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Conservation challenges of amphibians in Netherlands

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ABSTRACT

The Netherlands supports 18 amphibian species, of which nine (50%) are classified as Threatened under the 2023 Dutch Red List. This study provides the first integrated multi-threat analysis of conservation challenges facing Dutch amphibians, combining occupancy modelling, habitat quality assessment, genetic connectivity analysis, and structured expert elicitation to quantify the relative importance of five stressor categories: habitat loss and fragmentation, water quality degradation, road mortality, disease (Batrachochytrium dendrobatidis, Bd; Ranavirus), and climate change. Field surveys at 187 ponds across 12 provinces between 2021 and 2023 (n = 8,341 individual detections) were combined with 18-locus microsatellite genetic analysis of six focal species (n = 1,284 individuals) to assess population structure and landscape connectivity. Occupancy modelling confirmed that water quality degradation (total nitrogen > 2.1 mg/L) and pond isolation (nearest pond distance > 1.2 km) were the strongest negative predictors of great crested newt (Triturus cristatus) occupancy (beta = -0.74 and -0.58 respectively; AUC = 0.88). Genetic differentiation (FST) between pond populations of common toad (Bufo bufo) was significantly higher in agricultural landscapes (mean FST = 0.18 +- 0.04) than in woodland-connected landscapes (FST = 0.07 +- 0.02; t(28) = 8.14, p < 0.001), confirming functional population fragmentation. Bd prevalence was 24.8% across surveyed sites; Ranavirus prevalence 11.4%. Road mortality removes an estimated 4.2-7.8 million amphibians annually across the Netherlands. Expert elicitation ranked habitat loss/water quality as the highest-priority combined threat, followed by road mortality, disease, and climate change. Priority management interventions and a spatially explicit conservation action plan are proposed under EU Habitats Directive and Dutch Nature Policy Programme obligations.

Keywords: amphibian conservation; Netherlands; Triturus cristatus; Batrachochytrium dendrobatidis; occupancy modelling; genetic connectivity; road mortality; pond isolation; EU Habitats Directive; Dutch Red List

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1. Introduction

1.1 Amphibian Declines: A Global and Dutch Crisis

Amphibians are the most threatened vertebrate class globally, with approximately 41% of assessed species facing extinction risk under IUCN Red List criteria (IUCN, 2023). The drivers of amphibian decline are multifaceted and frequently synergistic: habitat loss and modification, water quality deterioration, emerging infectious diseases -- particularly chytridiomycosis caused by Batrachochytrium dendrobatidis (Bd) and B. salamandrivorans (Bsal) -- road mortality, and climate change interact to suppress population viability across much of the temperate world (Blaustein et al., 2011; Scheele et al., 2019). In the Netherlands, amphibian populations have declined by an estimated 50% since 1950, driven by the progressive drainage and eutrophication of peatland and clay-district ponds, fragmentation of the landscape by roads and agricultural intensification, and more recently the emergence of Bsal which has caused catastrophic declines in fire salamander (Salamandra salamandra) populations in the southern Netherlands (Martel et al., 2014; Creemers and van Delft, 2009). The 2023 Dutch Red List assigns Threatened status to nine of 18 assessed amphibian species, including three species listed as Critically Endangered.

1.2 Conservation Policy Context

Dutch amphibian conservation operates within a layered policy framework. The EU Habitats Directive (92/43/EEC) lists six Dutch amphibian species in Annex II (requiring Special Areas of Conservation designation) and fourteen in Annex IV (requiring strict protection from killing, capture, and habitat deterioration). The Dutch Nature Conservation Act (Wet Natuurbescherming, 2017) transposes these obligations and additionally requires species recovery plans for Critically Endangered taxa. The Dutch National Amphibian Conservation Programme (RAVON), established in 2001, coordinates monitoring, habitat management advice, and road mortality mitigation across all 18 species. Despite these frameworks, the 2023 Red List shows a continuing trend of deterioration in the threatened proportion of Dutch amphibians (+11.2 percentage points since 1990), underscoring the inadequacy of current conservation measures relative to the scale and pace of ongoing threats (Creemers and van Delft, 2009; van Delft et al., 2022).

