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Microhabitat Specialization in Amphibians

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ABSTRACT

Amphibians represent the most threatened vertebrate class globally, with approximately 41% of species assessed as threatened or extinct by IUCN, driven principally by habitat loss, chytridiomycosis, climate change, and pollution. Their biphasic life history -- requiring both aquatic and terrestrial habitats for reproduction, larval development, and adult survival -- makes amphibians exceptionally sensitive to the specific microhabitat conditions present in their home landscapes, and their strong site fidelity means that microhabitat degradation rapidly translates into population decline. This study quantified microhabitat specialisation across 48 amphibian species from 12 families in six European and Neotropical study regions, measuring 24 aquatic and terrestrial microhabitat variables at 312 occupied and 312 matched unoccupied sites. Multivariate habitat selection models -- fitted using conditional logistic regression with mixed effects -- identified pond surface area (R2 = 0.68, p < 0.001), canopy openness above breeding ponds (R2 = 0.64, p < 0.001), emergent vegetation cover (R2 = 0.61, p < 0.001), distance to nearest woodland edge (R2 = 0.58, p < 0.001), and soil moisture in terrestrial buffer zones (R2 = 0.54, p < 0.001) as the strongest predictors of amphibian occupancy. Microhabitat specialisation indices (Levins' B, niche breadth) ranged from 0.18 (Triturus cristatus, highly specialised) to 0.87 (Bufo bufo, habitat generalist) and were negatively correlated with IUCN threat category (Spearman rs = -0.72, p < 0.001), confirming that specialists face elevated extinction risk. Temperature sensitivity analyses demonstrated that breeding pond thermal regimes would shift beyond species-specific optimum ranges under RCP 4.5 and RCP 8.5 warming scenarios for 34.4% and 62.5% of species respectively by 2070. These findings provide quantitative microhabitat thresholds for amphibian conservation planning and restored habitat design.

Keywords: amphibian conservation; microhabitat selection; pond ecology; breeding site fidelity; niche breadth; habitat specialisation; chytrid; climate vulnerability

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1. Introduction

1.1 Amphibian Decline and Habitat Specificity

Amphibians are experiencing the most severe biodiversity crisis of any vertebrate class, with 41% of assessed species classified as Threatened or Extinct by the IUCN Red List -- a proportion exceeding that of mammals (26%), birds (14%), and reptiles (21%) (IUCN, 2023; Luedtke et al., 2023). The primary drivers of this crisis -- habitat destruction and degradation, the chytrid fungal pathogens Batrachochytrium dendrobatidis (Bd) and B. salamandrivorans (Bsal), climate change, invasive species, and chemical pollution -- are fundamentally microhabitat-mediated: they alter the specific microclimatic, hydrological, and biotic conditions that define suitable breeding and refugia sites, rather than acting uniformly across landscapes (Stuart et al., 2004; Scheele et al., 2019). Amphibians' permeable, desiccation-sensitive skin, ectothermic physiology, and biphasic aquatic-terrestrial life history make them far more dependent on precise microhabitat conditions -- particularly local moisture, temperature buffering, and water quality -- than most other vertebrate groups (Wells, 2007; Cushman, 2006).

1.2 Microhabitat Specialisation and Conservation Vulnerability

Microhabitat specialisation -- the restriction of habitat use to a narrow subset of available environmental conditions -- is both an ecological reality for many amphibian species and a primary driver of their conservation vulnerability. Specialised species cannot buffer population declines by colonising alternative habitats when their preferred conditions are degraded, making them acutely susceptible to habitat modification at scales as small as individual ponds (Cushman, 2006; Ficetola and Denooel, 2009). Levins' B niche breadth metric -- which quantifies the evenness of resource use across available habitat types -- has been used to compare microhabitat specialisation across taxa and predict extinction risk, with narrower niche breadths correlating with higher IUCN threat categories in multiple vertebrate assessments (Levins, 1968; Clavel et al., 2011). However, cross-species comparative analyses of amphibian microhabitat specialisation using standardised habitat assessment protocols across multiple study regions remain rare, limiting the generalisability of findings to inform conservation planning at landscape and national scales.

1.3 Objectives

This study provides the most comprehensive standardised cross-species analysis of amphibian microhabitat specialisation conducted in Europe and the Neotropics to date. Specific objectives were: (i) quantify 24 aquatic and terrestrial microhabitat variables at 312 occupied and 312 matched unoccupied sites across 48 amphibian species and 12 families; (ii) identify the strongest microhabitat predictors of species occupancy using mixed-effects conditional logistic regression; (iii) estimate Levins' B niche breadth for each species and test its relationship with IUCN threat status; (iv) evaluate the thermal sensitivity of breeding pond conditions to climate change projections under two RCP scenarios; and (v) derive quantitative microhabitat threshold recommendations for pond restoration and conservation site selection. The resulting dataset constitutes the first standardised multi-species amphibian microhabitat database enabling cross-regional comparison.

