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REVIEW

Development of transcriptional biomarkers for ammonia stress in fish

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Abstract

Ammonia loading is recognized as a pervasive constraint in intensive aquaculture and eutrophic waters, yet transcriptional indicators of ammonia stress have been reported across disparate species, tissues, and exposure regimes. In this review, fish studies linking ammonia exposure to mRNA responses are synthesized using a tissue-forward framework that prioritizes first-contact epithelia and downstream metabolic organs. In gills, recurring modulation is described for Rhesus glycoproteins, particularly *Rhbg* and *Rhcg*/*RhCG* isoforms, and associated acid–base and ionoregulatory machinery (V-type H + -ATPase, Na + /H + exchangers, carbonic anhydrase, Na + /K + -ATPase), alongside inflammatory and remodeling signals consistent with epithelial disturbance. In liver and other internal tissues, coherent shifts are reported in amino-acid and nitrogen metabolism, mitochondrial energy pathways, redox control, and cell-death programs, with representative candidate biomarkers including glutamine synthetase, carbamoyl phosphate synthetase I, antioxidant/redox genes, cytokine-related markers, and apoptosis/ferroptosis-associated transcripts. Because many oxidative and immune transcripts are shared among stressors, improved diagnostic value is expected when biomarker panels are derived from co-regulated modules using network-guided reduction approaches, including weighted gene co-expression network analysis (WGCNA), and validated against key confounders. Candidate gene sets are collated and a roadmap is outlined for translating tissue signatures into compact assays for aquaculture management and field surveillance.

Keywords Ammonia stress · Fish · Transcriptional biomarkers · Non-invasive biomonitoring · Environmental RNA · Eco-toxicology

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Introduction

Ammonia has been positioned at the intersection of reactive nitrogen enrichment, eutrophication, and fish health, and its monitoring has been treated as a core element of aquatic nitrogen management across freshwater, estuarine, and marine systems (Edwards et al. 2024; Zhou et al. 2024). Excess ammonia has been linked to multiple anthropogenic sources, including agricultural and wastewater discharge, and has been discussed as an increasingly salient stressor for aquatic biota as global nitrogen cycling is altered by human activity (Edwards et al. 2024). Additional attention has been drawn to ammonia because expanding industrial use, including proposed deployment as a low-carbon energy carrier, has been recognized as a plausible driver of new environmental release scenarios that would require robust ecological surveillance (Edwards et al. 2024). Such scenarios include accidental leakage or spills during expanded ammonia production, storage, transport, and terminal handling, as well as bunkering/refueling and ship-fuel-system releases in marine applications; atmospheric NH_3, NO_x, or N_2O emissions and subsequent deposition may also provide indirect pathways linking ammonia-energy infrastructure to aquatic nitrogen loading.

In intensive aquaculture, accumulation of total ammonia nitrogen (TAN) has been repeatedly identified as a dominant water-quality constraint, arising from protein catabolism and excretion, microbial mineralization of wastes, and incomplete nitrification or water exchange in production systems (Parvathy et al. 2023). As broad management context, well-functioning intensive or recirculating systems commonly aim to keep TAN below a few mg/L, often below approximately 5 mg/L, whereas sublethal concern is more directly linked to the unionized NH_3 fraction, which may become damaging when it approaches or exceeds approximately 0.05 mg/L (Lindholm-Lehto 2023; Parvathy et al. 2023). Sub-lethal exposure has been associated with depressed performance and altered physiological state, while sensitivity has been reported to vary widely across taxa and life stages, thereby complicating universal "safe" thresholds and motivating species-aware diagnostics (Parvathy et al. 2023). Consequently, ammonia has been framed not only as a toxicant but also as a management variable with direct implications for welfare, productivity, and disease vulnerability in cultured fish (Parvathy et al. 2023).

In aqueous systems, ammonia exists in equilibrium between unionized NH_3 and ionized NH_4^+. The NH_3 fraction has been considered as a key determinant of toxicity because the unionized form exhibits enhanced permeability and higher internal loading (Edwards et al. 2024; Parvathy et al. 2023). Interpretation of biological responses has therefore been strengthened when TAN has been reported jointly with pH and temperature (and salinity where relevant), because these parameters determine NH_3 availability and the directionality of diffusion gradients at exchange surfaces (e.g., higher NH_3 levels at higher pH) (Edwards et al. 2024; Parvathy et al. 2023). For biomarker development, this chemistry has practical consequences: "ammonia stress" has not been defined solely by TAN, but by the interaction of exposure concentration with water chemistry and exposure time (Edwards et al. 2024; Parvathy et al. 2023; Zimmer 2024).

Most teleosts are ammonotelic, and the gill has been established as the principal site of nitrogenous waste elimination alongside its roles in gas exchange and ionoregulation (Zimmer 2024). Mechanistic models of branchial ammonia excretion have converged on coordinated transport processes in which Rh glycoproteins facilitate NH_3 transport and boundary-layer acidification promotes diffusion trapping as NH_4^+, with functional coupling to ionocyte transporters such as V-type H^+-ATPase, Na^+/H^+ exchangers, carbonic anhydrase, and Na^+/K^+-ATPase (Nakada et al. 2007; Nawata et al. 2007; Zimmer 2024). The central

role of Rh-based pathways has been supported by seminal molecular evidence demonstrating Rh dependence of gill ammonia secretion and by subsequent work in salmonids linking Rh expression dynamics with ammonia excretion capacity and the acidification machinery (Nakada et al. 2007; Nawata et al. 2007). Under sustained high environmental ammonia, compensatory responses have been shown to differ among fish species and can include transcriptional modulation of Rh genes and associated ion-transport components in gills, reinforcing the relevance of these pathways as exposure-proximal biomarker candidates (Chen et al. 2017; Sinha et al. 2013).

Beyond the gill interface, ammonia stress has been associated with systemic metabolic remodeling, oxidative imbalance, inflammatory signaling, and tissue injury, indicating that internal organs integrate exposure consequences even when external gradients dominate initial loading (Parvathy et al. 2023). Omics-level studies in aquaculture-relevant fish have further indicated that ammonia exposure can produce time-structured transcriptional shifts in liver and/or gill that map to lipid and sterol metabolism, mitochondrial organization, amino-acid metabolism, and canonical stress-response signaling (Shang et al. 2021; Zhu et al. 2019). The applied interpretation of these signals has been complicated by environmental and husbandry factors (e.g., temperature, oxygen availability, handling intensity, and stocking density), which have been emphasized as modifiers of ammonia outcomes in aquaculture syntheses and therefore as confounders that must be addressed during biomarker validation (Edwards et al. 2024; Parvathy et al. 2023).

Transcriptional endpoints have been increasingly used for ammonia-stress assessment because pathway perturbations can be detected earlier than overt pathology and because targeted RT-qPCR panels can be derived from RNA-seq discovery frameworks (Shang et al. 2021; Zhu et al. 2019). However, many oxidative and immune transcripts responsive to ammonia have been shared with generalized stress programs, so improved diagnostic performance has been expected from tissue-aware multi-gene signatures that incorporate ammonia-transport and acid–base modules alongside downstream response genes (Parvathy et al. 2023; Zimmer 2024). Translation to routine surveillance has additionally been limited by sampling invasiveness, and feasibility of water-based transcript readouts has been advanced by recent demonstrations that environmental RNA sequencing from aquarium water can recover stress-responsive mRNA profiles in fish (Miyata et al. 2025). In this review, evidence for fish transcriptional responses to ammonia is synthesized using a tissue-forward framework that prioritizes environmentally exposed epithelia and metabolically integrative internal organs, with emphasis placed on candidate gene sets suited to targeted assays and non-invasive monitoring pathways (Parvathy et al. 2023; Zimmer 2024) (see the graphical summary of this study in Fig. 1).

