Quantifying Environmental Stressor Intensities in Ponds and Their Relationship to Tadpole Body Condition

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INTRODUCTION

Ponds and other small freshwater bodies are biodiversity hotspots, but their shallow depth, limited volume, and proximity to human activity, combined with seasonal groundwater and drainage inflows, make them highly vulnerable to pollution and environmental stress (Loose *et al.* 2024).

While Environmental Quality Standards (EQSs) and Regulatory Acceptable Concentrations (RACs) aim to protect aquatic ecosystems, studies show these thresholds are frequently exceeded in small water bodies (Lorenz et al. 2025). This raises concerns about real-world pesticide exposure, especially for sensitive species.

Amphibians, like the common frog (*Rana temporaria*), are especially vulnerable due to their permeable skin, aquatic life stages, and reliance on freshwater habitats for reproduction. Their decline serves as a key indicator of ecosystem health and cumulative stress.

This project investigates how multiple co-occurring stressors affect *R. temporaria* presence and condition across UK pond types. Using eDNA, water chemistry, and tadpole morphometrics, it aims to explore how stressor intensity and interactions shape amphibian resilience under current and future environmental conditions.

METHODS

STUDY SITES AND SAMPLE COLLECTION

Sites were prioritised across five UK regions: South-East England (SE), East Anglia (EA), South-West Scotland (D&G), Central-West Scotland (AYR), and Scotland's Central Belt (CB), based on recent *R. temporaria* records from the NBN Atlas, Freshwater Habitats Trust, Natural England's Priority Ponds Network, and surveys conducted in May 2023.

Water quality was measured and samples were collected for eDNA (R. temporaria and invasive crayfish, Pacifastacus leniusculus) and chemical analysis (pesticides, pharmaceuticals, metals).

Water samples were analysed for:

- 76 pesticides (UPLC-MS/MS)
- 74 pharmaceuticals (UPLC-MS/MS)
- 28 trace metals (ICP-MS)

When present, R. temporaria tadpoles were sampled (n = 3–20), morphometric recorded, and preserved in RNAlater for future analysis.

SCALED MASS INDEX (SMI)

Body condition was assessed using the Scaled Mass Index (SMI) following Peig & Green (2009), which standardizes body mass to a reference body length for cross-individual comparisons. SMI for each individual i was calculated as:

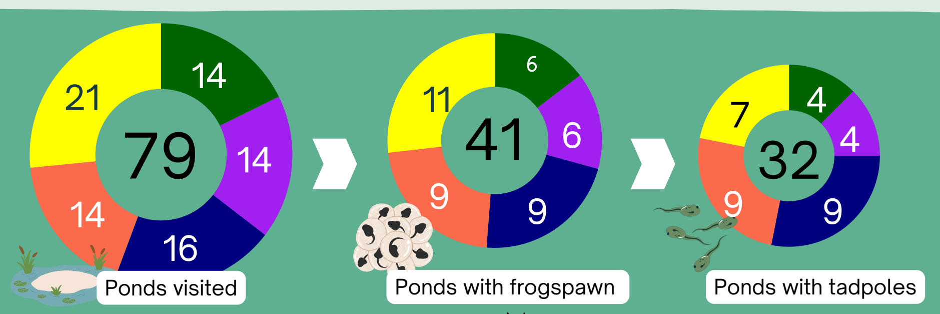
$$SMI = M_i \left[\frac{L_0}{L_i} \right]^{bSMA}$$

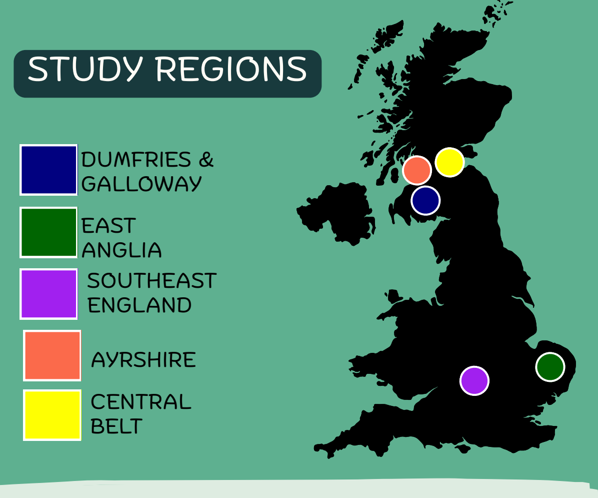
POLLUTION INDEX

Kaplan–Meier (KM) means were calculated per pollutant using survival analysis to account for censored values. KM means were then standardized across regions using z-scores, The pollution index for each region was calculated as the mean of all standardized pollutant values to provide a composite score representing overall chemical burden for each region.

