = \frac{G - B}{G + B} \tag{2.6}$$

In addition to the spectral indices, two topographic variables were derived from the Digital Elevation Model: slope and aspect, representing the steepness and orientation of the terrain, respectively.

All processing steps were performed using QGIS software (version 3.44.0).

2.3.4 Water bodies identification

Water bodies were identified by photointerpretation for each orthophoto. In QGIS, a polygon vector layer was created, and each water body was classified into one of the following five categories: on-trail, dune, temporary outside trail pond, permanent pond, or lateral stream. Due to dense canopy cover in some sections, it was impossible to detect ground-level water bodies below the forest canopy. Therefore, only water bodies visible in the orthophotos were mapped. In the forested section of the transect, water was present between the January and May sampling events. However, these water bodies were not included in the shapefile due to a lack of dimensional data.

The distribution of water bodies across the Reserve was uneven. Figure 3 shows all the water bodies identified along the nine months of sampling. The highest and most constant concentration of water was found on the trails marked for people and vehicles. The temporary pools in the dunes only kept water between March and May 2025. All orthophotos created can be viewed in Appendices 4 to 12, which show the identified water bodies and the distribution of amphibians observed for each sampling.

Figure 3 Map of the study area showing (in blue) all temporary pools found during the sampling period.

2.4 Statistical analysis

2.4.1 Statistical assessment of species–habitat associations

To evaluate the recorded species' preference for particular habitat types, the sampling transect was divided into three categories: dunes, trails (designated walking paths for visitors) and forests. A chi-squared test was performed to determine whether the frequency of species occurrence differed significantly between these habitat categories. Correspondence analysis was also conducted using R software (version 4.5.1) packages dplyr, ggplot2, FactoMineR and factoextra to explore and visualise the associations between species and habitat categories.

2.4.2 Influence of water availability on amphibian distribution

To investigate whether the water availability determined the spatial structure of the amphibian community, a series of generalised linear models (GLMs) was applied. First, the distance from each amphibian to the nearest body of water was obtained using QGIS. Additionally, mean distances to the nearest body of water were calculated for each species. GLMs were then fitted using all recorded distances and the percentage of water cover corresponding to each distance over the nine-month analysis period (October 2024 to June 2025). Time was not explicitly included as a predictor; all observations were treated as independent, and temporal variation in water availability is indirectly captured through the percentage of water cover corresponding to each observation. The GLM models were constructed hierarchically, starting with a general model and then incorporating taxonomic levels (order, family and species) to test for differential responses. Genera were not analysed because each genus contained only one species.

A Kruskal–Wallis test was performed to investigate whether there were differences in the distance to the nearest body of water among all species recorded. A Dunn test was applied to identify potential ecological groupings and to evaluate whether species could be clustered according to similar habitat-use strategies within the amphibian community. To further explore the influence of water availability on these spatial patterns, a Kruskal–Wallis test was performed separately for each species. For this analysis, the dataset was divided into two categories ('Dry' and 'Wet') based on the total amount of water accumulated in the Reserve, with a threshold of 300 m² being used. This value corresponds to the minimum water extent at which temporary pools first appeared in areas adjacent to the trail and, most notably, in the dune system. This categorisation enabled us to assess whether amphibians were found closer to or further away from bodies of water, depending on the total amount of water available. All analyses were performed using the R software (version 4.5.1) packages tidyverse, lubridate, viridis, dplyr, dunn.test and ggplot2.

2.4.3 Climatic variables' effects on amphibian aboveground activity

The influence of climatic variables on the aboveground activity of the amphibian community was evaluated. Temperature (ranging from 7.0°C to 18.0°C), relative humidity (52–99%), and accumulated precipitation over the past three days (0–22.7 mm) and five days (0–47 mm) were used as predictors of species richness and abundance, along with the distance to the nearest body of water.

Poisson regression models were fitted to the count-based response variables richness and abundance, while distance to water, a continuous response variable, was modelled using multiple linear regression with a Gaussian link. Community-level metrics (species richness and abundance) were analysed at the sampling event level, while habitat use (distance to the nearest body of water) was analysed at the individual level. The influence of environmental variables was also analysed for each species present in at least seven sampling occasions since October 2024. Redundancy analysis (RDA) was performed to test whether climatic variables explained the variation in amphibian community composition over time. All analyses were performed using R software (version 4.5.1) packages tidyverse, lubridate, dplyr, broom, vegan and ggplot2.

3. Results

3.1 Description of transect data

There was a total of 998 records during the survey, comprising 554 specimens of the order Anura and 444 specimens of the order Caudata, distributed across seven families. Ten species were recorded: *Alytes obstetricans*, *Bufo spinosus*, *Discoglossus galganoi*, *Epidalea calamita*, *Pelobates cultripes*, *Pelodytes atlanticus*, *Pelophylax perezi*, *Lissotriton helveticus*, *Salamandra salamandra* and *Triturus marmoratus*. Data on species richness and relative abundance are summarised in Appendix 2, which provides a comprehensive list of all recorded taxa along with their occurrence across sampling units.

Figure 4 illustrates the total species richness (line) and abundance (bars) recorded per sampling event. The highest species richness was observed in September, with ten species recorded, including *P. atlanticus* and *L. helveticus*, which were detected exclusively during this survey. *A. obstetricans* was found only during the July and September sampling, 2 specimens in both cases. The lowest richness was recorded in August and June, with only two species observed: *B. spinosus* and *P. cultripes*. In terms of total abundance, the highest numbers of individuals were observed in December and November, with 252 and 209 observations, respectively. This significant variation was primarily driven by the high abundance of *S. salamandra*, accounting for 55% (138 individuals) of all records in December and 81% (170 individuals) in November. The lowest abundance was recorded in June, with only nine specimens observed.

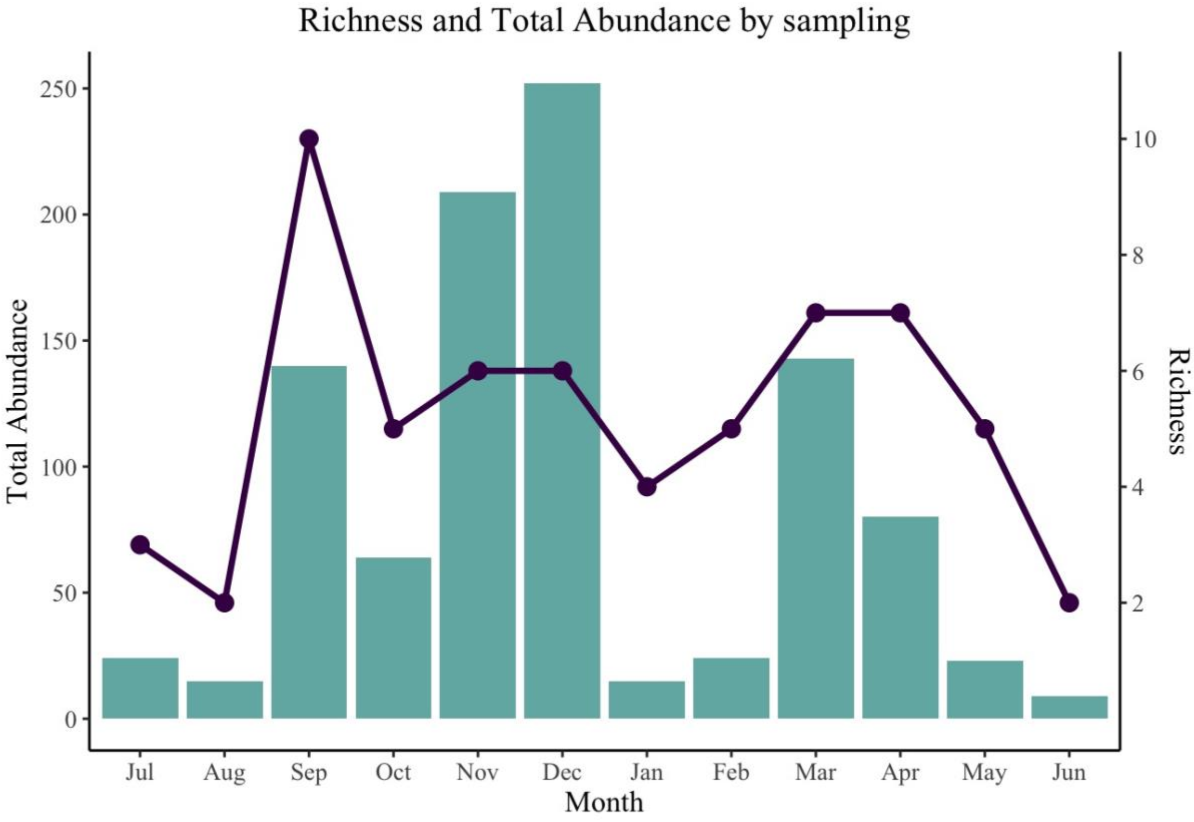


Figure 4 Species richness (line) and total abundance (bars) of amphibians observed in each sampling.

Figure 5 shows the activity patterns of the two most abundant species, *P. cultripes* and *S. salamandra*, presenting the number of observations for each species across all sampling months. *P. cultripes* was the only species recorded at every sampling event, and there was a considerable variation in the number of observations throughout the study period. *S. salamandra* was observed only between September and April, with an activity peak in November and December.

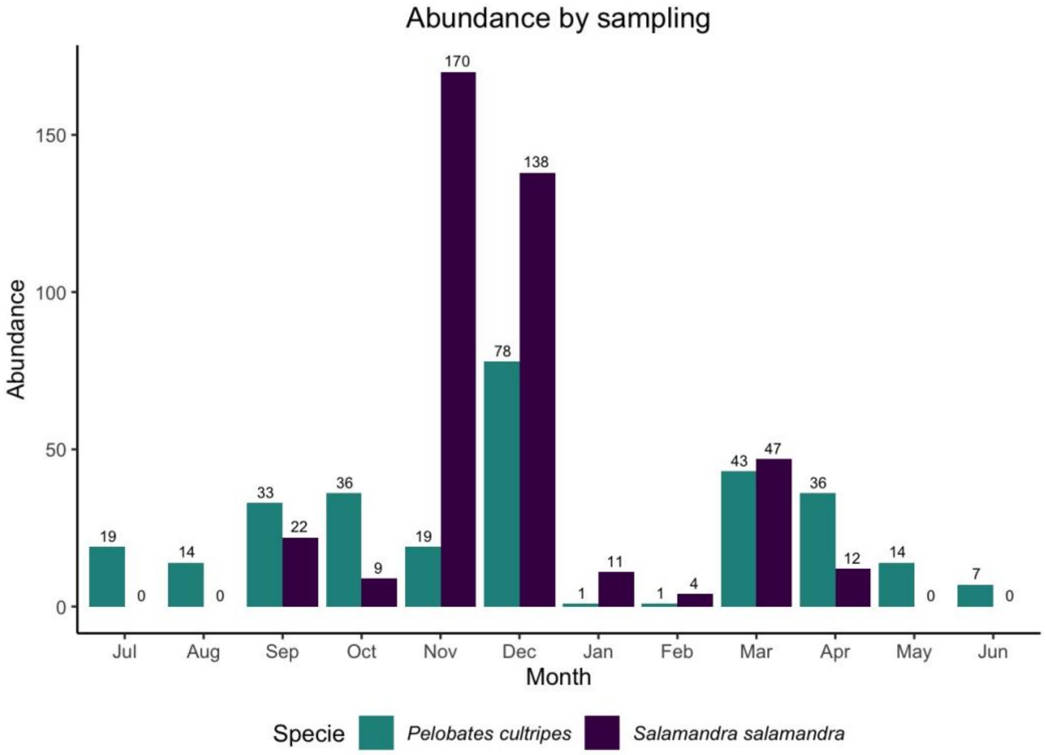


Figure 5 Abundance of *Pelobates cultripes* and *Salamandra salamandra* per sampling session.

3.2 Statistical assessment of species–habitat associations

There was a highly significant association between the species observed and the three habitat types (dune, forest and trail), indicating that the species were not randomly distributed across the landscape (Pearson $X^2 = 284.38$, df = 12, $p < 0.0001$).

The results of the correspondence analysis (CA) are presented in Figure 6. The total inertia of the model was 0.347, and the association between species and habitats was confirmed to be highly significant ($p < 0.0001$). The first two dimensions of the CA collectively explained 100% of the total inertia, providing a complete representation of the associations in two dimensions.

The first dimension (Dim 1), which accounted for 85.4% of the inertia, clearly describes a strong habitat gradient. As shown in Figure 6, this axis contrasts the 'Forest' habitat (coordinate = -0.75) with the 'Dune' habitat (coordinate = 0.92). *S. salamandra* (coordinate = -0.46) and *T. marmoratus* (coordinate = -0.43) are strongly associated with the 'Forest' habitat. In contrast, *P. cultripes* (coordinate = 0.75) and E. *calamita* (coordinate = 0.48) are closely associated with the 'Dune' habitat. These species were very well represented on Dimension 1 ($\cos^2 > 0.92$ for *S. salamandra*, *P. cultripes* and *E. calamita*), which confirms the robustness of their observed habitat preferences. Although *D. galganoi* also shows an affinity for the "Dune" habitat (coordinate = 0.58, cos² = 0.97), its relative contribution to the axis was lower (ctr = 2.22) compared to *E. calamita* (ctr = 5.45), probably due to the smaller sample size (n = 16).

The second dimension (Dim2), which explains the remaining 14.6% of the inertia, reveals a secondary pattern of community structure. This axis primarily contrasts the "Trail" habitat (coordinate = –0.17) with the "Forest" habitat (coordinate = 0.42). On the negative side of the axis, *P. perezi* (–0.62, cos² = 0.97) and *B. spinosus* (–0.53, cos² = 0.74) are clearly associated with "Trail", indicating that their distributions are best explained by this secondary gradient. In contrast, *S. salamandra* (0.13, cos² = 0.08), *P. cultripes* (0.06, cos² = 0.01) and *E. calamita* (–0.01, cos² < 0.01) occupy intermediate to slightly positive positions along Dim 2, showing a closer relationship with forested microhabitats. The position of *T. marmoratus* (-0.38, cos² = 0.44) and *D. galganoi* (-0.11, cos² = 0.03) indicate intermediate positions with little contribution to this axis.

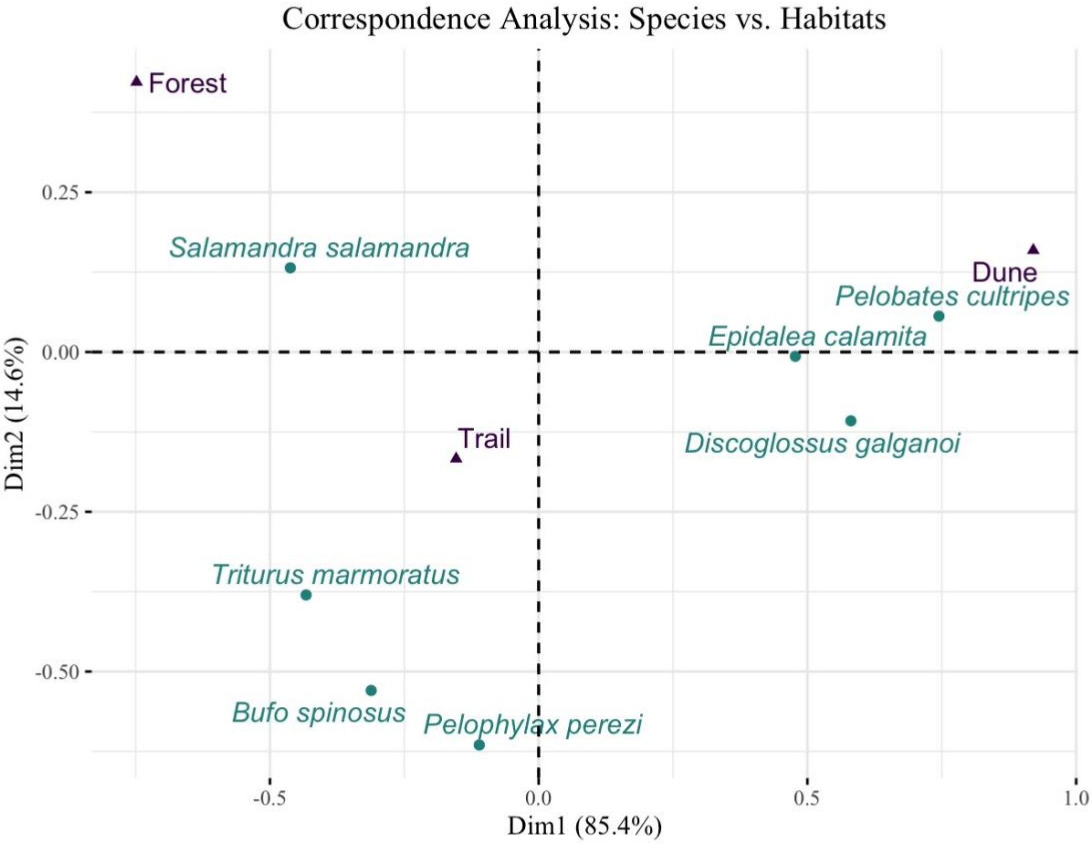


Figure 6 Correspondence analysis between types of habitats and amphibian species.