Evidence identification and extraction with a tissue-forward prioritization scheme

This article was prepared as a scoping evidence synthesis of experimental fish studies in which ammonia exposure was explicitly linked to transcriptional outcomes. A scoping design was selected because the evidence base is methodologically heterogeneous. Studies differed across fish taxa and life stages, exposure regimes, ammonia forms and reporting units, water chemistry, and transcript platforms. This heterogeneity limited the defensibility of pooled quantitative meta-analysis for most gene-level endpoints without extensive harmonization and reprocessing of primary data (Arksey and O'Malley 2005; Levac

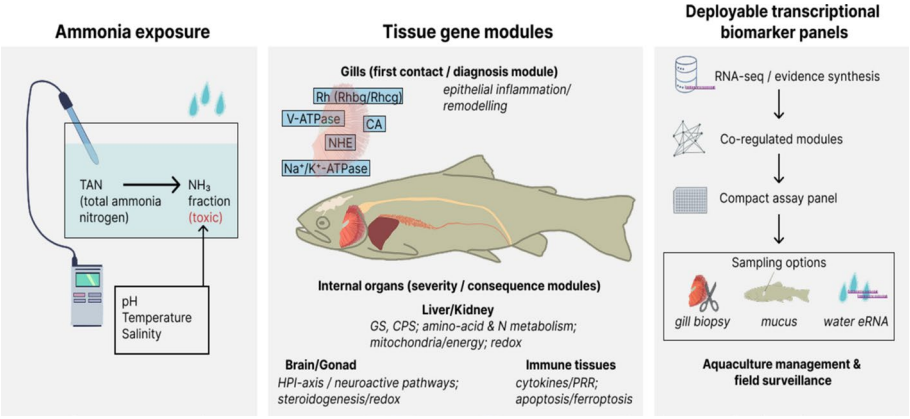

Fig. 1 Conceptual framework linking water chemistry to deployable transcriptional biomarkers of ammonia exposure. The left-to-right schematic illustrates the pipeline from environmental chemistry to field-ready monitoring tools. **(Left panel)** Ammonia exposure: The proportion of the toxic ammonia fraction (NH_3) of total ammonia nitrogen (TAN) is determined by pH, temperature, and salinity. **(Center panel)** Tissue gene modules: The gill represents the first point of contact and diagnostic response, characterized by a core acid–base and ionoregulatory module (Rhbg/Rhcg, V-ATPase, NHE, carbonic anhydrase, Na^+/K^+-ATPase) alongside epithelial inflammation and remodeling signals. Internal organs capture downstream severity and consequences, but are less specific by themselfs: liver and kidney (glutamine synthetase, carbamoyl phosphate synthetase; amino-acid and nitrogen metabolism, mitochondrial energy pathways, redox balance), immune tissues (cytokines and pattern-recognition receptors, apoptosis/ferroptosis), and brain and gonads (HPI-axis and neuroactive pathways, steroidogenesis, redox control). **(Right panel)** Deployable transcriptional biomarker panels/from discovery to monitoring: Transcriptomic evidence (RNA-seq and synthesis across studies) is distilled into co-regulated gene modules (e.g., WGCNA), validated with compact multigene assay panels (RT-qPCR). Sampling strategies range with gill biopsy, mucus, or water-borne environmental RNA (eRNA) from most to least invasive. Overall, the framework emphasizes a progression from ammonia exposure, to a gill-specific transport module, to broader systemic stress modules, enabling validated, nonlethal transcriptional panels for aquaculture management and field surveillance

et al. 2010; Peters et al. 2021, 2020). Contemporary methodological guidance for scoping reviews was followed to support transparent mapping of the available literature and to keep synthesis boundaries explicit (Levac et al. 2010; Peters et al. 2021, 2020). Reporting of identification, screening, eligibility decisions, and synthesis scope was aligned with PRISMA 2020 principles and the PRISMA extension for scoping reviews (PRISMA-ScR), so that record flow and inclusion logic could be traced reproducibly (Page et al. 2021; Tricco et al. 2018). Reporting of the search process (databases, query structure, run dates, and record-management decisions) was additionally aligned with PRISMA-S items to strengthen reproducibility of the identification workflow (Rethlefsen et al. 2021).

Terminology and exposure characterization were standardized as a prerequisite for cross-study interpretability. Total ammonia nitrogen (TAN) was treated as the primary reported metric when available, while unionized ammonia (NH_3, frequently reported as NH_3–N or UIA) was treated as the proximate toxic fraction whose magnitude varies with pH and temperature (and, in saline waters, ionic strength/salinity) (Batley and Simpson 2009; Emerson et al. 1975; Park et al. 2018). Accordingly, for each eligible record, the ammonia metric reported by authors (TAN, NH_3–N, NH_4^+–N, or UIA) was extracted alongside the accompanying water chemistry required to interpret speciation (pH and temperature at minimum; salinity where relevant) (Batley and Simpson 2009; Emerson et al. 1975; Park et al. 2018). Where reported, the pH scale, total alkalinity, and CO_2/

carbonate-system descriptors were also recorded because similar pH values can correspond to different CO_2 conditions and acid–base backgrounds depending on water chemistry. When sufficient parameters were reported, approximate NH_3 fractions were derived using established equilibrium relationships for zero-salinity conditions following Emerson et al. (1975) and saltwater contexts following the salinity-adjusted approach of Bower and Bidwell (1978), while conversions were not attempted when pH/temperature (or salinity for marine systems) were not reported at the exposure time scale (Batley and Simpson 2009; Bower and Bidwell 1978; Emerson et al. 1975). Because regulatory criteria and toxicity relationships for ammonia have been explicitly defined as pH- and temperature-dependent (and, in marine waters, salinity-sensitive), omission of these descriptors was treated as a major interpretability constraint during biomarker inference rather than as a minor reporting detail (Batley and Simpson 2009; Park et al. 2018).

Bibliographic identification was initiated using structured searches in PubMed and expanded through complementary searches in Web of Science Core Collection, Scopus, and Google Scholar to capture aquaculture and ecotoxicology journals with variable biomedical indexing. Database and repository searches were conducted from November 2025 to January 2026. Query strings were designed to reduce common retrieval gaps by combining three concept blocks: (i) ammonia terms and reporting variants (ammonia, total ammonia nitrogen, TAN, un-ionized/unionized ammonia, NH3, NH4 +, ammonium; and common experimental reagents such as ammonium chloride), (ii) fish descriptors (fish, teleost, and frequent model or aquaculture species terms), and (iii) transcriptional outcomes (gene expression, mRNA, RT-qPCR/qPCR, transcriptome, RNA-seq, microarray, differential expression) (Rethlefsen et al. 2021). Backward reference screening and forward citation chasing were applied from core mechanistic and transcriptome papers to identify additional studies not retrieved by keyword searches due to inconsistent indexing or inconsistent use of ammonia descriptors (Rethlefsen et al. 2021). To improve capture of transcriptome datasets and associated companion papers, targeted repository searches were additionally conducted in NCBI Gene Expression Omnibus (GEO), NCBI Sequence Read Archive (SRA), and the European Nucleotide Archive (ENA), where fish transcriptome studies are commonly deposited, and accession identifiers were recorded when provided (Edgar et al. 2002; Katz et al. 2022; Yuan et al. 2024).