RESULTS

A total of 79 ponds were surveyed across five UK regions. Frogspawn was observed in 52–64% of Scottish ponds and 42% of English ponds. *R. temporaria* eDNA was detected in 100% of ponds. On return visits, tadpoles were present in 78% of ponds with prior frogspawn; 64–100% in Scotland and 66% in England.





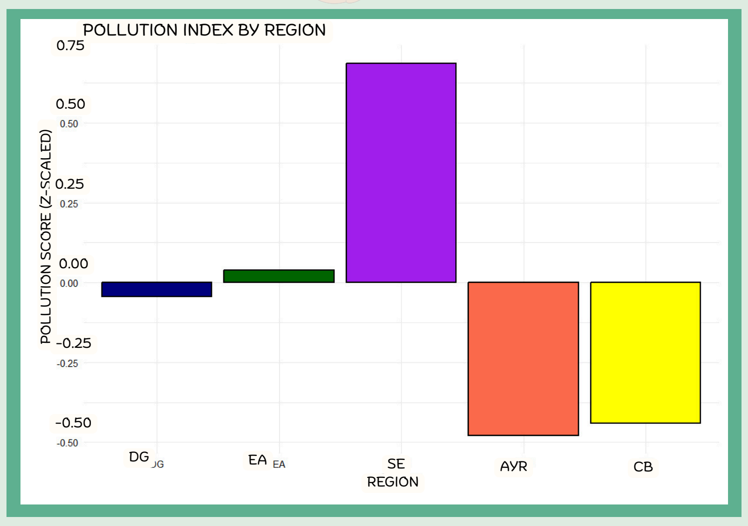
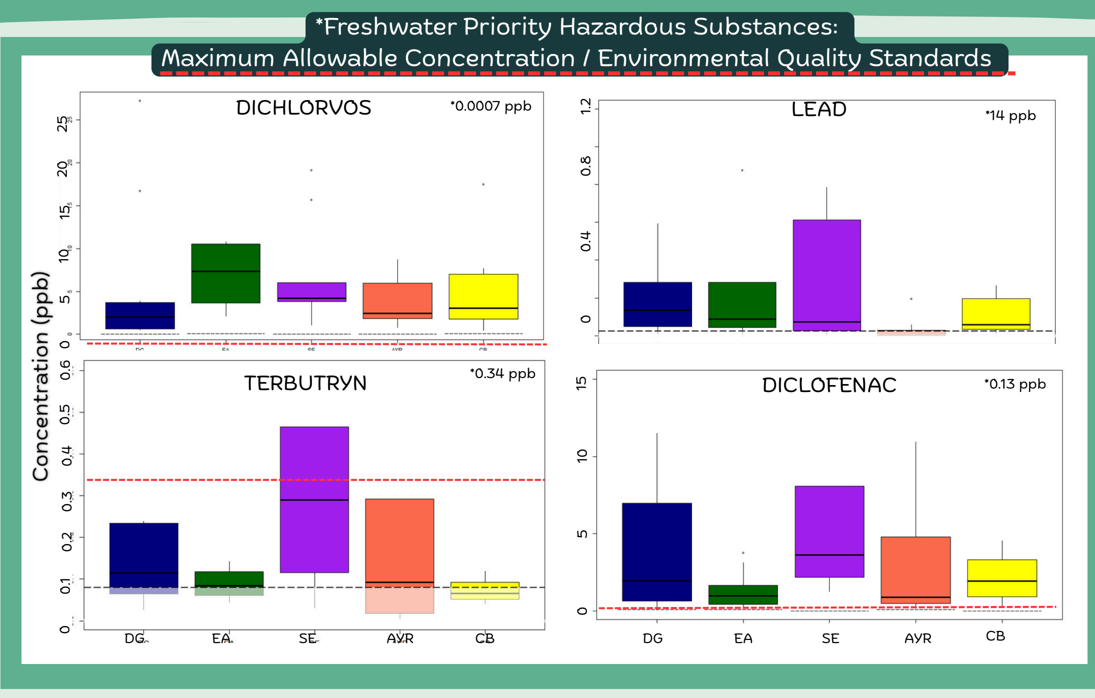


Figure 1. Standardised pollution index for each region. The South East (SE) showed the highest cumulative chemical burden, while Ayrshire (AYR) and Central Belt (CB) had the lowest. This index reflects relative contamination pressure across sites.



