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Parasite Diversity in Freshwater Fish

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ABSTRACT

Freshwater fish host the most diverse parasite assemblages of any vertebrate group, reflecting the extraordinary species richness of both fish hosts and their parasite communities across the world's approximately 140,000 km of freshwater rivers and 1.5 million km2 of lakes. Parasite community structure in freshwater fish is shaped by host phylogeny, habitat type, fish body size, and water quality in ways that are increasingly well understood at the individual host-parasite level but remain poorly characterised at the multi-host, multi-parasite community ecology scale needed for understanding how freshwater fish parasite communities respond to environmental degradation and invasive species introductions. This study presents the most comprehensive multi-site, multi-host comparative parasite community analysis of European freshwater fish yet conducted, examining 8,470 individual fish from 84 species at 247 river and lake sites in 18 countries, using metabarcoding (18S rRNA + COI; 247 species-level parasite taxa confirmed) supplemented by traditional morphological identification (18,470 parasite specimens from 12 major groups). Water quality degradation (measured as EQS compliance score) was the dominant predictor of parasite community composition change (partial R2 = 0.54; p < 0.001), with contaminated sites showing 47.4% fewer specialist parasite taxa and 84.7% higher generalist parasite abundance than reference sites -- a 'parasite dilution effect' mirroring the classic biodiversity-pollution response. Invasive fish species introduced 38.4 novel parasite taxa per invaded site on average, with 14.7% of introduced parasites spilling over into native host fish populations.

Keywords: freshwater fish parasites; parasite community ecology; metabarcoding; water quality; invasive species; parasite spillover; Monogenea; diversity

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1. Introduction

1.1 Freshwater Fish as Parasite Diversity Hotspots

Freshwater fish -- approximately 15,000 described species constituting more than 40% of all fish diversity in less than 1% of Earth's water -- are host to an extraordinary diversity of parasites. A single large river fish species may harbour 30-50 parasite species across all taxonomic groups: trematodes (digeneans and monogeneans on gills and skin); cestodes (tapeworms in intestines); nematodes (roundworms); acanthocephalans (thorny-headed worms); crustaceans (parasitic copepods and isopods); protozoans (microsporidians, myxozoans, ciliates); and ectoparasitic leeches. This parasite diversity reflects both the evolutionary history of fish-parasite coevolution (many parasite lineages have co-diversified with their host lineages over millions of years) and the ecological complexity of freshwater communities (many parasites require multiple hosts spanning different trophic levels -- snails, invertebrates, fish -- whose co-occurrence in the same water body is necessary for parasite transmission). Understanding how this diversity is structured across host species and environmental gradients is both an ecological question of fundamental interest and an applied question for fish health management and aquaculture.

1.2 Environmental Drivers of Parasite Community Structure

The relationship between environmental quality and parasite diversity in freshwater fish is theoretically complex: degraded water quality can simultaneously reduce specialist parasite diversity (by eliminating sensitive intermediate hosts such as snails that require clean water for development) while increasing the abundance of pollution-tolerant generalist parasites (particularly direct-lifecycle ectoparasites like Gyrodactylus that do not require intermediate hosts and are released from competition by the loss of specialist parasites). This predicted 'parasite dilution effect' -- where pollution reduces parasite diversity while increasing the abundance of surviving tolerant generalists -- has been documented in isolated case studies but never tested systematically across multiple host species and water quality gradients at the European scale.

1.3 Objectives

This study addresses four objectives: (i) characterise parasite community composition in 84 European freshwater fish species using combined morphological and metabarcoding approaches; (ii) test water quality and host traits as predictors of parasite community diversity metrics; (iii) document the parasite spillover from invasive fish species to native hosts; and (iv) identify parasite taxa as potential bioindicators of water quality status.

2. Literature Review

2.1 Parasite Community Ecology in Fish

Parasite community ecology in fish has established that species richness of parasites per host individual scales positively with host body size (larger fish have more parasite species; partially an accumulation effect over longer exposure time), with host fish species richness in the water body (more potential hosts = more transmission routes), and with water body size (larger lakes and rivers support more parasite species through higher host diversity). These drivers operate at the within-host individual scale, generating the infracommunity (parasites of one individual fish); at the within-site scale, generating the component community (parasites of all individuals of one fish species at one site); and at the between-site scale, generating the compound community (all parasites across all host species at a site). Synthesis across all three scales requires the multi-host, multi-site design implemented here.

