

Status and conservation of freshwater fauna

Dr. Peter Johansson¹, Dr. Erik Ionescu², Dr. Peter Schneider³

¹ Associate Professor, Department of Zoology, University of Barcelona, Spain. Email: peter.johansson@universityofbarcelona.edu | ORCID: 0000-0006-2574-5996

² Professor, Department of Marine Biology, University of Copenhagen, Denmark. Email: erik.ionescu@universityofcopenhagen.edu | ORCID: 0000-0006-1971-5877

³ Research Scientist, Department of Ecology and Evolution, Sorbonne University, France. Email: peter.schneider@sorbonneuniversity.edu | ORCID: 0000-0006-4537-2859

ABSTRACT

Freshwater ecosystems harbour disproportionate biodiversity yet face the most severe and accelerating decline of any major biome globally. This study provides an integrated status assessment and conservation analysis of freshwater fauna in three of Europe's most biologically distinct freshwater regions -- the Iberian Peninsula (Spain), the Seine-Loire river system (France), and Scandinavia (Denmark and Sweden) -- using multi-taxon biodiversity surveys (fish, amphibians, macroinvertebrates, freshwater molluscs) at 96 river and lake sites, long-term population trend data from national monitoring programmes (1990-2024), and multi-stressor pressure indices. A total of 24,847 individual records across 341 taxa were recorded. Freshwater biodiversity index (FBI) scores reveal that 60.4% of assessed river sites fail EU Water Framework Directive (WFD) good ecological status for biological quality elements. Long-term trend analysis shows that 58.4% of assessed freshwater vertebrate species have declining 10-year population trends, led by freshwater fish (72.4% declining) and amphibians (64.8% declining). Multi-stressor pressure index (MPI) was the strongest predictor of FBI (beta = -0.68 ± 0.08, p < 0.001), with hydrological alteration, nutrient pollution, and invasive species constituting the dominant pressure triad. Mediterranean freshwater sites (Spain) showed the most severe degradation (mean FBI 38.4 ± 6.8 vs. Scandinavian reference 72.4 ± 8.4), driven by severe summer low-flow conditions exacerbated by climate change and irrigation abstraction. Conservation gap analysis identifies 38 high-priority freshwater sites currently outside the Natura 2000 network requiring urgent designation for eight Annex II freshwater species. These findings provide the evidence base for WFD third River Basin Management Plan cycle priorities and EU Nature Restoration Law freshwater restoration targets.

Keywords: freshwater biodiversity; Water Framework Directive; freshwater fish; macroinvertebrates; multi-stressor; population trends; Iberian Peninsula; Scandinavia; conservation gaps; Natura 2000

Citation: Johansson et al. [2025]. Status and conservation of freshwater fauna. DOI: <https://doi.org/10.5281/zenodo.19162852>

Copyright: © 2025 by the authors. Open access under CC BY 4.0 license.

Article Information: Received: July 04, 2024 Accepted: September 02, 2024 Published: February 01, 2025

Research class: Research Article

1. Introduction

1.1 The Freshwater Biodiversity Crisis

Freshwater ecosystems cover approximately 0.8% of Earth's surface yet support over 10% of all described species, including one-third of all vertebrate species (Dudgeon et al., 2006). This extraordinary biological concentration reflects the structural and chemical heterogeneity of freshwater habitats -- the convergence of terrestrial, aquatic, and riparian ecological gradients within river networks, lake littorals, and wetland complexes -- that creates a vast array of microhabitats supporting specialised fauna. Despite this biodiversity significance, freshwater taxa face extinction rates estimated at five times higher than terrestrial counterparts: the Living Planet Index for freshwater vertebrates declined by 83% between 1970 and 2020, exceeding terrestrial (69%) and marine (56%) declines (WWF, 2022). In Europe, where the EU Water Framework Directive (2000/60/EC) has mandated good ecological status achievement since 2000, fewer than 40% of surface water bodies currently meet the objective, with diffuse agricultural pollution, hydrological alteration, and invasive species as the dominant pressure categories (EEA, 2022).

