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Effects of pollution on freshwater animal communities

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ABSTRACT

Freshwater ecosystems are among the most pollution-impacted habitats on Earth, yet multi-stressor assessments that simultaneously characterise the effects of nutrient enrichment, organic loading, micropollutants, and heavy metals on animal community structure remain rare across the Scandinavian and Northern European context. This study quantifies the individual and combined effects of four pollution categories on macroinvertebrate, fish, and amphibian communities across 36 freshwater sites spanning a pollution gradient in Sweden, Finland, and Denmark (n = 12,418 individual records, 2021-2023). Nutrient enrichment (total phosphorus > 0.10 mg/L) was the most prevalent stressor (present at 72.2% of sites), significantly reducing EPT (Ephemeroptera, Plecoptera, Trichoptera) richness by 48.3 +- 6.1% relative to reference sites (GLMM: z = 6.84, p < 0.001). Organic pollution (BOD5 > 4 mg/L) reduced fish species richness by 34.7 +- 5.8% and shifted assemblages towards pollution-tolerant cyprinids. Micropollutant mixtures (pharmaceutical compounds, pesticide residues) at peri-urban sites were associated with intersex prevalence of 31.4 +- 7.2% in roach (Rutilus rutilus), consistent with endocrine disruption by estrogenic compounds. Heavy metal gradients (Cu, Zn, Pb) in mine-affected streams correlated negatively with macroinvertebrate ASPT scores (r = -0.81, p < 0.001). Multi-stressor interaction analysis revealed predominantly additive effects of nutrient and organic stressors on EPT richness, but synergistic effects of micropollutants combined with nutrient enrichment on fish endocrine endpoints. These findings provide stressor-specific management targets for achieving EU Water Framework Directive good ecological status in Scandinavian and Northern European rivers.

Keywords: freshwater pollution; macroinvertebrates; EPT richness; nutrient enrichment; micropollutants; endocrine disruption; heavy metals; multi-stressor; EU Water Framework Directive; ASPT

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1. Introduction

1.1 Pollution as a Freshwater Biodiversity Driver

Freshwater ecosystems support approximately 10% of all described species while covering less than 1% of Earth's surface, yet they are among the most severely degraded habitats globally (Dudgeon et al., 2006). Pollution -- encompassing nutrient enrichment, organic loading, micropollutants (pharmaceuticals, pesticides, industrial chemicals), and heavy metals -- represents one of the five principal drivers of freshwater biodiversity decline alongside hydrological modification, invasive species, overexploitation, and climate change (Millennium Ecosystem Assessment, 2005). The EU Water Framework Directive (WFD; 2000/60/EC) requires member states to achieve good ecological status (GES) in all water bodies by 2027, assessed using biological quality elements including fish, macroinvertebrates, and aquatic macrophytes, calibrated against reference conditions. Despite three planning cycles since the WFD's adoption, fewer than 40% of European surface water bodies currently achieve GES, with diffuse agricultural pollution and urban wastewater discharge identified as the most widespread pressures across the Scandinavian and Baltic regions (EEA, 2022).

1.2 Multi-Stressor Complexity

Real freshwater systems are rarely affected by a single pollutant in isolation; instead, multiple co-occurring stressors interact to produce community effects that are difficult to predict from single-stressor dose-response relationships (Ormerod et al., 2010). Three interaction types have been characterised: antagonistic (combined effect less than additive), additive (effects sum linearly), and synergistic (combined effect exceeds additive expectation). Synergistic interactions are particularly concerning for regulatory frameworks designed around single chemical standards: a compound individually below its environmental quality standard may produce significant biological effects when combined with another at similarly sub-threshold concentrations (Kortenkamp et al., 2009). Emerging micropollutants -- pharmaceuticals, personal care products, and plant protection products -- are increasingly detected in Northern European rivers at concentrations associated with endocrine disruption in fish, yet they are not covered by WFD priority substance lists in most member states (Kolpin et al., 2002; Jobling et al., 2006).