3.3 Influence of water availability on amphibian distribution

The species were distributed differently throughout the PPRLVC-ROM, at varying distances from water. Table 1 shows the average distance between each specimens of each species of amphibian and the nearest body of water, along with the number of observations. The total amount of water available in the Reserve was not constant throughout the sampling period, as seen in Figure 7.

Table 1 Average distance (m) to the nearest water body and the abundance for each species.

Species	Average distance (m)	N
Bufo spinosus	43.98	44
Discoglossus galganoi	7.97	16
Epidalea calamita	111.94	58
Pelobates cultripes	192.84	235
Pelophylax perezi	5.88	46
Salamandra salamandra	50.64	391
Triturus marmoratus	13.51	29

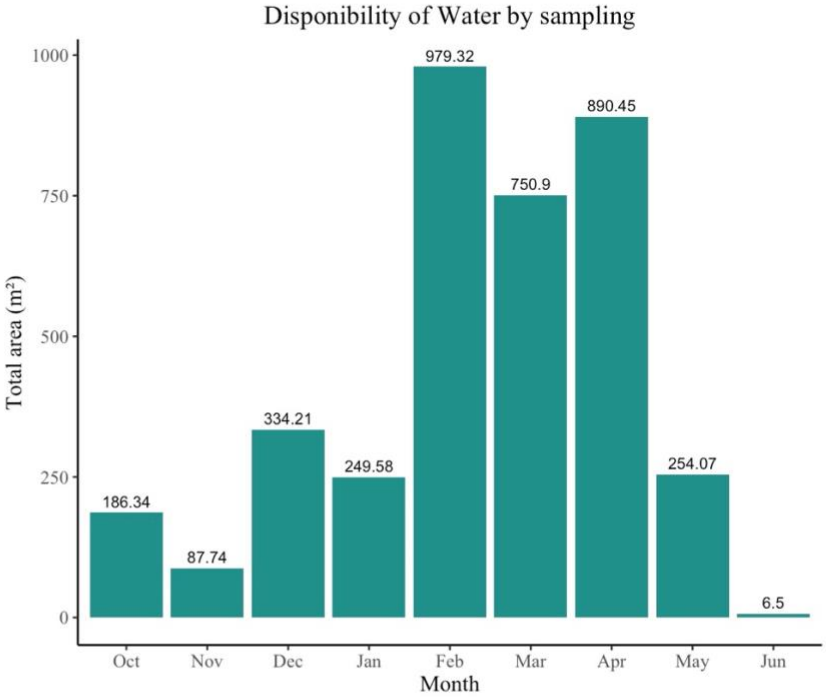


Figure 7 Total area (m^2) of water present in the orthophoto in each sampling session.

A GLM revealed a highly significant relationship between distance and water availability across all taxa ($\beta = -0.079$, SE = 0.011, $t = -7.10$, $p < 0.001$). As Figure 8A illustrates, there is a clear overall trend of amphibians being found closer to bodies of water with a larger total surface area.

This relationship was not uniform across all amphibians: the model incorporating the taxonomic order revealed a significant interaction between total water area and order ($\beta = 0.141$, $p < 0.001$), indicating significant behavioural differences between anurans and caudates. For anurans, the relationship was strongly negative ($\beta = -0.194$, $p < 0.001$), suggesting that their proximity to water increased with the extent of nearby water bodies. In contrast, for caudates, this relationship was significantly weaker

(Figure 8B). This model substantially improved the fit to the data (AIC decreased from 9778.8 to 9431.6).

Refining the analysis to the family level provided greater insight and a better model fit (AIC = 9120.8). The reference family, Bufonidae, showed a strong negative relationship between total water availability and distance to the nearest water source, indicating that individuals were found closer to water under wetter conditions. In contrast, the families Ranidae, Salamandridae, and Discoglossidae exhibited significantly weaker or reversed relationships ($\beta = 0.165$, $p < 0.001$; $\beta = 0.133$, $p < 0.001$; and $\beta = 0.179$, $p = 0.036$, respectively). Finally, the Pelobatidae family showed a response similar to that of Bufonidae, with no significant interaction detected ($\beta = 0.019$, $p = 0.478$). Figure 7C illustrates the contrasting responses among families.

The final, best-fitting model (AIC = 9104.0) examined these relationships at the species level (Figure 8D). *P. perezi*, *S. salamandra* and *T. marmoratus* were all significantly less sensitive to total water area in the landscape than *B. spinosus*, as indicated by their positive interaction terms ($\beta = 0.116$, $p = 0.018$; $\beta = 0.087$, $p = 0.021$; and $\beta = 0.104$, $p = 0.030$, respectively). Conversely, the spatial patterns of *D.galganoi*, *E. calamita* and *P. cultripes* in relation to water availability did not differ statistically from *B. spinosus* ($p > 0.05$ for all). The complete statistical output of this final model is presented in Table 2.

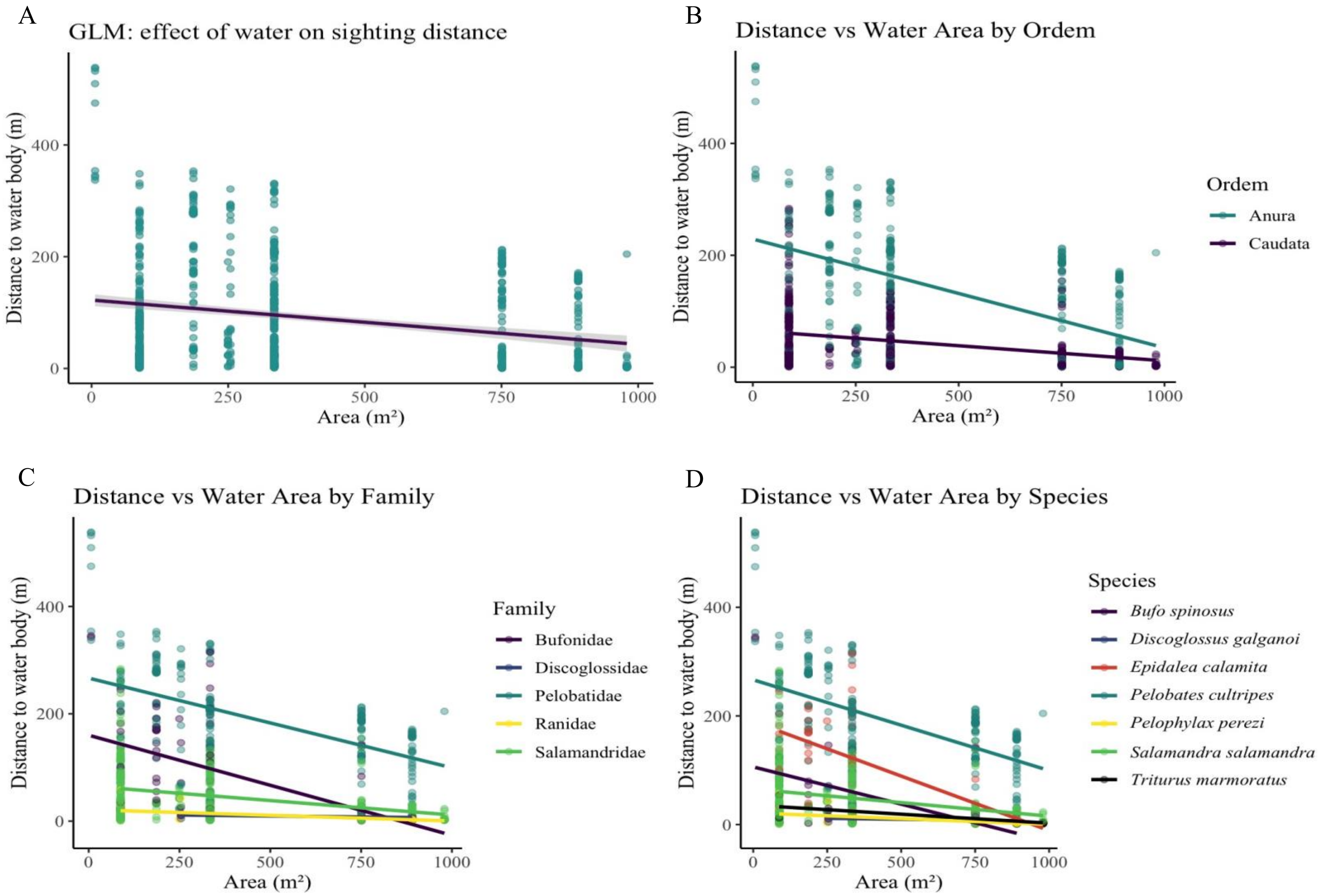


Figure 8 Generalised linear models relating the distance to the nearest water body (m) with total area (m_2) of water represented in orthophoto by: (A) all the data, (B) genus, (C) family and (D) species.

Table 2 GLM results from the distance to the nearest water body and the total surface area of water at the species level. Significance codes (p<): 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	Std. Error	t value	Pr(>|t|)	Signif. code
(Intercept)	106.12	18.62	5.70	< 0.0001	***
Total Water	-0.14	0.04	-3.86	0.0001	***
Species (Ref: *Bufo spinosus*)					
Discoglossus galganoi	-92.93	64.23	-1.45	0.1483	
Epidalea calamita	83.40	23.49	3.55	0.0004	***
Pelobates cultripes	160.38	20.06	7.99	< 0.0001	***
Pelophylax perezi	-84.76	32.35	-2.62	0.0090	**
Salamandra salamandra	-40.73	19.26	-2.12	0.0347	*
Triturus marmoratus	-70.49	30.57	-2.31	0.0214	*
Interaction: Total Water x Species					
Discoglossus galganoi	0.13	0.09	1.47	0.1426	
Epidalea calamita	-0.06	0.05	-1.35	0.1760	
Pelobates cultripes	-0.03	0.04	-0.78	0.4354	
Pelophylax perezi	0.12	0.05	2.37	0.0180	*
Salamandra salamandra	0.09	0.04	2.31	0.0212	*
Triturus marmoratus	0.10	0.05	2.18	0.0297	*

The null hypothesis of no difference in distance from water among species was rejected ($p < 0.0001$), indicating that at least one species uses the habitat differently from the others. Figure 9 shows the boxplot of the parameters for the distance to the nearest body of water for each species. The Dunn test, when comparing each pair of species, revealed three distinct groups. *P. perezi*, *D. galganoi* and *T. marmoratus* showed no statistical difference, revealing that they use similar habitats. *P. cultripes* and *E. calamita* differed greatly from the other species, placing them in a distinct ecological group. *S. salamandra* and *B. spinosus* exhibited intermediate values, falling between the previous two groups. The values resulting from the Dunn test can be seen in Table 3.

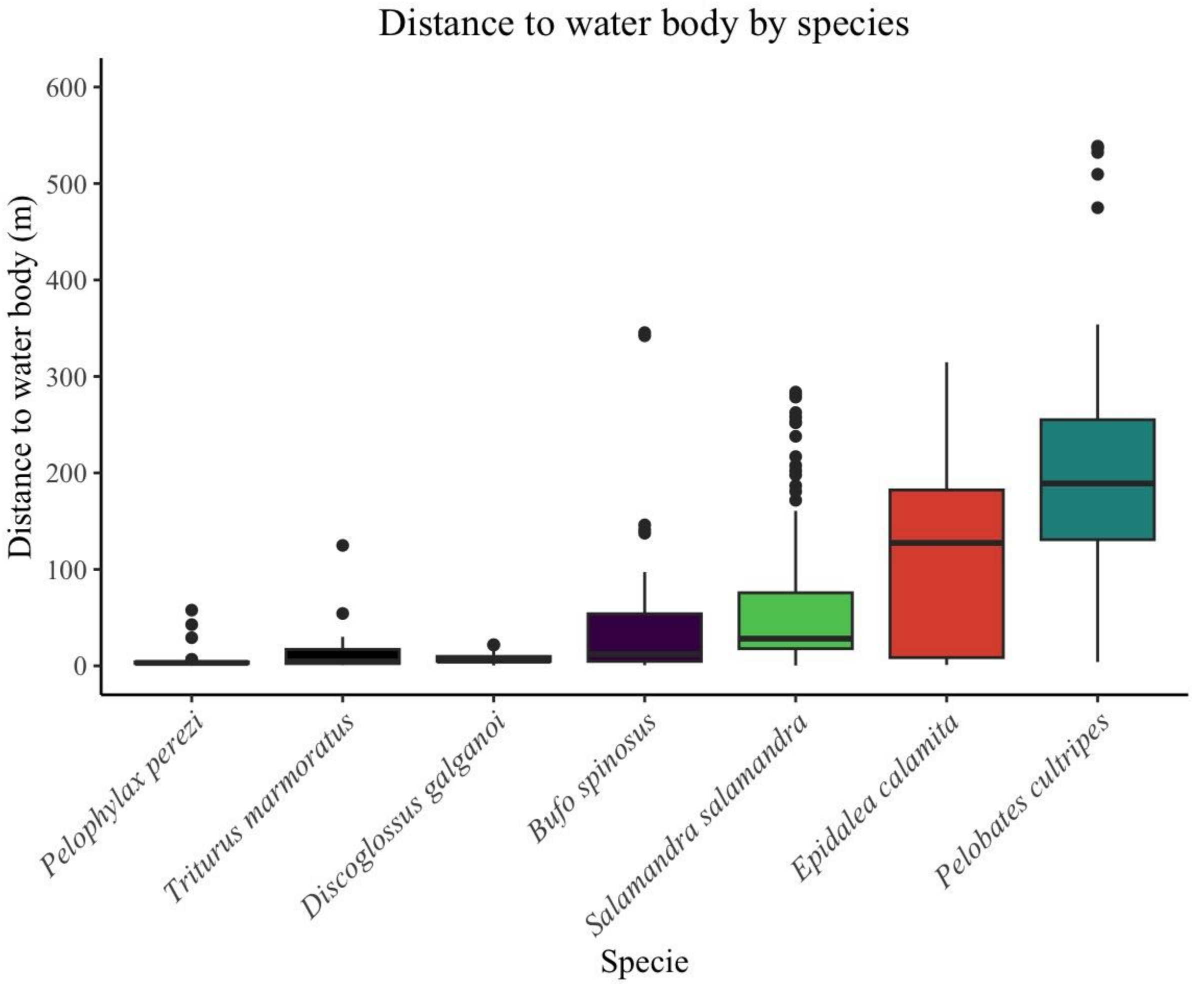


Figure 9 Boxplot presenting the distance to the nearest water body by each species.