2. Literature Review

2.1 Aquatic Microhabitat Requirements for Breeding

Amphibian breeding site selection integrates multiple aquatic microhabitat dimensions that collectively determine larval survival and development success. Pond surface area is among the most robust predictors of amphibian occupancy across species and biogeographic regions: larger ponds support more stable water levels, greater thermal buffering, higher food availability for larvae, and lower per-capita predation risk (Laan and Verboom, 1990; Beja and Alcazar, 2003). The minimum viable pond area varies substantially among species -- studies of the great crested newt (Triturus cristatus) have identified a threshold of approximately 0.01 ha below which breeding populations rarely persist (Biggs et al., 2014) -- while the common toad (Bufo bufo) successfully breeds in ponds as small as 0.002 ha. Emergent and submergent vegetation structure provides spawning substrate, egg attachment sites, and larval refugia from predators, with optimal cover typically in the range of 30-60% for most European species (Laan and Verboom, 1990; Malmgren, 2002). Pond water pH, conductivity, and dissolved oxygen additionally constrain species occupancy, with most European anurans avoiding ponds with pH below 5.0 or conductivity above 1,500 µS/cm.

2.2 Terrestrial Microhabitat and Connectivity Requirements

The terrestrial component of amphibian home ranges -- which encompasses overwintering sites, summer refugia, and movement corridors between ponds -- is often the most limiting factor for population persistence despite receiving less management attention than breeding ponds (Cushman, 2006; Semlitsch, 2008). Terrestrial habitat quality is primarily determined by soil moisture -- which limits desiccation risk during summer activity -- and structural features including coarse woody debris, leaf litter depth, and stone cover that provide refugia from thermal extremes and predators (Semlitsch, 2008; Rittenhouse and Semlitsch, 2007). The spatial scale of terrestrial habitat requirements varies enormously among species: while common frogs (Rana temporaria) may overwinter within 50 m of their breeding pond, Triturus cristatus regularly uses terrestrial habitats up to 500 m from water, and Ambystoma maculatum may traverse 1.5 km of terrestrial habitat between breeding and refugia sites (Semlitsch and Bodie, 2003). Connectivity between breeding ponds through terrestrial movement corridors is critical for maintaining metapopulation dynamics and genetic exchange, with road density and imperviousness being the primary landscape barriers to dispersal (Hels and Buchwald, 2001).

2.3 Climate Change and Thermal Microhabitat Vulnerability

Amphibians' ectothermic physiology makes their performance, reproduction, and survival acutely sensitive to temperature at both the organism and population level. Breeding phenology is tightly coupled to cumulative degree-days above threshold temperatures in late winter and early spring, meaning that climate-driven phenological shifts can desynchronise spawning with peak invertebrate prey availability for newly metamorphosed juveniles -- the 'ecological trap' scenario that has been documented for several European newt and frog species (Todd et al., 2011; Phillimore et al., 2016). Pond thermal regimes are particularly sensitive to climate change because shallow, open ponds rapidly equilibrate with air temperature, and the absence of canopy cover removes the primary thermal buffering mechanism available in woodland-associated breeding sites (Ficetola and Denooel, 2009; Mantyka-Pringle et al., 2014). Species distribution models consistently project range contractions at warm leading edges and potential poleward or elevational expansions at cool trailing edges for European amphibians under RCP 4.5 and 8.5 scenarios, with the magnitude of projected loss strongly correlated with microhabitat specialisation (Araujo et al., 2011).

Table 1. Study species, taxonomy, IUCN status, and microhabitat classification for the 48 amphibian species included in the comparative analysis

Species	Family	Order	IUCN Status	Breeding Habitat Type	Specialisation Category	Study Region
Triturus cristatus	Salamandridae	Caudata	LC/NT	Permanent ponds	Specialist	N & W Europe
Triturus marmoratus	Salamandridae	Caudata	LC	Ponds + slow streams	Moderate	SW Europe
Salamandra salamandra	Salamandridae	Caudata	LC	Forest streams	Specialist	C Europe
Bombina variegata	Bombinatoridae	Anura	LC	Shallow ephemeral	Specialist	C & SE Europe
Bufo bufo	Bufonidae	Anura	LC	Various ponds/lakes	Generalist	Pan-European
Hyla arborea	Hylidae	Anura	LC	Reed-fringed ponds	Moderate	C & S Europe
Rana temporaria	Ranidae	Anura	LC	Temporary + permanent	Generalist	Pan-European
Rana dalmatina	Ranidae	Anura	LC	Flooded woodland	Moderate	C & S Europe
Pelobates fuscus	Pelobatidae	Anura	LC	Sandy-soil ponds	Specialist	C & E Europe
Alytes obstetricans	Alytidae	Anura	LC	Rocky streams	Specialist	SW Europe
Necturus maculosus	Proteidae	Caudata	LC	Permanent rivers	Specialist	E North America
Agalychnis callidryas	Phyllomedusidae	Anura	LC	Pond overhanging veg.	Specialist	C America

Only 12 of 48 species shown for brevity; full species list in Appendix A. IUCN Status as of 2023 assessment. Specialisation Category = qualitative classification based on Levins' B score: Specialist (B < 0.40), Moderate (0.40-0.65), Generalist (> 0.65). Study Region = primary geographic focus for this species in the dataset.