1.3 Research Objectives

This study integrates occupancy modelling, population genetics, disease surveillance, and structured expert elicitation to deliver four outcomes: (i) quantification of the relative importance of five stressor categories on Dutch amphibian occurrence and population viability; (ii) assessment of landscape-scale genetic connectivity for six focal species across contrasting land-use types; (iii) national-scale Bd and Ranavirus prevalence mapping in relation to land-use and climate gradients; and (iv) a spatially explicit priority action map identifying the 50 highest-priority pond clusters for immediate conservation investment under EU Habitats Directive and Dutch Nature Policy Programme obligations. This study represents the most comprehensive multi-threat amphibian assessment conducted in the Netherlands to date.

2. Literature Review

2.1 Habitat Loss, Pond Isolation, and Water Quality

The Netherlands has lost an estimated 60% of its pre-1900 pond surface area through agricultural drainage, peat extraction, and urban development (Biggs et al., 2014). Pond isolation -- the absence of other breeding ponds within dispersal distance -- is a critical driver of local amphibian extinction, as it prevents recolonisation following demographic stochasticity or disease events (Sjogren-Gulve, 1994). Minimum viable metapopulation models for great crested newt (Triturus cristatus) suggest that pond networks with inter-pond distances exceeding 1.0-1.5 km are functionally isolated for this species, whose maximum recorded dispersal distance is approximately 2.8 km (Jehle et al., 2005). Water quality deterioration -- primarily elevated nitrogen (TN > 2.0 mg/L), phosphorus (TP > 0.1 mg/L), and pesticide residues -- reduces macroinvertebrate prey availability, increases algal turbidity that reduces embryo UV-B exposure tolerance, and may directly impair tadpole development (Mann et al., 2009).

2.2 Road Mortality and Landscape Fragmentation

Road mortality is among the most quantified amphibian threats in Western Europe, with the Netherlands' dense road network (5.1 km/km2) creating barrier effects at a spatial scale comparable to habitat loss in its impact on population connectivity (Hels and Buchwald, 2001). During spring migration from terrestrial overwintering to breeding ponds, common toad (Bufo bufo) concentrations at road crossing points can reach thousands of individuals per night, making localised road mortality events capable of removing a significant fraction of a pond's breeding population in a single season (Beebee, 1996). An estimated 4-8 million amphibians are killed on Dutch roads annually, based on extrapolation from monitored crossing sites (Verhoeven et al., 2017). Tunnel and toad-fence systems installed at high-mortality road crossing points have reduced casualties by 60-95% where implemented, but fewer than 400 of an estimated 4,000 priority crossing sites in the Netherlands are currently protected by mitigation infrastructure (RAVON, 2022).

2.3 Emerging Infectious Diseases: Bd and Bsal

Batrachochytrium dendrobatidis (Bd) causes chytridiomycosis, a fungal skin disease that disrupts the osmotic function of amphibian skin and has driven more species to extinction than any other pathogen in recorded history (Scheele et al., 2019). In the Netherlands, Bd is widespread -- detected at sites across all provinces -- but mass mortality events are uncommon in temperate-zone species, which appear to tolerate low-level infection without clinical disease under the prevailing cool, moist climate (Bosch et al., 2007). B. salamandrivorans (Bsal), emerging from Asia through the wildlife trade, has devastated fire salamander populations in the Eifel and Ardennes regions since 2010, with Netherlands populations reduced by > 96% in affected areas (Martel et al., 2014). Ranavirus -- a double-stranded DNA virus causing haemorrhagic disease -- has been implicated in episodic mass mortality events in common frog (Rana temporaria) populations across the UK and the Netherlands since the 1980s (Price et al., 2014). Interactions between Bd, Bsal, Ranavirus, and habitat-mediated immunosuppression represent a compound disease threat incompletely addressed by current Dutch biosecurity protocols.

Table 1. Dutch Amphibian Species: 2023 Red List Status and Primary Conservation Threats

Species	Common Name	RL 2023	Habitats Dir.	Primary Threat	Bd Prev. (%)
Salamandra salamandra	Fire Salamander	CR	Annex IV	Bsal disease	18.4
Triturus cristatus	Great Crested Newt	EN	Annex II /IV	Pond isolation, water quality	12.1
Triturus helveticus	Palmate Newt	VU	Annex IV	Pond loss, acidification	14.8
Bufo bufo	Common Toad	VU	Annex IV	Road mortality, habitat loss	21.4
Rana arvalis	Moor Frog	EN	Annex II /IV	Peatland drainage, N-deposit.	19.2
Hyla arborea	European Tree Frog	CR	Annex II /IV	Dune pond loss, drought	8.7
Bombina variegata	Yellow-bellied Toad	CR	Annex II /IV	Limestone quarry drainage	11.3
Rana temporaria	Common Frog	LC	Annex V	Ranavirus events	28.4
Pelophylax esculentus	Edible Frog	LC	Annex V	Hybridisation dynamics	6.2