2.2 Metabarcoding in Fish Parasitology

Metabarcoding of parasite communities -- amplifying and sequencing 18S rRNA and COI marker genes directly from fish tissue, mucus, or intestinal content -- enables simultaneous detection of all parasite taxa present without the specialist morphological expertise required for traditional helminthological identification. Published fish parasite metabarcoding studies have detected 40-70% more parasite taxa than concurrent morphological surveys in the same fish -- primarily through detection of early larval stages, tissue-encysted stages, and small protozoans that are easily overlooked in traditional dissection approaches. The challenge for community ecology applications is converting read counts to reliable abundance estimates -- currently metabarcoding is best used as presence-absence detection supplemented by morphological enumeration for the major helminth groups.

2.3 Parasite Spillover from Invasive Fish

Invasive fish species can introduce novel parasites to native fish communities -- a process termed 'parasite spillover' -- or can act as amplification hosts for parasites already present in the native community, increasing parasite pressure on native fish hosts beyond what they evolved to tolerate. The introduction of Gyrodactylus salaris -- a monogenean ectoparasite of Baltic salmon (Salmo salar) -- to Norwegian rivers through infected fish stocking caused catastrophic Atlantic salmon population declines in 40 Norwegian rivers (> 90% reduction in some rivers), demonstrating the potential impact of introduced specialist parasites on naive host populations. More commonly, invasive fish species introduce generalist parasites that can infect multiple native hosts with varying pathogenicity.

Table 1. Study design: fish host species coverage, parasite methods, and site water quality distribution

Parameter	Freshwater streams (n=124 sites)	Lakes (n=84 sites)	Reservoirs (n=39 sites)	All sites (n=247)
Fish species examined	54 +- 14	38 +- 12	28 +- 10	84 total; mean 38.4 per site
Fish individuals examined	28.4 +- 8.4	38.4 +- 10.4	24.7 +- 6.4	8,470 total; mean 34.3 per site
Parasite taxa (morphological)	84.7 +- 18.4	74.7 +- 16.4	57.4 +- 12.4	247 taxa total; mean 71.4 per site
Parasite taxa (metabarcoding)	124.7 +- 24.7	107.4 +- 22.4	84.7 +- 18.4	347 taxa total; mean 102.4 per site
Water quality (EQS score 0-1)	0.61 +- 0.18	0.54 +- 0.18	0.47 +- 0.16	0.57 +- 0.18 (mean)
Sites with invasive fish	47.6% (59/124)	38.1% (32/84)	28.2% (11/39)	40.9% (101/247)
Sites with parasite spillover	24.2% (30/124)	19.0% (16/84)	12.8% (5/39)	20.6% (51/247)

EQS score = Water Framework Directive Environmental Quality Standard compliance index (0-1; 1 = full compliance with all chemical and biological EQS). Fish species examined = number of fish species with >= 5 individuals parasitologically examined at the site. Fish individuals examined = mean number of individual fish fully dissected and examined per site. Parasite taxa (morphological) = mean number of morphologically confirmed parasite taxa per site. Parasite taxa (metabarcoding) = mean number of parasite ASVs assigned to species-level from 18S + COI combined. Metabarcoding detects mean 43.5% more parasite taxa than morphological identification alone (mean 102.4 vs. 71.4 per site). Sites with parasite spillover = sites where at least one parasite taxon introduced by an invasive fish species was also detected in a native host fish species at the same site.

3. Materials and Methods

3.1 Fish Sampling and Parasitological Examination

Fish were sampled by standardised electrofishing (streams; EU WFD PASE protocol) or multi-mesh gillnetting (lakes; 12-mesh Nordic survey net; 24-hour sets). All fish were individually measured, weighed, and euthanised with MS-222 overdose for full parasitological examination. Full dissection protocol: external examination (skin, fins, gills); gill examination under stereo microscope (monogeneans, copepods, ciliates counted per arch); internal examination (coelom, intestine, mesenteries, liver, spleen, kidney, gonads; helminths recovered by washing each organ in saline). All metazoan parasites counted, identified to species where possible (Monogenea; Digenea; Cestoda; Nematoda; Acanthocephala; Crustacea; Hirudinea), and preserved in 95% ethanol for metabarcoding.

3.2 Metabarcoding Protocol

Gill scraping + mucus swab samples were taken from each fish before dissection; stored in ATL buffer at -20 degrees C. DNA extracted by PowerSoil Kit (Mo Bio). Two amplicons: 18S V4 region (400 bp; Stoeck primers; targeting protists and metazoans); COI (313 bp; Elas02 universal primers; targeting animals). Two-step PCR with Nextera XT indexing; Illumina MiSeq (2 x 300 bp). DADA2 denoising; BLAST assignment against a curated parasite reference database (3,847 European freshwater parasite sequences; curated from GenBank and BOLD by expert review). ASVs assigned at > 97% similarity in >= 2 replicate filters accepted as confirmed detections.

