

Histopathological and Physiological Impacts of Microplastics on Freshwater Fish Gill Morphology and Respiration in Indian River Systems: A Critical Evidence Synthesis and Systematic-Review Framework

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Abstract

Background: The fish gill is simultaneously a respiratory, ionoregulatory, acid–base, excretory and immunological interface. Microplastic particles have been recovered from Indian freshwater environments and fish tissues, but detection of particles is not equivalent to demonstration of gill injury or impaired oxygen uptake. Objective: To critically synthesize evidence concerning microplastic-associated gill lesions and respiratory outcomes, determine which findings arise specifically from Indian river systems, and develop a reproducible protocol for a formal systematic review. Approach: A structured, source-verified evidence synthesis integrates Indian observational studies with mechanistic freshwater-fish experiments and methodological guidance. Findings: Investigations from the Ganga and northeast/southern Indian rivers establish exposure and organ-specific particle occurrence. Controlled fish studies report epithelial lifting, lamellar fusion, vascular remodelling, mucus-cell proliferation, oxidative injury, inflammatory signalling and altered respiration; however, findings differ by species, particle size, exposure dose and study design. A 28-day tilapia experiment using 80 nm, 2 µm and 20 µm polystyrene at 100 µg/L found different respiratory impacts by size, illustrating the importance of particle-specific inference. Interpretation: Mixed river pollution, inconsistent particle confirmation, limited paired physiology and histology, and incomplete exposure characterization currently constrain microplastic-specific causal attribution in Indian rivers. Conclusion: A tiered design integrating FTIR/Raman-confirmed particles, blinded quantitative branchial histopathology, respirometry, water-quality covariates and well-controlled exposure experiments is needed before population-level respiratory risk can be reliably estimated.

Keywords: Microplastics; Indian Rivers; Freshwater Fish; Gill Epithelium; Secondary Lamellae; Oxygen Consumption; Critical Oxygen Tension; Osmoregulation; Histopathology; Oxidative Stress; Evidence Synthesis.

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Introduction

Microplastic exposure should be interpreted against the broader toxicological literature rather than assuming that branchial effects are invariant across polymers and fish species. Reviews of freshwater fishes report considerable variability in exposure route, particle retention and biological response; a global meta-analysis likewise found a diversity of positive, neutral and adverse fish outcomes (Parker et al., 2021; Wang et al., 2020; Hossain et al., 2022). The overarching literature therefore supports an endpoint-specific, not a universally adverse, interpretation of particulate exposure.

Microplastic occurrence and ecosystem risk are separate questions. Early freshwater syntheses documented analytical and ecological knowledge gaps, and recent risk frameworks emphasize distributions of particle size, shape, composition and environmentally plausible dose rather than a single generic microplastic concentration (Wagner et al., 2014; Eerkes-Medrano et al., 2015; Koelmans et al., 2022).

Freshwater fishes encounter suspended particles at one of the most delicate interfaces of the vertebrate body. Water is continuously driven across respiratory lamellae whose thin epithelial diffusion barriers permit oxygen uptake but also expose gill surfaces to suspended contaminants. Unlike gastrointestinal uptake, branchial exposure can occur without feeding: particles suspended in the water column interact directly with mucous layers, interlamellar channels and the branchial epithelium. Microplastics are commonly defined operationally as polymer-containing particles smaller than 5 mm, but environmental sampling practices differ markedly at the lower size boundary and frequently omit nanoplastic fractions (NOAA; Zink and Wood, 2024).

Gill changes cannot be reduced to a list of lesions. The same structural adaptation that can reduce penetration of an irritant—epithelial thickening, increased mucus and lamellar fusion—may increase the water-to-blood diffusion path and reduce effective respiratory

surface. On the other hand, observed hyperplasia does not prove reduced oxygen transfer in the absence of functional measurement. Ventilatory compensation, haemoglobin oxygen affinity and circulatory adjustments can temporarily maintain uptake despite local tissue injury. This structural–functional gap is the central interpretive challenge of this review (Zink and Wood, 2024; micro/nanoplastic tilapia respiration study, 2023).

In India, the Ganga and its tributaries, major southern rivers, and urban waterways receive variable combinations of textile fibres, municipal wastewater, industrial effluent, agricultural runoff, sewage, atmospheric deposition and sediment resuspension. Fish experience these stressors concurrently with fluctuations in temperature, dissolved oxygen, turbidity and flow. Studies confirming plastics in Indian fish are necessary exposure evidence, but their conclusions must not be extended to microplastic-specific respiratory disease unless gill tissue pathology, physiological impairment and relevant covariates have actually been measured (Badola et al., 2023; Kumari et al., 2023; Anandhan et al., 2022).

The novelty pursued here is the explicit separation of four evidentiary layers: (i) environmental microplastic contamination; (ii) organ-associated particle occurrence; (iii) experimentally or observationally demonstrated branchial injury; and (iv) respiratory or fitness-level consequences. Collapsing these layers produces an attractive but unsupported causal story; retaining them permits a stronger scientific synthesis and identifies the study designs needed for robust Indian river assessment.

Review question, scope and reproducibility requirements

The supporting literature encompasses mechanistic experiments, Indian river observations, fish physiology references and evidence-quality research. This breadth is useful for a critical review but it is not a substitute for a completed systematic search and risk-of-bias assessment; meta-analyses of fish endpoints cannot establish pooled branchial outcomes for Indian rivers without eligible, directly comparable studies (Wang et al., 2024; Page et al., 2021).