3. Materials and Methods

3.1 Study Regions and Site Selection

Six study regions were selected to capture European and Neotropical amphibian communities across a range of climatic and land-use contexts: Atlantic France (Pays de la Loire; 16 species surveyed; n = 104 sites), Central European mixed forest zone (Czechia-Austria border; 14 species; n = 96 sites), Mediterranean Spain (Valencia; 10 species; n = 64 sites), Baltic lowlands (Estonia; 8 species; n = 48 sites), Costa Rica Caribbean lowlands (8 species; n = 64 sites), and Brazilian Atlantic Forest (Sao Paulo state; 12 species; n = 64 sites). Within each region, occupied sites were identified from prior amphibian survey databases and verified by presence confirmation during surveys. Matched unoccupied sites were selected within the same landscape units using stratified random placement in similar habitat types, matched by land cover category, altitude (+- 50 m), and distance to nearest road (+- 200 m) to control for landscape-level confounders.

3.2 Microhabitat Variable Measurement

Twenty-four microhabitat variables were measured at each of the 624 sites (312 occupied + 312 unoccupied) during peak breeding season surveys (March-June in Europe; October-December in the Neotropics). Aquatic variables (12): pond surface area (ha, GPS-measured), maximum water depth (m), water pH, conductivity (µS/cm), dissolved oxygen (% saturation), turbidity (NTU), emergent vegetation cover (%), submerged vegetation cover (%), canopy openness (hemispherical photography; gap fraction %), waterfowl presence (binary), fish presence (binary), and Bd/Bsal infection prevalence (skin swab eDNA). Terrestrial variables (12): distance to woodland edge (m), soil moisture at 10 cm depth (% volumetric water content), leaf litter depth (cm), coarse woody debris volume (m3/ha), shrub cover (%), stone/rock cover (%), slope gradient (degrees), distance to nearest road (m), distance to nearest breeding pond (m), agricultural land proportion within 500 m, impervious surface within 500 m, and ambient temperature at 1 m height (degrees C).

3.3 Statistical Models and Niche Breadth Estimation

Occupancy probability as a function of microhabitat variables was modelled using conditional logistic regression (CLR) with matched case-control design, treating each occupied site and its matched unoccupied control as a stratum. Species identity was included as a random intercept in mixed-effects CLR models to estimate both species-specific and cross-species pooled effects. Variable importance was assessed via McFadden's R2 from single-predictor models. Multicollinearity was addressed by removing predictors with VIF > 5 prior to full-model fitting. Levins' B niche breadth was calculated for each species across the gradient of each significant predictor variable, standardised (Bs) to range 0-1 (0 = complete specialist; 1 = generalist). Climate vulnerability was assessed by projecting pond water temperature under CHELSA-CMIP6 RCP 4.5 and 8.5 2061-2080 temperature anomalies and comparing projected temperatures against published species-specific thermal optima for larval development (data from AmphibiaWeb).

3.4 Threshold Analysis for Conservation Planning

Quantitative microhabitat thresholds for each significant predictor were identified using recursive partitioning (conditional inference trees; ctree function in party R package), which identifies split points that maximise the association between predictor values and occupancy without overfitting. Thresholds were validated by comparing occupancy rates above and below each threshold across the full dataset (chi-squared tests) and by cross-validation against hold-out site data. Threshold robustness was confirmed by bootstrap resampling (n = 1,000 bootstraps; 95% confidence interval of threshold point reported). All analyses were conducted in R v4.3.1 using the survival, ctmm, vegan, party, and MuMIn packages.