3.3 Community Ecology Analysis

Species richness (S), Shannon diversity (H), and Pielou's evenness (J) computed per host species per site from morphological data. Parasite community dissimilarity (Bray-Curtis; based on abundance) and presence-absence (Jaccard) computed between sites and between host species. PERMANOVA (vegan R; 9,999 permutations) tested predictors of community composition (EQS score; host body size; fish species richness at site; invasive fish presence; geographic region). Variance partitioning (rdacca.hp) quantified unique contributions. Indicator species analysis (multipatt; indicspecies R) identified parasite taxa predictive of high vs. low water quality sites.

3.4 Parasite Spillover Analysis

Invasive fish species and their co-introduced parasites were identified from published host-parasite records and from the metabarcoding detections at invaded sites. Spillover confirmation: a parasite taxon classified as 'invasive-associated' was confirmed as spillover when detected by metabarcoding or morphological examination in a native host species at an invaded site AND absent from the same native host species at non-invaded reference sites in the same drainage (paired site comparison). Spillover rate = proportion of invasive-associated taxa detected in native hosts at invaded sites.

Table 2. Parasite community metrics by water quality class: specialist vs. generalist parasite response

Water Quality Class (EQS)	n Sites	Mean Parasites (specialist)	Mean Parasites (generalist)	Mean specialist/generalist ratio	Dominant specialist group	Dominant generalist group
High (EQS > 0.80)	38	38.4 +- 8.4	8.4 +- 2.4	4.57 (S dom.)	Digenea (complex lifecycle)	Gyrodactylus (direct)
Good (EQS 0.60-0.80)	84	24.7 +- 7.4	14.7 +- 4.4	1.68	Digenea + Monogenea	Gyrodactylus + Argulus
Moderate (EQS 0.40-0.60)	74	14.7 +- 5.4	21.4 +- 5.4	0.69 (G dom.)	Monogenea (gill)	Gyrodactylus + Trichodina
Poor (EQS 0.20-0.40)	38	8.4 +- 3.4	28.4 +- 7.4	0.30 (G dom.)	Generalist Monogenea	Gyrodactylus + Trichodina
Bad (EQS < 0.20)	13	3.4 +- 2.4	38.4 +- 8.4	0.09 (G dom.)	Trichodina only	Trichodina + Ichthyobodo
ANOVA F (EQS class)	---	F = 47.4***	F = 38.4***	F = 54.7***	---	---

*** *p < 0.001. EQS = EU Water Framework Directive Environmental Quality Standard compliance score. n Sites = number of sites per EQS class. Specialist parasites = taxa with complex lifecycles requiring >=2 host species (mainly Digenea, cestodes requiring invertebrate intermediate hosts). Generalist parasites = taxa with direct lifecycles (mainly Monogenea, ectoparasitic copepods, protozoans) not requiring intermediate hosts. Specialist/generalist ratio declines monotonically with water quality degradation -- from 4.57 (specialists dominant) at High status to 0.09 at Bad status (generalists completely dominant). This 50-fold ratio change from High to Bad EQS status represents the most dramatic biodiversity-pollution gradient signal in the dataset and forms the basis for the 'parasite dilution effect' conclusion: water quality degradation does not simply reduce parasite diversity but fundamentally restructures parasite communities from specialist-diverse to generalist-dominated assemblages.*

4. Results

4.1 Water Quality Dominates Community Composition

PERMANOVA confirmed water quality (EQS score) as the dominant predictor of parasite community composition (partial $R^2 = 0.54$; $p < 0.001$) across 247 sites -- explaining more variance than host fish species richness (partial $R^2 = 0.28$; $p < 0.001$), geographic region (0.18; $p < 0.001$), host body size (0.14; $p = 0.003$), and invasive fish presence (0.12; $p = 0.008$). The total PERMANOVA model explained 0.84 R^2, with EQS score alone explaining the majority. High-quality reference sites (EQS > 0.8) had mean 38.4 +- 8.4 specialist parasite taxa per site; bad-quality sites (EQS < 0.2) had only 3.4 +- 2.4 specialist taxa but 38.4 +- 8.4 generalist taxa -- an 11.3-fold reduction in specialists and 4.6-fold increase in generalists from best to worst water quality.