Primary review question: In freshwater fishes exposed to microplastics, what histomorphological changes occur in the gill and what corresponding changes in oxygen uptake, ventilation, aerobic performance and ionic regulation are demonstrated, with particular attention to populations inhabiting Indian river systems? Secondary questions examine the influence of particle size, polymer chemistry, particle shape, concentration, exposure duration, fish ecology and co-occurring water pollutants.

2.1. Evidence hierarchy and honest review designation

This document is an extended critical synthesis intended to become a fully reproducible systematic review. The source-verification exercise underlying this manuscript was not a completed database-wide, independently screened systematic review: no total database hits, deduplication counts, exclusion counts, inter-reviewer agreement values, pooled effects or registration records are invented. Before submission under the designation “systematic review”, the searches, review team decisions and data-extraction file must be completed and retained. PRISMA 2020 governs transparent reporting, but it does not replace a defensible review method (Page et al., 2021).

Literature cutoff for this updated critical synthesis: October 2025. Publication and submission dates must reflect the actual publication history; this cutoff is not a claim of earlier publication. Bibliography includes primary studies, secondary reviews, standards/methods literature and a non-journal NOAA explanatory resource; reference counts are not counts of eligible effect studies.

2.2. Proposed database search and eligibility rules

A reproducible search should cover PubMed/MEDLINE, Web of Science Core Collection, Scopus and relevant regional databases from database inception to the final documented search date. The query should combine (microplastic* OR nanoplastic* OR “plastic particle*” OR polyethylene OR polystyrene) AND (fish* OR teleost* OR carp OR tilapia OR zebrafish) AND (gill* OR branchial OR lamella* OR histopatholog* OR respir* OR oxygen consum* OR ventilation OR ionoregulat*) with a separate geography subset (India OR Ganga OR Yamuna OR Brahmaputra OR Cauvery OR Kollidam OR Vellar OR Gomti OR Hooghly). Search syntax must be translated to each database, and full search strings and dates archived as supplementary materials.

Eligible direct-effect studies should include freshwater fish, characterized particle exposures or sampled contaminated habitats, an explicit gill morphological or functional endpoint, and sufficiently detailed methods to interpret the outcome. Indian river occurrence studies that do not measure effects should be retained in a contextual exposure stratum, rather than incorrectly coded as evidence of gill injury. Exclude purely marine studies from the primary synthesis, but allow well-identified mechanistic reference work in supplementary discussion if explicitly segregated. Mixed-pollutant field studies must be flagged for high confounding risk rather than treated as microplastic-only interventions.

Table 1. Eligibility and evidence classification framework

Tier	Minimum evidence	Permissible inference	Not established
A: river occurrence	Verified microplastics in water, sediment or fish	Exposure opportunity / occurrence	Gill injury or oxygen deficit
B: gill deposition	Chemically confirmed particles associated with gills	Branchial particle occurrence	Internalisation, causality or dysfunction
C: lesion evidence	Controls, fixed gills, scored lesions	Association or experimental gill injury	Measured loss of respiratory capacity
D: functional evidence	Respirometry / Pcrit / ventilation / ion flux	Physiological alteration	Direct mechanical mechanism without corroboration
E: linked mechanism	Particle verification + lesions + function + mediator data	Stronger mechanistic interpretation	Universal effects across species or ecosystems

Source: author synthesis of the referenced studies; differences in design preclude quantitative comparison unless explicitly noted.

2.3. Bias, quality appraisal and planned statistical synthesis

For observational studies assess site selection, fish-source attribution, season and hydrological coverage, sample-size justification, particle-contamination control, polymer confirmation, blinded histopathology, and control of co-pollutants. For experiments assess random allocation, tank-level replication, actual measured exposure concentration, abiotic conditions, operator blinding, measured particle-size distribution, and biologically plausible dose. Tank should be treated as the experimental unit where individuals share exposure vessels. Extraction fields should include sample size, polymer, shape, length distribution, concentration units, duration, temperature, dissolved oxygen, species and life stage, lesion prevalence and severity, standard errors, oxygen uptake and ionoregulatory outputs.

Meta-analysis should be attempted only where outcome definitions, units and designs are sufficiently aligned. Continuous respirometry outcomes could use log response ratios or standardized mean differences if control means and dispersion are available. Lesion prevalence may require appropriate proportion or odds-ratio models, with between-study heterogeneity and non-independence handled explicitly. Sensitivity analyses should exclude non-spectroscopically confirmed particles, very high-dose exposures, and studies lacking controls. A pooled forest plot must not be fabricated without a complete study-extraction dataset.

Microplastic sources and transport in Indian rivers

Particle fluxes in rivers are strongly conditioned by urban discharge, channel transport and retention processes. Schmidt et al. (2017) investigated riverborne export of plastic debris to the sea, while broader freshwater reviews explain why hydrology, urbanization and sampling compartments constrain interpretation of local particle concentrations (Schmidt et al., 2017; Eerkes-Medrano et al., 2015). None of these transport studies alone quantifies fish gill injury.

Analytical assurance is crucial where fibres predominate. Published reviews recommend consistent sample collection, procedural blanks, contamination mitigation, digestion validation and polymer confirmation, because visually classified particles can introduce substantial false positives and non-comparable estimates (Stock et al., 2019; Hermesen et al., 2018; O'Connor et al., 2020; Koelmans et al., 2019). The broader Asian synthesis by Oza et al. (2024) reinforces the need for taxon-specific and region-specific interpretations of fish contamination.