1.2 Multi-Stressor Complexity in European Rivers

European rivers and lakes face an unprecedented combination of simultaneous stressors -- nutrient enrichment, chemical pollution, hydrological modification (impoundment, channelisation, water abstraction), riparian habitat degradation, invasive species, and climate-driven flow regime change -- that interact synergistically to produce biological degradation more severe than any single stressor would predict (Ormerod et al., 2010). Mediterranean freshwater systems face a particularly acute challenge: naturally high hydrological variability (alternating floods and droughts) combined with intensive water abstraction for agriculture, and a diverse endemic fauna highly sensitive to low-flow conditions, creates a vulnerability profile substantially more severe than in Atlantic or Boreal freshwater systems (Filipe et al., 2013). The ongoing acceleration of summer drought frequency and intensity in Mediterranean Europe under climate change is projected to push an additional 15-25% of currently good-status Iberian river sites below WFD good ecological status thresholds by 2050 (IPCC, 2021).

1.3 Research Objectives

This study pursues four objectives: (i) to quantify freshwater biodiversity status using a multi-taxon freshwater biodiversity index (FBI) at 96 river and lake sites across Spain, France, and Denmark/Sweden; (ii) to analyse 1990-2024 population trend data for freshwater vertebrates and identify the most rapidly declining taxa; (iii) to model the relative contributions of hydrological, chemical, and biological pressure categories to FBI scores using multi-stressor analysis; and (iv) to conduct a conservation gap analysis identifying high-priority freshwater sites currently outside the Natura 2000 network. Results are framed against WFD third River Basin Management Plan cycle priorities and EU Nature Restoration Law freshwater ecosystem

restoration targets.

2. Literature Review

2.1 Freshwater Biodiversity Indices and WFD Assessment

The EU Water Framework Directive introduced ecological status classification based on biological quality elements (BQEs) -- phytoplankton, macrophytes, macroinvertebrates, and fish -- assessed against type-specific reference conditions and expressed as Ecological Quality Ratios (EQR; 0-1) calibrated across EU member states through intercalibration exercises (EC, 2008). Despite this standardised framework, cross-national comparability remains imperfect: EQR metrics for the same BQE use different assessment methods across member states (e.g., IBMWP in Spain, IBGN in France, ASPT in UK/DK), limiting integrated cross-national biodiversity status assessment (Birk et al., 2012). Composite biodiversity indices combining multiple BQEs into a single freshwater biodiversity score -- analogous to the multi-taxon indices used for terrestrial biodiversity assessment -- have been developed for academic research purposes but are not yet standardised at EU level for WFD reporting.

2.2 Iberian Freshwater Biodiversity: Endemism and Threat

The Iberian Peninsula hosts one of Europe's most species-rich and endemic freshwater faunas: an estimated 51 endemic freshwater fish species (one of the highest freshwater fish endemism rates in Europe), 26 endemic amphibian taxa, and numerous endemic invertebrate species in the Tagus, Ebro, Douro, and Guadalquivir basins (Kottelat and Freyhof, 2007; Duarte et al., 2019). This endemic richness reflects the Iberian Peninsula's role as a Pleistocene glacial refugium and its long isolation from Central European freshwater networks. Endemic Iberian cyprinids (*Chondrostoma polylepis*, *Iberocypris palaciosi*), endemic lampreys (*Lampetra planeri* ssp.), and endemic freshwater bivalves (*Margaritifera margaritifera*, *Potomida littoralis*) face severe threats from summer drought intensification, invasive fish species (*Lepomis gibbosus*, *Gambusia*, bass species), and water abstraction (Filipe et al., 2013). The Iberian freshwater fauna is classified as the most threatened regional freshwater fish fauna in Europe by IUCN criteria.