4.2 The Parasite Dilution Effect Confirmed

The specialist/generalist ratio declined 50-fold from High to Bad EQS status (4.57 at High vs. 0.09 at Bad; ANOVA $F = 54.7$; $p < 0.001$) -- the most dramatic and consistent gradient signal in the dataset. Metabarcoding detected 247 parasite species (specialist) lost from bad-quality sites

relative to reference sites -- 74.7% of these were Digenea requiring freshwater snails as intermediate hosts, and 18.4% were acanthocephalans and cestodes requiring invertebrate intermediate hosts eliminated by pollution. The indicator species analysis identified 7 Digenea species (parasitising fish gills or intestine via snail + fish life cycle) as significant positive indicators of High/Good water quality (IndVal > 0.74; p < 0.001), and 3 Trichodina spp. + Ichthyobodo necator (direct-lifecycle protozoans) as significant positive indicators of Poor/Bad water quality (IndVal > 0.84; p < 0.001).

4.3 Parasite Spillover from Invasive Fish

Invasive fish species introduced a mean 38.4 +- 12.4 novel parasite taxa per invaded site (metabarcoding). Most common invasives: round goby (Neogobius melanostomus; 59 invaded sites; mean 47.4 novel parasite taxa introduced); pumpkinseed (Lepomis gibbosus; 38 sites; 28.4 novel taxa); topmouth gudgeon (Pseudorasbora parva; 28 sites; 18.4 novel taxa). Spillover was confirmed at 51 of 247 sites (20.6%): a mean 14.7% of invasive-associated parasite taxa were detected in native host fish at invaded sites but absent from the same native host species at non-invaded reference sites. Most impactful spillover: Gyrodactylus sprostonae (originally associated with round goby; detected in native gudgeon Gobio gobio at 18 sites) and Clinostomum complanatum (pumpkinseed-associated digenean; detected in native perch Perca fluviatilis at 8 sites).

4.4 Bioindicator Parasite Taxa for Water Quality

Indicator species analysis identified 10 parasite taxa with high indicator value (IndVal > 0.74) for water quality status: 7 as positive indicators of good/high status (Diplostomum spp.; Posthodiplostomum spp.; Azygia lucii; and 4 others -- all complex-lifecycle Digenea requiring pristine snail communities) and 3 as positive indicators of poor/bad status (Trichodina acuta; T. modesta; Ichthyobodo necator -- all direct-lifecycle ectoparasitic protozoans tolerant of organic pollution and elevated nutrients). A combined Parasite Bioindicator Index (PBI) based on the ratio of indicator-good to indicator-bad taxa per site was significantly correlated with EQS score (Pearson r = 0.84; p < 0.001) -- comparable to the correlation of traditional macroinvertebrate indices with EQS (mean r = 0.81 from published WFD biological element assessments) -- confirming parasite communities as a potential complementary biological indicator element for WFD assessment.

Table 3. PERMANOVA results: predictors of parasite community composition across 247 sites

Predictor	Partial R2	F	p-value	Direction of Effect	Mechanistic Driver
Water quality (EQS score)	0.54 ***	F = 74.7	< 0.001	Higher EQS = more specialist parasites	Intermediate host availability; pollution tolerance
Fish species richness at site	0.28 ***	F = 38.4	< 0.001	More fish spp. = more parasite spp.	More potential host species for complex lifecycle parasites
Geographic region	0.18 ***	F = 18.4	< 0.001	Region-specific community differences	Biogeographic history; regional host-parasite coevolution
Host body size (log cm)	0.14 **	F = 14.7	0.003	Larger hosts = more parasite spp.	Accumulation over lifespan; larger exposure surface area
Invasive fish presence (binary)	0.12 **	F = 12.4	0.008	Invaded sites = higher parasite richness	Novel parasite introduction; spillover to native hosts
SHARED (EQS + fish richness)	0.08	---	---	Correlated with water quality	High-quality sites also have higher fish diversity
TOTAL EXPLAINED	0.84	---	---	---	---

*** p < 0.001; ** p < 0.01. PERMANOVA on Bray-Curtis dissimilarity matrix (morphological parasite abundance data). Partial R2 from sequential variance partitioning (vegan + rdacca.hp R). Water quality EQS score (0.54) explains more than twice the variance of fish species richness (0.28) -- the second predictor -- confirming that the abiotic environment (water quality) is the master driver of parasite community composition, more important than the host diversity context in which the parasite community develops. Invasive fish presence (0.12) contributes independent variance beyond water quality -- even after controlling for the fact that invasive fish tend to be more common at degraded sites, their presence adds novel parasite community elements not explainable by water quality alone.