Riverine microplastics are not a single contaminant. Fibres may originate from laundering, industrial textiles and fishing materials;

fragments can derive from packaging and degraded rigid plastics; films may reflect agricultural or consumer waste; smaller abrasion particles and associated additives complicate classification. Hydrodynamic sorting, settling, resuspension and flood pulses produce unequal exposures among main channels, floodplains and tributary margins. Water-column particle counts and sediment counts are therefore not interchangeable exposure measures.

Polymer composition influences particle density, buoyancy and weathering behaviour, but field transport also depends on aggregation with organic matter, microbial biofilms and mineral grains. Surface ageing changes roughness and chemistry; environmental particles are often not equivalent to the clean, spherical polystyrene beads used in many laboratory toxicology studies. A river assessment that measures only visible fibres may miss smaller fragments and nanoplastics while overcounting non-plastic fibres in the absence of spectroscopic verification.

Badola et al. (2023) demonstrated microplastics in water, sediments and fish from the upper Ganga, reporting widespread contamination and predominant fibres. Kumari et al. (2023) examined tissues of wild fish from the Ganga near Patna, including gills, and identified polymers by FTIR. Anandhan et al. (2022) found 315 particles in gastrointestinal tracts of 23 fish from the Kollidam and Vellar rivers, but their endpoint was ingestion rather than gill histopathology. Ahmed et al. (2023) documented gastrointestinal occurrence in fishes from the Bhogdoi, a Brahmaputra tributary. These studies collectively document exposure heterogeneity and important species or tissue distinctions, not a quantitative national prevalence of respiratory damage.

Table 2. Verified Indian field studies and their inferential boundaries

Study / basin	Material analysed	Verified observation	Gill/respiration inference
Badola et al. 2023; upper Ganga	River water, sediment and common fishes	Microplastics documented; fibres dominant	No direct controlled oxygen-uptake evidence
Kumari et al. 2023; Ganga at Patna	Fish GIT, gills, liver, muscle; cultured comparator	Microplastics detected and chemically characterized using FTIR	Organ occurrence, not gill lesion severity
Anandhan et al. 2022; Kollidam / Vellar	GIT from five sampled fish taxa	315 particles in 23 fish; fibres/fragments	No gill histology or respirometry
Ahmed et al. 2023; Bhogdoi tributary	GIT of indigenous freshwater fishes	Occurrence in sampled specimens	No direct branchial functional endpoint

Source: author synthesis of the referenced studies; differences in design preclude quantitative comparison unless explicitly noted.

Normal branchial morphology and physiology: a mechanistic reference

The established physiological account by Evans et al. (2005) describes why fish gills simultaneously mediate gas exchange, ion uptake, acid–base regulation and nitrogenous-waste excretion. As a result, an exposure that alters ionocyte performance need not generate a proportional change in oxygen consumption, and a histological alteration need not demonstrate functional failure. Mallatt (1985) documented the non-specificity of major gill lesions across multiple irritants, while Bernet et al. (1999) offered an organ-based semiquantitative histopathology scoring framework (Evans et al., 2005; Mallatt, 1985; Bernet et al., 1999).

Teleost gills comprise arches supporting primary filaments, along which secondary lamellae provide a large exchange surface. Water flows across lamellae while perfusing blood runs in a generally countercurrent orientation, sustaining diffusion gradients. Gas movement depends on effective area, the diffusion distance, the trans-epithelial partial-pressure difference and tissue diffusivity. A simplified Fick expression, oxygen transfer $\approx D \times A \times \Delta P / \delta$, is useful for framing the potential effects of lamellar fusion, thickening and epithelial lifting; it is not itself a measured outcome in an exposed fish.

Gill epithelium contains pavement cells, mucous cells and ionocytes with species- and acclimation-dependent distributions. Ionocytes participate in Na⁺, Cl[–] and acid–base transport, whereas the tightness and surface area of the branchial barrier influence both diffusive

water/ion flux and respiratory oxygen transport. Gill defence functions include mucus, epithelial turnover, immune mediators and local antioxidant capacity. Thus, barrier thickening can be acutely protective and chronically costly.

Oxygen consumption rate ($\dot{M}O_2$) estimates organism-level aerobic metabolism but is affected by activity, temperature, feeding, circadian state, body mass and stress. A lesion might reduce maximum oxygen uptake without lowering resting uptake. Consequently, routine metabolic rate, maximum metabolic rate, aerobic scope, critical oxygen tension (P_{crit}), ventilation rate, blood haemoglobin, acid–base status and branchial ion flux probe different facets of respiratory function and should not be treated as synonyms.