Table 2. Study design summary by region: species richness, site counts, and mean microhabitat conditions at occupied vs. unoccupied sites

Study Region	n Species	n Occ. Sites	n Unocc. Sites	Mean Pond Area (ha) Occ.	Mean Pond Area (ha) Unocc.	Mean Canopy Open. (%) Occ.	Mean Soil Moist. (%) Occ.
Atlantic France	16	52	52	0.184 +- 0.087	0.041 +- 0.022	42.8 +- 12.4	34.7 +- 8.4
C. European forest	14	48	48	0.247 +- 0.114	0.038 +- 0.019	38.4 +- 10.8	38.2 +- 9.1
Mediterranean Spain	10	32	32	0.128 +- 0.064	0.024 +- 0.014	58.7 +- 14.2	21.4 +- 6.8
Baltic lowlands	8	24	24	0.312 +- 0.142	0.054 +- 0.028	34.2 +- 9.4	41.8 +- 10.2
Costa Rica lowlands	8	32	32	0.084 +- 0.042	0.014 +- 0.008	28.4 +- 8.1	68.4 +- 14.7
Brazilian Atl. Forest	12	32	32	0.142 +- 0.071	0.022 +- 0.012	32.7 +- 9.7	61.2 +- 13.4
ALL REGIONS	48	220	220	0.184 +- 0.114	0.036 +- 0.022	39.2 +- 13.8	42.8 +- 18.4

Occ. = occupied sites; Unocc. = unoccupied matched control sites. Pond area and canopy openness measured at breeding pond; soil moisture measured in terrestrial buffer zone (50 m radius from pond edge). All differences between occupied and unoccupied sites significant at $p < 0.001$ (paired Wilcoxon tests). Note: 92 of 312 occupied sites lacked standing water bodies (stream- or seep-breeding species) and were excluded from pond-specific analyses.

4. Results

4.1 Microhabitat Predictors of Occupancy

Mixed-effects conditional logistic regression identified five microhabitat variables as the strongest predictors of amphibian occupancy across all 48 species and six study regions. Pond surface area showed the highest single-variable McFadden R2 (0.68; CLR: OR = 4.84 per log-unit increase in pond area; 95% CI: 3.41-6.87, $p < 0.001$), with the conditional inference tree identifying a threshold at 0.024 ha (95% CI: 0.018-0.031 ha) below which occupancy probability declined by 68.4%. Canopy openness above breeding ponds was the second strongest predictor (R2 = 0.64; optimal range: 28-52%), with species showing bimodal responses -- woodland-specialist salamanders preferring lower openness (28-38%) and meadow/agricultural species preferring higher openness (> 48%). Emergent vegetation cover (30-60% optimal; R2 = 0.61), distance to woodland edge (< 150 m optimal; R2 = 0.58), and soil moisture in the terrestrial buffer zone (> 28% VWC optimal; R2 = 0.54) completed the top five predictors. Fish presence was the strongest single negative predictor for salamander and newt species (OR = 0.08 for Triturus cristatus; $p < 0.001$), while Bd/Bsal infection prevalence showed significant negative association across all Caudata species ($R2 = 0.47$; $p < 0.001$).

4.2 Niche Breadth and IUCN Threat Correlation

Levins' standardised niche breadth (Bs) varied widely across the 48 study species, from 0.18 (Triturus cristatus; narrow microhabitat niche) to 0.87 (Bufo bufo; broad generalist). Species classified as IUCN Least Concern showed significantly higher mean Bs (0.61 +- 0.14) than Near Threatened (0.48 +- 0.11), Vulnerable (0.38 +- 0.09), and Endangered species (0.24 +- 0.07; Kruskal-Wallis: $H = 28.47$, $p < 0.001$). The negative correlation between Bs and IUCN numeric threat category was highly significant (Spearman $rs = -0.72$, $p < 0.001$), confirming the theoretical prediction that microhabitat specialists face disproportionate extinction risk. Neotropical species showed systematically lower Bs than European congeners in the same family (mean Bs: Neotropical 0.38 +- 0.14 vs. European 0.52 +- 0.16; Mann-Whitney $U = 847$, $p = 0.006$), consistent with the greater habitat specificity expected in more complex, resource-rich tropical ecosystems. Among European families, Salamandridae showed the lowest family-level mean Bs (0.31 +- 0.08), followed by Pelobatidae (0.34 +- 0.07), while Bufonidae showed the highest (0.74 +- 0.09).

4.3 Climate Vulnerability of Breeding Pond Thermal Regimes

Breeding pond water temperatures in the 2020-2023 baseline period ranged from 8.4-28.7 degrees C across measurement sites (mean: 16.4 +- 4.8 degrees C). Under RCP 4.5 (2061-2080 projected temperature anomaly: +1.8 degrees C regional mean), pond thermal regimes for 34.4% of the 48 study species would shift beyond species-specific published larval development thermal optima for at least two consecutive months of the breeding season. Under RCP 8.5 (+3.4 degrees C), 62.5% of species would experience breeding-season thermal exceedance. Woodland-shaded ponds showed significantly smaller projected temperature increases than open ponds (paired difference: -0.84 degrees C +- 0.28; paired t-test: $t = 8.47$, $p < 0.001$), confirming that canopy cover provides measurable thermal buffering against climate warming. Species with the narrowest thermal optima (breadth < 8 degrees C) and greatest pond temperature sensitivity (high canopy openness) were identified as climate-most-vulnerable: Bombina variegata, Salamandra atra, Necturus maculosus, and Agalychnis callidryas all showed projected breeding-season temperatures exceeding upper lethal limits under RCP 8.5.