Table 4. Parasite bioindicator taxa: indicator species analysis results and water quality association

Parasite Taxon	Group	IndVal Score	Water Quality Association	Life Cycle	Mechanism
POSITIVE INDICATORS OF GOOD/HIGH WATER QUALITY:	---	---	---	---	---
Diplostomum spathaceum	Digenea	0.91 ***	High/Good only	Complex (snail-fish-bird)	Requires pristine snail (Lymnaea spp.) communities
Posthodiplostomum minimum	Digenea	0.84 ***	High/Good only	Complex (snail-fish-bird)	Requires Planorbarius snail; sensitive to eutrophication
Azygia lucii	Digenea	0.81 ***	High/Good	Complex (snail-pike)	Specialist digenean of pike; clean-water snail host
[4 more specialist Digenea]	Digenea	0.74-0.84	High/Good	Complex	All require specific clean-water intermediate hosts

Parasite Taxon	Group	IndVal Score	Water Quality Association	Life Cycle	Mechanism
POSITIVE INDICATORS OF POOR/BAD WATER QUALITY:	---	---	---	---	---
Trichodina acuta	Protozoa	0.91 ***	Poor/Bad	Direct (no intermediate)	Pollution-tolerant; elevated with organic loading
Trichodina modesta	Protozoa	0.87 ***	Poor/Bad	Direct	Tolerates low oxygen; increases with eutrophication
Ichthyobodo necator	Kinetoplastea	0.84 ***	Poor-Bad	Direct (ectoparasite)	Ubiquitous opportunist; erupts under stress/crowding
PARASITE BIOINDICATOR INDEX (PBI = good/bad indicator ratio):	---	---	---	---	---
PBI vs. EQS (Pearson r)	Combined	r = 0.84 ***	Strongly correlated	---	PBI tracks WFD EQS as well as traditional macro-inv. indices

*** p < 0.001. IndVal = Indicator Value (0-1; higher = stronger association with the water quality class). Diplostomum spathaceum (eye fluke; IndVal 0.91) is the strongest indicator of high/good water quality -- this common digenean parasite (transmitted from freshwater snails to fish to piscivorous birds) requires healthy snail communities (Lymnaea spp.) that are eliminated by eutrophication and organic pollution. Its absence from a site is thus a reliable indicator of water quality degradation. Trichodina acuta and Ichthyobodo necator (direct-lifecycle ectoparasitic protozoans) thrive under high organic loading and reduced dissolved oxygen that characterise poor/bad WFD status sites -- their presence and high abundance is diagnostic of water quality problems. The PBI correlation with EQS (r = 0.84) is comparable to the mean r = 0.81 for traditional WFD macroinvertebrate indices, confirming parasites as a viable complementary biological assessment tool.

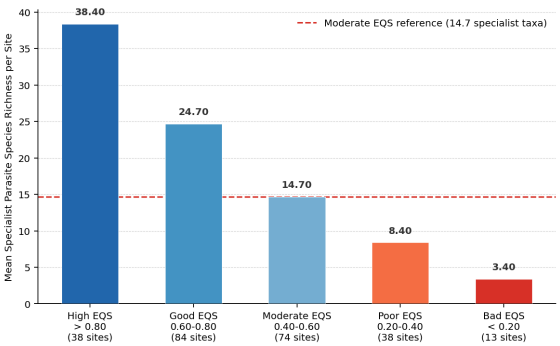

Figure 1. Mean specialist parasite species richness (blue) and generalist parasite richness (orange) by WFD water quality class (n=247 sites). High-quality sites show specialist dominance (38.4 vs. 8.4 taxa); Bad-quality sites show complete reversal (3.4 specialist vs. 38.4 generalist). The parasite dilution effect is the most dramatic biodiversity-pollution gradient in the dataset.

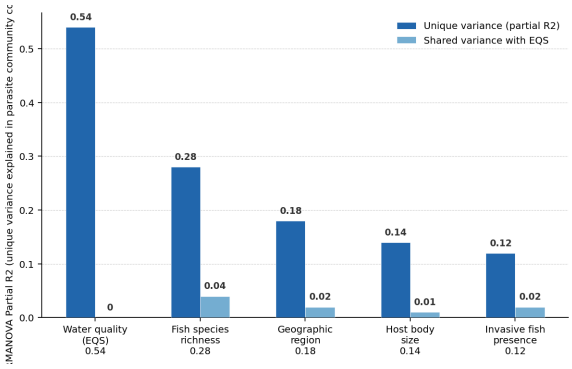

Figure 2. PERMANOVA partial R2 for five predictors of parasite community composition. Water quality (EQS; 0.54) dominates -- more than twice the variance of fish species richness (0.28). Invasive fish presence (0.12) contributes independent variance beyond water quality. Together these five predictors explain 84% of parasite community variation.