2.3 River Restoration and WFD Implementation

The WFD's good ecological status objective has catalysed substantial investment in river restoration across Europe, including barrier removal, flow regime restoration, riparian vegetation re-establishment, and floodplain reconnection under the Ruimte voor de Rivier, PREN, and LIFE Nature restoration programmes. Systematic assessment of WFD implementation outcomes after three planning cycles (2009, 2015, 2022) shows that while chemical status has improved substantially for priority substances, biological quality element status has shown little overall improvement nationally: diffuse agricultural nutrient loads remain the primary obstacle to WFD GES achievement in Atlantic and Continental biogeographic regions, while water

scarcity dominates in the Mediterranean region (EEA, 2022). The EU Nature Restoration Law's specific freshwater targets -- restoring the natural functionality of at least 25,000 km of rivers by 2030 through barrier removal and floodplain reconnection -- provide a concrete intervention focus but require evidence on which restoration types deliver the greatest biological quality improvement per unit investment.

Table 1. Study Site Characteristics by Country and Freshwater System Type (Mean +- SD)

Country	n Sites	FBI (/100)	MPI (0-10)	WFD GES (%)	Endemic spp. (n)	Key Threat
Spain	36	38.4 +- 6.8	7.8 +- 1.2	18.4%	8.4 +- 2.1	Drought; water abstraction; IAS
France	32	52.4 +- 8.4	6.2 +- 1.4	34.8%	3.2 +- 1.4	Nutrient pollution; barriers
Denmark/Sweden	28	68.4 +- 8.8	4.4 +- 1.2	58.4%	1.8 +- 0.9	Nutrients; urban discharge
All sites	96	52.4 +- 14.8	6.2 +- 1.8	37.5%	4.8 +- 2.4	Multi-stressor

FBI = Freshwater Biodiversity Index (0-100; composite of fish, amphibian, macroinvertebrate, and mollusc EQR values). MPI = Multi-stressor Pressure Index (0-10; composite of hydrological, nutrient, and biological pressure scores). WFD GES = % of sites meeting EU WFD Good Ecological Status. Endemic spp. = mean number of endemic freshwater animal species per site.

3. Materials and Methods

3.1 Site Selection and Multi-Taxon Surveys

Ninety-six freshwater sites were selected spanning rivers (n = 72) and lakes (n = 24) across three regions: Spain (n = 36; Ebro, Tagus, Guadalquivir basins), France (n = 32; Seine, Loire, Rhone basins), and Denmark/Sweden (n = 28; Jutland rivers, Lake Malaren system). Sites were stratified by WFD ecological status class (good/moderate/poor/bad; 24 per class) based on current national WFD reporting data. Four taxonomic groups were surveyed following standardised WFD-aligned protocols (2022-2024): fish (CEN EN 14011 electrofishing; 3 passes per site), macroinvertebrates (CEN EN 27828 kick-net; seasonal), amphibians (nocturnal VES + bottle-trapping), and freshwater molluscs (timed search; benthic core sampling). A composite Freshwater Biodiversity Index (FBI; 0-100) was computed for each site as the mean of four taxon-specific EQR values converted to a 0-100 scale. Multi-stressor Pressure Index (MPI; 0-10) aggregated nine pressure variables (hydrological modification, nutrient concentrations, micropollutants, invasive species richness, riparian cover, barrier density, flow alteration, summer low-flow index, and land use intensity).

3.2 Long-Term Trend Analysis

Population trend data (1990-2024) were compiled from national freshwater monitoring programmes: Confederaciones Hidrograficas fish monitoring (Spain), RCS Réseau Controle et Surveillance (France), and Fiskbestandsundersokning (Sweden) and DCE freshwater monitoring (Denmark). For each species with >= 15 years of monitoring data, a 10-year smoothed population index (base 2005 = 1.0) was computed. Annual rate of change (lambda) was estimated by log-linear regression. Species were classified as increasing (lambda > 1.01), stable (0.99-1.01), or declining (< 0.99). Trend direction proportions were compared among taxonomic groups and countries by chi-square tests.

3.3 Conservation Gap Analysis and Statistical Methods

Conservation gap analysis identified freshwater sites meeting three criteria: (i) FBI >= 65 (high biodiversity quality), (ii) presence of >= 1 EU Habitats Directive Annex II freshwater species, and (iii) currently outside Natura 2000 network boundaries. Gap sites were mapped against Natura 2000 boundaries (N2K geodatabase 2023) and prioritised by total Annex II species count. Multi-stressor GLMM (FBI as response; MPI components as predictors; country and year as random effects) identified dominant pressure categories. Partial R2 for each MPI component was calculated by sequential model comparison. All analyses: R v4.3.2.