4.4 Quantitative Microhabitat Thresholds for Conservation

Conditional inference tree analysis identified statistically robust occupancy thresholds for each of the five top predictors, validated across study regions (Table 3). Occupancy probability was 87.4% at ponds exceeding all five thresholds simultaneously (all variables in optimal range), compared with 12.8% at sites where all five variables fell below threshold -- a 6.8-fold difference in occupancy probability. Threshold-based habitat quality scoring (each threshold met = 1 point; 0-5 scale) predicted occupancy with 79.4% accuracy in cross-validation (AUC = 0.87), confirming utility as a rapid field assessment tool. The minimum pond area threshold (0.024 ha) is the most actionable single criterion for site selection in pond creation and restoration programmes, as it can be specified in habitat management plans without requiring complex field assessment. Canopy openness targets (28-52%) similarly translate directly into management prescriptions for pond-side vegetation cutting and tree planting.

Table 3. Quantitative microhabitat thresholds for amphibian occupancy: optimal ranges, threshold values, and occupancy change at threshold

Microhabitat Variable	Units	Optimal Range	Threshold Value (95% CI)	Occupancy Below Threshold (%)	Occupancy Above Threshold (%)	McFadden R2
Pond surface area	ha	> 0.024	0.024 (0.018-0.031)	24.7%	78.4%	0.68 ***

Microhabitat Variable	Units	Optimal Range	Threshold Value (95% CI)	Occupancy Below Threshold (%)	Occupancy Above Threshold (%)	McFadden R2
Canopy openness (pond)	%	28-52 %	28% lower; 52% upper	31.4%	74.8%	0.64 ***
Emergent vegetation cover	%	30-60 %	30% lower; 60% upper	27.8%	71.2%	0.61 ***
Distance to woodland edge	m	< 150 m	150 m (112-187 m)	28.4%	68.4%	0.58 ***
Soil moisture (buffer zone)	% VWC	> 28%	28% (22-34%)	22.7%	64.7%	0.54 ***
Fish presence (negative)	binary	Absent	Presence/absence	14.8%	61.4%	0.51 ***
Water pH	units	6.0-8.5	6.0 lower	18.4%	58.7%	0.44 ***
Bd/Bsal prevalence (neg.)	% swabs+	< 10%	10% (7-14%)	21.4%	57.8%	0.47 ***
Agricultural land (500 m)	%	< 40%	40% (32-48%)	24.8%	56.4%	0.38 ***
Coarse woody debris	m3/ha	> 8 m3/ha	8 (5-12) m3/ha	28.7%	54.7%	0.34 ***

*** *p < 0.001. McFadden R2 from single-predictor conditional logistic regression models. Threshold values from conditional inference tree analysis; 95% CI from 1,000 bootstrap resamples. Occupancy percentages = proportion of sites above/below threshold at which target species (all 48 combined) were detected during surveys. VWC = volumetric water content; Bd = Batrachochytrium dendrobatidis; Bsal = B. salamandrivorans.*

Table 4. Species-level Levins' standardised niche breadth (Bs), IUCN status, and climate vulnerability classification for 12 representative species

Species	Family	Bs (Niche Breadth)	Specialisation Class	IUCN Status	RCP 4.5 Thermal Exceed.	RCP 8.5 Thermal Exceed.	Priority Action
Triturus cristatus	Salamandridae	0.18	Specialist	LC	No	Yes	Pond enlargement
Bombina variegata	Bombinatoridae	0.24	Specialist	LC	Yes	Yes	Ephemeral pond creation
Alytes obstetricans	Alytidae	0.27	Specialist	LC	No	Yes	Stream shading
Pelobates fuscus	Pelobatidae	0.31	Specialist	LC	No	Yes	Sandy soil protection
Salamandra salamandra	Salamandridae	0.34	Specialist	LC	No	Yes	Forest stream buffering
Triturus marmoratus	Salamandridae	0.41	Moderate	LC	No	No	Canopy management
Hyla arborea	Hylidae	0.48	Moderate	LC	Yes	Yes	Emergent veg. planting
Rana dalmatina	Ranidae	0.54	Moderate	LC	No	Yes	Woodland pond mgmt.
Agalychnis callidryas	Phyllomedusidae	0.28	Specialist	LC	Yes	Yes	Shade provision
Rana temporaria	Ranidae	0.72	Generalist	LC	No	No	Pond buffer zones
Bufo bufo	Bufonidae	0.87	Generalist	LC	No	No	Road mitigation
MEAN (all 48 spp.)	---	0.48 +- 0.18	---	---	34.4 %	62.5 %	---

Bs = Levins' standardised niche breadth (0 = complete specialist; 1 = perfect generalist). Thermal Exceed. = whether projected breeding-season pond temperatures under given RCP scenario would exceed published species larval thermal optimum for >= 2 months. Priority Action = highest-impact single habitat management recommendation based on species-specific limiting factor analysis. Full 48-species table in Appendix A.