Table 3 Results from the Dunn test comparing the distance of the nearest water bodies between species.

Col Mean-Row Mean	*Bufo spinosus*	*Discoglossus galganoi*	*Epidalea calamita*	*Pelobates cultripes*	*Pelophylax perezi*	*Salamandra salamandra*
Discoglossus galganoi	1.8834 0.6263					
Epidalea calamita	-4.0228 0.0006*	-4.7952 0.0000*				
Pelobates cultripes	-9.7761 0.0000*	-8.3434 0.0000*	-5.4674 0.0000*			
Pelophylax perezi	3.5036 0.0048*	0.6511 1.0000	7.8155 0.0000*	14.543 0.0000*		
Salamandra salamandra	-2.0462 0.4277	-3.4313 0.0063*	3.4033 0.0070*	15.514 0.0000*	-6.8271 0.0000*	
Triturus marmoratus	2.1245 0.3531	-0.1338 1.0000	5.7706 0.0000*	10.741 0.0000*	-0.9727 1.0000	4.3309 0.0002*

The analysis to examine whether these spatial patterns varied under the total area with water bodies revealed significant differences for *B. spinosus* (p = 0.001), *E. calamita* (p = 0.002), *P. cultripes* (p < 0.001), *P. perezi* (p = 0.042) and *S. salamandra* (p < 0.001), indicating that these species adjusted their spatial distribution in response to recent rainfall. In general, individuals tended to be found farther from water during dry periods and closer during wet conditions. No significant effect was detected for *D. galganoi* (p = 0.874) or *T. marmoratus* (p = 0.088). These differences are illustrated in Figure 10, and Appendix 3 presents the main results of the analysis.

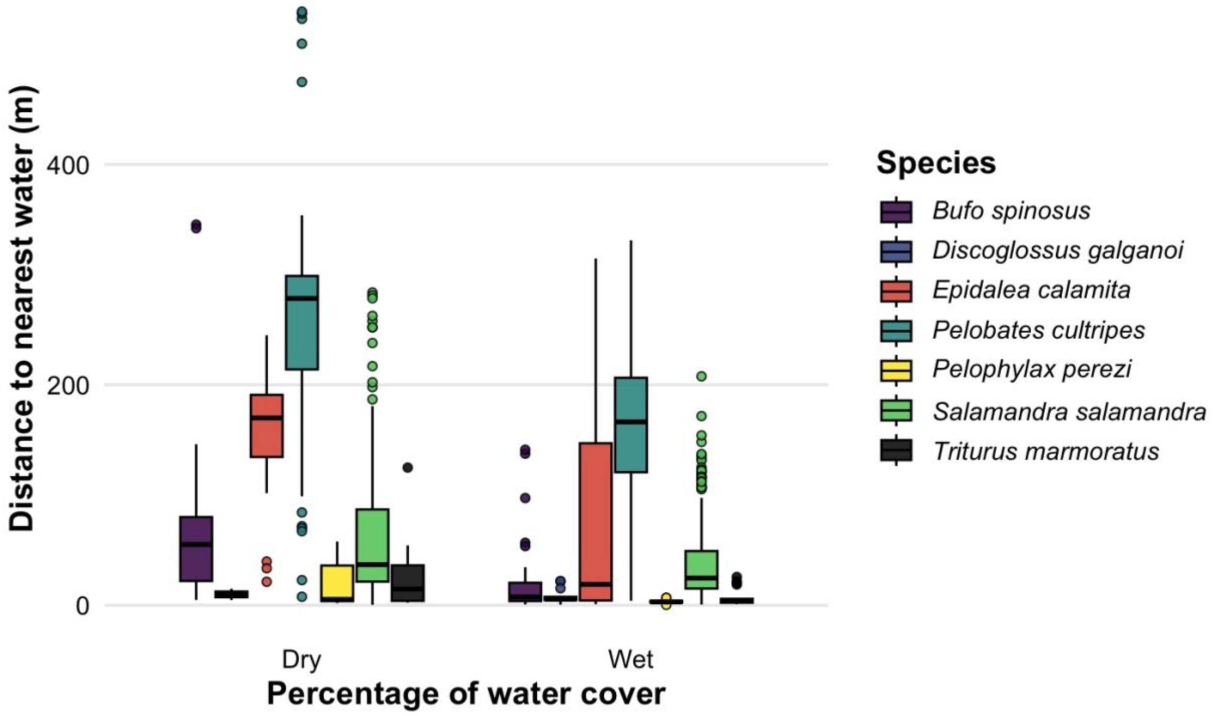


Figure 10 Variation in the distance to the nearest body of water (m) for each amphibian species divided into two categories ('Dry' and 'Wet') based on the total amount of water accumulated in the Reserve, with a threshold of 300 m².

3.4 Climatic variables effects on amphibian aboveground activity

Analysis of species richness using a Poisson regression model on the monthly survey data did not reveal a statistically significant relationship with any of the measured climatic variables. Mean temperature, humidity and precipitation over the preceding three or five days were not significant predictors of the number of species observed during a given survey night (all $p > 0.05$).

In contrast, total amphibian abundance was strongly and significantly influenced by climatic conditions. Recent precipitation had the most pronounced effect: the average precipitation over the last three days was a highly significant positive predictor of amphibian encounters ($p < 0.001$). However, accumulated precipitation over the last 5 days was found to be a significant negative predictor ($p < 0.001$), suggesting that, while light, recent rainfall may stimulate activity, prolonged rainy periods may suppress it. The model demonstrated that mean temperature was a highly significant positive predictor of abundance ($p < 0.001$), indicating that more individuals were active on warmer nights. Mean humidity also showed a significant positive relationship with abundance ($p < 0.05$). The values resulting from the Poisson regression analysis can be found in Table 4.

Table 4 Results of Poisson linear regression comparing the influence of the climatic variables on the abundance of amphibians. Significance codes (p<): 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	Std. Error	z value	Pr(>|z|)	Signif. code
(Intercept)	-1.01	0.61	-1.65	0.10	
Mean Temperature	0.24	0.03	8.86	0.001	***
Mean Humidity	0.07	0.03	2.52	0.01	*
Mean Precip. 3 days	0.49	0.03	18.06	0.001	***
Mean Precip. 5 days	-0.19	0.01	-17.80	0.001	***

A distinct model was used to assess how climatic variables influenced the distance of amphibians from the closest water bodies. Overall, the climatic variables demonstrated a weak influence on the spatial distribution of amphibians relative to water sources. Temperature was a strong positive predictor of distance, indicating that amphibians were found significantly farther from water on warmer nights (GLM, $p < 0.001$). The other factors did not achieve statistical significance.

At the species level, the analysis assessed how climatic variables affected each amphibian species individually. Relative humidity significantly ($p<0.001$) positively influences only *S. salamandra*, therefore, it was not included in the graphical presentation. Among the analysed parameters, relative humidity exhibited the greatest stability over the sampling period (Coefficient of Variation = 15%), followed by temperature with moderate variation (CV = 20%). In contrast, precipitation showed pronounced variability, with a coefficient of variation of 288%, indicating irregular rainfall patterns during the period.

As in the general analysis, the average precipitation over the last three days had a powerful positive influence on the abundance of all species. However, accumulation over the last five days had a negative influence, reducing the abundance of all species. Higher temperatures significantly affect all species, but in two different ways: a positive predictor for *B. spinosus*, *E. calamita*, *P. cultripes* and *S. salamandra*, and a negative predictor for *P. perezi* and *T. marmoratus*. The main results can be seen in the heatmap in Figure 11.

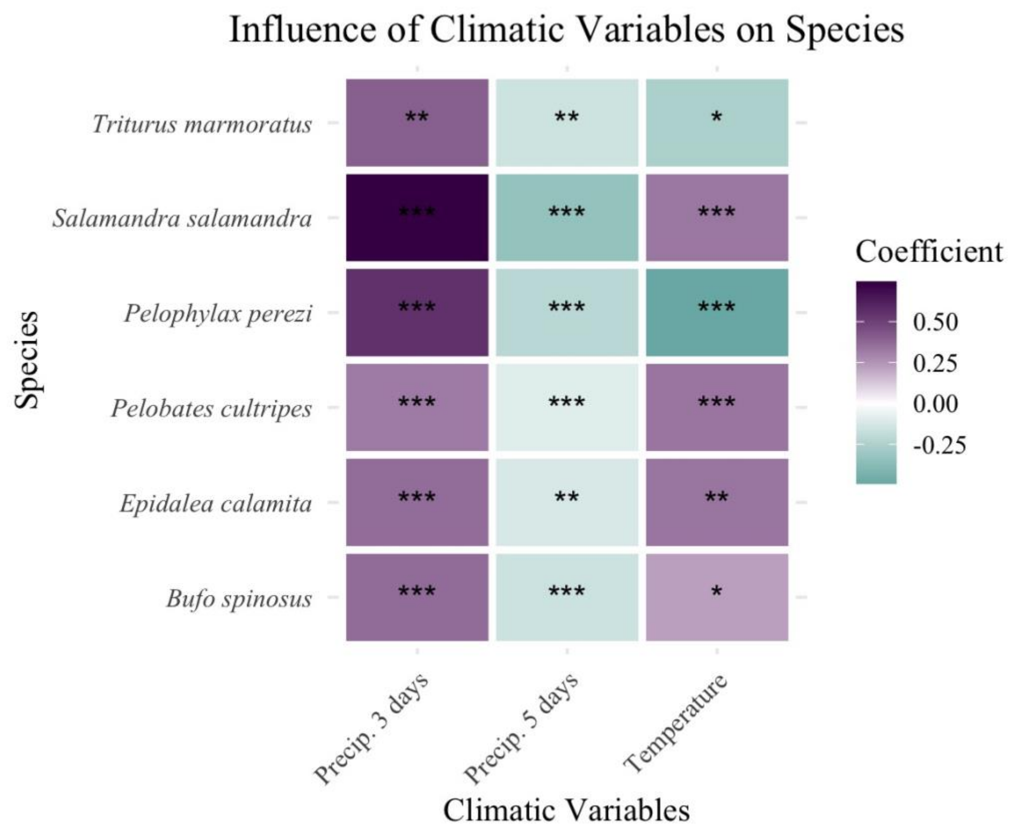


Figure 11 Model coefficients and significance of climatic variables in the abundance of amphibian species. Significance codes (p<): 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Redundancy analysis (RDA) of the influence of climatic factors on amphibian composition throughout the sampling period explained 50.7% of the variation in species composition ($R^2aj = 0.51$). However, the global permutation test for the model was not statistically significant ($F = 1.03$, $p = 0.516$). Consequently, no evidence was found that the measured climate variables were structuring the composition of the amphibian community at this temporal scale.

4. Discussion

This study aimed to assess the structure of amphibian communities in the PPRLVC-ROM and to identify the main environmental drivers influencing their distribution. Over the course of one year, ten species belonging to two orders and seven families were recorded, showing significant variations in species richness, abundance and activity throughout the sampling period. Community structure was strongly associated with habitat type: forest and dune environments supported distinct assemblages. Statistical models confirmed that water availability significantly influenced species distribution. Furthermore, climatic variables significantly affected amphibian aboveground activity, particularly temperature and recent precipitation. However, their influence on species richness and habitat use was comparatively weaker. Together, these results highlight the clear ecological gradients and interspecific differences that govern amphibian community dynamics in this landscape.

4.1 Medium-term community changes and ecological pressures

A comparison of the present survey with the previous investigation of amphibians in the Reserve, conducted by Velo-Antón (2020), reveals significant discrepancies in species occurrence. Two species previously documented—*L. boscai* and *H. molleri*—were not detected during the present study. The absence of *L. boscai*, which has already been described as a species of low abundance, and of *H. molleri*, which was considered rare in the area, may be partly explained by differences in the design of the sampling. Velo-Antón (2020) collected data over a longer period with sporadic visits without a defined methodology and over a wider spatial extent, which likely covered additional habitats suitable for these taxa, such as agricultural areas, open lands and permanent streams. However, ecological changes within the Reserve may have contributed to the absence of detection. The expansion of eucalyptus plantations, as evidenced by Velo-Antón (2020), has been demonstrated to have a detrimental effect on amphibians (Cruz *et al*., 2015). Furthermore, there is a possibility that this expansion may have a disproportionately negative impact on species that are sensitive to or specialist in their habitat. Like *Hyla arborea* and *H. meridionalis*, *H. molleri* exhibits a marked preference for permanent ponds over temporary ones, which are habitats that are undergoing constant deterioration in the Reserve (Cruz *et al*., 2015). Its behaviour, which is semi-arboreal, and its small body size (Martínez-Gil *et al*., 2023) further reduce detectability, potentially masking its true presence. To ascertain whether these absences are indicative of local population declines or are merely a consequence of the limitations of the present sampling methodology, it is recommended that future surveys extend their spatial coverage and incorporate a broader range of habitats within the Reserve.

Beyond the absence of *L. boscai* and *H. molleri*, differences in abundance were also evident when comparing the present results with those reported by Velo-Antón (2020). In the earlier survey, three species – *L. helveticus*, *A. obstetricans* and *P. atlanticus* – were recorded at a higher frequency than in the current study. The decline from high abundance (in the case of *L. helveticus*) and from low occurrence (*A. obstetricans* and *P. atlanticus*) to only a few encounters in this work may reflect the combined influence of the factors discussed above, including differences in sampling design, reduced detectability of small-bodied species, and potential ecological changes within the Reserve. In particular, *A. obstetricans* has been frequently observed by the present research group in the southern sector of the Reserve, an area not covered by this study, where agricultural activity is more pronounced and which provides anthropogenic habitats known to favour this species (Lange, Brischoux and Lourdais, 2020).

In contrast, two species categorised as rare by Velo-Antón (2020) were observed with higher frequency in the present survey. There was a higher number of records of *B. spinosus*, a finding that may be attributed to the sampling sites. This species is commonly associated with both fragmented and open habitats (Meek, 2022), including anthropogenic paths, which were well represented in the surveyed transects. The proximity of the study area to the Ribeira de Silvares, one of the few permanent water bodies in the Reserve, may also have been a contributing factor. The preference of *B. spinosus* for low-flow aquatic habitats (Santos *et al*., 2023) is consistent with this pattern and has been corroborated by informal observations of the species in PPRLVC-ROM. Furthermore, the results of this study demonstrate that *P. perezi* is more prevalent than was previously documented. This discrepancy is probably associated with its documented capacity to tolerate contaminated waters and its ability to persist under anthropogenic environmental alterations (Zamora-Camacho and Aragón, 2024), characteristics that may confer a competitive advantage under the current conditions in the Reserve.

4.2 Temporal dynamics and seasonal patterns

Throughout the sampling period, species richness and abundance exhibited considerable variation between months, reflecting strong seasonal dynamics. The highest richness was recorded in September, coinciding with the beginning of the rainy season. According to data from the IPMA, heavy and continuous rainfall occurred from 20 September 2024 onwards, providing suitable hydrological conditions for amphibian aboveground activity. Given that the Reserve is located in a temperate climate with cold, wet winters and hot, dry summers, precipitation plays a pivotal role in structuring amphibian phenology. The beginning of the activity season was therefore marked by the September rains, which functioned as a pivotal environmental trigger for amphibians inhabiting warm and dry landscapes (Ficetola and Maiorano, 2016).

Conversely, July and August exhibited the lowest richness and abundance, coinciding with the peak of the dry season. During this period, the combination of low water availability and high temperatures is likely to have constrained amphibian activity, as individuals reduce surface movements. Such extreme thermal conditions have been demonstrated to suppress activity and alter phenological patterns in ectothermic organisms, including amphibians (Ragland and Kingsolver, 2008). These seasonal patterns highlight the close relationship between climatic conditions and the dynamics of the amphibian community in the Reserve.