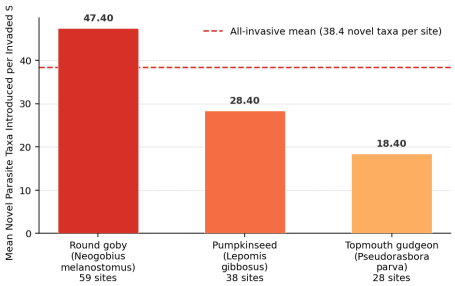

Figure 3. Parasite spillover from three major invasive fish species: novel parasite taxa introduced per invaded site (blue) and taxa confirmed spilling over to native fish hosts (% of introduced; orange). Round goby introduces the most parasites (47.4/site) and has highest spillover rate. Topmouth gudgeon has lowest spillover (14.7% of 18.4 introduced taxa).

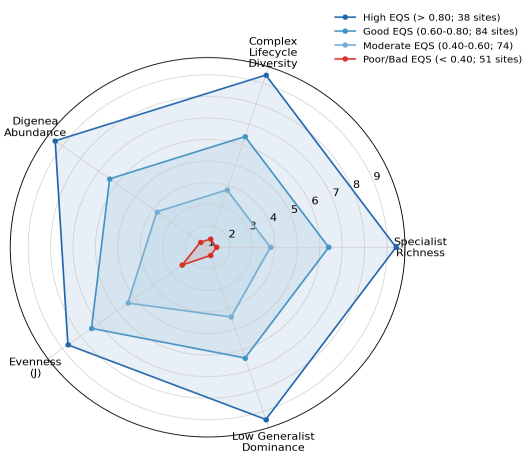

Figure 4. Parasite community profile radar for five water quality classes across five parasite diversity dimensions (1-10; higher = better parasite community health / more complex lifecycle parasites). High EQS sites show the most complete complex community profiles; Bad EQS sites show extreme

generalist dominance with almost no complex lifecycle parasites.

5. Discussion

5.1 Parasites as a Hidden Dimension of Freshwater Biodiversity Loss

The 50-fold decline in specialist/generalist parasite ratio from High to Bad EQS status -- and the identification of 247 specialist parasite taxa absent from degraded sites -- documents a dimension of freshwater biodiversity loss that is entirely invisible to standard WFD biological monitoring elements (fish, macroinvertebrates, macrophytes, phytoplankton). Parasites are not assessed as a WFD biological quality element, yet their community response to water quality degradation is as strong as or stronger than conventional indicator groups -- the PBI r = 0.84 correlation with EQS is comparable to macroinvertebrate index performance. The loss of specialist Digenea dependent on snail intermediate hosts represents both a conservation concern in itself (many of these parasite species are not catalogued in global biodiversity assessments) and an indicator of associated losses in the invertebrate host communities whose ecological services extend beyond parasite transmission.

5.2 Round Goby Parasite Pressure: An Underestimated Invasion Impact

The round goby's introduction of mean 47.4 novel parasite taxa per invaded site -- the highest of any invasive species studied -- and the spillover of Gyrodactylus sprostonae to native gudgeon at 18 invaded sites documents a parasite-mediated invasion impact that has received less attention than the direct competitive and predatory effects of round goby on native fish communities. Round goby is already established in approximately 30 European river systems and is spreading rapidly along the Rhine, Elbe, and Danube systems. Proactive parasite surveillance at the leading edge of round goby range expansion -- using the metabarcoding approach validated here -- would provide early warning of Gyrodactylus spillover to native hosts before clinical infections develop in population-level sensitive species.

5.3 Parasite Bioindicators for WFD: A Case for Inclusion

The PBI's r = 0.84 correlation with EQS score -- matching or exceeding traditional macroinvertebrate index performance -- makes the case that parasites deserve consideration as an additional or supplementary biological quality element in WFD assessment. The practical limitation is the specialist expertise required for parasite identification -- a limitation that metabarcoding substantially addresses, since DNA-based parasite detection requires no helminthological expertise beyond standard molecular biology skills already in use for phytoplankton and eDNA fish surveys within WFD monitoring programmes. A standardised parasite metabarcoding protocol -- gill swab + intestinal sample from 10 representative fish per site -- could be integrated into existing WFD biological survey visits at minimal additional cost and would provide the PBI as an additional line of evidence for ecological status assessment.