Eligibility was restricted to studies meeting all of the following conditions: (i) fish were the exposed organism; (ii) ammonia exposure was defined as a distinct treatment condition (including elevated TAN achieved via ammonium salts), with an interpretable exposure contrast relative to controls; and (iii) a transcriptional endpoint was reported (targeted mRNA measurements, microarray, bulk RNA-seq, or other transcriptomic profiling). Studies were excluded when transcriptional outcomes were absent, when ammonia could not be separated from other nitrogen toxicants or complex mixtures as an identifiable treatment contrast, when the exposed organism was not fish, or when the design lacked an exposure comparator adequate for causal interpretation. Field surveys were not treated as primary evidence for biomarker nomination unless an exposure contrast and supporting water chemistry were reported in a manner sufficient to attribute transcriptional differences to ammonia with reasonable confidence under the study design (Park et al. 2018; Rethlefsen et al. 2021; Tricco et al. 2018). Eligibility criteria and extracted variables were designed to support tissue-resolved synthesis and are summarized in Table 1.

For each eligible record, extraction was performed to support tissue-aware interpretation and biomarker nomination. Study descriptors were recorded (species, life stage, sex where reported, acclimation conditions, exposure route and duration, sampling time points, recovery designs, and control structure), alongside exposure descriptors (ammonia form

Table 1 Inclusion rules, extracted variables, interpretability flags, and tissue-tiering scheme used for this ammonia-focused synthesis

Domain	Operational approach used in this review	Key relevance for ammonia-stress biomarker inference	Ref
Review type and reporting	Evidence mapping performed as a scoping synthesis with PRISMA-aligned documentation of identification and screening decisions	Allows transparent handling of heterogeneous exposure metrics, platforms, and tissues without forcing meta-analysis where harmonization is limited	(Arksey and O'Malley 2005; Levac et al. 2010; Tricco et al. 2018)
Search concepts	Structured searches built from (i) ammonia descriptors (TAN, unionized NH_3, NH_4^+, ammonium salts), (ii) fish terms, and (iii) transcriptional endpoints (qPCR, microarray, RNA-seq)	Reduces retrieval loss caused by inconsistent indexing of ammonia form and reporting units	(Rethlefsen et al. 2021)
Eligibility	Fish exposure studies with a defined ammonia treatment contrast and a transcriptional endpoint (targeted or transcriptome-scale)	Ensures transcript changes can be attributed to ammonia exposure rather than background variability without a comparator	(Arksey and O'Malley 2005; Levac et al. 2010; Tricco et al. 2018)
Core extracted study metadata	Species, life stage, sex (if reported), acclimation, exposure duration, sampling timepoints, recovery design, and control structure	Time-structured signatures are common under ammonia; sampling design strongly shapes apparent "biomarker stability"	(Zimmer 2024)
Exposure characterization	Ammonia form/metric (TAN, NH_3–N/UIA, NH_4^+–N), nominal vs measured values, and water chemistry (pH and pH scale where reported, temperature; salinity, alkalinity, and CO_2/carbonate-system descriptors where relevant) extracted verbatim	NH_3 fraction and diffusion gradients depend on water chemistry; transcript interpretation is weakened when pH/temperature are missing	(Emerson et al. 1975; Park et al. 2018; Zimmer 2024)
Speciation handling	NH_3 fraction derived only when sufficient parameters are reported; otherwise not back-calculated	Avoids misleading standardization when key parameters are absent or mismatched to sampling	(Emerson et al. 1975; Park et al. 2018)
Transcript platform and accessibility	Platform, normalization/threshold reporting (where available), and public accession IDs captured when present	Supports reproducibility and future cross-study module building	(Edgar et al. 2002; Katz et al. 2022; Wilkinson et al. 2016)

Table 1 (continued)

Domain	Operational approach used in this review	Key relevance for ammonia-stress biomarker inference	Ref
Interpretability flags (study-level)	Reporting noted for ammonia measurement method, pH/temperature at exposure, pH scale, alkalinity, CO_2/carbonate-system descriptors, and key confounders (oxygen, density, feeding/fasting, handling)	These covariates can shift baseline expression and alter NH_3 bioavailability; flags inform confidence weighting	(Zimmer 2024)

and metric, nominal versus measured concentrations, pH, temperature, salinity, dissolved oxygen where reported, and water renewal or static/flow-through conditions) (Batley and Simpson 2009; Emerson et al. 1975; Park et al. 2018). Where authors described analytical methods for ammonia measurement, the method family was captured (e.g., phenate/colorimetric, electrode-based, automated methods) because method choice and interference control can influence comparability across matrices and concentration ranges (Lin et al. 2019). Transcript platform details were recorded (RT-qPCR, microarray, RNA-seq), including whether validation of discovery transcriptomics was performed by targeted assays, and whether sequence data accessions were reported to enable downstream reanalysis (Edgar et al. 2002; Katz et al. 2022; Wilkinson et al. 2016; Yuan et al. 2024). Principal genes and pathways emphasized by authors were extracted in a structured manner (transport and acid–base regulation, nitrogen metabolism, oxidative and inflammatory signaling, energy metabolism, cell-death programs), while interpretation of "candidate biomarkers" was intentionally constrained to tissue context to avoid overgeneralization from systemic stress genes.

A tissue prioritization framework was applied to keep the synthesis anchored to biological contact zones and ammonia-handling physiology rather than to organ lists alone. Three operational tissue groups were used. Well-exposed exchange interfaces were defined as tissues directly contacting water and plausibly representing first-contact sites for ammonia flux and early transcriptional response, with gills treated as the primary exchange and excretory surface and additional exposed mucosae (e.g., skin/mucus and olfactory epithelium) retained when explicitly sampled (Zimmer 2024). Metabolically integrative internal tissues were defined as organs expected to integrate downstream consequences of ammonia loading and compensatory nitrogen handling, including liver and kidney (and immune-relevant hematopoietic tissues such as head kidney when sampled), reflecting their recurrent use in ammonia toxicology for interpreting systemic injury and metabolic remodeling (Park et al. 2018; Zimmer 2024). Fitness and regulatory tissues were defined as brain and reproductive tissues (gonad, eggs) when assessed, because endocrine regulation and neurophysiology can be affected by ammonia stress and because such tissues are often used to bridge molecular change to functional outcomes in aquaculture and ecotoxicology designs (Park et al. 2018). This structure was selected to preserve a tissue-forward logic comparable across studies while allowing nonlethal matrices (e.g., mucus-associated sampling) to be retained as a biomonitoring-relevant layer when transcript data were available. Finally, because reproducible biomarker discovery increasingly depends on reusability of primary sequencing data and metadata, availability of public data accessions and minimum metadata elements was recorded whenever reported, and extracted variables were aligned to support future dataset integration under FAIR data principles (Ahi 2025; Wilkinson et al. 2016).

Transcriptional responses at first-contact exchange epithelia

In most teleosts, ammonia handling has been dominated by exchange epithelia, with the gill being treated as the primary surface for both nitrogen waste elimination and early sensing of adverse external gradients (Zimmer 2024). Because gill epithelia simultaneously support gas exchange, ionoregulation, and acid–base control, transcript shifts induced by ammonia have been expected to integrate transport remodeling with broader stress physiology rather than to appear as a single linear pathway (Ip and Chew 2010; Zimmer 2024).

Consequently, candidate transcriptional biomarkers at exchange surfaces have been most defensibly nominated when (i) linkage to established ammonia-excretion mechanisms has been maintained and (ii) tissue specificity and life-stage context have been retained during interpretation (Ip and Chew 2010; Wright and Wood 2009; Zimmer 2024). Representative fish studies supporting the synthesis in this section are summarized in Table 2.