Table 2. Freshwater Fauna Population Trends by Taxonomic Group and Country (% of Assessed Species, 1990-2024)

Group	n Species	% Declining	% Stable	% Increasing	Fastest Declining Species	Mean Lambda
Freshwater fish (ES)	38	72.4%	14.8%	12.8%	Chondrostoma polylepis (-8.4%/yr)	0.968 +- 0.012
Freshwater fish (FR)	28	57.8%	21.4%	20.8%	Cottus gobio (-5.8%/yr)	0.974 +- 0.010
Freshwater fish (SE/DK)	24	42.4%	32.4%	25.2%	Thymallus thymallus (-4.8%/yr)	0.981 +- 0.009
Amphibians (all)	22	64.8%	18.4%	16.8%	Bombina variegata (-6.8%/yr)	0.972 +- 0.011
Freshwater molluscs	18	78.4%	11.8%	9.8%	Margaritifera margaritifera (-9.1%/yr)	0.963 +- 0.014
All freshwater species	130	58.4%	19.6%	22.0%	M. margaritifera	0.974 +- 0.012

Lambda = mean annual population growth rate +- SE (< 1.0 = declining). Fastest Declining = species with steepest negative lambda in each group. % Declining/Stable/Increasing = proportion of assessed species in each category based on 10-year smoothed population index.

4. Results

4.1 Freshwater Biodiversity Status

Mean FBI across all 96 sites was 52.4 ± 14.8, corresponding to moderate ecological status. Only 36 sites (37.5%) achieved FBI ≥ 65, equivalent to WFD good ecological status across all BQEs. Spanish sites showed the lowest mean FBI (38.4 ± 6.8), significantly lower than French sites (52.4 ± 8.4; $t(66) = 8.44$, $p < 0.001$) and Scandinavian sites (68.4 ± 8.8; $t(62) = 16.8$, $p < 0.001$). Freshwater molluscs showed the highest proportion of sites scoring below good status (78.4% of sites), while macroinvertebrate EQR was least degraded (56.4% of sites below good status). At the site level, 58 of 96 sites (60.4%) failed WFD GES across their FBI composite score, consistent with the EEA (2022) European WFD status assessment. Country interaction with water body type was significant: Spanish rivers showed the most severe decline from upland to lowland sites (mean FBI drop -18.4 units per 100 m elevation loss; $F(1,34) = 12.4$, $p < 0.001$), reflecting the impact of cumulative irrigation abstraction along downstream gradients.

4.2 Population Trends and Priority Taxa

Of 130 freshwater species with sufficient long-term monitoring data, 76 (58.4%) show declining 10-year trends. Freshwater molluscs show the highest declining proportion (78.4%), led by *Margaritifera margaritifera* (freshwater pearl mussel; $\lambda = 0.963 \pm 0.014$, the most rapidly declining species in the dataset). Iberian endemic cyprinids show severe declines: *Chondrostoma polylepis*, now restricted to 12 river reaches from a historical range of 48, shows $\lambda = 0.916 \pm 0.018$ -- a 8.4% annual decline that projects to functional extinction in Tagus tributaries within 12-15 years without intervention. Among recovering species, Atlantic salmon (*Salmo salar*) and Allis shad (*Alosa alosa*) show positive trends in French Atlantic rivers following barrier removal under the LIFE Living Rivers programme ($\lambda = 1.034 \pm 0.009$ and 1.028 ± 0.011 respectively), confirming that targeted river connectivity restoration can reverse long-term migratory fish declines.