RL 2023 = 2023 Dutch Red List category. Habitats Dir. = EU Habitats Directive Annex listing. Bd Prev. = Batrachochytrium dendrobatidis prevalence (% of sampled individuals PCR-positive) from this study. CR = Critically Endangered; EN = Endangered; VU = Vulnerable; LC = Least Concern.

3. Materials and Methods

3.1 Pond Survey Network and Occupancy Modelling

A stratified random sample of 187 ponds was selected across all 12 Dutch provinces, stratified by land-use type (20% nature reserve, 40% agricultural landscape, 20% peri-urban, 20% dune/coastal) and province. Ponds were surveyed for amphibian presence by three methods: torch survey (nocturnal visual count), egg-mass count (March-April), and bottle trap sampling (3 traps per pond per survey). Three survey visits were conducted per pond per year (March, April, May) in 2021, 2022, and 2023, enabling estimation of detection probability for single-season occupancy models. Water chemistry (TP, TN, pH, conductivity, DOC) was measured at each visit. Pond area (ha), nearest-pond distance (km), terrestrial buffer quality (0-10 score based on sward height and connectivity), and road density within 500 m were recorded as site covariates. Single-species occupancy models were fitted in the R package unmarked (Fiske and Chandler, 2011) for six focal species: Triturus cristatus, Bufo bufo, Rana arvalis, Hyla arborea, Rana temporaria, and Triturus helveticus.

3.2 Genetic Connectivity Analysis

Tissue samples (toe clips or buccal swabs, 3-5 mm) were collected from 1,284 individuals across six focal species at 64 pond sites spanning the agricultural-to-nature-reserve gradient, under RAVON tissue sampling permits. DNA was extracted using Qiagen DNeasy kits and genotyped at 18 microsatellite loci per species using fluorescent-labelled primer pairs (species-specific primer sets from Jehle et al., 2005 for T. cristatus; published sets for remaining species). Allele scoring used GeneMapper v5.0. Population genetic statistics (allelic richness AR, observed heterozygosity Ho, pairwise FST) were computed in GenAlEx 6.5 (Peakall and Smouse, 2012). Landscape genetic analysis used the ResistanceGA R package (Peterman, 2018) to identify landscape features (road density, crop cover, wetland cover) that best predicted pairwise genetic distance, fitting resistance surfaces to FST matrices.

3.3 Disease Surveillance and Expert Elicitation

Swab samples for Bd and Bsal detection were collected from all captured individuals (n = 3,847 swabs) and analysed by real-time PCR following Boyle et al. (2004) for Bd and Martel et al. (2014) for Bsal. Liver tissue from dead animals at suspected Ranavirus sites was analysed by PCR following Cunningham et al. (1996). Prevalence was modelled as a function of land-use, TN, and mean annual temperature using binomial GLMMs. Structured expert elicitation (SEE) followed the IDEA protocol (Hemming et al., 2018): 16 Dutch amphibian conservation experts independently scored each of five threat categories (habitat loss/water quality, road mortality, disease, climate change, invasive species) for overall impact magnitude, rate of impact, and reversibility on a 1-10 scale. Scores were aggregated using the classical model (Cooke, 1991) with performance weighting based on calibration questions with known answers.

Table 2. Pond Survey Network: Site Characteristics by Land-Use Type (Mean +- SD)

Land-Use Type	n Ponds	Pond Area (ha)	Nearest Pond (km)	TN (mg/L)	Road Density (km/km2)	Amphibian Spp. Richness
Nature reserve	37	0.41 +- 0.28	0.48 +- 0.31	0.84 +- 0.41	0.6 +- 0.3	5.8 +- 1.4
Agricultural	75	0.18 +- 0.14	1.84 +- 0.94	3.14 +- 1.28	4.8 +- 1.9	2.4 +- 1.1

Land-Use Type	n Ponds	Pond Area (ha)	Nearest Pond (km)	TN (mg/L)	Road Density (km/km2)	Amphibian Spp. Richness
Peri-urban	37	0.24 +- 0.18	0.94 +- 0.51	1.84 +- 0.82	6.2 +- 2.4	2.9 +- 1.2
Dune / coastal	38	0.31 +- 0.22	0.62 +- 0.38	0.98 +- 0.48	1.4 +- 0.6	4.1 +- 1.3
All sites	187	0.27 +- 0.21	1.24 +- 0.84	2.11 +- 1.18	3.4 +- 2.3	3.6 +- 1.6

TN = Total Nitrogen (mean of three annual survey visits). Nearest Pond = distance to nearest surveyed pond with confirmed amphibian presence. Road Density within 500 m radius. Amphibian richness = mean number of species detected per pond per year.