The activity period of *S. salamandra* documented in this study, which extends from September to April, is consistent with findings from other regions of Portugal. As Rebelo and Leclair (2003) reported, based on 49 visits conducted between 1991 and 1997 in the Sintra-Cascais Natural Park, the species was never observed to be active before the last week of September, nor after April. This matches the temporal window documented here. During this period, *S. salamandra* typically exhibits two peaks of activity: one in November and another during spring (Marques, Mata and Velo-Antón, 2022), with reduced activity in December and January due to low temperatures (Rebelo and Leclair, 2003). The species is distinguished by its sedentary lifestyle and strong site fidelity, with adults generally remaining within small home ranges. Individuals have been observed to be frequently associated with tree root systems, rock crevices and underground tunnels (Rebelo and Leclair, 2003), which serve as essential refuges against heat stress and desiccation.

P. cultripes is a terrestrial and fossorial anuran that exhibits remarkable ecological adaptations, enabling its persistence in environments subject to prolonged drought and elevated temperatures. During periods of extreme aridity and the consequent high daytime temperatures experienced in July and August, the animals enter a state of physiological lethargy while remaining buried at considerable depths in the soil (Cei and Crespo, 1971; Burraco, Duarte and Gomez-Mestre, 2013). In order to achieve this, the toads excavate vertical burrows or cylindrical tunnels, preferentially in loose substrates such as the sandy soils that are characteristic of dune habitats. This fossorial lifestyle is complemented by physiological mechanisms of internal osmotic regulation that minimise the risk of dehydration. The species exhibits an intermediate level of tolerance to water loss, situated between the strictly aquatic amphibians and the desert specialists (Cei and Crespo, 1971). Furthermore, *P. cultripes* has been shown to absorb water efficiently from sandy soils and to rapidly rehydrate in running water, with full recovery from dehydration occurring within a few hours (Cei and Crespo, 1971). These adaptations enable the species to resist the most severe summer conditions while remaining present in the habitats. The coastal location of the Reserve may create favourable microclimatic conditions, as certain summer nights remain relatively mild and windy, which may explain the detection of individuals during every month of sampling.

4.3 Species–habitat affinities

The correspondence analysis conducted in this study revealed clear associations between the recorded amphibian species and the surveyed habitats. The strongest gradient was observed between forest and dune habitats, with *S. salamandra* and *T. marmoratus* showing a stronger affinity for forested areas. Following metamorphosis, *S. salamandra* predominantly inhabits forest floors and woodland ecosystems (Alcobendas, Dopazo and Alberch, 1996; Bani *et al.*, 2015), where leaf litter, root systems, and stones provide essential shelter (Rebelo and Leclair, 2003). While also semi-aquatic, *T. marmoratus* is comparatively more adapted to terrestrial environments and shows a marked preference for forest habitats with shrubs, hedgerows, and trees, actively avoiding open pastures and grasslands (Jehle and Arntzen, 2000; Préau *et al.*, 2021). This species is highly dependent on the availability of refuges and often occupies underground shelters, such as burrows of small mammals (Préau *et al.*, 2021).

At the opposite end of the gradient, *P. cultripes* and *E. calamita* were more strongly associated with dune habitats. Due to its fossorial behaviour, *P. cultripes* is well adapted to environments with soft, easily excavated soils. It shows a clear preference for sandy substrates with low vegetation cover and tends to be scarce in compact or calcareous soils (Cei and Crespo, 1971; Crottini, Galán and Vences, 2010). Similarly, *E. calamita* has been consistently reported in the literature as being closely associated with open, sandy habitats characterised by sparse vegetation (Smith and Skelcher, 2019; Reyne *et al.*, 2021; Rannap *et al.*, 2024). In contrast, *D. galganoi* did not show a clear affinity for any specific habitat type in the present analysis. This lack of pattern may be attributed to the relatively small number of records obtained for this species (only 16 observations), which limited the statistical power of the analysis. Alternatively, this could reflect the ecological flexibility of *D. galganoi*, a species often described as a habitat generalist (Sánchez, Talavera and Hinckley, 2015).

Although representing a weaker gradient, the second dimension of the correspondence analysis revealed an association of *B. spinosus* and *P. perezi* with the "Trail" habitat. Strictly speaking, trails do not represent a natural habitat, but rather anthropogenically constructed pathways that exert significant ecological impacts. Previous studies have shown that trails can alter ecosystem functioning, causing soil compaction and erosion, as well as increasing muddiness due to vegetation removal (Svajda *et al.*, 2016; Chang *et al.*, 2023). They can also modify the soil's chemical and physical properties (Svajda *et al.*, 2016) and change the composition of the vegetation in the surrounding area (Eisenlohr *et al.*, 2011). One of the main effects of trail networks is habitat fragmentation and the creation of edges, which in turn affect population dynamics (Bötsch *et al.*, 2018; Shorb, Freymiller and Hernandez, 2020).

The association of *B. spinosus* with trails can be explained by its generalist ecology. This species occupies a variety of environments, including wetlands, forests, rural areas and human-modified landscapes (Guillot *et al.*, 2016; Meek, 2022; Santos *et al.*, 2023). Primarily terrestrial, it uses aquatic habitats mainly during the breeding season (Petrovan *et al.*, 2022). It shows a preference for wooded habitats and is capable of climbing arboreal environments, probably in search of cavities and nests offering safe, humid refuges (Gosá, 2003). Its higher occurrence along trails may be related to the open spaces with reduced vegetation cover, which could facilitate encounters between individuals during the breeding season. At the same time, the immediate presence of arboreal borders provides shelter opportunities, making trails a potentially suitable movement corridor for this generalist species.

In contrast, the association of *P. perezi* with trails appears to be linked to the presence of water bodies. Within the study area, the largest temporary ponds were concentrated along trails (see Figure 6) due to reduced soil permeability and vegetation removal. These ponds play a critical role in amphibian reproduction, but they also face significant anthropogenic pressures, including contamination and physical disturbances caused by their use as crossing points. However, *P. perezi* is known for its relatively high tolerance of organic pollution (Egea-Serrano, Tejedo and Torralva, 2008), which may explain its persistence in such habitats. Nevertheless, the presence of vehicles within the PPRLVC-ROM poses a significant threat as it alters the structure and stability of these ponds, a pattern frequently observed during surveys. This affects the survival of amphibians and their offspring.

The distribution of amphibians in nature is not random but is instead strongly influenced by habitat preferences and the complex interaction of environmental, biological and anthropogenic factors (Vasconcelos *et al.*, 2014; Thorpe *et al.*, 2018). These patterns are particularly evident in global biodiversity hotspots, where amphibian diversity is highest in habitats such as tropical montane forests, following latitudinal gradient patterns (Willig, Kaufman and Stevens, 2003). In contrast, extreme environments such as deserts and polar regions are largely devoid of amphibians. A consistent relationship emerges between microhabitat diversity and amphibian distribution: environmental heterogeneity promotes higher local richness. Landscapes with a greater variety of microhabitats support more diverse amphibian communities (Figueiredo *et al.*, 2019).

This reflects the fact that different species depend on specific environmental conditions for reproduction, with the species composition of aquatic systems often reflecting the dominant landscape matrix, whether agricultural, forest, forest edge or urban (Figueiredo *et al.*, 2019). Microhabitat diversity provides calling sites, shelter, thermal and desiccation refuges, and protection from predators for both larvae and adults. These factors facilitate higher species richness (Thorpe *et al.*, 2018; Figueiredo *et al.*, 2019). The presence and structure of marginal and aquatic vegetation also play a key role in shaping species distribution (Hamer and McDonnell, 2008; Figueiredo *et al.*, 2019), while the type of substrate influences humidity and generates distinct niches for ground-dwelling amphibians (Oliveira *et al.*, 2021). Spatial distribution patterns are strongly influenced by highly specific microhabitat preferences at the species level (Ficetola *et al.*, 2018). Even closely related taxa within the same geographic area may display distinct habitat-use strategies, which can shift seasonally in response to changing environmental conditions. Amphibians have habitat preferences that encompass both terrestrial and aquatic environments, with distinct requirements across different life stages (Bani *et al.*, 2015).

4.4 Water availability as a driver of amphibian spatial distribution

The results of this study demonstrate that proximity to water sources is a major factor in structuring the distribution of amphibians and in determining habitat quality. This is consistent with previous findings (Contardo and Labra, 2024; Ma *et al.*, 2024). A strong negative relationship was observed across all taxa between the total surface area of water and the distance at which amphibians were recorded. This negative association reflects the fact that an increase in water availability within the PPRLVC-ROM leads to the formation of more water bodies, thereby promoting a broader distributed network of aquatic habitats. These findings are also consistent with the well-documented dependence of amphibians on aquatic environments for reproduction, larval development and physiological processes such as hydration (Walls, Barichivich and Brown, 2013). In Portuguese coastal landscapes, where hydrological regimes are highly seasonal and unpredictable, the strength of this association is particularly notable, as temporary ponds may be the only suitable breeding sites for many species (Gutiérrez-Rodríguez *et al.*, 2022; Segura and Palomar, 2023).

However, despite this general trend, marked differences were observed across taxonomic groups, indicating that not all amphibians rely on water to the same degree. Both anurans and caudates were found closer to water sources in wetter landscapes. However, anurans were found to have a much stronger negative correlation with distance and total water availability. This pattern likely reflects fundamental differences in life history. In anurans, aquatic environments are critical for reproduction and early development: eggs are deposited in water, and the larvae go through dramatic metamorphosis before reaching adulthood (Brooks and Kindsvater, 2022). Nevertheless, many anuran species have adapted to drier conditions and are considered terrestrial or semi-aquatic, often migrating seasonally to bodies of water exclusively for breeding (Castro *et al.*, 2021; Çömden, Yenmiş and Çakır, 2023).

By contrast, caudates display greater variability in reproductive strategies, but generally maintain a more continuous dependence on moist habitats or aquatic environments (Brooks and Kindsvater, 2022). As caudates rarely leave the water bodies, their spatial distribution is less influenced by the total volume of available water than that of anurans. In other words, increased water availability in the Reserve brings anurans closer to aquatic habitats, whereas caudates remain tightly bound to water regardless of fluctuations in water abundance.

At finer taxonomic scales, additional nuances were detected. For families such as Ranidae, Salamandridae and Discoglossidae, dependence on total water surface area was weaker than for Bufonidae, while Pelobatidae exhibited patterns more similar to bufonids. These findings align with the respective groups' ecological profiles: for example, *P. perezi* (Ranidae) is highly aquatic and remains close to bodies of water, whereas *P. cultripes* (Pelobatidae) can disperse over long distances into drier environments, as has been reported previously.

At the species level, three distinct strategies were evident. Firstly, strongly aquatic species such as P. *perezi, D. galganoi* and *T. marmoratus* remained closely associated with bodies of water (Sánchez-Montes and Martínez-Solano, 2011; Escoriza and Boix, 2014; López-Delgado, Riemsdijk and Arntzen, 2020; Reyes-Moya, Sánchez-Montes and Martínez-Solano, 2022). Secondly, species that were more tolerant of a terrestrial environment, such as *P. cultripes* and *E. calamita*, were found at greater distances from water. This reflects their capacity to exploit drier habitats and reproduce opportunistically in temporary pools (Cei and Crespo, 1971; Arntzen *et al.*, 2017). Finally, intermediate species such as *B. spinosus* and *S. salamandra* occupied a middle position, suggesting more flexible habitat use (Rebelo and Leclair, 2003; Janin, Léna and Joly, 2011). Non-parametric analyses further supported this differentiation in habitat use, identifying three statistically distinct ecological groups and providing strong evidence for niche partitioning within the amphibian community.

When comparing “wet” (total water surface > 300 m^2) and “dry” ($\leq$ 300 m^2) conditions, several species exhibited significant shifts in their proximity to water. *B. spinosus*, *S. salamandra*, *E. calamita*, *P. cultripes* and *P. perezi* were found significantly closer to water bodies when overall water availability was higher, suggesting behavioural plasticity in response to hydric availability. In contrast, *D. galganoi* and *T. marmoratus* maintained similar distances regardless of water availability, consistent with their stronger and more stable association with aquatic environments.

These results reinforce the idea that local hydrological dynamics modulate habitat use and species coexistence within the amphibian community of the PPRLVC-ROM. Seasonal water availability is critical for maintaining ecosystems and providing the necessary aquatic habitats for amphibian reproduction. Temporary water bodies were concentrated mainly along trails, while temporary dune ponds were only available between March and May. The hydrological period under analysis (October 2024 to June 2025) was shorter and drier than the previous year's. Compared to the average precipitation recorded between 1991 and 2020, total mean rainfall across the whole mainland Portugal during this hydrological cycle was slightly above average. However, strong regional differences were noted: in the northern coastal region where the PPRLVC-ROM is located, cumulative precipitation represented only 75–100% of the long-term mean (APA, 2025). In previous years, a greater number of temporary ponds were observed in dune habitats, with water accumulation starting earlier, persisting for longer and reaching larger volumes (informal observations from the research group).

4.5 Effects of climatic conditions on amphibian abundance, activity and community structure

The results of this study demonstrate that amphibian activity is strongly influenced by climatic conditions. Due to the relatively high distance between the reserve and the methodological station, the raw values of the environmental factors were disregarded. Furthermore, the results may have been influenced by the distance and the fact that the weather record was not from the microhabitat.

Unlike many previous studies, which identified climate as a key factor in determining species richness (Qian *et al.*, 2007; Soares and Brito, 2007; Miller *et al.*, 2018), no significant correlation was found between richness and the measured climatic variables. Several factors may account for this outcome. The relatively small size of the amphibian community within the Reserve may have reduced the statistical power to detect patterns. Furthermore, species richness tends to be less responsive to short-term environmental fluctuations than activity or abundance metrics, as climatic variability primarily impacts detectability and individual behaviour rather than the presence or absence of species within an ecological community (Buckley and Jetz, 2007). Indeed, other studies have shown that richness is more strongly influenced by habitat availability and local environmental characteristics than by short-term climatic variation (Behangana, Kasoma and Luiselli, 2009; Carvalho-Rocha, Peres and Neckel-Oliveira, 2020). Together, these results suggest that, while climatic fluctuations can alter amphibian activity levels and abundance, the underlying species pool within the Reserve remains stable across survey months.

Temperature emerged as a strong positive predictor of total abundance, indicating that warmer nights were associated with increased amphibian activity. This pattern is consistent with the ectothermic physiology of amphibians, since temperature regulates vital processes such as oxygen consumption, heart rate, locomotion, digestion and the development and survival of eggs and larvae (Blaustein *et al.*, 2010). Temperature is also a recognised driver of phenological shifts in amphibians (Ficetola and Maiorano, 2016) and of the timing and intensity of anuran breeding calls (Saenz *et al.*, 2006). However, the magnitude and direction of temperature effects were species-specific in this study. While *B. spinosus*, *E. calamita, P. cultripes* and *S. salamandra* were positively associated with higher temperatures, *P. perezi* and *T. marmoratus* displayed the opposite trend. These divergent responses likely reflect interspecific differences in thermal tolerance and ecological strategies. The two taxa that were negatively associated are both highly aquatic, and their reduced activity under warmer conditions may represent behavioural avoidance of desiccation risk, responses to lower water availability during hotter periods, or shifts in microhabitat use that reduce detectability (Rödder *et al.*, 2017).

Precipitation was another key climatic driver of amphibian abundance, exerting both positive and negative influences depending on the temporal scale. Short-term rainfall over the preceding three days strongly increased abundance across all species, which is consistent with the well-documented role of precipitation in stimulating amphibian activity, reproduction and movement (Oseen and Wassersug, 2002; Richter-Boix, Llorente and Montori, 2006; Ficetola and Maiorano, 2016; Beranek *et al.*, 2022). However, cumulative rainfall over the previous five days negatively affected abundance. Continuous rainfall can drastically alter the structure of the Reserve's habitats by transforming temporary ponds into flowing streams. These changes increase the risk of egg and larval washout mortality, ultimately reducing recruitment to adult populations (Walls, Barichivich and Brown, 2013). Furthermore, prolonged rainfall can create more water bodies, providing widespread suitable habitats and dispersal opportunities, and thereby reducing the detectability of individuals at any given sampling site.