Gill exchange epithelium: transport-centered transcriptional responses

Branchial ammonia excretion has been increasingly explained by models in which Rh glycoproteins participate in transcellular NH_3 movement and "acid trapping" is enhanced by coordinated H^+ secretion and boundary-layer acidification, with additional support provided by carbonic anhydrase–dependent acid–base chemistry and classic ion-transport machinery (Wright and Wood 2009; Zimmer 2024). Because carbonic anhydrase and V-type H^+-ATPase act at different points in this coupled acid-trapping module, current evidence does not support assigning a single universal rate-limiting role to either component. Carbonic anhydrase supports rapid local H^+/HCO_3^- generation from CO_2 hydration, whereas V-type H^+-ATPase and associated proton-extrusion pathways contribute to maintaining an acidified boundary layer; the dominant constraint is therefore expected to vary with species, ionocyte phenotype, external buffering capacity, pH/CO_2 conditions, and exposure duration (Nawata et al. 2007; Wright and Wood 2009; Zimmer 2024). The molecular basis for this framework has been supported by early localization and functional evidence in pufferfish gill, where multiple Rh glycoproteins were reported to be expressed in gill tissue and to mediate ammonium analog transport in heterologous systems, thereby challenging purely passive diffusion views of branchial ammonia elimination (Nakada et al. 2007). In freshwater rainbow trout, high external ammonia (HEA) exposure was associated with time-structured changes in gill Rh transcript abundance and with modulation of acidification-related machinery, with upregulation of V-type H^+-ATPase transcripts being reported alongside changes in *Rhbg* and *Rhcg* isoforms and concurrent evidence for boundary-layer acidification as a facilitating mechanism (Nawata et al. 2007). In follow-up physiology–molecular integration, feeding-driven endogenous ammonia loading was reported to increase branchial mRNA levels of Rh genes, NHE-2, and H^+-ATPase, reinforcing that candidate "ammonia stress" transcripts at the gill can be induced by internal nitrogen turnover as well as by external ammonia challenge (Zimmer et al. 2010).

Life-stage dependence has been repeatedly emphasized in zebrafish, where HEA exposure was associated with elevated Rh mRNA expression in embryos and larvae, whereas adult gills were described as relying more strongly on increased transcription of V-type H^+-ATPase under the same excretory constraint, indicating that the same stressor can engage different transcriptional solutions across development (Braun et al. 2009). In embryos, the chorion may further modify the timing and local microenvironment of ammonia exposure by influencing diffusion conditions around the embryo and the perivitelline space, although direct ammonia-specific evidence for chorion-controlled transcriptional responses remains limited; therefore, embryo–larva–adult differences should be interpreted as the combined outcome of exposure geometry, chorion/perivitelline conditions, and developmental maturation of ionocytes, gill structures, and acid–base transport capacity (Kais et al. 2013)(Pelka et al. 2017). A broader interspecific perspective has been provided by chronic HEA experiments in trout, carp, and goldfish, in which species-specific patterns were reported for Rh isoforms, H^+-ATPase, and Na^+-handling genes, together with physiological evidence that compensatory trajectories differ markedly among freshwater teleosts

Table 2 Representative fish studies reporting transcriptional responses at first-contact exchange epithelia under ammonia exposure

Tissue	Species and life stage	Ammonia exposure context	Platform	Main transcriptional endpoints emphasized	Ref
Gill	Rainbow trout, juvenile/adult	High environmental ammonia challenge; time-structured sampling	Targeted mRNA	Rh glycoprotein isoforms and acidification-linked machinery (e.g., V-type H^+-ATPase) discussed in relation to branchial excretion capacity	(Nawata et al. 2007)
Gill	Rainbow trout, juvenile/adult	Feeding × high environmental ammonia interaction	Targeted mRNA	Coordinated modulation of Rh genes with NHE/H^+-ATPase-associated transport components under endo/exogenous exposure	(Zimmer et al. 2010)
Gill	Zebrafish, embryo/larva/adult	High external ammonia exposure; developmental comparisons	Targeted mRNA	Stage-dependent induction of Rh transcripts and/or H^+-ATPase-linked components; nitrogen-excretion strategy shifts across development	(Braun et al. 2009)
Gill	Multiple teleosts (trout, carp, goldfish)/NR; 132 ± 22/16 ± 4/15 ± 5 g BW	Chronic high environmental ammonia; interspecific comparison	Targeted mRNA	Species-specific regulation of Rh isoforms and ion-transport genes with different compensatory strategies under sustained ammonia	(Sinha et al. 2013)
Gill	Zebrafish, adult	Genetic perturbation under ammonia challenge	Targeted mRNA	Compensatory Rh-family responses reported after disruption of a Rh paralog; implications for single-gene "diagnostic" instability	(Zimmer and Perry 2020)
Gill	Climbing perch/ NR; 25–45 g BW	External ammonia exposure	Targeted mRNA + protein	Rh gene and protein expression changes in gill under ammonia exposure; emphasis placed on specific Rh members as responsive targets	(Chen et al. 2017)

Table 2 (continued)

Tissue	Species and life stage	Ammonia exposure context	Platform	Main transcriptional endpoints emphasized	Ref
Gill + skin	Mangrove killifish/adult	Elevated environmental ammonia and air exposure	Targeted mRNA	Strong induction of RhCG transcripts in gill and skin under ammonia/air conditions; multi-epithelium compensation highlighted	(Hung et al. 2007)
Gill	Large-scale loach/NR	Ammonia loading (acute and/or time series)	Targeted mRNA	Rh-family–related gene regulation described as exposure- and time-dependent; directionality differed with exposure phase	(Huang et al. 2022)
Gill	Yellow catfish/ juvenile	Chronic ammonia nitrogen stress	Multi-omics	Inflammation/remodeling modules emphasized; IL17 signaling highlighted as a candidate pathway linked to hyperplasia	(Zhong et al. 2023)
Gill	Spotted scat/NR	Ammonia stress	RNA-seq	Immune signaling and metabolic pathway enrichment (e.g., cytokine-related and energy metabolism categories) reported in gill	(Xu et al. 2025)
Intestine	Yellow catfish/ NR; 53.67 ± 4.90 g BW	Ammonia nitrogen stress	Targeted mRNA	Tight-junction markers and inflammatory cytokine transcripts altered in association with barrier damage and inflammation	(Liu et al. 2023)
Gill	Large-scale loach/juvenile	Acute ammonia stress (with parallel stress context in study)	RNA-seq	Gill transport-related modules (Rh, aquaporins/ion transport) contrasted with hepatic metabolic/stress programs	(Shang et al. 2021)

Life stage is reported as stated in the original article. When a formal life stage was not explicitly provided, the available size class, body mass, or reproductive/tissue maturity stage is reported instead to avoid over-interpretation. Life stage is reported as stated in the original article. *NR*, not reported or not explicitly stated; *BW*, body weight

under sustained environmental ammonia loading (Sinha et al. 2013). Mechanistic plasticity has also been highlighted by gene-editing work in zebrafish, in which CRISPR/Cas9 disruption of *rhcgb* was not associated with the predicted loss of ammonia excretion capacity across several challenge contexts and compensatory changes (including increased *rhbg* transcript abundance) were described, implying that biomarker panels centered on a single Rh paralog may be unstable across genetic backgrounds or compensatory states (Zimmer and Perry 2020).