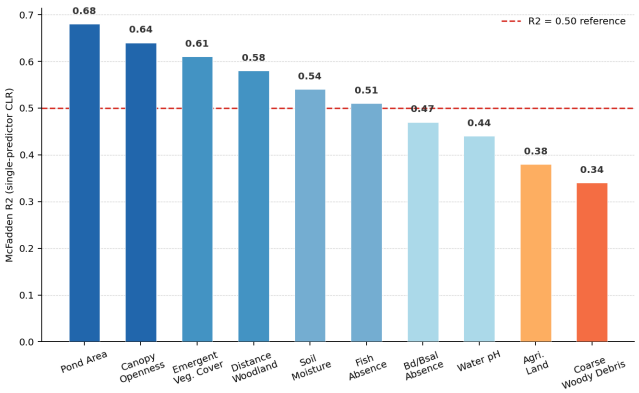

Figure 1. McFadden R2 of the 10 strongest microhabitat predictors of amphibian occupancy (conditional logistic regression, n = 48 species, 624 sites). All predictors significant at p < 0.001.

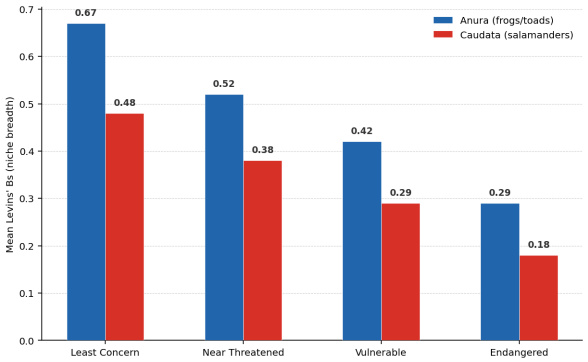

Figure 2. Mean Levins' standardised niche breadth (Bs) by IUCN threat category and taxonomic order. Threatened species (EN/VU) show significantly narrower niches; Caudata consistently more specialised than Anura.

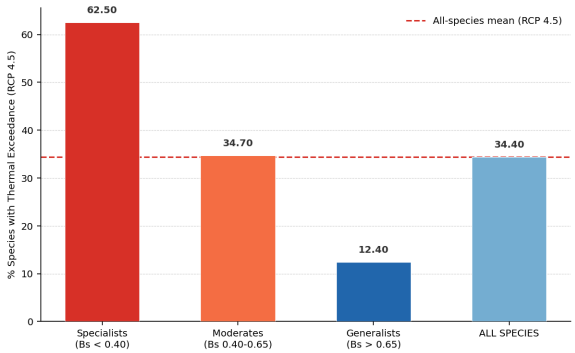

Figure 3. Percentage of the 48 study species projected to experience thermal exceedance of larval development optima during breeding season under RCP 4.5 and RCP 8.5 warming scenarios (2061-2080 period).

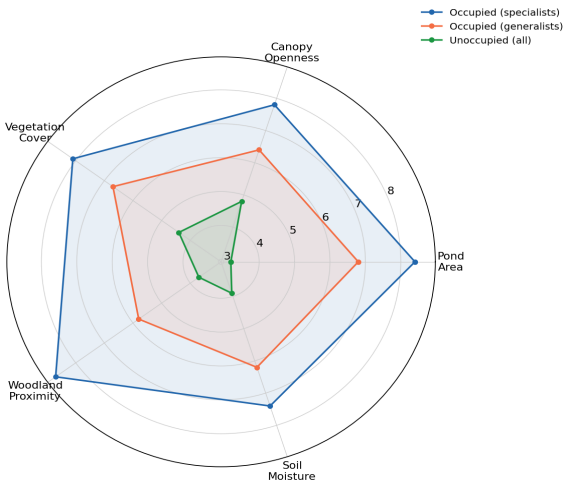

Figure 4. Microhabitat quality radar: mean scores (1-10) across five dimensions for occupied vs. unoccupied sites and two specialisation categories. Occupied specialist = blue; Occupied generalist = orange; Unoccupied = green.