Although relative humidity is often considered a critical determinant of amphibian ecology, it showed only a weak overall influence in this study. Humidity plays a multifaceted role in amphibian physiology, behaviour, reproduction, survival and community structure (Qian *et al.*, 2007; Blaustein *et al.*, 2010; Beranek *et al.*, 2022). Soil moisture, for instance, is a vital resource for species with terrestrial reproduction and direct development, as well as helping to mitigate the risk of desiccation (Walls, Barichivich and Brown, 2013; Ficetola and Maiorano, 2016). In these analyses, humidity was significantly associated only with *S. salamandra*. The absence of stronger relationships with other species can be explained by the lower variability of relative humidity values compared to other climatic factors. Furthermore, this may be due to the generally high levels of nocturnal humidity in the study area, which reduces its role as a limiting factor. The Portuguese coast has strong Atlantic influence (Leira *et al.*, 2018), which results in persistently high humidity levels throughout much of the year.

Climatic conditions had relatively minor effects on the spatial distribution of amphibians in relation to aquatic habitats. Temperature was the only significant predictor, with individuals being found farther from water on warmer nights. Higher temperatures favour locomotor activity in ectotherms, enabling amphibians to explore upland or drier habitats. Although temperatures in PPRLVC-ROM are rarely low enough to severely constrain amphibian movement, relatively cooler conditions may still lead individuals to remain closer to water sources (Oseen and Wassersug, 2002; Aquino and Nkomo, 2021). This finding highlights the dual role of aquatic habitats as breeding grounds and refuges under unfavourable conditions. However, it is important to note that extreme heat may also drive amphibians to seek shelter and reduce activity to avoid desiccation stress (Blaustein *et al.*, 2010; Beranek *et al.*, 2022). Such conditions were not predominant in the PPRLVC-ROM, as even during the summer months, nocturnal temperatures remained relatively cool and strong coastal winds provided additional protection against desiccation.

Despite the strong influence of climatic conditions on total abundance and habitat use, the multivariate analysis (RDA) did not reveal a significant effect on community composition. This suggests that, at the monthly temporal scale considered here, climate did not restructure amphibian assemblages. The most likely explanation for this result is the limited statistical power associated with the small number of surveys (N = 9) and the distance from the source of the climatic data, which may have been insufficient to detect subtle patterns in community composition. Alternatively, unmeasured factors such as habitat structure, hydrological dynamics and biotic interactions may play a greater role in shaping community assembly than short-term climatic fluctuations.

4.6 Conservation implications

Amphibian populations worldwide are experiencing unprecedented declines, highlighting the urgent need for effective conservation strategies. The PPRLVC-ROM hosts one of the most diverse amphibian communities in Portugal, with 10 and potentially up to 12 species recorded (Velo-Antón, 2020). Two species detected in this study are of particular biogeographic importance: the Iberian endemic *D. galganoi* and the Portuguese endemic *P. atlanticus*. According to IUCN criteria, three species present in the Reserve are classified as vulnerable: *P. cultripes*, *S. salamandra* and *T. marmoratus*, while the remaining species are currently listed as least concern. The results of this research demonstrate that the composition of the amphibian community in the Reserve is strongly influenced by habitat type, water availability and short-term climatic variability.

Although the PPRLVC-ROM is formally protected, its management has not explicitly prioritised amphibian conservation. Despite their ecological importance, amphibians face multiple pressures that threaten their survival and reproductive success. One of the most severe threats is the expansion of eucalyptus plantations, which negatively affect amphibian populations through soil acidification, alteration of understory vegetation and reduction of aquatic habitats (Cruz *et al.*, 2015). Habitat fragmentation caused by construction and the expansion of trail networks increases these pressures further by compacting the soil, destroying marginal ponds and increasing the risk of road mortality. Equally concerning is the degradation of permanent and temporary ponds, which are critical breeding sites but are increasingly vulnerable to natural desiccation and human disturbance. In addition, pronounced climatic variability — especially recurrent droughts and episodes of heavy rainfall — intensifies the instability of amphibian breeding habitats.

Effective amphibian conservation requires a multifaceted approach that addresses threats to both their aquatic and terrestrial habitats throughout their life cycle. Hydroperiod plays a pivotal role in shaping amphibian movement, reproduction, and survival, as demonstrated in this and other studies. The orthophoto analysis conducted here revealed that most temporary ponds form alongside trails, thereby exposing them to increased anthropogenic disturbance. Conservation measures in the Reserve should therefore prioritise pond protection to ensure the availability of suitable breeding habitats. This includes safeguarding existing ponds, restoring degraded ones, and maintaining hydroperiods long enough to support reproductive success (Wu *et al.*, 2025). Pond creation and restoration enhance habitat availability, restore ecological dynamics and increase connectivity, and have been successful elsewhere (Brown *et al.*, 2012). For instance, a 20-year monitoring programme in northern Switzerland showed that pond restoration successfully stagnated and even reversed amphibian declines (Moor *et al.*, 2022). Therefore, maintaining a network of both permanent and temporary ponds connected across the landscape is essential for sustaining the Reserve's amphibian diversity.

However, increasing aquatic habitat alone is insufficient. Conservation must also ensure connectivity between breeding and non-breeding habitats by establishing ecological corridors (Becker *et al.*, 2007) and restoring connectivity with other natural areas currently isolated by urban expansion. The PPRLVC-ROM encompasses a variety of terrestrial habitats that support amphibian diversity, yet their integrity is threatened by exotic plantations. Restricting the spread of eucalyptus and other non-native species while promoting native vegetation would preserve the microhabitat diversity necessary for shelter, thermoregulation and access to refuges during droughts (Cruz *et al.*, 2015; Thorpe *et al.*, 2018). Human activities within the Reserve must also be carefully managed, with increased surveillance and the enforcement of new rules to reduce the impacts of tourism. Construction projects must be preceded by environmental impact assessments, and trail networks must be regulated to reduce vehicle access and preserve natural soil, especially during the breeding season, when temporary ponds form along the trails.

Finally, active community involvement will be crucial for long-term conservation success. Promoting awareness of the ecological value of amphibians and the importance of temporary ponds could encourage local stewardship. Citizen science initiatives involving residents, schools and visitors could increase monitoring capacity while strengthening support for conservation efforts.

In summary, the persistence of amphibian communities in the PPRLVC-ROM depends on protecting aquatic habitats, maintaining terrestrial habitat diversity, mitigating human pressures and fostering community engagement. Conservation efforts should be supported by long-term monitoring programmes and further research to track population trends and inform adaptive management strategies.

4.7 Limitations and future directions

Despite the valuable insights provided by this study, several limitations must be acknowledged. Firstly, the survey only covered a single year and involved one sampling event per month. As amphibian populations are known to fluctuate considerably from year to year, the patterns reported in this work represent a single snapshot of the Reserve's ecological dynamics. Secondly, while the survey covered a large area of the Reserve, not all habitat types were sampled. Consequently, certain areas remain unrepresented, which could result in important populations, habitat associations or variations in species richness and abundance being overlooked. Thirdly, the investigation focused solely on abiotic factors, including habitat type, water availability and climatic variables. While climatic variables were important for the analyses, the raw data could not be used as they were derived from a weather station located relatively far from the Reserve. Finally, the methodology employed — nocturnal visual transects — may have reduced the detectability of some species without complementary approaches. The use of UAV/drones enabled the creation of high-quality orthophotos for identifying bodies of water, but only in open areas.

These limitations highlight several opportunities for future research. Long-term monitoring programmes are essential to capture interannual variability and detect population trends in response to changes in PPRLVC-ROM characteristics and climate. Expanding spatial coverage, particularly to under-sampled areas, would provide a more complete understanding of species distributions. Future studies could incorporate additional ecological factors, such as biotic interactions, disease dynamics and water quality, to offer further insights into community structuring. Furthermore, obtaining climatic data directly from the Reserve would enable the inclusion of raw measurements and improve species-specific analyses. Finally, incorporating additional survey methods, such as acoustic monitoring, sampling ponds with a net, environmental DNA sampling or automated recording devices, could improve the detection of elusive species. In order to include all available water in the Reserve, a more comprehensive methodology should be included to incorporate water bodies present below the vegetation cover.

5. Conclusion

This study provided a systematic and updated assessment of the amphibian community in the PPRLVC-ROM. It highlighted the diversity and seasonal dynamics of the community, as well as the key environmental factors that influence the presence and abundance of species. Over a full annual cycle, clear patterns in community composition were observed, with strongly significant associations with habitat types and water availability, as well as an influence of climatic variables on amphibian activity. Comparisons with historical data suggest that, while overall diversity has remained stable, local populations are under pressure, which could affect their long-term survival.

The findings reinforce the ecological importance of temporary ponds and small aquatic habitats, which are critical for reproduction and seasonal activity. The amphibians' strong dependence on aquatic and terrestrial resources confirms their role as indicators of habitat quality, while also exposing them to the risks of habitat fragmentation, water contamination, and climate change.

From a conservation perspective, the results emphasise the need to preserve habitat heterogeneity within the PPRLVC-ROM and ensure connectivity between aquatic and terrestrial environments. Essential steps to safeguard amphibian populations in the PPRLVC-ROM include mitigating urban and agricultural pressures, increasing water availability and maintaining water quality, and controlling invasive species. Continuous monitoring is necessary to detect temporal trends and anticipate the impacts of environmental changes. In addition, it is necessary to propose strengthening the integration of amphibian conservation into regional planning strategies.

Beyond its local scope, this study contributes to a broader understanding of amphibian ecology in Portuguese coastal landscapes, where populations are under increasing pressure from human activities. By integrating ecological knowledge with conservation management, the PPRLVC-ROM reserve can serve as a reference site for amphibian conservation in Portugal and the wider region.

In conclusion, the amphibian population in PPRLVC-ROM is diverse but vulnerable. Their persistence will depend on proactive conservation measures based on ecological research, such as that conducted in this study, to ensure that this historically significant Reserve continues to function as a refuge for biodiversity in an increasingly altered landscape.

6. References

Alagador, D. (2022) ‘Effective conservation planning of Iberian amphibians based on a regionalization of climate-driven range shifts’, *Conservation Biology*, 37(2). doi:https://doi.org/10.1111/cobi.14026.

Alarcón-Ríos, L. *et al.* (2019) ‘Evolutionary history and not heterochronic modifications associated with viviparity drive head shape differentiation in a reproductive polymorphic species, *Salamandra salamandra*’, *Evolutionary Biology*, 47(1), pp. 43–55. doi:https://doi.org/10.1007/s11692-019-09489-3.

Alcobendas, M., Dopazo, H. and Alberch, P. (1996) ‘Geographic variation in allozymes of populations of *Salamandra salamandra* (Amphibia: Urodela) exhibiting distinct reproductive modes’, *Journal of Evolutionary Biology*, 9(1), pp. 83–102. doi:https://doi.org/10.1046/j.1420-9101.1996.9010083.x.

APA – Agência Portuguesa do Ambiente (2025). *Monitorização agrometeorológica e hidrológica. Ano hidrológico 2024/2025. Relatório do Grupo de Trabalho de assessoria técnica à Comissão Permanente de Prevenção, Monitorização e Acompanhamento dos Efeitos da Seca*. Lisboa: APA. Available at: https://apambiente.pt/sites/default/files/_SNIAMB_Agua/DRH/OrgaosConsultivos/ComissaoPermanenteSeca/Relatorios/104_Relatorio_Monitorizacao_30Jun2025.pdf (Accessed: 28 Ago 2025).

Aquino, A.G.H.E.O. and Nkomo, S.L. (2021) ‘Spatio-Temporal Patterns and Consequences of Road Kills: A review’, *Animals*, 11(3), p. 799. doi:https://doi.org/10.3390/ani11030799.

Arntzen, J.W. *et al.* (2017) ‘Amphibian decline, pond loss and reduced population connectivity under agricultural intensification over a 38 year period’, *Biodiversity and Conservation*, 26(6), pp. 1411–1430. doi:https://doi.org/10.1007/s10531-017-1307-y.

Bani, L. *et al.* (2015) ‘Ecological connectivity assessment in a strongly structured fire salamander (*Salamandra salamandra*) population’, *Ecology and Evolution*, 5(16), pp. 3472–3485. doi:https://doi.org/10.1002/ece3.1617.

Becker, C.G. *et al.* (2007) ‘Habitat split and the global decline of amphibians’, *Science*, 318(5857), pp. 1775–1777. doi:https://doi.org/10.1126/science.1149374.

Behangana, M., Kasoma, P.M.B. and Luiselli, L. (2009) ‘Ecological correlates of species richness and population abundance patterns in the amphibian communities from the Albertine Rift, East Africa’, *Biodiversity and Conservation*, 18(11), pp. 2855–2873. doi:https://doi.org/10.1007/s10531-009-9611-9.

Beranek, C.T. *et al.* (2022) ‘Factors influencing persistence of a threatened amphibian in restored wetlands despite severe population decline during climate change driven weather extremes’, *Biodiversity and Conservation*, 31(4), pp. 1267–1287. doi:https://doi.org/10.1007/s10531-022-02387-9.

Blaustein, A.R. *et al.* (2010) ‘Direct and indirect effects of climate change on amphibian populations’, *Diversity*, 2(2), pp. 281–313. doi:https://doi.org/10.3390/d2020281.

Bötsch, Y. *et al.* (2018) ‘Effect of recreational trails on forest birds: Human presence matters’, *Frontiers in Ecology and Evolution*, 6. doi:https://doi.org/10.3389/fevo.2018.00175.

Brooks, G.C. and Kindsvater, H.K. (2022) ‘Early development drives variation in amphibian vulnerability to global change’, *Frontiers in Ecology and Evolution*, 10. doi:https://doi.org/10.3389/fevo.2022.813414.

Brown, D.J. *et al*. (2012) ‘A place to call home: amphibian use of created and restored wetlands’, *International Journal of Ecology*, 2012, pp. 1–11. doi:https://doi.org/10.1155/2012/989872.

Buckley, L.B. and Jetz, W. (2007) ‘Environmental and historical constraints on global patterns of amphibian richness’, *Proceedings of the Royal Society B Biological Sciences*, 274(1614), pp. 1167–1173. doi:https://doi.org/10.1098/rspb.2006.0436.

Burraco, P., Duarte, L.J. and Gomez-Mestre, I. (2013) ‘Predator-induced physiological responses in tadpoles challenged with herbicide pollution’, *Current Zoology*, 59(4), pp. 475–484. doi:https://doi.org/10.1093/czoolo/59.4.475.

Cabral, M.J. *et al*. (2005). *Livro Vermelho dos Vertebrados de Portugal*. Instituto da Conservação da Natureza, Lisboa.

Carvalho-Rocha, V., Peres, C.A. and Neckel-Oliveira, S. (2020) ‘Habitat amount and ambient temperature dictate patterns of anuran diversity along a subtropical elevational gradient’, *Diversity and Distributions*, 27(2), pp. 344–359. doi:https://doi.org/10.1111/ddi.13187.

Castro, K.M.S.A. *et al*. (2021) ‘Water constraints drive allometric patterns in the body shape of tree frogs’, *Scientific Reports*, 11(1). doi:https://doi.org/10.1038/s41598-020-80456-1.

Cei, J.M. and Crespo, E.G. (1971) ‘Remarks on some adaptive ecological trends of *Pelobates cultripes* from Portugal: thermal requirement, rate of development and water regulation’, *Arquivos do Museu Bocage*, 2, pp. 9-36.

Chang, H.C. *et al*. (2023) ‘Ecological carrying capacity estimation of the trails in a protected area: integrating a path analysis model and the stakeholders’ evaluation’, *Forests*, 14(12), p. 2400. doi:https://doi.org/10.3390/f14122400.