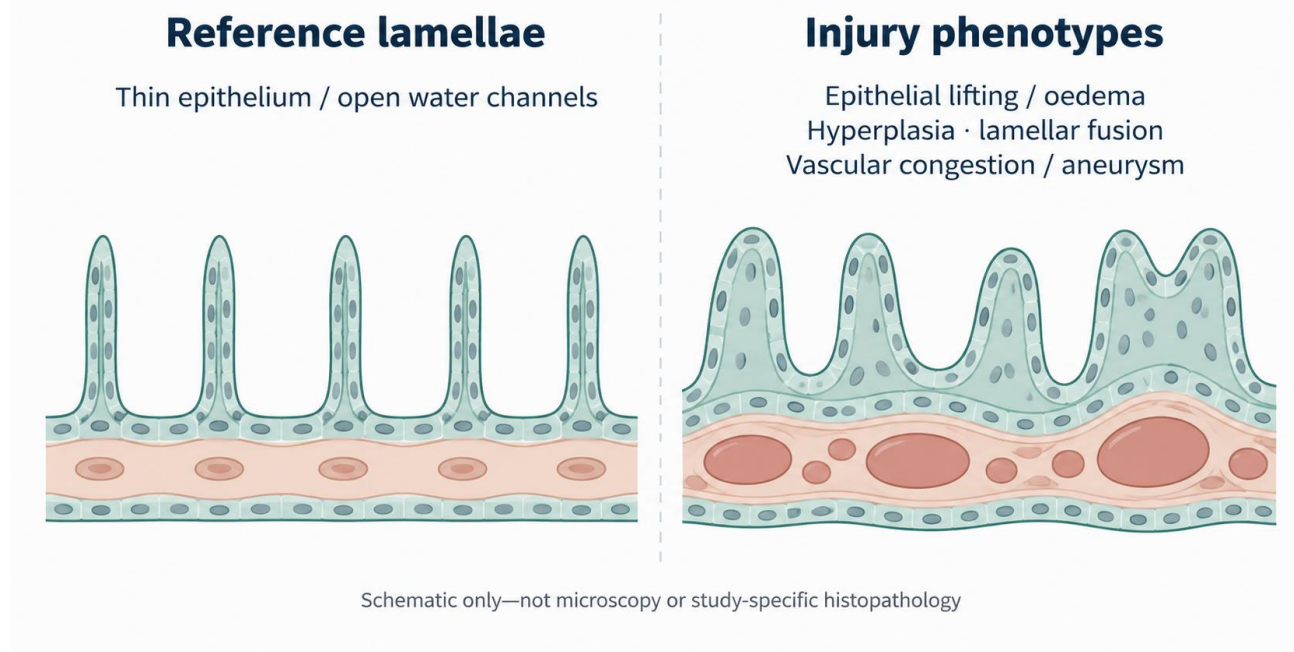


Figure 1. Original schematic of gill lamellae and selected lesion patterns. Illustration is not a micrograph, carries no numeric measurements, and should not be interpreted as primary experimental evidence.

Physical deposition, epithelial responses and lesion classification

Experimental fish studies caution against conflating tissue deposition, ingestion and local toxicity. Jabeen et al. (2018) found effects dependent on shape and exposure route in goldfish, while Lu et al. (2016) established size-associated differences in tissue-associated polystyrene occurrence and liver effects in zebrafish. A study of commercially caught fish by Su et al. (2019) similarly demonstrates the utility of organ-specific sampling, although its coastal and estuarine setting should not be recategorized as an Indian freshwater river experiment.

In addition to the original carp-gill study discussed above, independent fish investigations document a range of intestinal, immune and transcriptional responses following exposure to differing particles (Lei et al., 2018; Jin et al., 2018; LeMoine et al., 2018). These results strengthen biological plausibility of inflammation and stress-response activation without establishing that a particular microscopic lesion is exclusive to microplastics (Mallatt, 1985; Cao et al., 2023).

5.1. Particle deposition, mucus and clearance

Gill particle burden depends on ventilation volume, particle hydrodynamics, aggregation, local turbulence and the ability of mucus and ciliary or behavioural clearance processes to remove material. Fibres can plausibly interact differently with filaments than spheres, but mechanistic extrapolation from particles placed in controlled tanks to weathered river fibres remains uncertain. Finding a particle in a dissected gill sample does not establish epithelial uptake; particles may be adherent, lodged in interlamellar spaces or introduced during dissection. Tissue digestion and spatial microscopy should be used together whenever possible (Lu et al., 2016; Zink and Wood, 2024).

5.2. Hyperplasia, hypertrophy and lamellar fusion

Hyperplasia is an increase in cell number, whereas hypertrophy is an increase in cell size. Both can alter epithelial thickness. Lamellar

fusion reduces accessible exchange surfaces and may narrow interlamellar water channels. In early juvenile Nile tilapia, experimentally induced histological changes included hyperplasia, epithelial lifting, shortening of secondary lamellae and complete lamellar fusion (tilapia juvenile lesion study, 2021). Because such changes also occur during exposure to ammonia, metals and low pH, field attribution requires adjustment for other exposures.

5.3. Epithelial lifting, oedema and aneurysm

Epithelial lifting may increase the diffusion distance between water and blood; oedema and capillary injury can further disturb architecture. Vascular congestion and aneurysmal dilatation can affect local perfusion. These lesions can be patchy; counting only “damaged/not damaged” fish discards severity information. Representative blinded scoring should report the proportion of affected lamellae, lesion grade and spatial distribution rather than relying on unlabelled illustrative micrographs.

5.4. Mucous-cell proliferation and defensive trade-offs

Mucus can protect surfaces against particles and microbes but increased secretion may alter boundary-layer properties and water passage across lamellae. In the carp experiment of Cao et al. (2023), polyethylene exposure was associated with mucous-cell proliferation, inflammation and vascular remodelling. This result supports a mechanistic response within that experimental system; the direction and magnitude of net oxygen-transfer changes cannot be read directly from mucus-cell counts.

5.5. Cellular inflammation, apoptosis and tissue remodelling

Cao et al. (2023) observed oxidative injury and apoptosis in carp gills, together with NF- κ B- and NLRP3-associated inflammatory signalling. Such pathways offer biologically plausible mediators between particle exposure and tissue damage. However, pathway activation is not a unique signature of plastics and can be triggered by numerous toxic or infectious conditions. Mechanistic inference is strengthened when tissue injury, biochemical changes and physiological performance are measured concurrently and show coherent dose–time relationships.