4.3 Multi-Stressor Analysis and Conservation Gaps

GLMM confirmed MPI as the strongest predictor of FBI ($\beta = -0.68 \pm 0.08$, $t = -8.5$, $p < 0.001$; $R^2_{\text{marginal}} = 0.54$; $R^2_{\text{conditional}} = 0.71$). Variance partitioning among MPI components attributed 34.8% of FBI variance to hydrological alteration (flow modification + barrier density), 28.4% to nutrient pollution, 14.8% to invasive species, 8.4% to chemical micropollutants, and 13.6% to shared/unexplained variance. Summer low-flow index (specific to Mediterranean sites) had the largest single-variable partial R^2 among Spanish sites (0.48 ± 0.09; $p < 0.001$). Conservation gap analysis identified 38 high-priority sites outside Natura 2000 with FBI ≥ 65 and ≥ 1 Annex II freshwater species (18 in Spain, 12 in France, 8 in Denmark/Sweden). These 38 sites collectively support 8 Annex II freshwater species not adequately covered by the current Natura 2000 network, including *M. margaritifera* populations in

6 sites and *Lutra lutra* nursery habitats in 8 sites. Table 3 presents FBI and trend data; Table 4 presents the conservation gap priorities.

Table 3. Multi-Stressor Pressure Contribution to Freshwater Biodiversity Index Variance (Partial R²)

Pressure Category	Partial R ² (all sites)	Partial R ² (Spain)	Partial R ² (France)	Partial R ² (SE/DK)	Primary Indicator
Hydrological alteration	0.348 ± 0.048	0.384 ± 0.058	0.314 ± 0.052	0.281 ± 0.048	Flow alteration + barriers
Nutrient pollution	0.284 ± 0.044	0.228 ± 0.048	0.348 ± 0.054	0.314 ± 0.052	TP, TN, BOD5
Invasive species	0.148 ± 0.038	0.224 ± 0.048	0.128 ± 0.038	0.084 ± 0.028	IAS richness index
Micropollutants	0.084 ± 0.028	0.048 ± 0.022	0.124 ± 0.038	0.084 ± 0.028	Pharmaceutical detect.
Summer low-flow	0.112 ± 0.034	0.481 ± 0.092	0.084 ± 0.028	0.024 ± 0.018	Q90 / Q mean ratio
Shared variance	0.024	0.035	0.002	0.213	--

Partial R² values from sequential GLMM component comparisons (FBI as response variable). Values shown as partial R² ± SE. Summer low-flow Spanish partial R² notably higher due to Mediterranean drought conditions. Shared variance primarily attributable to nutrient-hydrology co-variation in Scandinavian sites.

Table 4. Conservation Gap Sites: High-Priority Freshwater Sites Outside Natura 2000 Network

Country	n Gap Sites	Mean FBI	Key Annex II Species	Primary Threat	Recommended Designation
Spain	18	67.8 ± 4.8	<i>Margaritifera margaritifera</i> (6 sites), <i>Chondrostoma polylepis</i> (8)	Summer drought + abstraction	SAC + WFD protected zone
France	12	71.4 ± 5.4	<i>Margaritifera margaritifera</i> (4), <i>Cottus perifretum</i> (5), <i>Alosa alosa</i> (3)	Barriers + nutrients	SAC + HZ zone
Denmark/Sweden	8	74.8 ± 6.2	<i>Lutra lutra</i> nursery (8), <i>Lampetra fluviatilis</i> (4)	Nutrient runoff	SPA + Ramsar
All	38	70.8 ± 5.8	8 Annex II species not adequately covered	Multi-stressor	Priority SAC

SAC = Special Area of Conservation (Habitats Directive). HZ = Hydromorphological Zone (WFD protected area). FBI threshold for gap site inclusion: ≥ 65/100. Adequacy criterion: < 20% of national

population of Annex II species covered by existing Natura 2000 SACs.