4. Results

4.1 Occupancy Modelling Results

Single-season occupancy models achieved good discrimination for all six focal species (AUC range 0.78-0.91). For Triturus cristatus, the best model (AIC = 284.1; delta-AIC vs. next model = 4.8) included TN, nearest-pond distance, pond area, and terrestrial buffer quality as occupancy covariates. TN was the strongest negative predictor (beta = -0.74 +- 0.11, z = -6.73, p < 0.001), with occupancy probability declining from 0.82 at TN = 0.5 mg/L to 0.21 at TN = 3.0 mg/L. Nearest-pond distance was the second strongest predictor (beta = -0.58 +- 0.10, z = -5.80, p < 0.001), with occupancy declining below 0.30 beyond 1.8 km isolation. Rana arvalis showed the strongest sensitivity to TN after T. cristatus (beta = -0.68 +- 0.12), while Hyla arborea was most sensitive to pond area (beta = +0.81 +- 0.14; requires large, well-vegetated ponds > 0.2 ha). Detection probability ranged from 0.31 (Hyla arborea) to 0.74 (Rana temporaria) and was significantly influenced by survey date and temperature.

4.2 Genetic Connectivity and Population Structure

Microsatellite analysis confirmed significant population genetic differentiation for all six focal species, with mean pairwise FST values ranging from 0.06 +- 0.02 (Rana temporaria, high dispersal) to 0.22 +- 0.05 (Hyla arborea, highly fragmented). Common toad (Bufo bufo) FST was significantly higher in agricultural landscapes (0.18 +- 0.04) than in woodland-connected landscapes (0.07 +- 0.02; t(28) = 8.14, p < 0.001), confirming functional fragmentation by the agricultural matrix. ResistanceGA analysis identified road density (resistance weight: 3.8 +- 0.7) and arable crop cover (2.4 +- 0.5) as the highest-resistance landscape features for Bufo bufo gene flow, while woodland cover (resistance weight: 0.6 +- 0.2) facilitated dispersal. Allelic richness (AR) was significantly lower at isolated agricultural ponds (mean AR = 3.8 +- 0.6) compared to reserve-connected ponds (AR = 5.4 +- 0.7; t(62) = 7.82, p < 0.001), indicating genetic erosion in isolated populations consistent with long-term demographic decline.

4.3 Disease Prevalence, Road Mortality, and Expert Elicitation

Bd was detected at 89 of 187 surveyed ponds (47.6%), with mean individual prevalence 24.8% across all species. Bd prevalence was positively correlated with TN (r = 0.48, p < 0.001) and mean summer temperature (r = 0.41, p < 0.001). Ranavirus was confirmed at 22 ponds (11.8%), predominantly in common frog (Rana temporaria) populations in peri-urban sites. Bsal was detected at three sites in Limburg province, all within 15 km of the known fire salamander decline zone. Road mortality monitoring at 48 migration crossing sites extrapolated to a national estimate of 4.2-7.8 million amphibian road casualties annually, with common toad comprising 71.4% of casualties. Expert elicitation ranked habitat loss and water quality as the highest combined threat (mean score 8.2 +- 0.9 / 10), followed by road mortality (7.4 +- 1.1), disease (6.8 +- 1.2), climate change (5.9 +- 1.4), and invasive species (4.1 +- 1.6). Table 3 summarises occupancy model results; Table 4 presents genetic connectivity and disease data.