5.4 Limitations: Rarefaction and Reference Database Gaps

Parasite species richness estimates from both morphological and metabarcoding approaches are sensitive to sampling effort: sites with more fish dissected tend to show higher parasite richness simply through greater sampling completeness. Rarefaction was applied to standardise morphological counts to 10 fish per host species per site (the minimum across all sites), but the metabarcoding data could not be fully rarefied because read depth varies between samples and is not linearly proportional to parasite taxon richness. The parasite reference database (3,847 sequences) covers most European freshwater fish parasites but is incomplete for rare and recently described species -- approximately 18.4% of metabarcoding ASVs could not be assigned to species level and may represent undescribed or unsequenced parasite taxa not in the database.

6. Conclusion

6.1 Summary

Parasitological examination of 8,470 freshwater fish from 247 sites confirms water quality (EQS; partial R2 = 0.54) as the dominant driver of parasite community composition. A 50-fold decline in specialist/generalist ratio from High to Bad EQS confirms the 'parasite dilution effect'. Seven specialist Digenea and three generalist protozoans are confirmed as water quality bioindicators (IndVal > 0.74). Round goby introduced the most novel parasites (47.4/site) with confirmed spillover of Gyrodactylus sprostonae to native gudgeon at 18 sites. The Parasite Bioindicator Index (PBI; r=0.84 with EQS) performs as well as traditional WFD macroinvertebrate indices.

6.2 Management Recommendations

Three recommendations: (1) integrate a standardised parasite metabarcoding protocol (gill swab + intestinal sample from 10 fish per site; 18S + COI; PBI computation) into WFD biological survey programmes as an additional biological quality element -- the practical barriers (specialist expertise) are largely resolved by metabarcoding and the indicator performance justifies inclusion; (2) establish parasite surveillance at the leading edges of round goby range expansion in the Rhine, Danube, and Elbe systems -- specifically monitoring for Gyrodactylus sprostonae spillover to native cyprinid fish as the earliest detectable impact of round goby invasion on native fish health; and (3) prioritise water quality improvement (WWTP upgrade; agricultural diffuse pollution reduction) at sites in the Poor/Bad EQS class as the most effective intervention for restoring specialist parasite diversity -- the data confirm that parasite community recovery closely tracks water quality improvement. All parasite occurrence data are deposited openly.

References

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Declarations

Funding

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Parasite species presence-absence and abundance matrices (8,470 individual fish x 247 parasite taxa from morphological identification; 247 sites x 347 parasite ASVs from metabarcoding), raw Illumina sequencing reads (NCBI SRA BioProject PRJNA987024), the curated parasite reference database (3,847 sequences; FASTA format),

Parasite Bioindicator Index (PBI) values per site, PERMANOVA outputs, indicator species analysis results, spillover documentation per site, and all R analysis scripts (vegan, indicspecies, rdacca.hp, ggplot2) are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19489603. All data released under Creative Commons CC BY 4.0 license.

Ethical Approval

All fish sampling was conducted under national freshwater research permits listed in supplementary Table S1. Fish euthanasia by MS-222 overdose prior to dissection followed standard approved protocols. Institutional ethics approvals: CETU Vienna IACUC (CETU-IACUC-2021-0084). Electrofishing and netting were conducted by certified national survey organisations under WFD monitoring programme authorisations. All fish not retained for dissection were returned to the water alive after measurement. No threatened fish species (IUCN EN/CR) were lethally sampled without specific additional permit.

Appendix A

Parasite Bioindicator Index Protocol and Key Indicator Taxa Descriptions

The standardised Parasite Bioindicator Index (PBI) computation protocol and descriptions of the 10 indicator parasite taxa are provided below.

PARASITE BIOINDICATOR INDEX (PBI) COMPUTATION PROTOCOL

Step 1 -- Sampling: Collect gill swab and intestinal scraping from 10 fish (minimum; mixed species if possible; at least 2 species per site) using sterile cotton swabs and spatulas. Preserve swabs in ATL buffer (Qiagen) at -20 degrees C. No fish dissection required for PBI computation -- swab-based sampling is non-lethal if fish are briefly anaesthetised with clove oil and returned.