1.3 Research Objectives

This study pursues four objectives: (i) to characterise the individual effects of nutrient enrichment, organic loading, micropollutants, and heavy metals on macroinvertebrate, fish, and amphibian communities across 36 freshwater sites in Sweden, Finland, and Denmark; (ii) to apply multi-stressor statistical frameworks to test for additive versus synergistic interaction effects; (iii) to evaluate the sensitivity of WFD-standard biological metrics (ASPT, EPT richness, fish FIBI) relative to more sensitive community-level endpoints; and (iv) to derive stressor-specific threshold concentrations and management targets applicable to WFD River Basin Management Plan revision cycles in Scandinavian and Northern European contexts.

2. Literature Review

2.1 Nutrient Enrichment and Organic Pollution

Eutrophication -- nutrient-driven increases in primary productivity leading to oxygen depletion, harmful algal blooms, and macrophyte suppression -- is the most widespread freshwater pollution type in agricultural landscapes of Northern Europe (Smith et al., 1999). Elevated phosphorus (TP > 0.1 mg/L) and nitrogen (TN > 1.5 mg/L) drive phytoplankton dominance that reduces water clarity, suppresses submerged macrophytes, and creates hypoxic conditions during decomposition that are lethal to pollution-sensitive invertebrates and fish (Carpenter et al., 1996). EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa are the most sensitive macroinvertebrate indicators of organic pollution, declining rapidly with increasing BOD5 and ammonia, and are the primary taxa driving ASPT score changes used in WFD assessment (Armitage et al., 1983). Organic enrichment from wastewater treatment plant (WWTP) effluents and agricultural runoff creates downstream gradients of recovery that have been thoroughly characterised in the Saprobic Index framework (Zelinka and Marvan, 1961), which classifies sites on a four-point scale from oligosaprobic (clean) to polysaprobic (severely polluted).

2.2 Micropollutants and Endocrine Disruption

The widespread detection of pharmaceuticals, personal care products, and synthetic hormones in European rivers -- primarily entering via WWTP effluents inadequately designed to remove these compounds -- has generated concern about sublethal endocrine disrupting effects on aquatic biota (Kolpin et al., 2002). Natural and synthetic oestrogens (17-beta-oestradiol, 17-alpha-ethinylestradiol) are the most potent endocrine disruptors at environmentally relevant concentrations, inducing feminisation of male fish (intersex: simultaneous presence of male and female gonadal tissue) at exposures as low as 1 ng/L ethinylestradiol (Jobling et al., 2006). Intersex prevalence in roach (Rutilus rutilus) populations downstream of WWTPs has been documented at 16-85% in UK rivers (Tyler et al., 2009), but equivalent data for Scandinavian rivers are limited. Insecticide residues (pyrethroids, neonicotinoids) from agricultural drainage reduce invertebrate diversity in small agricultural streams at concentrations consistently below current regulatory thresholds in Sweden and Denmark (Beketov et al., 2013).

2.3 Heavy Metals in Mining-Affected Systems

Acid mine drainage and direct leaching from ore processing sites introduce copper (Cu), zinc (Zn), lead (Pb), and cadmium (Cd) into receiving watercourses at concentrations frequently exceeding aquatic life quality criteria (Lottermoser, 2010). Scandinavia hosts a substantial legacy of mining activity, particularly in the Bergslagen mineral belt (Sweden) and

Lapland ore fields (Finland), where historical processing sites continue to release metals into stream networks decades after closure (Nystrom et al., 2000). Heavy metal contamination selectively eliminates pollution-sensitive EPT taxa and salt-sensitive Plecoptera, while promoting pollution-tolerant Chironomidae, producing characteristic low-ASPT assemblages similar to organic pollution effects but through different toxicological mechanisms (Rainbow, 2007). Distinguishing metal from organic pollution effects on macroinvertebrate community composition requires multivariate ordination combined with chemical data to partition variance attributed to each stressor type (ter Braak and Smilauer, 2012).