Çömden, E.A., Yenmiş, M. and Çakır, B. (2023) ‘The Complex Bridge between Aquatic and Terrestrial Life: Skin Changes during Development of Amphibians’, *Journal of Developmental Biology*, 11(1), p. 6. doi:https://doi.org/10.3390/jdb11010006.

Contardo, J. and Labra, A. (2024) ‘Mapping an endangered living fossil’s distribution, threats, and protection coverage’, *Conservation Science and Practice*, 6(4). doi:https://doi.org/10.1111/csp2.13106.

Crottini, A., Galán, P. and Vences, M. (2010) ‘Mitochondrial diversity of Western spadefoot toads, *Pelobates cultripes*, in northwestern Spain’, *Amphibia-Reptilia*, 31(3), pp. 443–448. doi:https://doi.org/10.1163/156853810791769527.

Cruz, J. *et al*. (2015) ‘Exotic Fish in Exotic Plantations: A Multi-Scale Approach to Understand Amphibian Occurrence in the Mediterranean Region’, *PLoS ONE*, 10(6), p. e0129891. doi:https://doi.org/10.1371/journal.pone.0129891.

Du, M. and Noguchi, N. (2017) ‘Monitoring of wheat growth status and mapping of wheat yield’s within-field spatial variations using color images acquired from UAV-camera system’, *Remote Sensing*, 9(3), p. 289. doi:https://doi.org/10.3390/rs9030289.

Egea-Serrano, A., Tejedo, M. and Torralva, M. (2008) ‘Analysis of the Avoidance of Nitrogen Fertilizers in the Water Column by Juvenile Iberian Water Frog, *Pelophylax perezi* (Seoane, 1885), in Laboratory Conditions’, *Bulletin of Environmental Contamination and Toxicology*, 80(2), pp. 178–183. doi:https://doi.org/10.1007/s00128-007-9341-1.

Eisenlohr, P.V. *et al.* (2011) 'Floristic variations in a woody plant community along a trail in a Semideciduous Seasonal Forest, Viçosa, Minas Gerais State, Brazil', *Hoehnea*, 38(1), pp. 61–71. doi:https://doi.org/10.1590/s2236-89062011000100006.

Escoriza, D. and Boix, D. (2014) 'Reproductive habitat selection in alien and native populations of the genus *Discoglossus*', *Acta Oecologica*, 59, pp. 97–103. doi:https://doi.org/10.1016/j.actao.2014.06.005.

Ficetola, G.F. and Maiorano, L. (2016) 'Contrasting effects of temperature and precipitation change on amphibian phenology, abundance and performance', *Oecologia*, 181(3), pp. 683–693. doi:https://doi.org/10.1007/s00442-016-3610-9.

Ficetola, G.F. *et al.* (2018) 'Differences between microhabitat and broad-scale patterns of niche evolution in terrestrial salamanders', *Scientific Reports*, 8(1). doi:https://doi.org/10.1038/s41598-018-28796-x.

Figueiredo, G.T. *et al.* (2019) 'Influence of microhabitat on the richness of anuran species: a case study of different landscapes in the Atlantic Forest of southern Brazil', *Anais Da Academia Brasileira De Ciências*, 91(2). doi:https://doi.org/10.1590/0001-3765201920171023.

Fisher, M.C. and Garner, T.W.J. (2020) 'Chytrid fungi and global amphibian declines', *Nature Reviews Microbiology*, 18(6), pp. 332–343. doi:https://doi.org/10.1038/s41579-020-0335-x.

Forio, M.A.E. and Goethals, P.L.M. (2020) 'An integrated approach of Multi-Community monitoring and assessment of aquatic ecosystems to support sustainable development', *Sustainability*, 12(14), p. 5603. doi:https://doi.org/10.3390/su12145603.

Gitelson, A.A. *et al.* (2002) 'Novel algorithms for remote estimation of vegetation fraction', *Remote Sensing of Environment,* 80(1), pp. 76–87. doi:https://doi.org/10.1016/s0034-4257(01)00289-9.

Gonçalves, H. *et al.* (2009) 'High levels of population subdivision in a morphologically conserved Mediterranean toad (*Alytes cisternasii*) result from recent, multiple refugia: evidence from mtDNA, microsatellites and nuclear genealogies', *Molecular Ecology*, 18(24), pp. 5143–5160. doi:https://doi.org/10.1111/j.1365-294x.2009.04426.x.

Gosá, A. (2003) '*Bufo bufo* (common toad), *Alytes obstetricans* (midwife toad), and *Rana temporaria* (common frog). Tree climbing', *Herpetological Review*, 34(4), p. 355.

Guillot, H. *et al.* (2016) 'Landscape influences the morphology of male common toads (*Bufo bufo*)', *Agriculture Ecosystems & Environment*, 233, pp. 106–110. doi:https://doi.org/10.1016/j.agee.2016.08.032.

Gutiérrez-Rodríguez, J. *et al.* (2022) 'The role of habitat features in patterns of population connectivity of two Mediterranean amphibians in arid landscapes of central Iberia', *Landscape Ecology*, 38(1), pp. 99–116. doi:https://doi.org/10.1007/s10980-022-01548-z.

Hamer, A.J. and McDonnell, M.J. (2008) 'Amphibian ecology and conservation in the urbanising world: A review', *Biological Conservation*, 141(10), pp. 2432–2449. doi:https://doi.org/10.1016/j.biocon.2008.07.020.

Honrado, J. *et al.* (2005). Estudo Prévio para a Elaboração de um Plano Estratégico com vista ao Ordenamento e Gestão da Reserva Ornitológica do Mindelo e Área Envolvente do Concelho de Vila do Conde, Relatórios Sectoriais. Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto.

Hopkins, W.A. (2007) 'Amphibians as models for studying environmental change', *ILAR Journal*, 48(3), pp. 270–277.

Janin, A., Léna, J.P. and Joly, P. (2011) 'Beyond occurrence: Body condition and stress hormone as integrative indicators of habitat availability and fragmentation in the common toad', *Biological Conservation*, 144(3), pp. 1008–1016. doi:https://doi.org/10.1016/j.biocon.2010.12.009.

Jehle, R. and Arntzen, J.W. (2000) 'Post-breeding migrations of newts (*Triturus cristatus* and *T. marmoratus*) with contrasting ecological requirements', *Journal of Zoology*, 251(3), pp. 297–306. doi:https://doi.org/10.1017/s0952836900007032.

Lange, L., Brischoux, F. and Lourdais, O. (2020) 'Midwife toads (*Alytes obstetricans*) select their diurnal refuges based on hydric and thermal properties', *Amphibia-Reptilia*, 41(2), pp. 275–280. doi:https://doi.org/10.1163/15685381-20201246.

Leira, M. *et al.* (2018) 'Holocene sea level and climate interactions on wet dune slack evolution in SW Portugal: A model for future scenarios?', *The Holocene*, 29(1), pp. 26–44. doi:https://doi.org/10.1177/0959683618804633.

Lertzman-Lepofsky, G.F. *et al.* (2020) 'Water loss and temperature interact to compound amphibian vulnerability to climate change', *Global Change Biology*, 26(9), pp. 4868–4879. doi:https://doi.org/10.1111/gcb.15231.

López-Delgado, J., Riemsdijk, I.V. and Arntzen, J.W. (2020) 'Tracing species replacement in Iberian marbled newts', *Ecology and Evolution*, 11(1), pp. 402–414. doi:https://doi.org/10.1002/ece3.7060.

Louhaichi, M., Borman, M.M. and Johnson, D.E. (2001) 'Spatially located platform and aerial photography for documentation of grazing impacts on wheat', *Geocarto International*, 16(1), pp. 65–70. doi:https://doi.org/10.1080/10106040108542184.

Luedtke, J.A. *et al.* (2023) 'Ongoing declines for the world's amphibians in the face of emerging threats', *Nature*, 622(7982), pp. 308–314. doi:https://doi.org/10.1038/s41586-023-06578-4.

Ma, Q. *et al.* (2024) 'Impact of Climate Change on the Distribution of Three Rare Salamanders (*Liua shihi, Pseudohynobius jinfo*, and *Tylototriton wenxianensis*) in Chongqing, China, and Their Conservation Implications', *Animals*, 14(5), p. 672. doi:https://doi.org/10.3390/ani14050672.

Macedo, P.A.C. (2002) 'Land Use Change in Mindelo Ornithological Reserve', *Littoral the Changing Coast*, pp. 95-100.

Marques, A.J.D., Mata, V.A. and Velo-Antón, G. (2022) 'COI Metabarcoding Provides Insights into the Highly Diverse Diet of a Generalist Salamander, *Salamandra salamandra* (Caudata: Salamandridae)', *Diversity*, 14(2), p. 89. doi:https://doi.org/10.3390/d14020089.

Martínez-Gil, H. *et al.* (2023) 'Fine-scale functional connectivity of two syntopic pond-breeding amphibians with contrasting life-history traits: an integrative assessment of direct and indirect estimates of dispersal', *Conservation Genetics*, 24(3), pp. 361–374. doi:https://doi.org/10.1007/s10592-023-01506-5.

Mathwin, R. *et al.* (2020) 'Manipulating water for amphibian conservation', *Conservation Biology*, 35(1), pp. 24–34. doi:https://doi.org/10.1111/cobi.13501.

Meek, R. (2022) ‘Long-Term Changes in Four Populations of the Spiny Toad, *Bufo spinosus*, in Western France; Data from Road Mortalities’, *Conservation*, 2(2), pp. 248–261. doi:https://doi.org/10.3390/conservation2020017.

Miller, D.W. *et al*. (2018) ‘Quantifying climate sensitivity and climate-driven change in North American amphibian communities’, *Nature Communications*, 9(1). doi:https://doi.org/10.1038/s41467-018-06157-6.

Moor, H. *et al*. (2022) ‘Bending the curve: Simple but massive conservation action leads to landscape-scale recovery of amphibians’, *Proceedings of the National Academy of Sciences*, 119(42). doi:https://doi.org/10.1073/pnas.2123070119.

Mulder, K.P. *et al*. (2016) ‘The complete mitochondrial genome of *Salamandra salamandra* (Amphibia: Urodela: Salamandridae)’, *Mitochondrial DNA Part B*, 1(1), pp. 880–882. doi:https://doi.org/10.1080/23802359.2016.1253042.

Oliveira, J.C.F. *et al*. (2021) ‘Amphibians of Serra das Torres Natural Monument: a reservoir of biodiversity in the Atlantic Forest of southeastern Brazil’, *Biota Neotropica*, 21(3). doi:https://doi.org/10.1590/1676-0611-bn-2020-1085.

Oseen, K.L. and Wassersug, R.J. (2002) ‘Environmental factors influencing calling in sympatric anurans’, *Oecologia*, 133(4), pp. 616–625. doi:https://doi.org/10.1007/s00442-002-1067-5.

Peixoto, S. *et al*. (2020) ‘Species detection from aquatic eDNA: Assessing the importance of capture methods’, *Environmental DNA*, 3(2), pp. 435–448. doi:https://doi.org/10.1002/edn3.130.

Petrovan, S.O. *et al*. (2022) ‘Why link diverse citizen science surveys? Widespread arboreal habits of a terrestrial amphibian revealed by mammalian tree surveys in Britain’, *PLoS ONE*, 17(7), p. e0265156. doi:https://doi.org/10.1371/journal.pone.0265156.

Préau, C. *et al*. (2021) ‘Climate change would prevail over land use change in shaping the future distribution of *Triturus marmoratus* in France’, *Animal Conservation*, 25(2), pp. 221–232. doi:https://doi.org/10.1111/acv.12733.

Qian, H. *et al*. (2007) ‘Environmental determinants of amphibian and reptile species richness in China’, *Ecography*, 30(4), pp. 471–482. doi:https://doi.org/10.1111/j.2007.0906-7590.05025.x.

Ragland, G.J. and Kingsolver, J.G. (2008) ‘Evolution of thermotolerance in seasonal environments: the effects of annual temperature variation and life-history timing in Wyeomyia Smithii’, *Evolution*, 62(6), pp. 1345–1357. doi:https://doi.org/10.1111/j.1558-5646.2008.00367.x.

Rannap, R. *et al*. (2024) ‘Extensive restoration of the entire habitat complex is key to the successful recovery of threatened species: The case of the natterjack toad *Epidalea calamita* at the northern range margin’, *Journal for Nature Conservation*, 82, p. 126707. doi:https://doi.org/10.1016/j.jnc.2024.126707.

Rebelo, R. and Leclair, M.H. (2003) ‘Site Tenacity in the Terrestrial Salamandrid *Salamandra salamandra*’, *Journal of Herpetology*, 37(2), pp. 440-445. doi: http://dx.doi.org/10.1670/0022-1511(2003)037%5B0440:STITTS%5D2.0.CO;2.

Reyes-Moya, I., Sánchez-Montes, G. and Martínez-Solano, Í. (2022) ‘Integrating dispersal, breeding and abundance data with graph theory for the characterization and management of functional connectivity in amphibian pondscapes', *Landscape Ecology*, 37(12), pp. 3159–3177. doi:https://doi.org/10.1007/s10980-022-01520-x.

Reyne, M. *et al*. (2021) 'Will predicted positive effects of climate change be enough to reverse declines of the regionally Endangered Natterjack toad in Ireland?', *Ecology and Evolution*, 11(10), pp. 5049–5064. doi:https://doi.org/10.1002/ece3.7362.

Ribeiro, C. *et al*. (2014) 'Estudo da variação de propriedades de sedimentos com objetivos forenses', *Comunicações Geológicas*, 101, pp. 1349-1353.

Richardson, A.D. *et al*. (2007) 'Use of digital webcam images to track spring green-up in a deciduous broadleaf forest', *Oecologia*, 152(2), pp. 323–334. doi:https://doi.org/10.1007/s00442-006-0657-z.

Richter-Boix, A., Llorente, G.A. and Montori, A. (2006) 'Breeding phenology of an amphibian community in a Mediterranean area', *Amphibia-Reptilia*, 27(4), pp. 549–559. doi:https://doi.org/10.1163/156853806778877149.

Rödder, D. *et al*. (2017) 'Global realized niche divergence in the African clawed frog *Xenopus laevis*', *Ecology and Evolution*, 7(11), pp. 4044–4058. doi:https://doi.org/10.1002/ece3.3010.

Saenz, D. *et al*. (2006) 'Abiotic Correlates of Anuran Calling Phenology: The Importance of Rain, Temperature, and Season', *Herpetological Monographs*, 20(1), p. 64. doi:https://doi.org/10.1655/0733-1347(2007)20.

Sánchez-Montes, G. and Martínez-Solano, I. (2011) 'Population size, habitat use and movement patterns during the breeding season in a population of Perez's frog (*Pelophylax perezi*) in central Spain', Basic and Applied Herpetology, 25, pp. 81-96. doi:https://doi.org/10.11160/bah.11013.

Sánchez, A., Talavera, A. and Hinckley, A. (2015) 'Descripción y conservación de una población urbana de *Discoglossus galganoi*', *Boletín De La Asociación Herpetológica Española*, 26(1), pp. 72–75.

Santos, B. *et al*. (2023) 'Skin and gut microbiomes of tadpoles vary differently with host and water environment: a short-term experiment using 16S metabarcoding', *Scientific Reports*, 13(1). doi:https://doi.org/10.1038/s41598-023-43340-2.

Segura, A. and Palomar, G. (2023) 'Hydroperiod of temporary ponds threats amphibian recruitment in Mediterranean environments', *Amphibia-Reptilia*, 44(3), pp. 375–384. doi:https://doi.org/10.1163/15685381-bja10146.

Shoab, M., Singh, V.K. and Ravibabu, M.V. (2021) 'High-Precise True Digital Orthoimage Generation and Accuracy Assessment based on UAV Images', *Journal of the Indian Society of Remote Sensing*, 50(4), pp. 613–622. doi:https://doi.org/10.1007/s12524-021-01364-z.