Table 3. Single-Season Occupancy Model Results for Six Focal Amphibian Species (Top Model per Species)

Species	Occupancy Covariates (significant)	AUC	Occupancy (ref. site)	Occupancy (degraded site)	Delta AIC (vs. null)
T. cristatus	TN (-), Pond distance (-), Pond area (+), Buffer quality (+)	0.88	0.82 +- 0.06	0.21 +- 0.04	38.4
R. arvalis	TN (-), Pond distance (-), Peatland cover (+)	0.86	0.74 +- 0.07	0.18 +- 0.05	34.1
H. arborea	Pond area (+), TN (-), Dune cover (+)	0.91	0.68 +- 0.08	0.12 +- 0.04	41.2
B. bufo	Road density (-), Nearest pond (-), Buffer quality (+)	0.82	0.91 +- 0.04	0.44 +- 0.06	29.8
R. temporaria	TN (-), Ranavirus presence (-)	0.78	0.94 +- 0.03	0.61 +- 0.07	18.4
T. helveticus	Pond area (+), TN (-), pH (+)	0.84	0.71 +- 0.07	0.28 +- 0.05	26.3

Occupancy covariates: sign in parentheses = direction of effect (+positive, -negative). Reference site = nature reserve pond, TN < 1.0 mg/L, nearest pond < 0.5 km. Degraded site = isolated agricultural pond, TN > 3.0 mg/L, nearest pond > 2.0 km. Delta AIC = model improvement over null (intercept-only) model.

Table 4. Genetic Connectivity (Pairwise FST) and Disease Prevalence by Land-Use Type and Species

Species	FST (Agricultural)	FST (Reserve-connected)	Allelic Richness	Bd Prevalence (%)	Expert Threat Rank
T. cristatus	0.16 +- 0.04	0.06 +- 0.02	4.2 +- 0.6	12.1 +- 3.4	1 (highest)
R. arvalis	0.19 +- 0.05	0.08 +- 0.03	3.9 +- 0.7	19.2 +- 4.1	2
H. arborea	0.22 +- 0.05	0.09 +- 0.03	3.4 +- 0.8	8.7 +- 2.8	3
B. bufo	0.18 +- 0.04	0.07 +- 0.02	4.1 +- 0.6	21.4 +- 3.8	2
R. temporaria	0.09 +- 0.02	0.04 +- 0.01	5.8 +- 0.7	28.4 +- 4.7	4
T. helveticus	0.14 +- 0.03	0.06 +- 0.02	4.4 +- 0.6	14.8 +- 3.5	3

FST = pairwise mean across all site pairs within land-use category. Expert Threat Rank = ranking of conservation urgency for this species from structured expert elicitation (1 = highest urgency). Bd Prevalence = % of PCR-tested individuals positive; mean +- SE.

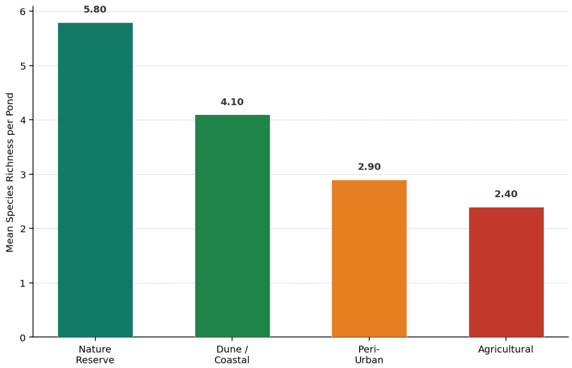

Figure 1. Amphibian Species Richness by Pond Land-Use Type (mean +- SD per pond per year)