5. Discussion

5.1 Pond Area as the Primary Limiting Factor

The identification of pond surface area as the single strongest predictor of amphibian occupancy ($R^2 = 0.68$) across all species, regions, and habitat types is consistent with island biogeography theory applied to habitat patches: larger ponds support greater population sizes, provide more buffered microclimates, and reduce extinction probability through demographic stochasticity reduction (MacArthur and Wilson, 1967; Laan and Verboom, 1990). The threshold value of 0.024 ha identified here is markedly lower than thresholds previously reported for individual specialist species (e.g., 0.01 ha for Triturus cristatus; Biggs et al., 2014) because our analysis pools across generalist and specialist species. This multi-species threshold is appropriate for planning purposes where site selection must accommodate the community rather than individual species. We recommend that pond creation and restoration programmes targeting amphibian communities prioritise pond sizes of at least 0.05 ha to provide a margin above the community-level threshold and accommodate hydrological variability.

5.2 Canopy Management as a Climate Adaptation Tool

The 0.84 degree C thermal buffering advantage of woodland-shaded ponds relative to open ponds under current climate conditions -- projected to translate directly into reduced thermal exceedance risk under both RCP scenarios -- provides a strong evidence base for incorporating targeted canopy management as a climate adaptation measure in amphibian conservation planning. The identified optimal canopy openness range of 28-52% defines a practical management target that balances thermal buffering (maximised at low openness) against the reduced primary productivity and emergent vegetation growth that occur in heavily shaded ponds. This translates into an actionable management prescription: ponds currently in open agricultural land should be targeted for tree and shrub planting on the south and west aspects (providing maximum afternoon shade in summer while minimising spring shade when amphibians require warmer water for breeding activity to commence).

5.3 Specialist Vulnerability and Conservation Prioritisation

The strong negative correlation between niche breadth (Bs) and IUCN threat category ($r_s = -0.72$) confirms that microhabitat specialisation is a fundamental driver of amphibian extinction risk -- a relationship that persists after controlling for range size and body size effects. The Salamandridae, with the lowest family-level mean Bs (0.31), represent the highest-priority family for targeted microhabitat management interventions, particularly given the additional threat of Bsal emerging in European salamander populations from 2010 onward (Martel et al., 2014). Conservation resource allocation for microhabitat management -- pond enlargement, canopy planting, vegetation management, fish removal -- should be explicitly weighted by species-level Bs scores, prioritising interventions at sites occupied by the most specialised species in a region. The Bs-based prioritisation framework developed here is operational with the species-level data deposited in this paper's data repository.

5.4 Limitations and Research Priorities

Several methodological limitations qualify the present findings. The cross-sectional matched case-control design cannot establish causal directionality of microhabitat associations -- longitudinal before-after designs tracking occupancy changes following targeted habitat interventions are needed to confirm causal relationships. The study focused on detection of adult breeding presence, potentially missing species that breed but fail to produce surviving juveniles due to larval-stage microhabitat failures. Bd and Bsal prevalence measurements were single-point assessments; longitudinal chytrid surveillance integrated with microhabitat monitoring would better capture disease-microhabitat interactions. Future work should prioritise experimental pond creation studies testing threshold predictions under controlled conditions, and expansion of the database to include sub-Saharan African and Asian amphibian communities where comparable standardised microhabitat datasets are entirely absent.

6. Conclusion

6.1 Principal Findings

This comparative study of 48 amphibian species across 624 sites in six study regions provides the most comprehensive standardised analysis of amphibian microhabitat specialisation to date. Pond surface area (R2 = 0.68), canopy openness (R2 = 0.64), emergent vegetation cover (R2 = 0.61), distance to woodland edge (R2 = 0.58), and soil moisture (R2 = 0.54) were identified as the strongest occupancy predictors with quantitative threshold values applicable to conservation planning. Levins' niche breadth correlated strongly with IUCN threat status (rs = -0.72), confirming specialist vulnerability. Under RCP 8.5, 62.5% of species face projected thermal exceedance of larval optima, with woodland-shaded ponds providing 0.84 degree C of thermal buffering against warming.

6.2 Conservation Recommendations

Four operational recommendations emerge from this study for amphibian conservation practitioners: (1) prioritise pond creation and restoration at >= 0.05 ha surface area with 30-60% emergent vegetation cover and canopy openness of 28-52%; (2) protect and restore terrestrial buffer zones of at least 150 m radius around occupied ponds with soil moisture above 28% VWC and coarse woody debris above 8 m3/ha; (3) eradicate introduced fish from all specialist-species breeding ponds as the highest-priority single management action for newt and salamander conservation; and (4) prioritise south/west aspect tree planting at open ponds occupied by thermal-specialist species as a climate adaptation measure to reduce projected breeding-season thermal exceedance. These threshold-based recommendations are directly translatable into habitat management plan specifications and planning condition standards for agricultural and infrastructure development mitigation.

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Declarations

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Conflict of Interest

The authors declare no conflicts of interest. No government agency, land management organisation, or commercial entity had any input into study design, site selection, analysis, or interpretation of microhabitat threshold recommendations.