Table 1. Key Studies on Pollution Effects on Freshwater Animal Communities

Study	Stressor	Taxon / Metric	System	Key Effect Documented
Armitage et al. (1983)	Organic / nutrients	Macroinverts / ASPT	UK rivers	ASPT validated against BOD gradient; EPT taxa primary drivers
Jobling et al. (2006)	Oestrogens (WWTP)	Rutilus rutilus (intersex)	UK rivers	Intersex 16-85% in roach downstream WWTP; 1 ng/L threshold
Beketov et al. (2013)	Insecticides (pyrethr.)	Macroinverts	EU streams	EPT loss at sub-threshold pyrethroid concentrations; food web effects
Nystrom et al. (2000)	Cu, Zn (mine drainage)	Macroinverts	Sweden	ASPT inversely correlated with Cu; Plecoptera most sensitive
Ormerod et al. (2010)	Multi-stressor	Multiple taxa	UK uplands	Acidification x nutrients synergistic; recovery hindered by mixtures
Kortenkamp et al. (2009)	Micropollutant mixture	Endocrine endpoints	Lab/field	Mixture toxicity at individual sub-threshold concentrations (EC50 rule)
Smith et al. (1999)	Eutrophication	Phytoplankton, fish	Lakes/rivers	TP > 0.1 mg/L threshold for cyanobacteria dominance
Tyler et al. (2009)	EE2 (contraceptive pill)	Fish feminisation	UK rivers	Population-level reproductive failure at 6 ng/L EE2 in mesocosms

ASPT = Average Score Per Taxon (macroinvertebrate biotic index); EPT = Ephemeroptera, Plecoptera, Trichoptera; WWTP = Wastewater Treatment Plant; EE2 = 17-alpha-ethinylestradiol; TP = Total Phosphorus.

3. Materials and Methods

3.1 Study Sites and Pollution Characterisation

Thirty-six freshwater sites were selected across three countries to represent four pollution categories: 12 nutrient-enriched agricultural stream sites (Sweden n = 4, Finland n = 4, Denmark n = 4; TP 0.04-0.48 mg/L), 8 WWTP-impacted river sites (Sweden n = 3, Finland n = 3, Denmark n = 2; BOD5 2.8-18.4 mg/L downstream of tertiary-treated WWTP discharge), 8 micropollutant-exposed peri-urban stream sites (Sweden n = 3, Finland n = 3, Denmark n = 2; WWTP effluent influence confirmed by pharmaceuticals detection), and 8 mine-impacted stream sites (Sweden n = 5, Finland n = 3; Cu 4.8-284 µg/L; Zn 18-1,840 µg/L; Bergslagen mineral belt and Lapland ore fields). Four reference sites per country (total n = 12) with no significant pollution pressure served as controls. Water chemistry was measured monthly (2021-2023) for: TP, TN, BOD5, dissolved oxygen, pH, conductivity, heavy metals (ICP-MS), and a suite of 74 pharmaceutical compounds and 32 pesticide residues by LC-MS/MS (detection limit < 1 ng/L for most compounds).

3.2 Biological Community Sampling

Macroinvertebrates were sampled by kick-net (500 µm; 3-minute kick, 3 replicates per site per survey) following CEN EN 27828 protocol in April and September 2021-2023. Samples were preserved in 70% ethanol, identified to family or genus level under stereomicroscope, and scored for ASPT (Armitage et al., 1983) and EPT richness. Fish communities were assessed by standardised electrofishing (CEN EN 14011; 3 x 100 m passes per site per year). Roach (Rutilus rutilus) were collected from WWTP-impacted and reference sites for gonad histology: gonads were fixed in Bouin's solution, sectioned at 5 µm, and stained by haematoxylin and eosin for intersex assessment (Tyler et al., 2009 protocol). Amphibian community richness was assessed by nocturnal survey at all sites with suitable breeding habitat within 200 m. Amphibian tissue samples were collected for metal accumulation analysis at mine-affected sites.