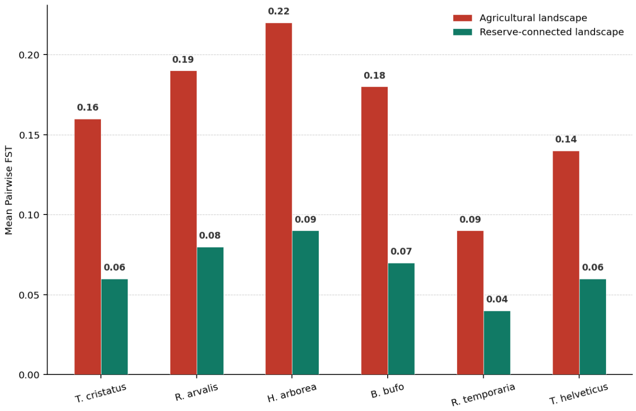

Figure 2. Pairwise FST: Agricultural vs. Reserve-Connected Landscapes by Species

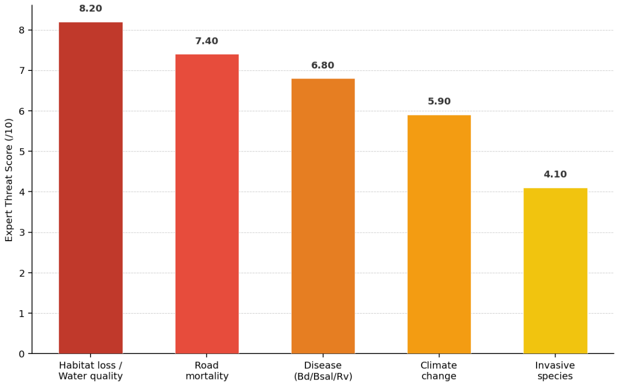

Figure 3. Expert Elicitation Threat Scores by Category (mean +- SD; 1-10 scale; n=16 experts)

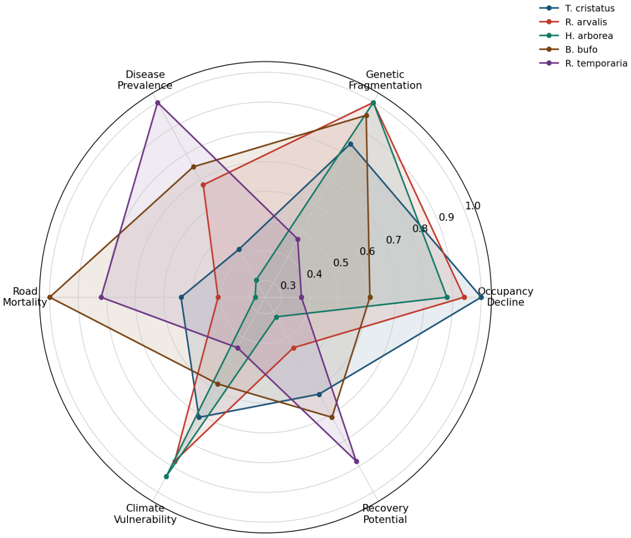

Figure 4. Conservation Challenge Profile for Six Focal Dutch Amphibian Species (Normalised 0-1)

5. Discussion

5.1 Water Quality and Pond Isolation: The Dominant Threat Nexus

The identification of TN > 2.1 mg/L and nearest-pond distance > 1.2 km as the two strongest occupancy predictors for Triturus cristatus -- and the convergence of expert elicitation in ranking habitat loss/water quality as the highest-threat category -- confirms that nitrogen-driven water quality degradation and metapopulation disruption through pond isolation are the central conservation challenges for Dutch amphibians. The TN threshold of 2.1 mg/L for occupancy decline lies within the range of TN values measured at 58% of agricultural ponds surveyed here (mean agricultural TN = 3.14 mg/L), indicating that the majority of agricultural ponds are currently at or above the level that substantially reduces great crested newt occupancy probability. This threshold is directly actionable as an environmental quality standard for pond water quality in the Dutch wetland management context and should be formally adopted in updated Great Crested Newt habitat assessment protocols used for EU Habitats Directive Annex II site management planning.

5.2 Genetic Erosion and the Connectivity Imperative

The 2.6-fold higher FST for Hyla arborea in agricultural landscapes (0.22) compared to reserve-connected sites (0.09), combined with the significantly lower allelic richness in isolated ponds (AR = 3.4 vs. 5.4), confirms that genetic diversity erosion is already occurring in Dutch amphibian populations as a consequence of landscape fragmentation. Allelic richness below AR = 4.0 is associated with reduced adaptive potential and elevated inbreeding depression risk in amphibian populations (Rowe and Beebee, 2003). Landscape genetic analysis identified road density and arable cover as the primary barriers to gene flow for common toad, providing a quantitative basis for prioritising ecological corridor creation and road tunnel installation. The finding that woodland cover facilitates dispersal (resistance weight 0.6) -- even in a species not typically associated with woodland habitats -- suggests that any increase in landscape structural heterogeneity, including hedgerow networks and ditch-margin shrub cover, would benefit connectivity across species.