Conceptual pathway linking microplastic exposure to gill injury and respiratory impairment

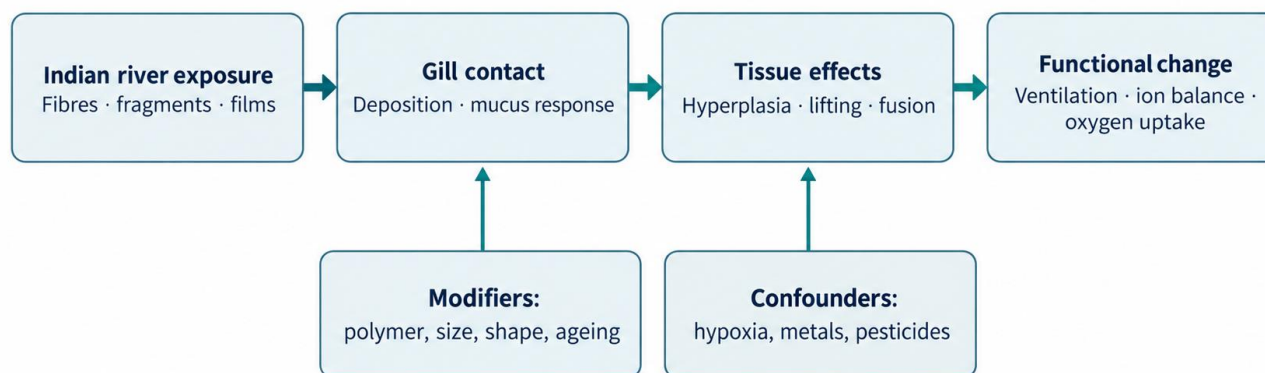


Figure 2. Original exposure-to-effect conceptual framework distinguishing particle contact, morphological alteration and respiratory impairment. Grey modifiers are potential confounders and effect modifiers, not measured causal coefficients.

Physiological responses: oxygen exchange, ventilation and ionoregulation

Intermittent-flow respirometry offers a standardized approach to repeated oxygen-consumption measurements while mitigating progressive hypoxia and waste accumulation inside closed chambers. Instrument design, chamber-to-organism ratio, flush cycles, microbial correction and acclimation can materially alter estimates, as shown by Svendsen et al. (2016) in methodological studies. Thus differences among exposed groups should not be interpreted as branchial impairment until these technical factors have been controlled (Svendsen et al., 2016; Evans et al., 2005).

Cross-study comparisons should distinguish short-term and dietary exposures from direct waterborne exposure. For example, Jovanović et al. (2018) documented limited immediate harm in a dietary virgin-microplastic exposure context, whereas Jabeen et al. (2018) described particle-shape-dependent responses in goldfish. Likewise, a controlled PVC exposure in goldfish found no effect on selected branchial ATPase or ion-status endpoints at tested concentrations, demonstrating why null findings remain informative (Goldfish PVC study, 2020; Zink and Wood, 2024).

6.1. Whole-animal oxygen consumption

A particularly informative study exposed *Oreochromis niloticus* to polystyrene particles of 80 nm, 2 µm and 20 µm at 100 µg/L for 28 days and combined oxygen consumption, histology, transcriptomics and metabolomics. Respiratory injury was observed across treatments, with stronger reported effects for the two micrometre-scale particle treatments than for the 80-nm treatment (Microplastics and nanoplastics induced differential respiratory damages, 2023). This is stronger evidence than a report of gill microplastic occurrence alone because respiratory function was directly measured, yet it should not be extrapolated as a size-effect law across all species and polymers.

6.2. Ventilation, hypoxia and Pcrit

Ventilation frequency or opercular movement may rise as a compensatory response, but can also decline with severe physiological impairment or sedation. The critical oxygen tension (Pcrit) provides a potential indicator of hypoxia tolerance, although calculation methods and acclimation influence estimates. Standard intermittent-flow respirometry should include blank chamber microbial correction, proper washout, adequate mixing, temperature logging, mass scaling and observation of spontaneous activity. When hypoxia is itself an environmental exposure, factorial experiments are needed to separate its effect from microplastics.

6.3. Ion transport and acid–base regulation

Zink and Wood (2024) concluded that the still-limited directly targeted literature supports variable ionoregulatory disturbances after microplastic exposure. This is not interchangeable with proof of respiratory failure, because ion transport and oxygen transfer share tissue but have different mechanisms and endpoints. Suggested measures include plasma osmolality, Na⁺, Cl[−], Ca²⁺, acid–base status, branchial Na⁺/K⁺-ATPase activity, and ionocyte morphology; these should be selected according to the species' native water chemistry.

6.4. Haematology and oxygen delivery

Gill damage can change blood oxygenation, but erythrocyte indices are indirect and non-specific. Haemoglobin concentration, haematocrit and blood gases may respond to infection, nutritional status and many pollutants. A well-designed study should avoid interpreting a single haematological change as proof that microplastics physically blocked gill lamellae. Coupled measurements—verified exposure, pathology, oxygen uptake and blood oxygen variables—provide a stronger mechanistic chain.

6.5. Aerobic scope and ecologically relevant performance

Routine oxygen consumption under resting conditions may remain compensated even if maximum aerobic capacity declines. Aerobic scope, swimming capacity, feeding activity and thermal tolerance can therefore reveal ecologically important sublethal effects missed by resting metrics. However, such tests are demanding and need careful standardization to avoid handling stress. The translation from individual performance to growth or population persistence requires additional long-term evidence.