Shorb, C.M., Freymiller, L.A. and Hernandez, D.L. (2020) 'Differential responses of prairie rodents to edge effects from recreational trails', *Nature Conservation*, 41, pp. 113–140. doi:https://doi.org/10.3897/natureconservation.41.52100.

Sillero, N. *et al*. (2021) 'Want to model a species niche? A step-by-step guideline on correlative ecological niche modelling', *Ecological Modelling*, 456, p. 109671. doi:https://doi.org/10.1016/j.ecolmodel.2021.109671.

Silva, H.M.O. (2017) *Sistemas de Informação Geográfica no estudo da Paisagem Protegida Regional do Litoral de Vila do Conde e Reserva Ornitológica de Mindelo*. Master's thesis, Universidade do Porto.

Smith, P.H. and Skelcher, G. (2019) ‘Effects of environmental factors and conservation measures on a sand-dune population of the natterjack toad (*Epidalea calamita*) in north-west England: a 31-year study’, *Herpetological Journal*, 29(3), pp. 146–154. doi:https://doi.org/10.33256/hj29.3.146154.

Soares, C. and Brito, J.C. (2007) ‘Environmental Correlates for Species Richness among Amphibians and Reptiles in a Climate Transition Area’, *Biodiversity and Conservation*, 16(4), pp. 1087–1102. doi:https://doi.org/10.1007/s10531-006-9070-5.

Svajda, J. *et al*. (2016) ‘Trail impact monitoring in Rocky Mountain National Park, USA’, *Solid Earth*, 7(1), pp. 115–128. doi:https://doi.org/10.5194/se-7-115-2016.

Thorpe, C.J. *et al*. (2018) ‘Micro-habitat distribution drives patch quality for sub-tropical rocky plateau amphibians in the northern Western Ghats, India’, *PLoS ONE*, 13(3), p. e0194810. doi:https://doi.org/10.1371/journal.pone.0194810.

Tong, Q. *et al*. (2023) ‘Effects of fine-scale habitat quality on activity, dormancy, habitat use, and survival after reproduction in *Rana dybowskii* (Chordata, Amphibia)’, *BMC Zoology*, 8(1). doi:https://doi.org/10.1186/s40850-022-00163-4.

Vasconcelos, T.S. *et al*. (2014) ‘Biogeographic distribution patterns and their correlates in the diverse frog fauna of the Atlantic Forest hotspot’, *PLoS ONE*, 9(8), p. e104130. doi:https://doi.org/10.1371/journal.pone.0104130.

Velo-Antón, G. (2020) ‘Amphibian micro-hotspot at the Mindelo Ornithological Reserve (Porto, Portugal)’, *Boletín de la Asociación Herpetológica Espanola*, 31(2), pp. 120-124.

Walls, S., Barichivich, W. and Brown, M. (2013) ‘Drought, Deluge and Declines: The impact of precipitation extremes on amphibians in a changing climate’, *Biology*, 2(1), pp. 399–418. doi:https://doi.org/10.3390/biology2010399.

West, J. (2018). *Importance of Amphibians: A Synthesis of Their Environmental Functions, Benefits to Humans, and Need for Conservation.* In BSU Honors Program Theses and Projects, Bridgewater State University.

Willig, M.R., Kaufman, D.M. and Stevens, R.D. (2003) ‘Latitudinal Gradients of Biodiversity: pattern, process, scale, and synthesis’, *Annual Review of Ecology, Evolution and Systematics*, 34(1), pp. 273–309. doi:https://doi.org/10.1146/annurev.ecolsys.34.012103.144032.

Woebbecke, N.D.M. *et al*. (1995) ‘Color indices for weed identification under various soil, residue, and lighting conditions’, *Transactions of the ASAE*, 38(1), pp. 259–269. doi:https://doi.org/10.13031/2013.27838.

Wu, N.C. *et al*. (2025) ‘A user’s guide for understanding reptile and amphibian hydroregulation and climate change impacts’, *Conservation Physiology*, 13(1). doi:https://doi.org/10.1093/conphys/coaf038.

Zamora-Camacho, F.J. and Aragón, P. (2024) ‘Increased temperature and exposure to ammonium alter the life cycle of an anuran species’, *Ecology and Evolution*, 14(12). doi:https://doi.org/10.1002/ece3.70685.

7. Appendix

Appendix 1 Information for each sampling.

Date	Start time	End time	Duration	Rain obs	Observants	Notes	Abundance	Richness
08/07/2024	21:57	23:15	1h18	Mild rain	Bruna, Marisa		24	3
12/08/2024	21:31	22:35	1h04		Bruna, Marisa		15	2
26/09/2024	20:35	23:15	2h40	Heavy rain on the day before	Bruna, Miguel	Start of the rains and first ponds appearing	140	10
21/10/2024	20:40	22:26	1h46		Bruna, Miguel		64	5
20/11/2024	19:00	20:55	1h55	Mild rain	Bruna, Luca	Passage of heavy vehicles on the trails	209	6
17/12/2024	18:32	20:33	2h01	Heavy rain	Bruna, Marisa	Passage of heavy vehicles on the trails	252	6
15/01/2025	18:42	19:50	1h08		Bruna, Marisa	Start of the pond in the forest area	15	4
26/02/2025	19:04	20:14	1h10		Bruna, Marisa		24	5
18/03/2025	19:41	21:45	2h04		Bruna, Miguel	Start of the ponds in the dunes Side stream on the trail	143	7
21/04/2025	21:05	22:33	1h28		Bruna, Marisa		80	7
26/05/2025	22:12	23:21	1h09		Bruna, Luca	Passage of heavy vehicles on the trails Drying up of dune and forest ponds Tall vegetation in the dune area Very dense forest and fallen vegetation	23	5
26/06/2025	22:18	23:30	1h12		Bruna, Luca	All ponds dry up Tall vegetation in the dune area Very dense forest and fallen vegetation	9	2

Appendix 2 Description of night transect sample data.

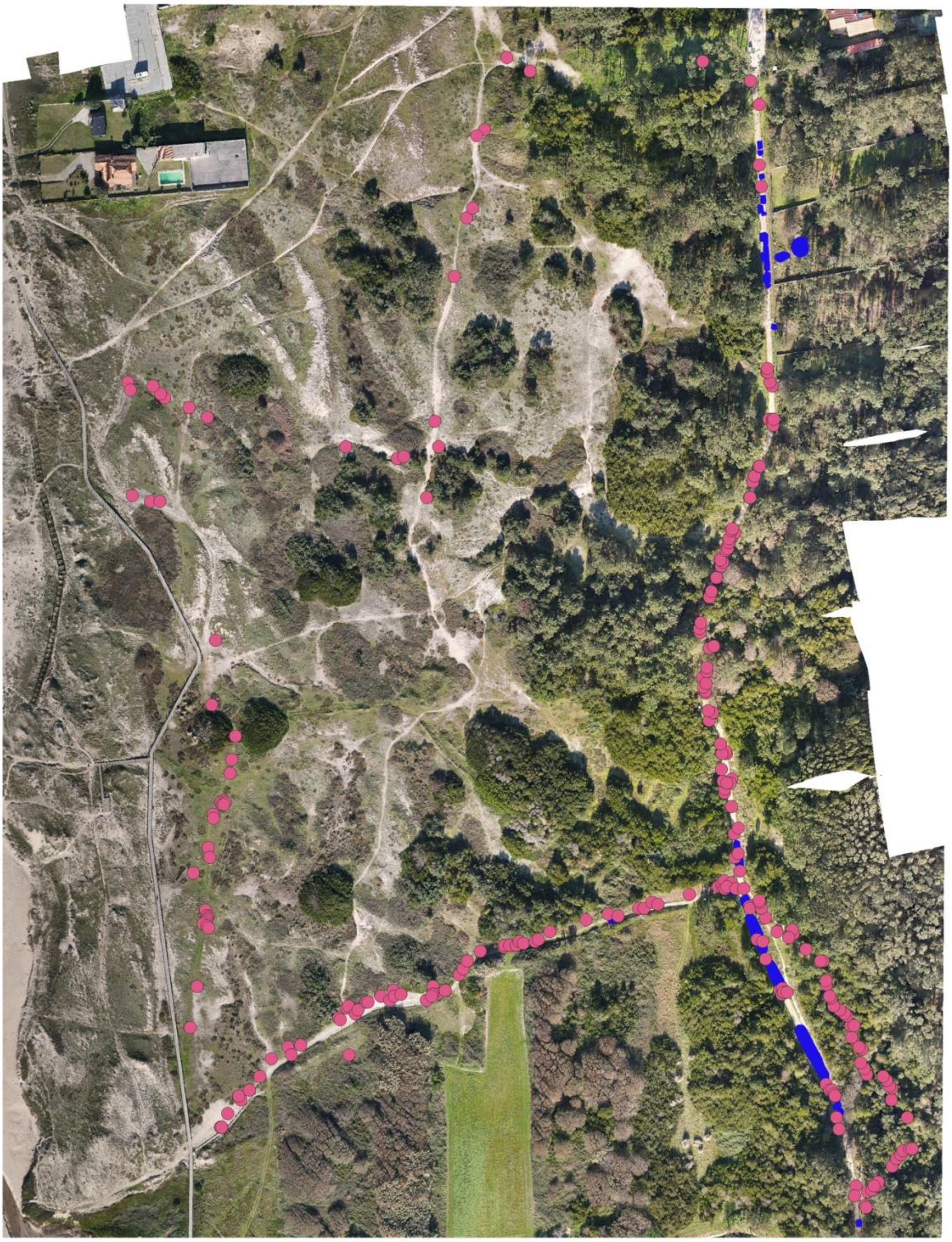
Taxa	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Anura												
Alytidae												
Alytes												
Alytes obstetricans	2	-	2	-	-	-	-	-	-	-	-	-
Bufonidae												
Bufo												
Bufo spinosus	3	1	3	4	2	14	-	-	15	2	5	2
Epidalea												
Epidalea calamita	-	-	16	14	8	20	1	1	9	5	-	-
Discoglossidae												
Discoglossus												
Discoglossus galganoi	-	-	5	-	-	-	-	-	9	5	2	-
Pelobatidae												
Pelobates												
Pelobates cultripes	19	14	33	36	19	78	1	1	43	36	14	7
Pelodytidae												
Pelodytes												
Pelodytes atlanticus	-	-	1	-	-	-	-	-	-	-	-	-
Ranidae												
Pelophylax												
Pelophylax perezi	-	-	56	-	4	1	2	9	16	13	1	-
Caudata												
Salamandridae												
Lissotriton												
Lissotriton helveticus	-	-	1	-	-	-	-	-	-	-	-	-
Salamandra												
Salamandra salamandra	-	-	22	9	170	138	11	4	47	12	-	-
Triturus												
Triturus marmoratus	-	-	1	1	6	1	-	9	4	7	1	-

Appendix 3 Results of Kruskal–Wallis tests comparing the distance to the nearest water body between “Dry” (total water area ≤ 300 m²) and “Wet” (total water area > 300 m²) conditions for each amphibian species in the PPRLVC-ROM. Significant p-values ($p < 0.05$).

Species	Mean distance (m)		N		Kruskal-Wallis χ^2	p-value	Significant
	Dry	Wet	Dry	Wet			
Bufo spinosus	93.8	23.1	13	31	10.26	0.0014	Yes
Discoglossus galganoi	23.1	9.8	2	14	0.03	0.8738	No
Epidalea calamita	155.0	83.6	23	35	9.86	0.0017	Yes
Pelobates cultripes	261.0	160.0	77	158	57.96	<0.0001	Yes
Pelophylax perezi	20.6	3.24	7	39	4.14	0.0420	Yes
Salamandra salamandra	61.0	40.8	190	201	12.07	0.0005	Yes
Triturus marmoratus	30.9	6.87	8	21	2.92	0.0877	No

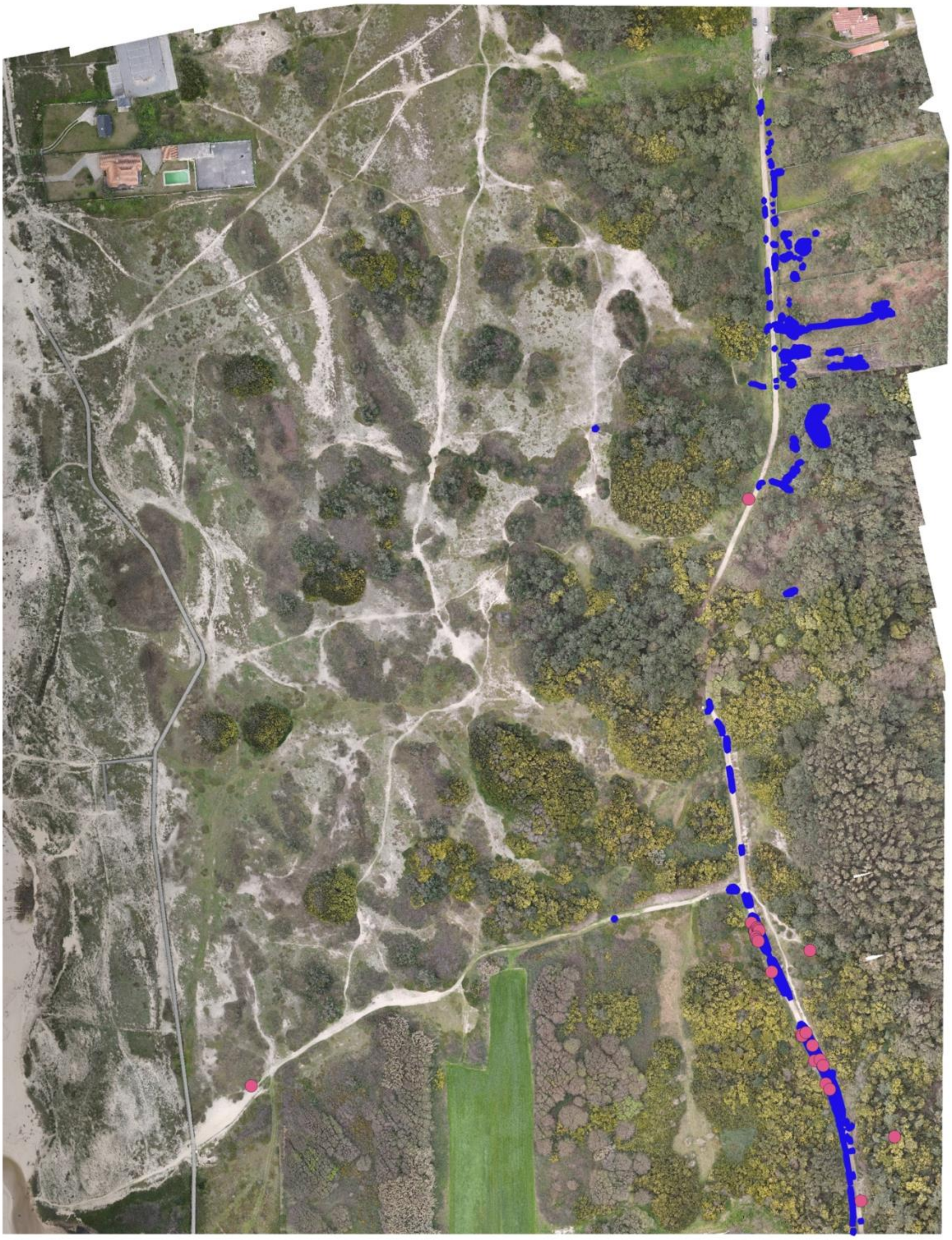
Appendix 4 Orthophoto from October 2024 with the identification of water bodies in blue and amphibian observations in pink dots.

Appendix 5 Orthophoto from November 2024 with the identification of water bodies in blue and amphibian observations in pink dots.