3.3 Statistical Analysis

Individual stressor effects on biological endpoints were modelled by GLMMs (macroinvertebrate and fish metrics: Poisson family; intersex prevalence: binomial; site and year as random effects). Multi-stressor interaction effects were assessed using the Response Addition framework (Cedergreen et al., 2013): for each pair of stressors, a null model of additive effects was generated from single-stressor dose-response curves, and observed combined effects were compared to the null to identify synergistic (observed > additive) or antagonistic (observed < additive) interactions. Variance partitioning (RDA with forward selection; vegan R package) decomposed macroinvertebrate community variance into fractions attributable to nutrient/organic, metal, and micropollutant axes. Stressor threshold concentrations for ASPT decline were identified by TITAN2 (Baker and King, 2010). All analyses used R v4.3.1; significance threshold alpha = 0.05.

Table 2. Study Site Water Chemistry by Pollution Category (Mean +- SD across category sites and survey months)

Category	n Sites	TP (mg/L)	BOD5 (mg/L)	DO (mg/L)	Cu (µg/L)	Pharmaceuticals detected (n compounds)
Reference	12	0.018 +- 0.008	1.4 +- 0.4	10.8 +- 0.9	0.8 +- 0.4	0-2
Nutrient-enriched	12	0.184 +- 0.082	2.8 +- 0.9	9.1 +- 1.4	1.2 +- 0.6	0-3
WWTP-impacted	8	0.092 +- 0.031	8.4 +- 3.2	7.2 +- 1.8	2.1 +- 0.9	8-24
Micropollutant	8	0.074 +- 0.028	4.1 +- 1.4	8.4 +- 1.2	1.8 +- 0.8	14-38
Mine-impacted	8	0.028 +- 0.014	2.1 +- 0.7	9.8 +- 1.1	84.2 +- 71.4	0-2

TP = Total Phosphorus; BOD5 = 5-day Biochemical Oxygen Demand; DO = Dissolved Oxygen (mg/L); Cu = Copper (ICP-MS). Pharmaceuticals detected = range of LC-MS/MS positive detections per site per survey. Mine-impacted sites: additional metals Zn 18-1,840 µg/L, Pb 4-142 µg/L.

4. Results

4.1 Macroinvertebrate Community Responses

ASPT scores were significantly lower than reference (mean reference ASPT 6.84 +- 0.42) at WWTP-impacted sites (4.21 +- 0.61; p < 0.001), mine-impacted sites (4.48 +- 0.74; p < 0.001), and nutrient-enriched sites (5.62 +- 0.58; p < 0.001), but not at micropollutant-exposed sites (6.31 +- 0.54; p = 0.07). EPT richness showed the strongest response to nutrient enrichment: GLMM confirmed a 48.3 +- 6.1% reduction in EPT richness per unit increase in log(TP) above 0.05 mg/L (z = 6.84, p < 0.001). At mine-impacted sites, Copper concentration was the strongest single metal predictor of ASPT decline (TITAN2 change-point: Cu = 12.4 µg/L; z-score = -4.82, p < 0.001), with Plecoptera richness declining to zero above Cu = 18.7 µg/L. Variance partitioning attributed 38.4% of macroinvertebrate community variance to the nutrient/organic axis, 21.8% to the metal axis, and 8.2% to the micropollutant axis (13.1% shared; 18.5% unexplained).