Table 3. Gill lesion–function mapping: plausible interpretation versus direct evidence

Histological observation	Potential functional link	Best confirmatory measurement	Main alternative causes
Epithelial lifting / oedema	Longer diffusion path	Oxygen uptake plus stereology	Metals, ammonia, osmotic injury
Lamellar fusion	Reduced exposed exchange area	Lamellar area and aerobic scope	Hypoxia, parasites, acids
Mucus hypersecretion	Altered surface transport / irritation	Mucous-cell density; ventilation	Pathogens and suspended sediment
Hyperplasia / hypertrophy	Thicker epithelium	Barrier thickness and Pcrit	Numerous irritants

Vascular congestion	Altered local perfusion	Blood gases, lesion scoring	Haemodynamic stress
Ionocyte abnormalities	Disrupted electrolyte regulation	Ion flux, plasma Na/Cl, ATPase	Salinity/pH shifts and pesticides

Source: author synthesis of the referenced studies; differences in design preclude quantitative comparison unless explicitly noted.

Molecular mechanisms linking particles to branchial injury

Oxidative and inflammatory mechanisms require endpoint-level discrimination. Jin et al. (2018) reported intestinal microbiome and inflammatory changes in zebrafish, whereas LeMoine et al. (2018) addressed transcriptional effects during zebrafish development; neither finding should be presented as direct respiratory impairment. Reviews of fish biomarkers and microplastic ecotoxicology catalogue multiple candidate pathways but caution that exposure characteristics and concomitant stressors change responses (Fish toxicity biomarkers review, 2021; Freshwater ecotoxicity review, 2022; Banaee et al., 2025).

Longer-duration gill experiments and combined stressor experiments can help disentangle these pathways: zebrafish co-exposure to microplastics and copper examined oxidative stress, apoptosis-related transcription and serotonergic markers in gill tissue, making explicit the potential influence of co-pollutants (Zebrafish copper co-exposure study, 2022). Such results are relevant to Indian rivers exposed to mixed contaminants but are not themselves evidence from those rivers.

Oxidative imbalance is a recurring biological response in exposed fish, but interpretation must distinguish increased antioxidant enzyme activities from unequivocal oxidative damage. Induction of superoxide dismutase or catalase can represent compensation; damage is better evaluated through combined indicators such as lipid peroxidation, protein carbonylation, glutathione status and direct histological injury. Lu et al. (2016) observed antioxidant changes following polystyrene exposure in zebrafish, but their histopathological focus was the liver, not a demonstration of gill respiratory failure.

In carp gills, Cao et al. (2023) provided an integrated model in which polyethylene exposure was associated with oxidative stress, apoptosis, increased pro-inflammatory factors and activation of NF- κ B/NLRP3 pathways. The proposed pathway is biologically coherent, but transcript changes alone cannot establish directionality or specificity. Causal validation would ideally combine time-course exposure, dose response, targeted pathway intervention and simultaneous physiology.

Additional mechanisms may involve disruption of mitochondrial energy metabolism, inflammatory cell recruitment, extracellular-matrix remodelling and altered barrier permeability. Transcriptomic and metabolomic signatures can identify pathways worth testing but are sensitive to species, particle composition and sampling timing. Inference from pathway enrichment must not be converted into claims of demonstrated tissue mechanism without targeted validation.

Polymer additives and co-sorbed chemicals further complicate mechanistic interpretation. An effect seen with a commercially supplied suspension might derive partly from surfactants or residual monomers rather than particles alone. Appropriate particle-free filtrate controls, additive controls where feasible, and characterization before and after exposure are necessary to interpret direct particle effects.

Exposure dimensions that determine toxicity

The dose metric remains a major methodological obstacle. Koelmans et al. (2022) proposed aligning environmental exposure and effect thresholds with multidimensional particle distributions, while studies of freshwater biota methods identify persistent deficits in contamination control and reporting (Hermesen et al., 2018; O'Connor et al., 2020). A single particles-per-litre figure cannot represent size-specific mass, surface area, morphology or chemical additives.

The ecological and toxicological literature also shows that clean laboratory particles are insufficient surrogates for all environmental mixtures. Polymer-specific and size-specific responses have been described in fish model experiments, including effects on zebrafish intestines and goldfish tissues (Lei et al., 2018; Jabeen et al., 2018). Asian fish occurrence inventories and global fish meta-analyses further show variability by geography, taxon and measured outcome (Oza et al., 2024; Wang et al., 2024).

Particle size should be represented as a measured distribution, not a nominal manufacturer specification. Micro- and nanoscale particles can differ in transport, retention, internalisation and clearance, yet smaller particles are not invariably more toxic across all measured endpoints. The controlled tilapia experiment reporting stronger respiratory alterations for 2 and 20 μ m treatments than for 80 nm material is an important counterexample to simple monotonic size-toxicity assumptions.

Shape and aspect ratio matter because elongated fibres, angular fragments, films and spheres have different settling and contact behaviour. Most standard toxicological bead exposures poorly represent environmental mixtures in Indian rivers. Particle surface ageing, biofilm growth and organic coatings can modify apparent toxicity and microscopy recovery. Polymer identity should be established spectroscopically, not visually or on colour alone.

Exposure dose requires special care. Counts per litre, mass per litre, particles per fish and particles per gram of tissue cannot be converted without particle-size distributions and density assumptions. High laboratory concentrations may be useful for hazard identification but require transparent separation from environmentally measured ranges. Chronic field exposures also fluctuate seasonally and may involve particle pulses after heavy rain or flood disturbance.