Species- and habitat-linked modifiers at the gill surface

In fishes that routinely encounter extreme ammonia or acid–base constraints (e.g., amphibious or alkali-tolerant taxa), transcriptional regulation of ammonia transporters has been shown to extend beyond canonical "teleost averages," thereby providing useful natural experiments for biomarker nomination. In the mangrove killifish, gill *RhCG2* and skin *RhCG1* mRNA were strongly upregulated under elevated environmental ammonia, and air exposure was associated with induction of RhCG transcripts in skin, supporting the view that transcriptional upregulation of Rh genes can be expressed across multiple excretory epithelia when conventional diffusion gradients are disrupted (Hung et al. 2007). In large-scale loach, acute ammonia loading was associated with increased gill *Rhcg*, *Rhbg*, and *Rhag* mRNA abundance, whereas longer exposures were reported to downshift expression of several Rh-related genes, and the absence of key Rh components in skin was interpreted as evidence that cutaneous ammonia excretion is not universally required even in air-breathing fishes (Huang et al. 2022). In Amur ide, which persists in highly alkaline waters that intensify ammonia toxification risk, gill Rh proteins and H^+ transporters were investigated as a coordinated excretory module and were proposed to facilitate active ammonia elimination while supporting acid–base balance under alkali exposure (Zhao et al. 2024). Collectively, these habitat-linked studies have suggested that "Rh + acidification" gene modules are conserved as candidates, but directionality and persistence of transcriptional change are shaped by exposure duration and by evolved physiological context rather than by ammonia concentration alone.

Gill remodeling, inflammation, and energetic reprogramming under ammonia

Although transport-centered transcripts provide mechanistic specificity, ammonia exposure has also been associated with transcriptional programs consistent with epithelial injury, proliferative remodeling, and immune activation at the gill interface. In yellow catfish subjected to chronic ammonia nitrogen stress, combined transcriptomic and proteomic profiling identified coordinated activation of inflammation-linked signaling pathways in gill tissue and highlighted IL17 signaling as a putative mediator of hyperplasia and inflammatory remodeling, with parallel evidence of oxidative stress and tissue pathology (Zhong et al. 2023). Bulk RNA-seq in *Scatophagus argus* gills under ammonia stress has similarly shown that gill transcription can shift toward immune and cellular-defense pathways (e.g., NOD-like receptor signaling, lysosome, cytokine–cytokine receptor interactions) while metabolic pathway genes (glycolysis-related enzymes, TCA-cycle components, and mTOR-associated regulators) are concurrently modulated, supporting the view that barrier remodeling and energetic reallocation co-occur at the primary exchange surface (Xu et al. 2025). These patterns have reinforced that transport-module transcripts alone are unlikely to capture the full gill phenotype under ammonia stress, while also indicating that immune

and metabolic readouts require careful specificity testing because they can be shared across diverse stressors.

An additional, emerging modifier has been suggested by gill microbiome synthesis, in which ammonia-oxidizing bacteria were reported to be present in more than half of publicly available teleost gill microbiome datasets and were proposed to influence host nitrogen excretion by altering local ammonia availability at the gill surface (Mes et al. 2024). While direct connections between gill microbiome composition and host transcriptional ammonia signatures remain to be tested systematically, this evidence has indicated that microenvironmental ammonia processing at the gill boundary may represent an underappreciated source of cross-study variability in gill transcriptional readouts under comparable water-column ammonia metrics (Mes et al. 2024). At present, direct evidence that gill-associated ammonia-oxidizing bacteria independently regulate host immune transcripts remains limited. However, broader gill-microbiome studies indicate that resident microbial communities can interact with epithelial immune, Toll/integrin, and permeability-related pathways; therefore, gill microbiota should be considered a plausible confounder when interpreting inflammation and remodeling markers under ammonia exposure (Mes et al. 2024)(François-Étienne et al. 2023).

Other exposed mucosae: intestinal barrier transcription as a complementary readout

Compared with gill tissue, fewer fish studies have quantified transcriptional responses to ammonia in other exposed mucosae using marker sets explicitly aligned to barrier integrity. In yellow catfish intestine, ammonia nitrogen stress was associated with structural and ultrastructural barrier disruption and with qRT-PCR evidence for altered tight-junction gene expression (including reduced *ZO-1*, *occludin*, and *claudin-1* transcripts and increased *claudin-2*), alongside increased expression of inflammatory cytokine genes, indicating that mucosal barrier gene panels can provide complementary exposure-relevant signals even when ammonia entry is presumed to be gill-dominated (Liu et al. 2023). The limited number of such studies has supported inclusion of intestinal barrier markers as a secondary interface panel rather than as a primary ammonia-diagnosis target, while leaving clear scope for expanding transcriptomic coverage of additional exposed mucosae (skin/mucus and olfactory epithelium) under ammonia challenge. Although direct ammonia-specific transcriptomic evidence from fish olfactory epithelium remains limited, olfactory profiling is justified as a future direction because contaminant-mediated disruption of fish olfaction can impair behaviors related to feeding, alarm responses, predator avoidance, homing, and reproduction (Kermen et al. 2013) (Tierney et al. 2010).

Neuroendocrine, somatotropic, and reproductive transcriptional responses under ammonia challenge

Ammonia is a potent neuroactive stressor in fish. Acute toxicity has therefore often been framed around central nervous system disruption, with downstream effects on behavior and fitness-relevant physiology (Randall and Tsui 2002). Endocrine regulation is also implicated, because ammonia exposure can activate the hypothalamic–pituitary–interrenal (HPI)/corticotropic stress axis and alter growth- and immune-related endpoints under aquaculture-relevant conditions (Randall and Tsui 2002; Xu et al. 2021). For transcriptional

biomarker development, brain and endocrine-axis markers can provide useful context for gill or liver signatures. However, HPI/corticotropic stress-axis, somatotropic growth-axis, and reproductive/gonadotropic-axis responses should be interpreted as related but distinct regulatory layers. These transcripts also respond to many non-ammonia stressors and therefore require context-aware interpretation (Xu et al. 2021). Illustrative studies underpinning the synthesis in this section are compiled in Table 3.

Central stress-axis activation and neuroendocrine control

In rainbow trout, HPI-axis stimulation by high environmental ammonia has recently been supported by combined endocrine and transcriptional evidence, with dose-dependent cortisol elevation and brain-region-specific shifts reported in serotonergic pathway genes (e.g., tryptophan hydroxylase and serotonin receptors) together with altered expression of corticotropin-releasing factor and nonapeptide signaling components (arginine vasotocin and isotocin systems) (Chivite-Alcalde et al. 2025). Pharmacological manipulation of serotonergic signaling has further been used to show that the ammonia-linked neuroendocrine response can be modified by serotonin receptor antagonism, supporting a mechanistic bridge between monoaminergic signaling and endocrine output under hyperammonemic conditions (Chivite-Alcalde et al. 2025). Earlier work in rainbow trout has also shown that appetite suppression during ammonia exposure can be accompanied by temporal and region-specific activation of brain monoaminergic systems and by altered abundance of forebrain corticotropin-releasing factor (*CRF*) and urotensin I (*UI*) transcripts, linking transcriptional modulation of neuropeptide systems to feeding phenotypes under ammonia challenge (Ortega et al. 2005).

Developmental state has been shown to modulate these endocrine responses in zebrafish. Under high environmental ammonia, life-stage-specific patterns have been described in which CRF-system transcripts and whole-body cortisol responses were differentially affected across embryonic and larval stages, and persistent programming effects on cortisol responsiveness to later stressors were reported following larval exposure (Williams et al. 2017). In juvenile turbot, short-term exposure across a graded TAN series has been reported to activate the HPI axis while suppressing growth- and immunity-associated endpoints, consistent with stress-axis recruitment as a component of ammonia-induced growth limitation (Jia et al. 2017). Concordant with this endocrine-growth linkage, acute ammonia exposure in pufferfish has been shown to reduce expression of GH/IGF-axis genes, including tissue-resolved effects in which hepatic *ghr2* and *igf-1* mRNA were downregulated over time under acute exposure while *ghr1* showed high baseline abundance in brain (Cheng et al. 2015). In a marine aquaculture model (gilthead seabream), acute ammonia challenge has similarly been shown to elevate cortisol and to modulate growth and stress-marker transcripts (including *igf2*, *mstn*, *hsp70*, and *glucocorticoid receptor*), indicating that endocrine and growth-marker gene panels can register ammonia as a time-structured acute stressor even when exposures are short (Zarantoniello et al. 2021).