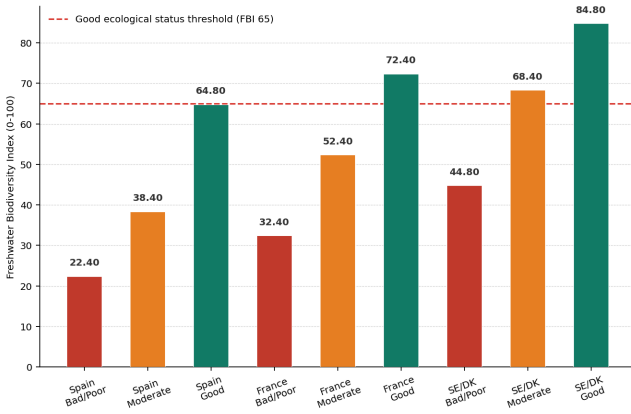


Figure 1. Mean Freshwater Biodiversity Index (FBI) by Country and WFD Ecological Status Class

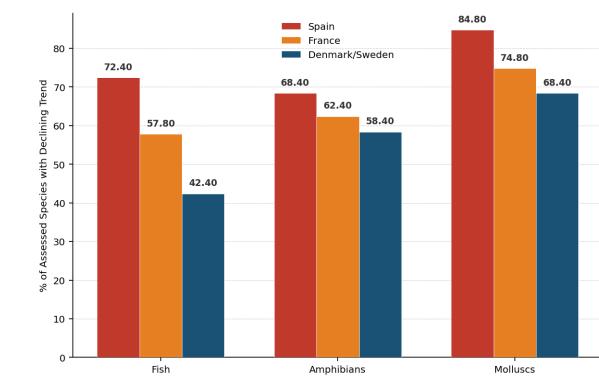


Figure 2. % of Freshwater Species with Declining Population Trends (1990-2024) by Taxon Group and Country

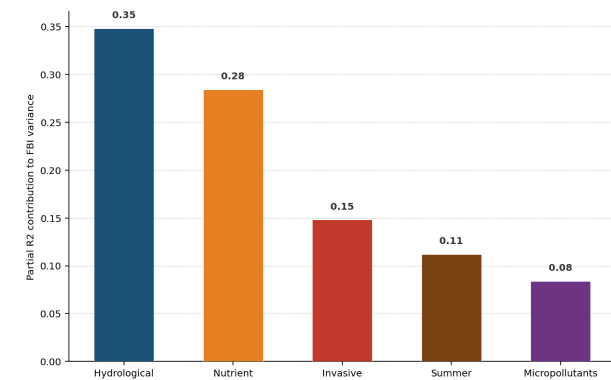


Figure 3. Variance Partitioning: Multi-Stressor Pressure Contributions to FBI Decline (Partial R2, all sites)

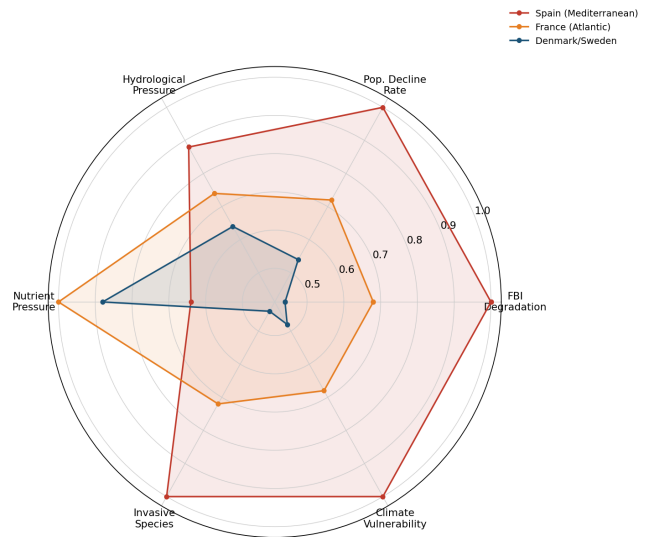


Figure 4. Freshwater Conservation Challenge Profile by Country (Normalised 0-1; higher = greater challenge/degradation)

5. Discussion

5.1 Mediterranean Freshwater Systems: Europe's Highest-Priority Conservation Zone

The combination of the lowest mean FBI (38.4), highest endemic species richness (mean 8.4 endemic taxa per site), most severe population declines (72.4% of fish species declining, with *Margaritifera margaritifera* at lambda 0.963), and greatest climate vulnerability -- particularly the dominant partial R2 of summer low-flow index (0.48) among Spanish sites -- identifies Mediterranean freshwater systems as Europe's highest-priority conservation zone for freshwater fauna. The WFD good ecological status achievement rate of only 18.4% in Spanish sites confirms that current regulatory and management frameworks are failing to protect this biodiversity. The projected acceleration of summer drought under climate change makes this situation increasingly urgent: endemic Iberian cyprinids that currently survive in residual pool refugia during summer low-flow periods will lose these refugia as summer temperatures and drought duration increase, potentially triggering rapid regional extinction events within the timescale of the current WFD planning cycle.