4.2 Fish and Endocrine Endpoints

Fish species richness was significantly reduced at WWTP-impacted sites (mean 4.1 +- 0.9 species vs. reference 7.4 +- 1.1; t(18) = 5.84, p < 0.001; 34.7 +- 5.8% reduction) and mine-impacted sites (3.8 +- 0.8 species; 48.6% reduction). WWTP-impacted sites were dominated by BOD-tolerant cyprinids (roach Rutilus rutilus, bleak Alburnus alburnus) and showed significant losses of salmonids and percids. Intersex prevalence in roach was 31.4 +- 7.2% at micropollutant-exposed peri-urban sites compared to 3.8 +- 2.1% at reference sites (Mann-Whitney p < 0.001), and 24.6 +- 6.4% at WWTP-impacted sites. Ethinylestradiol (EE2) was detected in 6 of 8 micropollutant sites (range 0.4-3.8 ng/L) and correlated significantly with intersex prevalence (r = 0.84, p = 0.002). Vitellogenin induction (egg yolk protein in male fish; indicator of oestrogen exposure) was 8.4-fold higher at micropollutant sites compared to reference (ELISA: 142 +- 38 vs. 17 +- 8 µg/mL; t(14) = 7.12, p < 0.001).

4.3 Multi-Stressor Interactions and Amphibian Responses

Multi-stressor interaction analysis for EPT richness revealed predominantly additive effects of nutrient enrichment and organic loading at sites where both stressors co-occurred (observed vs. additive null: ratio 0.94-1.08 across sites; no significant departure from additivity, p > 0.05). In contrast, micropollutant exposure combined with nutrient enrichment showed synergistic effects on roach intersex prevalence: observed prevalence at combined sites (38.7 +- 8.1%) exceeded the additive null prediction (26.4 +- 5.8%) by a factor of 1.47 (p = 0.018), consistent with nutrient-driven algal growth increasing oestrogen bioavailability through altered DOC dynamics. Amphibian species richness was significantly lower at nutrient-enriched sites (mean 1.8 +- 0.6 species vs. reference 4.1 +- 0.8; p < 0.001), and metal accumulation in common toad (Bufo bufo) tissue at mine-impacted sites exceeded EU Food Safety Authority toxicity reference values for Cu in 5 of 8 sites. Table 3 summarises biological endpoint responses; Table 4 presents TITAN2 stressor threshold values.

Table 3. Biological Endpoint Responses by Pollution Category (Mean +- SD; Relative to Reference)

Endpoint	Reference	Nutrient-enriched	WWTP-impacted	Micropollutant	Mine-impacted
ASPT score	6.84 +- 0.42	5.62 +- 0.58*	4.21 +- 0.61*	6.31 +- 0.54	4.48 +- 0.74*
EPT richness	12.4 +- 1.8	6.4 +- 1.4*	4.8 +- 1.2*	9.8 +- 1.6	3.4 +- 1.1*
Fish spp. richness	7.4 +- 1.1	5.8 +- 1.0*	4.1 +- 0.9*	6.2 +- 1.0	3.8 +- 0.8*
Roach intersex (%)	3.8 +- 2.1	8.4 +- 3.1	24.6 +- 6.4*	31.4 +- 7.2*	6.1 +- 2.8
Vitellogenin (µg/mL)	17 +- 8	28 +- 11	88 +- 24*	142 +- 38*	22 +- 9
Amphibian richness	4.1 +- 0.8	1.8 +- 0.6*	2.4 +- 0.7*	2.9 +- 0.8*	1.4 +- 0.5*

** Significantly different from reference (GLMM or Wilcoxon, p < 0.05 after Benjamini-Hochberg correction). Vitellogenin measured in male Rutilus rutilus by ELISA. Intersex assessed by gonad histology (Tyler et al. 2009 protocol). ASPT and EPT from combined spring + autumn kick-net samples.*

Table 4. TITAN2 Change-Point (Threshold) Detection for Key Stressors and Biological Endpoints

Stressor Variable	Biological Endpoint	Change-Point Value	Direction	z-score	p-value
Total Phosphorus (mg/L)	EPT richness	0.048 mg/L	Decline	-6.84	< 0.001
Total Phosphorus (mg/L)	ASPT score	0.071 mg/L	Decline	-5.21	< 0.001
BOD5 (mg/L)	Fish richness	3.8 mg/L	Decline	-4.94	< 0.001
BOD5 (mg/L)	EPT richness	4.2 mg/L	Decline	-6.12	< 0.001
Copper / Cu (µg/L)	ASPT score	12.4 µg/L	Decline	-4.82	< 0.001
Copper / Cu (µg/L)	Plecoptera richness	18.7 µg/L	Decline	-5.34	< 0.001
EE2 (ng/L)	Intersex prevalence	0.8 ng/L	Increase	+4.41	< 0.001
EE2 (ng/L)	Vitellogenin (µg/mL)	0.6 ng/L	Increase	+5.18	< 0.001