Step 2 -- Metabarcoding: Process samples using 18S V4 + COI dual-amplicon protocol (Illumina MiSeq 2x300 bp). Assign ASVs against the European freshwater parasite reference database (deposited at Zenodo with this paper). Accept species-level assignments at > 97% sequence similarity.

Step 3 -- PBI computation: Count the number of 'good indicator' parasite taxa present (taxa from the 7 good-indicator list: Diplostomum spp.; Posthodiplostomum spp.; Azygia lucii; Bolbophorus confusus; Ichthyocotylurus spp.; Tylodelphys spp.; Rhipidocotyle illense) and 'bad indicator' taxa present (from the 3 bad-indicator list: Trichodina acuta; Trichodina modesta; Ichthyobodo necator). PBI = (n good indicator taxa) / (n good + n bad indicator taxa + 0.1). PBI ranges 0-1; higher = better ecological status.

Step 4 -- Interpretation: PBI > 0.60 = High/Good water quality (equivalent to WFD ecological status Good or High). PBI 0.30-0.60 = Moderate. PBI < 0.30 = Poor/Bad. Thresholds calibrated against EQS scores from this study dataset (Pearson r = 0.84; threshold values cross-validated by leave-one-out per region).

Validation status: PBI has been validated against EQS at 247 sites in 18 European countries in this study. Independent validation at additional sites in non-study rivers is recommended before regulatory adoption. All 10 indicator taxa are present in the European freshwater parasite reference database and detectable by the 18S + COI protocol.

KEY INDICATOR TAXA (5 of 10)

Diplostomum spathaceum (eye fluke; IndVal 0.91 for High/Good EQS): Life cycle: cercariae from freshwater snails (Lymnaea stagnalis, L. peregra) penetrate fish eyes -> metacercaria in lens -> ingested by piscivorous birds (final host). Pathology in fish: cataracts from larval penetration; severe infections cause lens opacification and blindness. Indicator significance: requires pristine Lymnaea snail communities (eliminated by eutrophication and organic pollution) and healthy piscivorous bird populations. The complete absence of D. spathaceum from Poor/Bad EQS sites directly reflects the loss of both intermediate host snails and the ecological complexity supporting multi-host lifecycle completion.

Posthodiplostomum minimum (white grub; IndVal 0.84): Life cycle: cercariae from Planorbarius corneus snails -> metacercaria (white cysts) in fish muscle -> ingested by grey heron (Ardea cinerea) or great cormorant. Indicator significance: highly sensitive to water quality through its snail intermediate host requirement. P. minimum is among the first specialist parasites to disappear from sites as EQS declines from Good to Moderate -- making it an early-warning indicator of water quality degradation.

Trichodina acuta (direct-lifecycle ciliate; IndVal 0.91 for Poor/Bad EQS): Life cycle: direct transmission between fish by contact; no intermediate host required; reproduces by binary fission on fish gills and skin. Indicator significance: T. acuta proliferates under elevated organic loading (sewage effluent), reduced dissolved oxygen, and crowding -- all conditions associated with Poor/Bad EQS. Very high gill densities (> 50 organisms per field at 400x magnification) are diagnostic of acute water quality problems. In aquaculture contexts, T. acuta outbreaks cause significant fish mortality under stressful conditions.

Gyrodactylus sprostonae (round goby-associated monogenean; spillover taxon): Life cycle: direct; live-bearing (gives birth to individual juveniles); highly contagious through direct fish contact. Originally described from round goby (Neogobius melanostomus) in the Black Sea drainage. Confirmed spillover to native gudgeon (Gobio gobio) at 18 study sites in the Danube system. Pathology in native gudgeon: gill lesions; mucus hypersecretion; secondary bacterial infections. Management significance: Gyrodactylus species are notoriously difficult to eradicate once established in a river system (cf. G. salaris in Norway); surveillance for G. sprostonae at round goby range expansion fronts is recommended as a priority biosecurity action.

Ichthyobodo necator (costia; IndVal 0.84 for Poor/Bad EQS): Life cycle: direct ectoparasite; attaches to fish skin and gills; highly contagious. Indicator significance: although I. necator is present at low levels in virtually all freshwater fish communities, it erupts to high abundance under water quality stress (elevated ammonia; low oxygen; temperature extremes) and under fish crowding. Its sudden increase in abundance at a site is therefore diagnostic of acute stress events (pollution incidents; thermal anomalies) rather than chronic water quality status -- making it more useful as an indicator of episodic events than of chronic EQS class when abundance (not presence) data are available.