Brain-centered molecular correlates of ammonia neurotoxicity

Mechanistic interpretations of ammonia neurotoxicity have frequently converged on excitotoxicity-linked pathways, with early synthesis proposing that elevated NH_4^+ can disturb neuronal excitability and promote NMDA receptor–mediated cascades that culminate in neuronal injury (Randall and Tsui 2002). At the cellular level, such excitotoxic signaling

Table 3 Representative fish studies reporting transcriptional outcomes in neuroendocrine stress-axis, somatotropic/growth-axis, and reproductive tissues under ammonia exposure

Tissue	Species and life stage	Ammonia exposure context	Platform	Main transcriptional endpoints emphasized	Ref
Brain	Rainbow trout/NR	Ammonia exposure with neuroendocrine profiling	Targeted mRNA + endocrine measures	Serotonergic and hypophysiotropic peptide systems (CRF-related and nonapeptides) associated with stress-axis output under ammonia	(Chivite-Alcalde et al. 2025)
Brain	Rainbow trout/NR	Ammonia-associated appetite suppression paradigm	Targeted mRNA	CRF/UI system transcripts and monoaminergic activation patterns linked to feeding depression	(Ortega et al. 2005)
Whole-body	Zebrafish/embryo/larva	High environmental ammonia during development	Targeted mRNA + cortisol	Developmental-stage-specific CRF-system transcription and long-term effects on cortisol responsiveness	(Williams et al. 2017)
Brain	Zebrafish/adult	Sublethal ammonia exposure	Targeted mRNA + behavior	Glutamatergic metabolism genes and behavior-linked brain transcription changes reported under short exposure	(Lin et al. 2022)
Brain	Zebrafish/larva	Chronic hyperammonemia model	Targeted mRNA + neurochemistry	Neurotransmission module shift (GABA/glutamate handling) with receptor subunit transcription changes	(Probst et al. 2020)
Brain	Pufferfish/NR	Acute ammonia exposure	Targeted mRNA	GH/IGF-axis gene expression changes (GHRs, IGF-1) aligned with growth suppression	(Cheng et al. 2015)
Ovary	Goldfish/adult	High ammonia nitrogen exposure	RNA-seq (with pathology)	Steroidogenesis-associated genes and injury-related pathways altered in ovary	(Qi et al. 2024)

Table 3 (continued)

Tissue	Species and life stage	Ammonia exposure context	Platform	Main transcriptional endpoints emphasized	Ref
Eggs	Siberian sturgeon/adult	Ammonia stress	RNA-seq + WGCNA	Steroidogenesis enzymes and antioxidation genes down-regulated; exposure-associated modules nominated	(Qi et al. 2025)

Life stage is reported as stated in the original article. When a formal life stage was not explicitly provided, the available size class, body mass, or reproductive/tissue maturity stage is reported instead to avoid over-interpretation. Life stage is reported as stated in the original article. *NR*, not reported or not explicitly stated

is expected to involve excessive Ca^{2+} influx through NMDA receptors, followed by mitochondrial dysfunction, impaired ATP production, reactive oxygen species generation, and activation of necrotic or mixed cell-death pathways. In goldfish, which exhibit high tolerance to both hypoxia and ammonia, protective adjustment has been supported by evidence that NMDA receptor subunit abundance can be reduced during sustained high ammonia exposure, while NMDA receptor antagonism has prolonged survival in ammonia-challenged trout, thereby linking receptor-level modulation to differential sensitivity across species (Wilkie et al. 2011). Complementary work in an amphibious teleost (swamp eel) has shown that high brain ammonia tolerance can be accompanied by downregulation of *nkcc1b* mRNA and protein abundance in brain during ammonia exposure, a response that has been discussed as potentially reducing susceptibility to swelling-related pathology under hyperammonemic conditions (Ip et al. 2013).

Zebrafish models have enabled the experimental dissection of these brain-linked mechanisms under controlled exposure. A larval zebrafish hyperammonemia model has been established in which elevated ammonium acetate in water rapidly induced lethality and brain necrosis, and survival was prolonged by NMDA receptor antagonists and by the inhibition of glutamine synthetase, reinforcing the utility of zebrafish for screening neuroprotective interventions relevant to ammonia toxicity (Feldman et al. 2014). Chronic hyperammonemia in zebrafish larvae has additionally been associated with locomotor and feeding abnormalities alongside biochemical and transcriptional features consistent with altered neurotransmission, including increased activity of glutamate decarboxylase isoforms (GAD1/2), elevated GABA, depleted glutamate, and increased expression of GABA-a receptor subunits (Probst et al. 2020). At sublethal exposure levels in adult zebrafish, behavioral alterations in social phenotypes have been reported together with increased brain mRNA expression of glutaminase and glutamate dehydrogenase, supporting the interpretation that glutamatergic metabolism is transcriptionally responsive even during short exposures that do not produce broad cognitive deficits (Lin et al. 2022). Overall, brain-focused candidates have been most defensibly framed as modules—glutamate/GABA handling, NMDA-related signaling, and ion–water balance mediators—rather than as single genes expected to behave uniformly across species and exposure duration (Y. He et al. 2024a, b).

Ovarian and gamete transcription: steroidogenesis and redox vulnerability

Although ammonia effects on growth and survival have been more frequently emphasized than reproduction in routine monitoring, transcriptomic evidence has begun to clarify how ammonia can compromise female reproductive tissues and gamete quality. In goldfish mature ovary, short-term high ammonia nitrogen exposure has been profiled by RNA sequencing with concurrent histological evidence of ovarian injury, and disruption of steroidogenesis-linked transcription has been reported in association with oxidative and atresia-related pathology (Qi et al. 2024). In Siberian sturgeon mature eggs, ammonia stress has recently been shown to generate extensive differential expression accompanied by enrichment of amino-acid metabolism pathways and signatures affecting steroid biosynthesis and antioxidation; suppression of antioxidation-related genes (e.g., *GST* and *GCLM*) and downregulation of key steroidogenic enzymes (*CYP11A1*, *CYP17*, *CYP19A1*) have been reported under high ammonia, and WGCNA has been used to nominate modules and candidate genes associated with exposure severity (Qi et al. 2025). These ovarian and egg signatures are biologically relevant because fish egg quality reflects the capacity of

the egg to be fertilized and develop into a normal embryo, and egg transcriptome profiles have been linked to embryo viability in aquaculture species (Bobe 2015; Ma et al. 2019). Ammonia-specific early-life studies further show that elevated unionized ammonia can impair fertilized eggs and newly hatched larvae, including effects on hatching, deformity, and larval size/survival endpoints (El-Greisy et al. 2016). However, current ammonia-specific evidence has not yet directly established whether maternal ovarian or egg transcriptomic shifts generate persistent inherited F1 stress signatures; this remains a key validation gap for reproductive biomarker development (Qi et al. 2025, 2024). Because reproductive-axis transcripts are also responsive to nutritional status, temperature, reproductive stage, and other environmental or physiological stressors, their highest diagnostic value has been expected when they are interpreted alongside first-contact and metabolic-organ signatures rather than as standalone indicators.

Transcriptional responses in less exposed/internal tissues

Internal organs have been treated as integrative readouts of ammonia challenge because circulating nitrogen load, compensatory nitrogen handling, and secondary oxidative–inflammatory signaling have been expressed more strongly in metabolically active tissues than at the external interface alone (Parvathy et al. 2023; Xu et al. 2021). In aquaculture-oriented syntheses, hepatic injury, immunosuppression, and growth depression have been repeatedly associated with sustained ammonia exposure, while substantial response variation has been attributed to exposure duration, unionized ammonia fraction, and interacting husbandry stressors (Parvathy et al. 2023; Xu et al. 2021). Representative studies for this synthesis are compiled in Table 4.