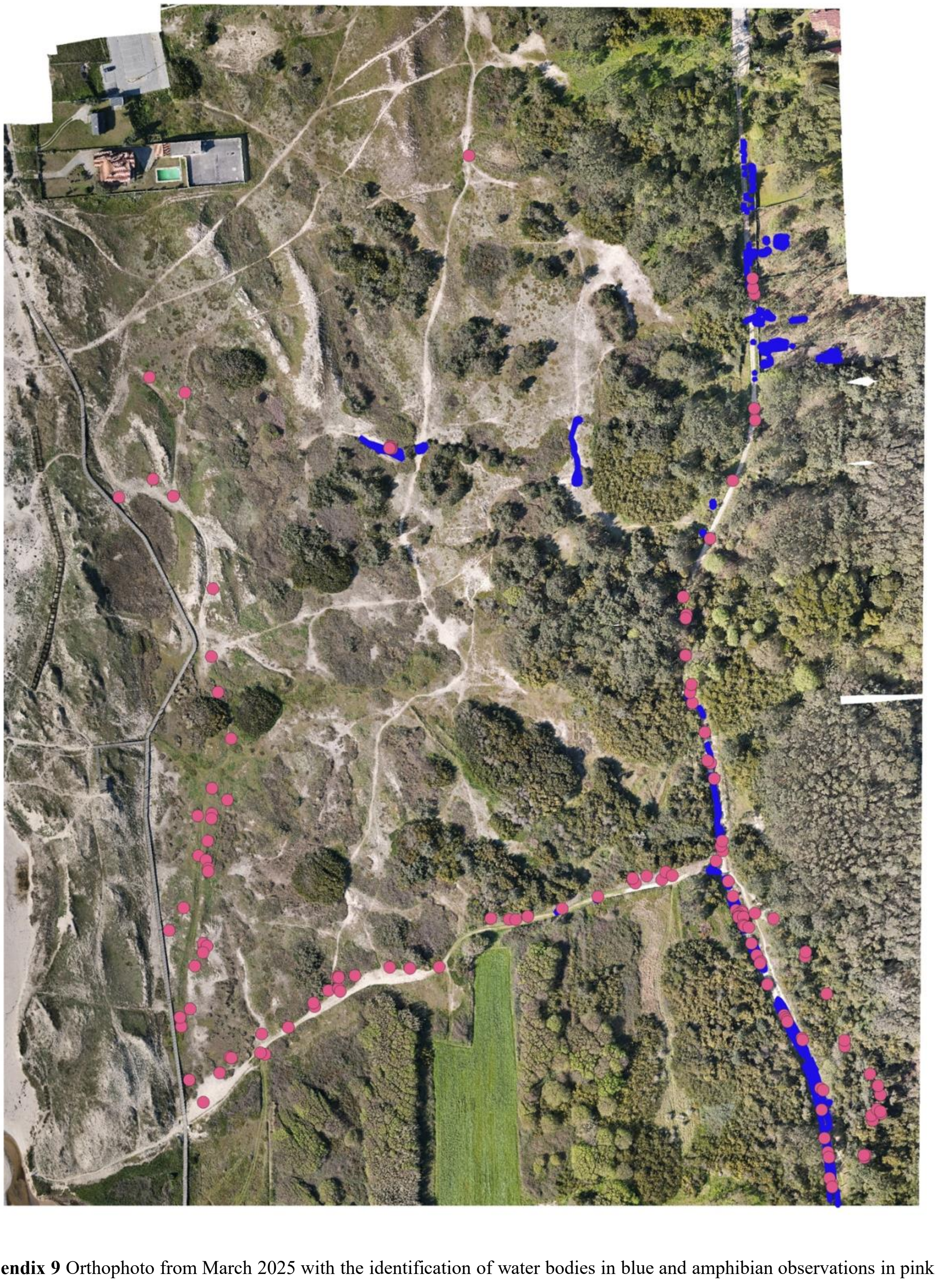


Appendix 6 Orthophoto from December 2024 with the identification of water bodies in blue and amphibian observations in pink dots.

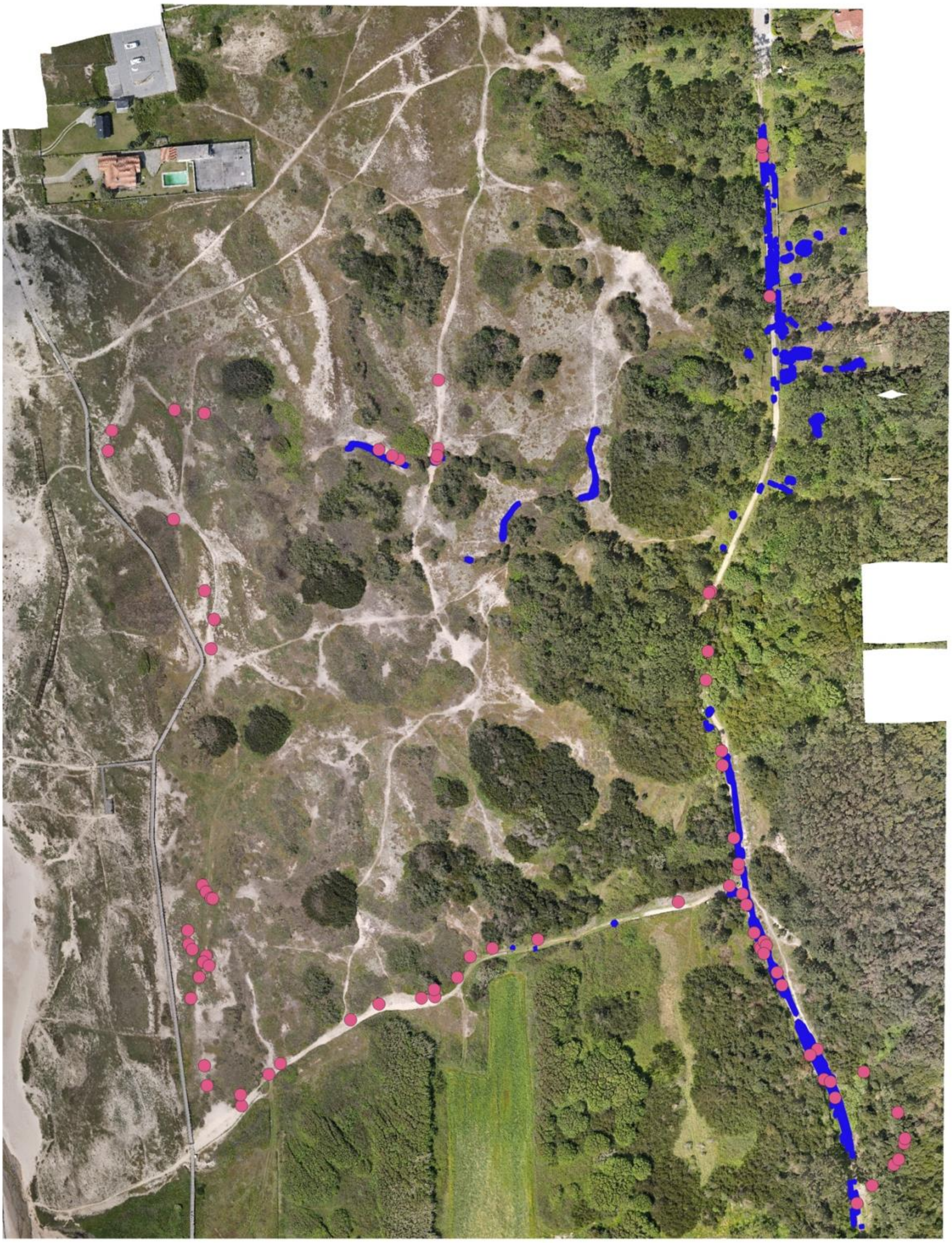
Appendix 7 Orthophoto from January 2025 with the identification of water bodies in blue and amphibian observations in pink dots.



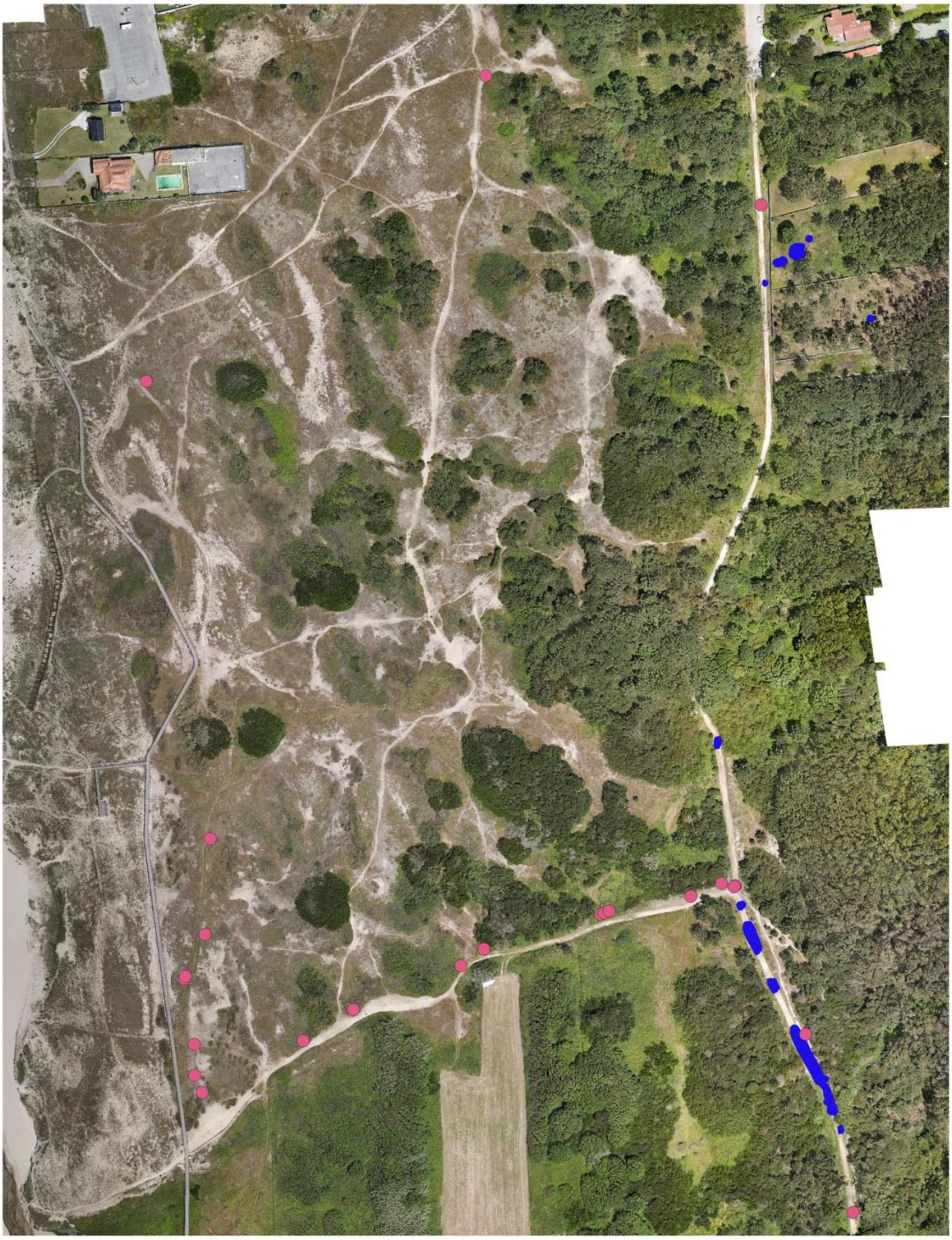
Appendix 8 Orthophoto from February 2025 with the identification of water bodies in blue and amphibian observations in pink dots.



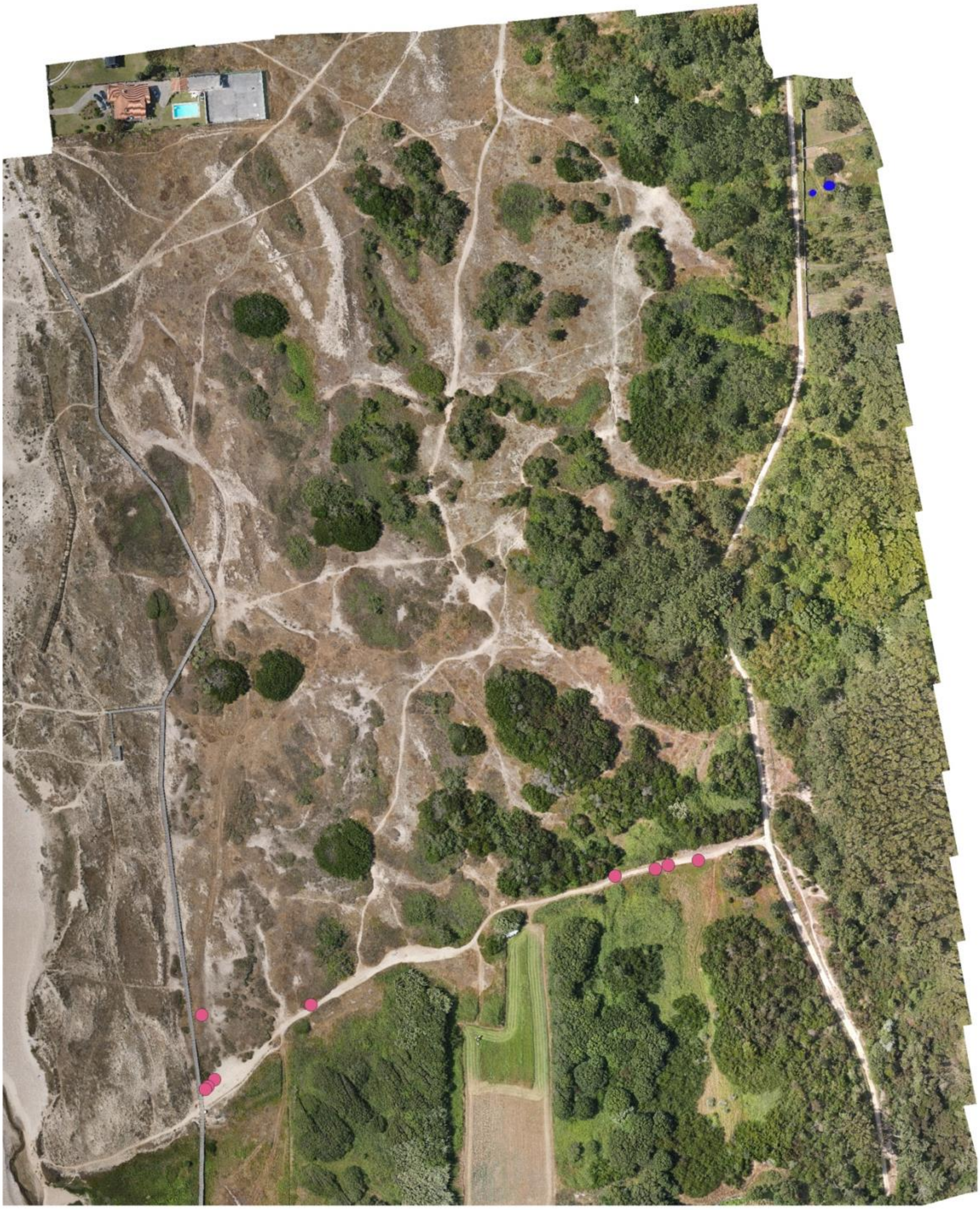
Appendix 9 Orthophoto from March 2025 with the identification of water bodies in blue and amphibian observations in pink dots.



Appendix 10 Orthophoto from April 2025 with the identification of water bodies in blue and amphibian observations in pink dots.



Appendix 11 Orthophoto from May 2025 with the identification of water bodies in blue and amphibian observations in pink dots.



Appendix 12 Orthophoto from June 2025 with the identification of water bodies in blue and amphibian observations in pink dots.