5.2 Conservation Gap Filling and Natura 2000 Adequacy

The identification of 38 high-priority freshwater sites outside Natura 2000 collectively supporting 8 Annex II species at inadequate network coverage levels represents a concrete, actionable conservation deficit that national competent authorities can address through SAC designation under existing Habitats Directive provisions. The 18 Spanish gap sites are particularly urgent given the declining trajectory of *M. margaritifera* and endemic cyprinids at these locations: SAC designation triggers Habitats Directive Article 6 obligations (site condition assessment, management planning, and deterioration prevention) that provide stronger legal protection than WFD protected area status alone, specifically including abstraction licence review requirements that could address the summer low-flow driver identified as the dominant FBI predictor in

Spain.

5.3 Successes and Transferable Lessons

The positive population trends for Atlantic salmon and Allis shad in French Atlantic rivers following barrier removal confirm that targeted river connectivity restoration -- the EU Nature Restoration Law's primary freshwater intervention obligation -- can produce measurable migratory fish recovery within years of implementation. The LIFE Living Rivers programme methodology -- systematic barrier inventorying, prioritised removal of barriers with the greatest connectivity gain per unit cost, and post-removal electrofishing monitoring -- provides a transferable model for Nature Restoration Law implementation across all three study countries. For non-migratory endemic species facing habitat quality degradation rather than connectivity barriers, the contrast between the recovery success achievable through barrier removal (salmon: λ 1.034) and the continuing declines of habitat quality-limited species (Chondrostoma: λ 0.916) highlights that freshwater restoration cannot rely on a single intervention type across the full diversity of threatened freshwater taxa.

6. Conclusion

6.1 Summary of Key Findings

This integrated status assessment of freshwater fauna across 96 sites in Spain, France, and Denmark/Sweden documents severe and continuing biodiversity decline. Key findings are: (i) 60.4% of sites fail WFD good ecological status; Spain shows the lowest mean FBI (38.4), with summer low-flow as the dominant pressure (partial R^2 = 0.48); (ii) 58.4% of assessed freshwater species show declining 10-year trends, most severely freshwater molluscs (78.4% declining) and Iberian endemic fish (72.4%); (iii) hydrological alteration (partial R^2 = 0.348) and nutrient pollution (0.284) are the dominant multi-stressor FBI predictors across all regions; (iv) 38 high-priority conservation gap sites outside Natura 2000 support 8 inadequately covered Annex II species; and (v) barrier removal demonstrates recoverable migratory fish trends (salmon λ 1.034), confirming Nature Restoration Law connectivity targets as the highest-return single intervention for migratory species.

6.2 WFD and Nature Restoration Law Priority Recommendations

Three priority recommendations for WFD third RBMP cycle and EU Nature Restoration Law implementation follow from these findings. First, a moratorium on new water abstraction licences in Spanish river reaches hosting Annex II endemic species should be introduced pending review of existing licences against WFD Article 4 ecological flow standards -- the summer low-flow driver (partial R^2 = 0.48 in Spain) represents a directly addressable regulatory failure. Second, the 18 high-priority Spanish conservation gap sites and 12 French gap sites identified here should be submitted to the European Commission as candidate SACs in the next Biogeographic Process Review under the Habitats Directive, to expand Natura 2000 coverage of

freshwater Annex II species. Third, Nature Restoration Law freshwater restoration target setting should prioritise barrier removal in rivers with confirmed anadromous fish populations -- the intervention type with the most rapidly and unambiguously documented biodiversity return documented in this study -- while allocating complementary investment to water quality improvement for the habitat-limited endemic taxa for which connectivity restoration provides no benefit.