Liver: detoxification programs and exposure-driven metabolic rewiring

Hepatic transcriptional responses to ammonia have been anchored to the diversion of nitrogen into glutamine via glutamine synthetase and, in some taxa or contexts, to conditional engagement of ureagenic steps when excretion gradients are constrained (Parvathy et al. 2023; Xu et al. 2021). Induction of glutamine synthetase expression under exogenous ammonia exposure has been demonstrated at the tissue level, with increased transcript abundance reported across liver and other internal tissues in a teleost exposed to high ammonia, supporting glutamine synthetase–linked transcripts as mechanistically grounded candidates rather than incidental correlates (Anderson et al. 2002). Yellow catfish experiments provide functional support for glutamine synthetase as an ammonia-tolerance marker. Acute ammonia exposure increased glutamine synthetase mRNA abundance, whereas experimental suppression of glutamine synthetase expression reduced ammonia tolerance (Li et al. 2022).

Complementary evidence has been reported for the ureagenic branch. Carbamoyl phosphate synthetase I (*CPS-I*) mRNA has been shown to increase in liver and kidney following acute ammonia stress in yellow catfish, and compensatory routing toward glutamine synthesis has been described when *CPS-I* function was perturbed, consistent with a regulated balance between nitrogen sequestration and ureagenic processing under ammonia load (Zhang et al. 2022). This balance also has an energetic dimension. Glutamine synthetase provides a relatively direct ATP-dependent route for incorporating ammonia into glutamine, which can rapidly lower free ammonia but retains nitrogen within the body

Table 4 Representative fish studies reporting transcriptional responses in metabolic and immune internal organs under ammonia exposure

Tissue	Species and life stage	Ammonia exposure context	Platform	Main transcriptional endpoints emphasized	Ref
Liver	Nile tilapia/NR	Acute ammonia exposure; time series	RNA-seq + metabolomics	Strong early DEG response; enrichment in lipid/sterol pathways, mitochondrial organization, and intermediary metabolism	(Zhu et al. 2019)
Liver	Fat greenling	Ammonia exposure	RNA-seq	MAPK/FOXO-linked enrichment; immune/redox pathway modulation reported	(Li et al. 2020)
Liver	Yellow catfish/NR	Ammonia stress	Targeted mRNA + functional perturbation	Glutamine synthetase induction; reduced tolerance reported when GS was experimentally suppressed	(Li et al. 2022)
Liver + kidney	Yellow catfish/NR	Acute ammonia exposure	Targeted mRNA + mechanistic assays	CPS I regulation; coordination between ureagenic components and glutamine synthesis network discussed	(Zhang et al. 2022)
Liver	Yellow catfish/juvenile	Ammonia stress	Multi-omics/targeted validation	Autophagy pathway modulation; amino-acid sensing (SLC38A9–mTOR) highlighted	(Li et al. 2024)
Liver	Pseudobagrus ussuriensis/juvenile	Acute ammonia stress	RNA-seq	PI3K–Akt and cell-fate pathway emphasis; injury-associated signatures reported	(Shi et al. 2025)
Kidney	Yellowfin tuna/juvenile; 260.4 ± 56.0 g BW	Acute ammonia nitrogen stress	Targeted mRNA + biochemistry	Antioxidant/immune gene expression shifts in kidney-associated immune tissue reported with enzyme activity changes	(Sun et al. 2024)

Table 4 (continued)

Tissue	Species and life stage	Ammonia exposure context	Platform	Main transcriptional endpoints emphasized	Ref
Spleen + head kidney	Wuchang bream/NR; 9.98 ± 0.48 g BW	Chronic TAN exposure	Targeted mRNA (with pathology)	Cytokine and immunoglobulin markers downregulated; impaired immune signaling described	(Guo et al. 2022)
Head kidney	Yellow catfish/NR	Acute ammonia exposure ± mitigation	Targeted mRNA	Inflammation, oxidative stress, polarization, and apoptosis gene modules modulated; partial alleviation described	(He et al. 2021)
Macrophages	Yellow catfish/NR	Ammonia exposure	Targeted mRNA	Ferroptosis-like transcriptional signature proposed in immune cells	(Gan et al. 2022)
Skeletal muscle	Nile tilapia/juvenile male; ~ 25 g BW	Chronic stress regime including ammonia	RNA-seq	Growth/energy allocation pathways enriched; stress-associated suppression of growth programs inferred	(Mwaura et al. 2023)
Liver + muscle	Mudskipper-related teleost model/NR	High exogenous ammonia	Targeted mRNA	Glutamine synthetase expression induced across multiple internal tissues under ammonia loading	(Anderson et al. 2002)

Life stage is reported as stated in the original article. When a formal life stage was not explicitly provided, the available size class, body mass, or reproductive/tissue maturity stage is reported instead to avoid over-interpretation. Life stage is reported as stated in the original article. *NR*, not reported or not explicitly stated; *BW*, body weight

and may become limited by glutamate availability, osmotic consequences, or downstream glutamine handling. In contrast, ureagenic routing through CPS-I/CPS-III and the ornithine–urea cycle requires higher ATP investment but converts nitrogen into urea, a less toxic and more readily excretable end product. Therefore, pathway use during severe or prolonged ammonia stress is likely shaped by both energetic state and physiological context: glutamine synthesis may dominate as an immediate sequestration response, whereas ureagenic components become more relevant in species or exposure conditions where excretory gradients are constrained and urea-cycle capacity is inducible (Anderson et al. 2002; Ip and Chew 2010; Zhang et al. 2022). Transcriptome-scale studies have indicated that these detoxification responses are embedded within broader metabolic restructuring. In Nile tilapia liver, combined RNA-seq and metabolomics have revealed strongly time-structured differential expression, with enrichment in cholesterol and lipid pathways, mitochondrial organization, and intermediary metabolism, accompanied by coordinated metabolite shifts across exposure time points (Zhu et al. 2019). In fat greenling, liver transcriptome profiling under ammonia exposure has likewise highlighted oxidative- and immune-linked signaling categories (including MAPK- and FOXO-associated enrichment), reinforcing that redox and inflammatory programs can be co-modulated with metabolic remodeling in marine fish (Li et al. 2020). In yellow catfish, ammonia challenge has additionally been linked to autophagy-related pathway modulation, and an amino-acid–sensing axis (SLC38A9–mTOR) has been proposed to shape autophagic dynamics under ammonia stress, with time-dependent regulation of canonical autophagy markers reported by targeted assays (Li et al. 2024). Mechanistically, this axis should not be interpreted as a glutamine-specific ammonia sensor. SLC38A9 is better understood as a lysosomal amino-acid sensor/transporter coupled to Rag–Ragulator–mTORC1 signaling, with particularly strong evidence for arginine-dependent regulation. Therefore, ammonia-derived glutamine may affect SLC38A9–mTOR signaling indirectly by contributing to broader changes in lysosomal and cytosolic amino-acid availability, rather than by being selectively prioritized over other amino acids. This distinction is important when using SLC38A9–mTOR and autophagy markers as ammonia-responsive candidates, because their activation may reflect integrated amino-acid imbalance rather than glutamine accumulation alone (Li et al. 2024; Wang et al. 2015; Wyant et al. 2017). In *Pseudobagrus ussuriensis*, acute ammonia exposure has been associated with extensive hepatic differential expression and pronounced histopathology, with prominent representation of PI3K–Akt–related signaling and cell-fate pathways, illustrating how internal-organ transcriptomes can register ammonia challenge through both signaling and injury-linked gene programs (Shi et al. 2025).