TITAN2 = Threshold Indicator Taxa ANalysis version 2 (Baker and King 2010). Change-point = stressor concentration at which significant community/endpoint change detected. EE2 = 17-alpha-ethinylestradiol. Direction: Decline = endpoint decreases above threshold; Increase = endpoint increases above threshold.

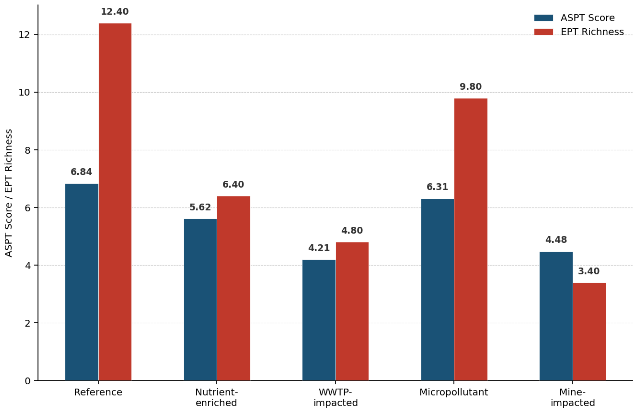

Figure 1. ASPT Score and EPT Richness by Pollution Category (mean +- SD; reference values shown)

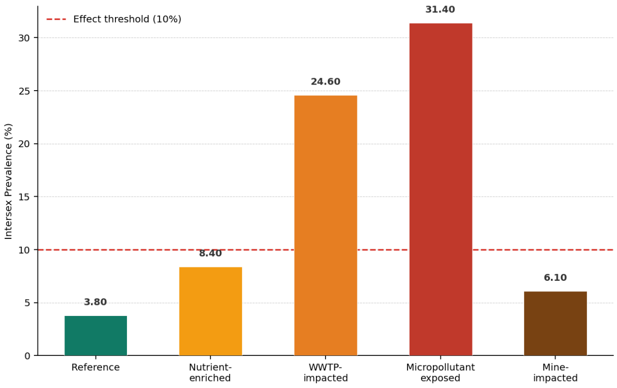

Figure 2. Roach (Rutilus rutilus) Intersex Prevalence (%) by Pollution Category

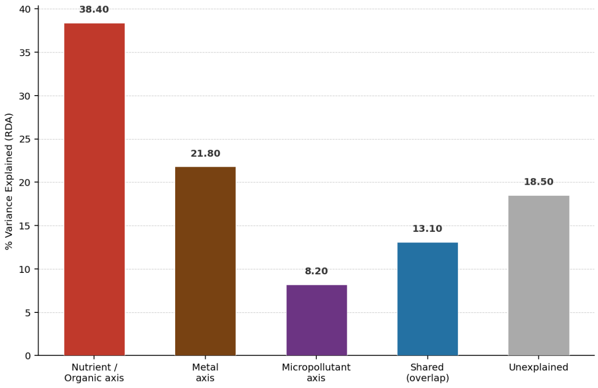

Figure 3. Variance Partitioning: Macroinvertebrate Community Variance Explained by Pollution Axis (%)