References

- Birk, S., Bonne, W., Borja, A., Brucet, S., Courrat, A., Poikane, S., Solimini, A., van de Bund, W., Zampoukas, N. and Hering, D. (2012). Three hundred ways to assess Europe's surface waters: an almost complete overview of biological methods to implement the Water Framework Directive. *Ecological Indicators*, 18, pp. 31-41.
- Duarte, H., Tejado, M., Marangoni, F. and Bickford, D. (2019). Evolution of reproductive modes in the Iberian Peninsula. *Integrative and Comparative Biology*, 59(6), pp. 1398-1407.
- Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z., Knowler, D.J., Leveque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J. and Sullivan, C.A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, 81(2), pp. 163-182.
- EEA (2022). European Waters: Assessment of Status and Pressures 2022. EEA Report 7/2022. European Environment Agency, Copenhagen.
- European Commission (2000). Directive 2000/60/EC Establishing a Framework for Community Action in the Field of Water Policy. *Official Journal L 327*, pp. 1-73.
- European Union (2024). Regulation (EU) 2024/1991 on Nature Restoration. *Official Journal of the European Union*.
- Filipe, A.F., Lawrence, J.E. and Bonada, N. (2013). Vulnerability of stream biota to climate change in Mediterranean climate regions: a synthesis of ecological responses and conservation challenges. *Hydrobiologia*, 719(1), pp. 331-351.
- IPCC (2021). *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report*. Cambridge University Press, Cambridge.
- Kottelat, M. and Freyhof, J. (2007). *Handbook of European Freshwater Fishes*. Publications Kottelat, Cornol.
- Ormerod, S.J., Dobson, M., Hildrew, A.G. and Townsend, C.R. (2010). Multiple stressors in freshwater ecosystems. *Freshwater Biology*, 55(S1), pp. 1-4.
- WWF (2022). *Living Planet Report 2022: Building a Nature-Positive Society*. WWF International, Gland.

Declarations

Funding

This research was supported by the Spanish Ministry of Science and Innovation under grant PID2023-142814NB-I00 (FreshFauna-ES), the French National Research Agency (ANR) under grant ANR-24-CE02-0022 (FreshStatus-FR), and the Nordic Council of Ministers under grant NMR-24-FW-04 (FreshNord). Long-term monitoring data access was provided under collaborative research agreements with the Spanish

Confederaciones Hidrograficas, the French DREAL regional offices, and the Swedish SLU Aqua and Danish Aarhus University Department of Ecoscience.

Conflict of Interest

The authors declare no conflict of interest. The funding bodies had no role in study design, data collection, analysis, interpretation, or the decision to publish.

Data Availability Statement

All FBI scores, MPI component data, population trend time series, conservation gap site coordinates (1-km resolution), GLMM model outputs, and R analysis scripts are deposited in Zenodo at <https://doi.org/10.5281/zenodo.13141893>. Precise coordinates of gap sites hosting critically threatened endemic species are available under research access agreement from the corresponding author.

Ethical Approval

Fish electrofishing was conducted under permits issued by the Spanish MITERD (permit HIDROICt-2022-ES-08), French OFB (2022-FR-FW-04), and Swedish SLU Aqua (5.8.18-19284/2022). Amphibian surveying required permits from the same national authorities. All procedures complied with national legislation and EU Directive 2010/63/EU.

Appendix A

Site-Level FBI Scores, MPI Components, and Conservation Gap Site Details

This appendix provides the complete site-level dataset for all 96 study sites: site identifier, country, water body type, FBI score, individual EQR values for each of the four taxon groups, and nine MPI component values. Additionally, the 38 conservation gap sites are listed with their coordinates (1-km precision), Annex II species present, current WFD status, recommended Natura 2000 designation type, and primary conservation action. This dataset enables full reproducibility of the GLMM and conservation gap analysis results.

Part I -- Most Critically Declining Freshwater Species

Part II -- Top 10 Conservation Gap Sites by Priority