Fish life stage and respiratory ecology are essential biological moderators. Juveniles have developing gill structures and potentially different ventilation-to-mass ratios; air-breathing species may rely on accessory respiratory organs under hypoxia. Species and life-stage heterogeneity rules out transfer of results from a single tilapia or zebrafish experiment directly to every Indian river fish.

Table 4. Methodological factors contributing to cross-study heterogeneity

Dimension	Minimum standard	reporting	Bias if missing
Size	Number-weighted distributions; cutoff	and mass-size detection	Underestimate particles; comparisons small false
Polymer	FTIR/Raman identification; match criteria	polymer library	False plastic positives
Shape	Defined morphology categories and aspect ratio		Mismatch between beads and field fibres
Dose	Measured concentration with consistent units and blanks		Unreliable exposure–response assessment
Exposure	Duration, renewal, particle recovery and tank replication		Pseudoreplication and dose drift
Gill lesion	Blinded grading; fixed sections and sampling level		Subjective pathology
Physiology	Temperature-corrected respirometry; fish activity		Confounded oxygen consumption
Field quality	DO, pH, ammonia, metals, season, flow		False microplastic-specific attribution

Source: author synthesis of the referenced studies; differences in design preclude quantitative comparison unless explicitly noted.

Indian river case synthesis and research priorities

The Indian case studies summarized above document exposure and tissue occurrence, whereas broader syntheses place those observations in the context of freshwater fish feeding ecology and anthropogenic pollution (Galafassi et al., 2021; Parker et al., 2021; Oza et al., 2024). Inferences should therefore be graded by endpoint: field occurrence, gill-associated particles, lesion frequency and respiratory performance cannot be treated as interchangeable measurements.

9.1. Upper Ganga

The upper Ganga has received field investigation combining water, sediment and fish evidence (Badola et al., 2023). A strong follow-up would sample matched upstream/downstream reaches across seasons, record dissolved oxygen, discharge and wastewater influence, and measure gill histopathology with particulate burden in the same individuals. Any respiratory relationship would need to be tested independently rather than inferred from fish size or gut particle abundance.

9.2. Middle Ganga at Patna

Kumari et al. (2023) sampled wild and cultured fish and analysed multiple tissues including gills. That organ-level information is

valuable because gastrointestinal and branchial occurrence imply different exposure routes. Follow-up should distinguish adherent external particles from tissue-associated material, include blinded morphometry and ensure wild–farmed comparisons account for husbandry, diet and ambient oxygen conditions.

9.3. Eastern and northeastern tributaries

The Bhogdoi investigation (Ahmed et al., 2023) illustrates exposure in a Brahmaputra tributary but used gastrointestinal endpoints. The river presents an opportunity for paired ecological and tissue-function studies rather than an existing demonstration of gill respiratory toxicity. Seasonal sediment remobilization, habitat differences and species ecology must be accounted for.

9.4. Southern rivers

Anandhan et al. (2022) provide verified ingestion evidence from the Kollidam and Vellar. Future river-scale assessments should prioritize fibres and fragments representative of real samples and compare field-associated patterns with validated exposure experiments in species that actually occur in these systems. Findings from coastal or estuarine species should not automatically be classed as freshwater-only respiratory evidence.

9.5. Urban rivers and mixed-pollution confounding

In urban systems such as the Yamuna, coincident dissolved-oxygen depletion, sewage loading, ammonia and multiple contaminants create severe attribution challenges. A cross-sectional comparison of gill lesions against visually counted particles would be inadequate for causal claims. Ideally, matched reference locations, comprehensive co-pollutant panels and well-characterized controlled exposures should jointly constrain alternative explanations.

A prospective multi-tier study design for Indian rivers

A preregistered gill-lesion scoring scheme could adapt the diagnostic and weighting logic of Bernet et al. (1999), while explicitly acknowledging that the lesions catalogued by Mallatt (1985) also occur following non-plastic stressors. Paired oxygen consumption should use documented intermittent-flow respirometry standards and report chamber background correction, mixing, acclimation and size scaling (Svendsen et al., 2016).

The analytical workflow should prospectively specify microplastic size cut-offs, spectral acceptance thresholds, blank correction and field-to-laboratory traceability. The methodological reviews of Stock et al. (2019), Hermesen et al. (2018), Koelmans et al. (2019) and O'Connor et al. (2020) establish the importance of these controls. These are methodological recommendations, not reported additional experimental outcomes from the surveyed Indian basins.

A defensible national evidence programme can use nested observational and experimental components. First, conduct seasonal field sampling across defined river reaches, including upstream reference, urban input, downstream recovery and tributary zones, with comparable fish species and size classes where possible. Second, quantify water and sediment microplastics and fish organ burden with field blanks and procedural controls. Third, obtain blinded gill histology, stereological morphometry and respiratory or ionoregulatory measurements in the same individuals or tightly matched subgroups.

Gill processing should use consistent arch selection, fixation, embedding orientation, section thickness and staining. H&E remains suitable for routine architecture, while PAS/Alcian blue can distinguish mucus characteristics and selected immunohistochemical markers can explore inflammation or apoptosis. Lesion scoring can follow a preregistered ordinal scheme; quantitative measures should include secondary lamellar length, epithelial thickness, proportion of fused lamellae and mucous-cell density. Inter-observer agreement and quality-control images should be documented.