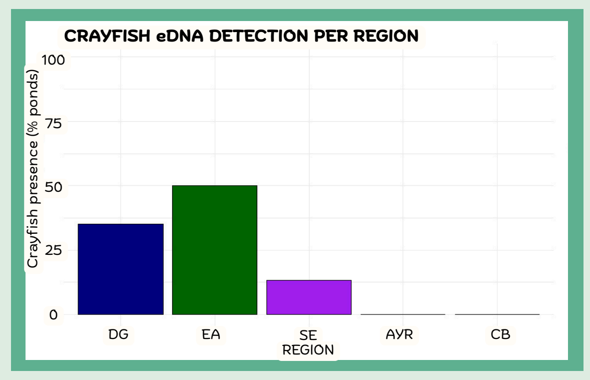


Figure 2. Proportion of ponds in each region where invasive crayfish eDNA was detected. Crayfish presence was highest in EA and DG, with detection in 50% and 35% of sampled ponds, respectively. No crayfish were detected in AYR or CB.

Figure 3. Boxplots showing concentrations (ppb) of selected pollutants grouped by category: pesticides (dichlorvos, terbutryn), pharmaceuticals (ketamine, diclofenac), and metals (lead, zinc). Horizontal lines indicate relevant Environmental Quality Standards (EQSs) where available. Several substances, notably dichlorvos, terbutryn, and diclofenac, exceeded maximum allowable concentration (*) thresholds in multiple regions, highlighting potential ecological and regulatory concerns.

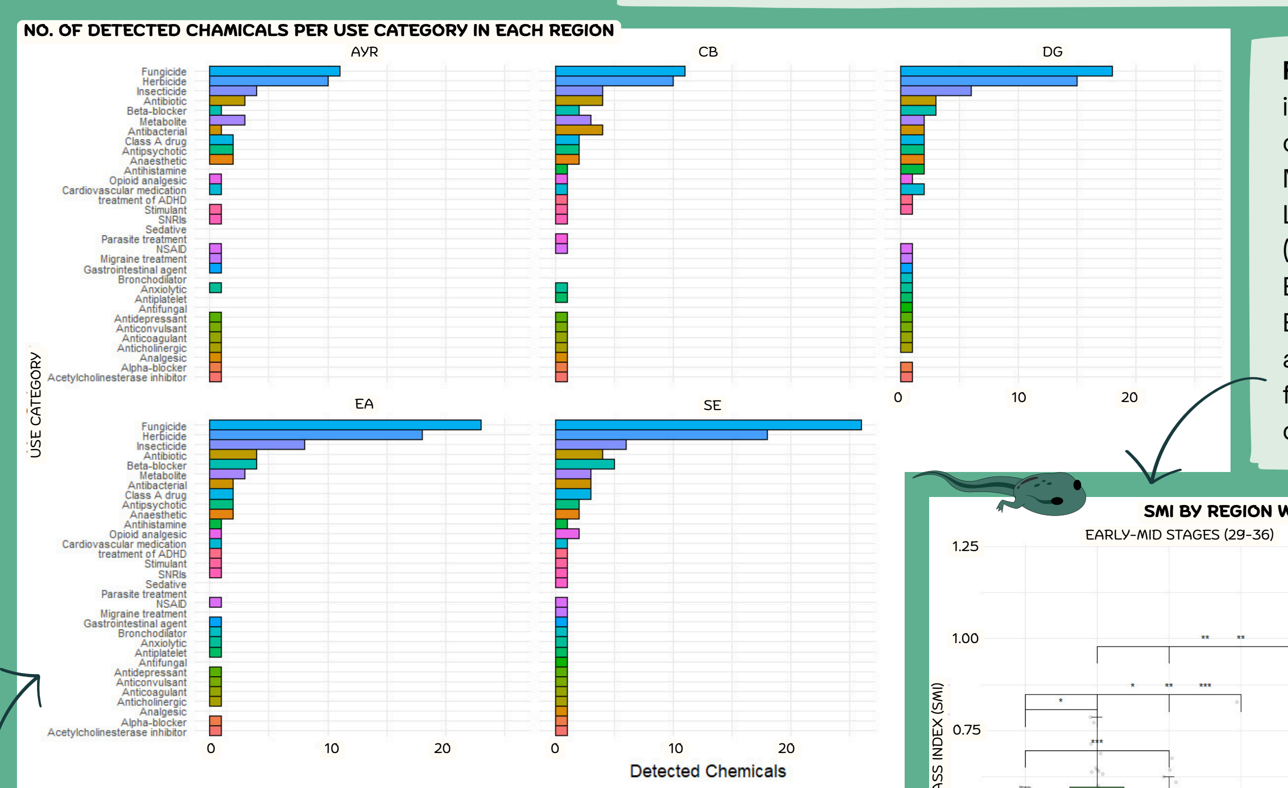
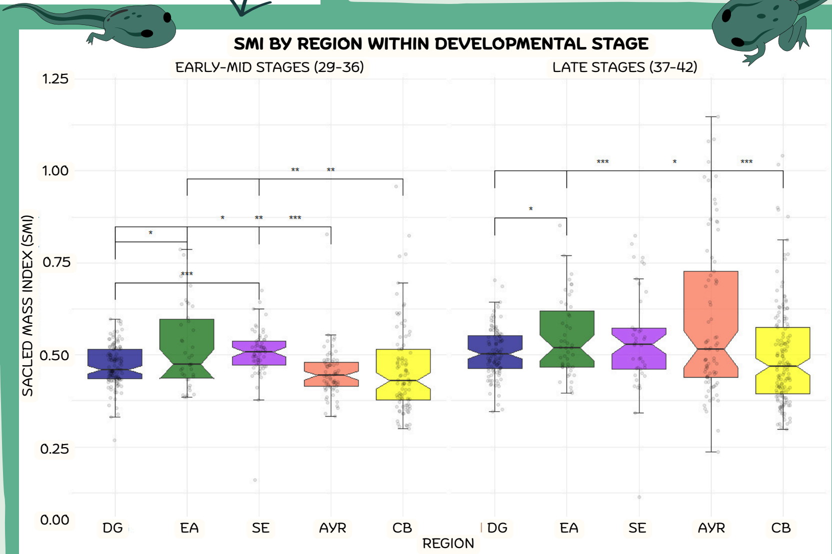