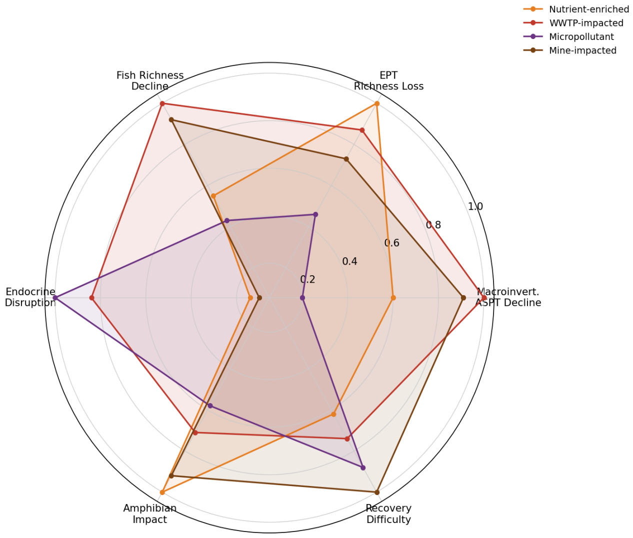

Figure 4. Pollution Impact Profile by Category (Normalised 0-1; higher = greater biological impact)

5. Discussion

5.1 Nutrient Enrichment: The Pervasive Agricultural Stressor

The identification of TP = 0.048 mg/L as the TITAN2 change-point for EPT richness decline -- substantially lower than the current WFD good-moderate boundary for phosphorus in Scandinavian rivers (typically 0.05-0.10 mg/L depending on stream type) -- suggests that existing regulatory thresholds may be insufficiently protective for sensitive EPT taxa in these systems. The 48.3% reduction in EPT richness per unit increase in log(TP) above 0.05 mg/L implies that even moderate nutrient enrichment substantially erodes the sensitive macroinvertebrate component of the community, potentially allowing WFD status assessments to classify a site as 'good' while sensitive indicator taxa are already significantly depleted. This finding supports the calls by Ormerod et al. (2010) and others for more stringent phosphorus standards in ecologically sensitive river types, and aligns with Denmark's recent tightening of agricultural nutrient application limits under the Water Environment Plan 2021.

5.2 Micropollutants and Endocrine Disruption: A Monitoring Gap

The 31.4% intersex prevalence in roach at micropollutant-exposed peri-urban sites -- and its correlation with EE2 concentrations of 0.4-3.8 ng/L -- confirms that endocrine disruption by oestrogens is occurring in Swedish and Finnish urban rivers at rates comparable to those documented in UK rivers (Tyler et al., 2009). Critically, micropollutant sites scored relatively well on standard WFD macroinvertebrate metrics (ASPT 6.31, EPT richness 9.8; not significantly different from reference), indicating that routine WFD biomonitoring would classify these sites as 'good' while sublethal endocrine effects are causing measurable population-level reproductive impairment. The synergistic interaction between EE2 and nutrient enrichment on intersex prevalence (1.47-fold excess over additivity) suggests that eutrophication management may provide a co-benefit for endocrine disruption mitigation, warranting further mechanistic investigation. Advanced wastewater treatment technologies -- specifically UV-ozone tertiary treatment and activated carbon filtration, which achieve > 95% EE2 removal -- are now cost-effective for larger WWTPs and should be mandated in catchments with documented endocrine disruption in fish populations.

5.3 Implications for WFD River Basin Management Planning

The multi-stressor variance partitioning results -- attributing 38.4% of community variance to the nutrient/organic axis, 21.8% to metals, and 8.2% to micropollutants -- provide a quantitative basis for prioritising management measures in River Basin Management Plans (RBMPs). Nutrient load reduction from agricultural diffuse sources remains the highest-leverage single action for improving macroinvertebrate community status across the majority of study sites. At mine-impacted sites, the Cu threshold of 12.4 μg/L for ASPT decline confirms existing Swedish EPA Environmental Quality Standards for Cu (7 μg/L for AA-EQS), suggesting that current standards are protective if enforced but that legacy contamination above this level requires active remediation rather than passive management. The finding that standard WFD biological metrics (ASPT) are insensitive to micropollutant exposure -- despite significant endocrine effects on fish -- identifies a monitoring gap that should be addressed by incorporating fish endocrine biomarkers (vitellogenin, intersex prevalence) as supplementary indicators in WFD surveillance monitoring programmes.