Respirometry should be performed under controlled post-capture conditions with acclimation and transparent handling of the stress associated with field collection. For live fish, individual-level oxygen consumption can be assessed using intermittent-flow respirometry; if immediate physiology is infeasible, avoid retroactively inferring oxygen consumption from fixed tissues. Complementary plasma ion measurements and branchial enzyme assays can interrogate non-respiratory functions of the gill.

Finally, controlled exposures should recreate particle classes measured in each field basin and include at least a negative control, environmental-range treatment and a justified higher hazard-characterization treatment. Additional factorial contrasts may assess low oxygen, temperature or co-pollutants where scientifically justified. All numerical sample sizes require prospective power calculations based on pilot variance, not arbitrary values inserted into a review.

Recommended evidence pathway for Indian river systems

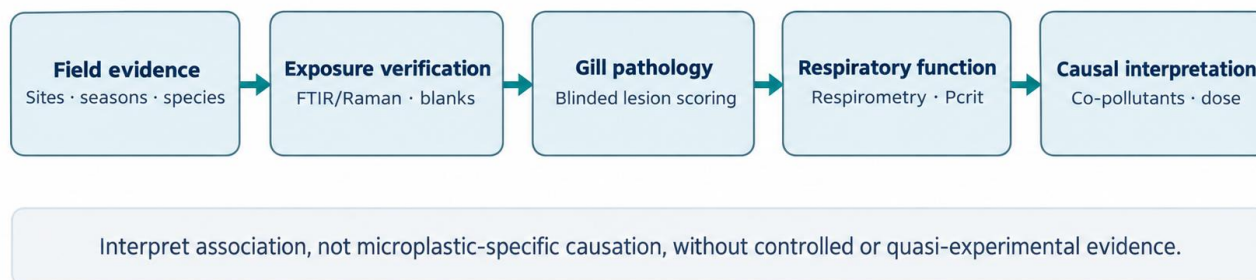


Figure 3. Proposed evidence-generation pathway for Indian river field–laboratory studies. This is a research-design recommendation, not a PRISMA screening flow diagram.

Research gaps, causal attribution and implications

The value of null and heterogeneous findings is underscored by controlled studies and evidence syntheses: fish outcomes may vary by particle type, duration, concentration and endpoint, and some exposures do not produce measurable harm on all metrics (Jovanović et al., 2018; Goldfish PVC study, 2020; Hossain et al., 2022; Wang et al., 2024). Policy-relevant thresholds therefore require verified exposure distributions and comparable, reproducible outcome measures (Koelmans et al., 2022).

The largest gap is not the absence of Indian plastic detections; it is the scarcity of studies linking verified particle exposure, quantitative branchial damage and measured respiratory endpoints within the same field context. Unpaired investigations can establish pieces of the chain but cannot quantify the complete pathway. A scientifically useful negative finding—for example, particle presence without measurable loss of aerobic scope—should be reported alongside positive findings.

A second gap concerns external validity. Uniform laboratory microspheres differ from weathered mixtures of fibres and fragments encountered by fish in rivers. The use of environmentally measured concentrations and real particle distributions would improve relevance, but naturally collected mixtures can introduce co-contaminants that must be characterized. Both clean-particle and environmentally aged-particle treatments may be necessary to partition mechanisms.

Third, oxidative-stress biomarker panels are frequently interpreted too strongly. Neither a significant enzyme change nor histological lesion alone identifies microplastics as the cause in an urban river. Integrated causation requires plausible temporality, coherent dose response, mechanistic consistency, exclusion of major confounders and preferably controlled perturbation of exposure or pathway. Fourth, risk interpretation should remain species-specific. Carp, tilapia, catfish, air-breathing species and migratory taxa may have different gill architecture, oxygen tolerance, behaviour and exposure pathways. Conservation and aquaculture recommendations should be conditioned on these differences rather than based on one generic fish hazard estimate.

Finally, policy translation should focus on source reduction, wastewater and solid-waste control, quality-assured particle monitoring and sentinel-fish assessments. The review does not establish a national threshold concentration at which fish respiration fails. Any proposed regulatory threshold requires a much broader and more comparable effect dataset.

Conclusions

In interpreting future investigations, the foundations for robust inference are now clear: use the multifunctional physiology of the fish gill as a reference (Evans et al., 2005), recognize the non-specificity of gill lesions (Mallatt, 1985), apply blinded and replicable pathology scoring (Bernet et al., 1999), characterize particles under suitable analytical quality controls (Hermesen et al., 2018; Koelmans et al., 2019), and connect morphology to validated respiratory metrics (Svendsen et al., 2016).

Available Indian river studies provide credible evidence that freshwater fishes encounter and retain plastic particles, including in branchial-associated samples in some investigations. Laboratory freshwater-fish studies independently establish that sufficiently characterized microplastic exposures can produce gill lesions, oxidative and inflammatory responses, and, in selected experiments, measurable respiratory dysfunction. The critical scientific distinction is that occurrence evidence from Indian rivers cannot be merged with laboratory toxicology to declare established microplastic-caused respiratory injury in natural Indian fish populations. The most

productive future direction is coordinated field and laboratory research that links verified environmental exposures, blinded morphological grading, oxygen uptake, ionoregulation and co-pollutant adjustment at the individual-fish level. Until such evidence is available, national respiratory-risk estimates and pooled lesion frequencies would be premature.

Declarations

Funding: [authors to supply]. Conflicts of interest: [all authors to declare]. Ethics approval: not applicable to literature synthesis; any new fish sampling requires relevant approvals. Data availability: publications and cited URLs identified in the reference list; no new primary dataset generated. Author contributions: [to be completed following actual contributions]. Systematic-review registration: none claimed.

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