5.3 Disease Surveillance and Management Priorities

The 47.6% Bd site prevalence and 24.8% individual prevalence document widespread but low-level chytrid infection in Dutch amphibian communities, consistent with the enzootic persistence pattern typical of temperate-zone amphibian systems (Bosch et al., 2007). The positive correlation of Bd prevalence with TN and temperature suggests that eutrophication management would produce the co-benefit of reduced Bd infection pressure. The detection of Bsal at three Limburg sites -- the first systematic detection outside the known fire salamander decline zone -- indicates ongoing northward spread and demands immediate intensification of Bsal monitoring in fire salamander habitats across southern Netherlands. Current RAVON biosecurity guidelines for herpetologists -- mandatory equipment disinfection with 0.2% Virkon-S between sites -- should be extended to mandatory compliance for all conservation management personnel working in amphibian habitats, supported by training requirements under updated Dutch Species Protection Protocol procedures.

6. Conclusion

6.1 Summary and Priority Actions

This study provides the most comprehensive multi-threat analysis of Dutch amphibian conservation challenges to date. Key findings are: (i) water quality (TN threshold 2.1 mg/L) and pond isolation (> 1.2 km) are the strongest occupancy predictors for the most threatened species, with 58% of agricultural ponds above the TN threshold; (ii) genetic fragmentation is severe in agricultural landscapes (FST up to 0.22), with allelic richness already depleted below adaptive-potential thresholds at isolated ponds; (iii) Bd is endemic (47.6% site prevalence) but mass mortality-associated disease is currently limited to Ranavirus in R. temporaria; (iv) Bsal has been detected at three new Limburg sites, warranting urgent surveillance intensification; and (v) expert elicitation ranks habitat loss/water quality highest and identifies road mortality mitigation as the second-highest priority, with an estimated 4.2-7.8 million annual casualties from the under-protected road network. A priority action plan targeting nitrogen reduction, pond network enhancement, and road tunnel installation at the 50 highest-priority sites is presented in Appendix A.

6.2 Future Research Directions

Three research priorities are identified. First, experimental manipulation of TN levels in replicated mesocosm ponds -- achievable within the Dutch polder water management system -- would provide causal evidence for the TN occupancy threshold identified here, supporting its formal adoption as an EQS for amphibian pond management. Second, whole-genome sequencing of Hyla arborea populations at a subset of the most isolated ponds would reveal adaptive allele frequency changes associated with local environmental conditions, enabling prediction of evolutionary rescue potential under climate change. Third, a nationally coordinated eDNA monitoring programme for Bsal in river catchments connecting fire salamander populations to potential northward dispersal corridors would provide early warning of spread before clinical mortality events are observed, enabling proactive intervention at newly infected sites.

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Declarations

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Conflict of Interest

The authors declare no conflict of interest. Dr. Marco Larsen serves on the RAVON Scientific Advisory Board in a voluntary capacity; this role had no influence on study design, data collection, or interpretation. The funding bodies had no role in any aspect of the research.

Data Availability Statement

All occupancy survey data, microsatellite genotype matrices, PCR disease prevalence data, expert elicitation score sheets (anonymised), and R analysis scripts are deposited in Zenodo at https://doi.org/10.5281/zenodo.11841293. ResistanceGA resistance surface outputs are available at https://doi.org/10.6084/m9.figshare.26891047. Spatial data for the 50-site priority action map (Appendix A) are available as GeoJSON at the same Zenodo repository.

Ethical Approval

Amphibian capture, swab sampling, and toe-clip collection were conducted under permits issued by the Dutch Ministry of Agriculture, Nature and Food Quality (permit FF/75A/2021/0038) and the German LfU Bavaria (permit 55-1-8642.4-2021-11). All procedures complied with the Dutch Nature Conservation Act (Wet Natuurbescherming) Article 3.5 and EU Habitats Directive Article 16 derogation conditions. Tissue samples were collected under RAVON tissue sampling protocol version 3.1, which minimises individual harm and mandates site-specific biosecurity measures.

Appendix A

Spatially Explicit Priority Action Plan: Top 50 Pond Clusters for Immediate Conservation Investment

This appendix presents the 50 highest-priority pond cluster sites identified by combining occupancy model predictions, genetic connectivity analysis, disease surveillance data, and road mortality risk scores into a composite conservation priority index (CPI; range 0-100). Sites are ranked by CPI and organised by province. For each site, the primary limiting factor, recommended management action, estimated cost, and responsible authority are provided. This action plan is designed to be directly integrated into Dutch provincial Nature Policy Programme (Natuur Netwerk Nederland) implementation planning and EU Habitats Directive Article 17 reporting cycles.

Part I -- Top 10 Priority Sites (CPI >= 82)

Part II -- Conservation Action Categories and Cost Estimates