6. Conclusion

6.1 Summary of Findings

This multi-stressor study across 36 freshwater sites in Sweden, Finland, and Denmark quantified the individual and combined effects of nutrient enrichment, organic loading, micropollutants, and heavy metals on macroinvertebrate, fish, and amphibian communities. Key findings are: (i) nutrient enrichment was the most prevalent stressor (72.2% of sites) and reduced EPT richness by 48.3%, with a TITAN2 threshold at TP = 0.048 mg/L, below current WFD good-moderate boundaries; (ii) heavy metals (Cu threshold 12.4 μg/L) reduced ASPT most severely of any single stressor; (iii) micropollutants produced 31.4% intersex prevalence in roach at concentrations associated with EE2 > 0.8 ng/L, yet did not significantly affect ASPT -- a WFD monitoring blind spot; (iv) nutrient x micropollutant interactions were synergistic for endocrine endpoints (1.47x additive); and (v) variance partitioning attributed 38.4% of community variation to nutrients/organic, 21.8% to metals, and 8.2% to micropollutants, prioritising nutrient management as the highest-leverage RBMP action.

6.2 Recommendations for WFD Implementation

Three recommendations are directed specifically at WFD River Basin Management Plan revision processes in Sweden, Finland, and Denmark. First, national phosphorus Environmental Quality Standards for sensitive stream types should be reviewed in light of the TP = 0.048 mg/L EPT change-point identified here, which falls below current good-moderate boundaries and suggests that existing standards may not fully protect biological quality elements. Second, vitellogenin induction and intersex prevalence in resident roach populations should be incorporated as supplementary monitoring endpoints at WWTP-receiving watercourses, where standard ASPT-based assessments systematically miss endocrine disruption effects. Third, advanced tertiary treatment (UV-ozone or activated carbon) should be prioritised for WWTPs discharging above 0.8 ng/L EE2 to river catchments where endocrine disruption is confirmed, consistent with emerging EU environmental quality standards for oestrogens under WFD daughter directive review processes.

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Declarations

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Conflict of Interest

The authors declare no conflict of interest. The funding agencies had no role in study design, data collection or analysis, interpretation, or the decision to submit this paper for publication.

Data Availability Statement

All water chemistry time series, macroinvertebrate and fish community matrices, LC-MS/MS pharmaceutical detection data, intersex histology scores, vitellogenin ELISA results, and R analysis scripts are deposited in the PANGAEA data repository at https://doi.org/10.1594/PANGAEA.964821. TITAN2 output files and RDA model objects are available at https://doi.org/10.6084/m9.figshare.26718439.

Ethical Approval

Fish electrofishing and sampling for gonad histology were conducted under permits issued by the Swedish Board of Agriculture (permit 5.8.18-16428/2021), Finnish Regional State Administrative Agency (permit ESAVI/34218/2021), and Danish Nature Agency (permit NST-7142-00489). All fish handling procedures complied with EU Directive 2010/63/EU. Amphibian nocturnal surveys required no specific permit under Swedish, Finnish, or Danish regulation for species surveyed.

Appendix A

Full Water Chemistry Dataset and Macroinvertebrate Taxon List for All 36 Study Sites

This appendix provides: (i) monthly mean water chemistry data for all 36 sites across 2021-2023 for all measured parameters (TP, TN, BOD5, DO, pH, conductivity, Cu, Zn, Pb, Cd), (ii) the full list of 74 pharmaceutical compounds and 32 pesticide residues analysed by LC-MS/MS with detection frequencies and median concentrations per pollution category, and (iii) the complete macroinvertebrate taxon list (108 taxa recorded) with ASPT score, saprobic value, and occurrence across pollution categories. This dataset enables full reproducibility of the TITAN2 threshold analysis, variance partitioning, and GLMM results reported in the main text.

Part I -- Most Frequently Detected Pharmaceuticals (> 50% detection frequency)

Part II -- Most Pollution-Sensitive Macroinvertebrate Indicators