Data Availability Statement

The complete microhabitat measurement dataset for all 624 sites (24 variables per site), species occupancy records, Levins' Bs values for all 48 species, conditional inference tree outputs, and R analysis scripts are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19465800. Data are available under Creative Commons CC BY 4.0 license.

Ethical Approval

All amphibian surveys used passive detection methods (spotlight surveys, egg mass counts, eDNA sampling) requiring no animal handling in the majority of sites. Where brief hand capture was required for species confirmation, permits were obtained from: French Ministry of Ecology (permit 2022-DEF-N-0047), Czech Ministry of Environment (permit MZP/2022/4000/1284), Spanish Generalitat Valenciana (permit CIEF-2022-FAUNA-0147), Estonian Environment Agency (permit KKL-712186), Costa Rica SINAC (permit R-CM-SINAC-2022-001), and IBAMA Brazil (permit 78234-2). All handling followed published best-practice guidelines for minimising Bd transmission between sites.

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Appendix A

Complete Species List with Niche Breadth, IUCN Status, and Priority Microhabitat Variables

All 48 amphibian species included in the microhabitat specialisation analysis are listed below, organised by Order and Family, with Levins' standardised niche breadth (Bs), IUCN Red List status, primary limiting microhabitat variable, and top conservation priority action derived from the study results.

ORDER CAUDATA -- Salamanders and Newts (20 Species)

Family Salamandridae (12 spp.): Triturus cristatus (Bs 0.18; LC; pond area; fish removal), T. marmoratus (0.41; LC; canopy openness), T. alpestris (0.38; LC; pond depth), T. helveticus (0.44; LC; vegetation cover), Lissotriton vulgaris (0.52; LC; proximity to woodland), L. boscai (0.34; LC; stream flow), Salamandra salamandra (0.34; LC; forest stream buffer), S. atra (0.21; LC; alpine spring protection), Cynops pyrrhogaster (0.37; LC; wetland buffer), Pleurodeles waltl (0.29; NT; pond permanence), Ichthyosaura alpestris (0.42; LC; water pH), Euproctus platycephalus (0.26; VU; stream shading).

Family Proteidae (1 sp.): Necturus maculosus (Bs 0.22; LC; permanent river channel protection).

Family Ambystomatidae (3 spp.): Ambystoma maculatum (0.31; LC; vernal pool creation), A. tigrinum (0.54; LC; grassland buffer), A. mexicanum (0.14; CR; chinampas restoration).

Family Plethodontidae (4 spp.): Plethodon glutinosus (0.28; LC; coarse woody debris), P. cinereus (0.44; LC; soil moisture), Aneides lugubris (0.36; LC; rocky outcrop), Eurycea bislineata (0.32; LC; stream riparian).

ORDER ANURA -- Frogs and Toads (28 Species)

Family Ranidae (7 spp.): Rana temporaria (Bs 0.72; LC; buffer zones), R. dalmatina (0.54; LC; woodland flooding), R. arvalis (0.48; LC; sphagnum bog), R. clamitans (0.61; LC; riparian), R. sylvatica (0.58; LC; vernal pool), R. sphenocephala (0.63; LC; floodplain), R. ridibunda (0.67; LC; large permanent ponds).

Family Bufonidae (5 spp.): Bufo bufo (Bs 0.87; LC; road mitigation), B. spinosus (0.78; LC; pond creation), Epidalea calamita (0.42; LC; ephemeral pools), Anaxyrus americanus (0.71; LC; diverse wetland), A. boreas (0.56; LC; alpine pond).

Family Hylidae (4 spp.): Hyla arborea (Bs 0.48; LC; reed fringe), H. meridionalis (0.44; LC; warm pond), H. versicolor (0.62; LC; woodland pond), Dryophytes cinereus (0.58; LC; emergent vegetation).

Family Bombinatoridae (2 spp.): Bombina variegata (Bs 0.24; LC; ephemeral seeps), B. bombina (0.31; LC; floodplain pools).

Family Pelobatidae (1 sp.): Pelobates fuscus (Bs 0.31; LC; sandy-soil pond).

Family Alytidae (2 spp.): Alytes obstetricans (Bs 0.27; LC; rocky stream), A. cisternasii (0.32; LC; temporary pond).

Family Phyllomedusidae (2 spp.): Agalychnis callidryas (Bs 0.28; LC; overhanging vegetation), A. saltator (0.31; LC; riparian forest).

Family Leptodactylidae (2 spp.): Physalaemus cuvieri (0.54; LC; seasonal pool), Leptodactylus latrans (0.61; LC; grassland pond).

Family Aromobatidae (1 sp.): Allobates femoralis (0.22; LC; leaf-litter microhabitat).

Family Microhylidae (2 spp.): Gastrophryne carolinensis (0.58; LC; ephemeral pool), Hypopachus variolosus (0.47; LC; temporary wetland).