Figure 5. The number of detected chemicals per use category across regions. Across all regions, fungicides were the most frequently detected group overall, followed by herbicides. A notably higher number of chemicals under these use categories were detected in the English regions (SE and EA) compared to the Scottish regions (CB, AYR and DG).

Figure 4. Boxplots showing variation in SMI across regions within developmental stages: Early and Mid (Gosner stages 29–36) and Late (37–42). Significant differences ($p < 0.05$) were observed between English and Scottish regions in the Early–Mid stage group. Notably, Ayr and D&G were significantly different from one another in both developmental stage categories.



TAKEAWAYS

- The pollution index revealed regional variation in cumulative chemical burden. Southeast England (SE) had the highest levels, while Ayrshire (AYR) and Central Belt (CB) showed the lowest.
- Fungicides were the most frequently detected pollutant class across all regions, with particularly high prevalence in SE and East Anglia (EA), suggesting stronger agricultural influence in southern areas.
- Tadpole presence was lowest in the English regions, where only 66% of ponds with frogspawn later contained tadpoles (compared to 86% in Scotland) suggesting potential impacts of regional contamination on recruitment success.
- Tadpole body condition (SMI) varied significantly by region. Lower SMI in some English ponds during early development suggests that contaminant exposure may be affecting amphibian health.
- Several chemicals—such as dichlorvos and terbutryn—exceeded Environmental Quality Standards (EQS) in certain ponds. However, EQSs are based on larger, flowing systems and may not adequately reflect risks in small, enclosed ponds (Lorenz *et al.*, 2025).
- Due to their limited dilution capacity and proximity to pollution sources, ponds may experience ecologically significant contaminant spikes (Loose *et al.*, 2024), even below EQS thresholds.

NEXT STEPS

The current pollution index is based on z-scored concentrations and does not account for differences in toxicity or ecological impact. Future work will incorporate risk-weighted approaches which combine pollutant concentrations with regulatory limits and toxic-response factors (Egbueri, 2020) This will support more meaningful, pond-specific risk assessments that better reflect the vulnerability of small freshwater ecosystems.

Future work will integrate tadpole morphometrics and gene expression data (from liver, gonads, and brain tissues) to explore the physiological impacts of multiple, co-occurring stressors. These analyses will be combined with existing data on pollutant concentrations, crayfish presence, habitat type, and body condition to assess how complex environmental exposures influence amphibian development and resilience.

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