Kidney and head kidney: redox, immune signaling, and nitrogen-handling links

Although the gill has remained the dominant excretory surface in most teleosts, kidney-associated tissues have been used as secondary targets for ammonia-stress transcription because acid–base homeostasis, nitrogen metabolism enzymes, and immune–endocrine coupling converge in renal and hematopoietic compartments (Parvathy et al. 2023; Xu et al. 2021). In yellow catfish, the CPS I response described above has explicitly included kidney upregulation under acute ammonia challenge, indicating that detoxification-linked transcripts are not restricted to liver (Zhang et al. 2022). In juvenile yellowfin tuna, acute NH_3–N exposure has been associated with altered antioxidant and phosphatase activities in trunk kidney, while head-kidney transcription of immune-related genes has been reported to shift with

concentration and time, supporting the use of kidney/head-kidney panels for capturing oxidative balance and immune modulation under ammonia (Sun et al. 2024).

Immune organs and macrophage-centered signatures

Chronic ammonia exposure has been summarized as a driver of impaired innate immune competence in fish, and transcriptional changes in cytokine and pattern-recognition pathways have been interpreted as a mechanistic layer linking ammonia stress to disease vulnerability (Parvathy et al. 2023; Xu et al. 2021). In Wuchang bream exposed for 30 days across a TAN gradient, pathological changes in spleen and head kidney have been reported together with downregulation of cytokine and immunoglobulin markers and altered expression of Toll-like receptor–associated signaling components, with stronger impairment described in spleen than in head kidney (Guo et al. 2022). Cell-resolved approaches have provided additional detail that is often obscured in whole-organ homogenates. In primary head-kidney macrophages of yellow catfish, acute ammonia exposure has been shown to modulate coordinated transcriptional programs spanning inflammation, antioxidation, polarization, and apoptosis, and mitigation experiments have indicated that the magnitude of inflammatory and apoptotic gene responses can be reduced pharmacologically (He et al. 2021). A distinct cell-death module has also been proposed through ferroptosis-oriented work, where ammonia exposure has been reported to trigger ferroptosis-like responses in fish macrophages with accompanying shifts in iron-handling and lipid-peroxidation–related transcripts (Gan et al. 2022). Mechanistically, ammonia may initiate this module by increasing ROS production and depleting GSH, thereby weakening GPX4-linked detoxification of lipid hydroperoxides. In parallel, altered expression of iron-handling genes, including transferrin receptor-, ferritin-, ferroportin-, and ferritinophagy-related targets, may expand or redistribute the labile iron pool. This redox-active iron can promote Fenton-type reactions and accelerate peroxidation of polyunsaturated membrane lipids, linking ammonia-induced oxidative imbalance to ferroptotic membrane injury. Because direct causal ordering among ammonia accumulation, iron redistribution, lipid peroxidation, and immune-cell death remains incompletely resolved, these markers are best interpreted as an emerging ferroptosis-associated module rather than as a single validated pathway endpoint (Gan et al. 2022; K. He et al. 2024a, b).

Muscle as a performance-linked transcript matrix under chronic stress

Skeletal muscle has not been treated as a primary site of ammonia interaction, yet chronic exposure has been reflected in pathways tied to energy allocation and growth (Wu et al. 2024). In Nile tilapia subjected to prolonged husbandry stressors including ammonia, muscle RNA-seq has identified differential expression enriched in pathways associated with muscle activity, energy mobilization, and immunity, consistent with energetic diversion away from growth under sustained stress (Mwaura et al. 2023).

From tissue markers to deployable panels: cross-tissue integration and noninvasive monitoring

Transcriptional responses to ammonia have been repeatedly described as multi-domain phenotypes in which transport remodeling, acid–base control, energy allocation, redox balance, and inflammatory signaling are co-modulated, while directionality and persistence

are strongly conditioned by exposure duration and by the unionized ammonia fraction set by water chemistry (Parvathy et al. 2023; Zimmer 2024). As a result, high diagnostic confidence has rarely been achieved with single transcripts, even when mechanistically specific candidates (e.g., Rh glycoproteins or V-type H+-ATPase subunits) have been targeted, because compensatory substitutions among paralogs and pathway-level redundancy have been documented across taxa and contexts (Qi et al. 2025; Zimmer 2024). Greater robustness has therefore been expected when candidate biomarkers have been treated as tissue-aware gene sets rather than isolated genes, with panels being anchored to (i) gill ammonia handling modules and (ii) liver- and immune-organ integrative stress modules (Parvathy et al. 2023; Zimmer 2024).

Building signatures that remain interpretable across experiments

For cross-study synthesis and panel nomination, gene-set–level interpretation has been widely used to reduce overreliance on DEG lists that may not replicate under modest shifts in dose, timing, or background physiology. Pathway enrichment methods such as Gene Set Enrichment Analysis have been established for extracting reproducible biological programs from genome-scale expression data, and these approaches have been well suited to ammonia datasets where coordinated regulation of transport and metabolism is more informative than any single fold-change value (Subramanian et al. 2005). Candidate panels have been strengthened when first-contact tissues have been prioritized for exposure diagnosis and internal tissues have been used to capture systemic consequences, rather than mixing tissues without a physiological rationale (Parvathy et al. 2023; Zimmer 2024).

Network-guided panel reduction and hub-gene nomination

Module inference has provided a practical route for shrinking transcriptome-scale responses into compact, assay-ready panels. Weighted gene co-expression network analysis (WGCNA) has been widely adopted for resolving co-regulated modules, module–trait associations, and highly connected "hub" genes that can serve as parsimonious proxies for broader pathway activity (Langfelder and Horvath 2008). In fish toxicogenomics, co-expression network construction has been used to derive chemical-class–responsive modules in zebrafish, providing a proof-of-principle that exposure-relevant modules can be recovered even when gene-level overlap is modest across experiments (Shankar et al. 2021). Concentration–response network approaches have also been used to identify chemical-specific gene modules in zebrafish datasets, supporting the feasibility of network-guided feature selection beyond simple DEG ranking (Wilson et al. 2022). Within ammonia-focused fish studies, WGCNA has already been applied in reproductive tissues to prioritize ammonia-associated modules and candidate genes, illustrating that module-level inference is technically feasible in this stressor context and can be extended to gill and liver datasets as public RNA-seq resources expand (Qi et al. 2025).

From panels to classifiers: specificity under confounders

Panel performance has been improved when classification has been treated as a validation problem rather than as an afterthought. In salmonids, nonlethal gill-biopsy gene-expression panels have been curated and validated to discriminate environmental stressors under

multi-stressor experimental designs, and classifier development has been used to show that stressor specificity can be recovered from compact gene sets even when physiological overlap is expected (Akbarzadeh et al. 2024; Houde et al. 2019). Such frameworks have been directly relevant to ammonia biomonitoring because pH, temperature, salinity, oxygen availability, and handling stress can all modulate transcriptional baselines in aquaculture and field settings, thereby creating realistic confounding structure that must be included during training and external validation rather than being ignored (Ahi et al. 2025; Akbarzadeh et al. 2024; Houde et al. 2019; Parvathy et al. 2023; Tang et al. 2024). Model interpretability has also been advanced by feature-attribution approaches such as SHAP, which has provided a formal method for ranking genes by contribution to predictions and for documenting why a classifier relies on particular transcript features (Lundberg et al. 2017).

Portability across species and assay design

A recurring constraint for ammonia biomarker deployment has been the diversity of sentinel species used across aquaculture sectors and regions. Cross-species portability has been improved when ortholog-aware mapping has been used to define conserved candidate genes and when primer design has explicitly accommodated teleost gene duplication and sequence divergence (Pashay Ahi 2025). Comparative transcriptomic work has demonstrated that "universal" fish biomarker candidates can be nominated and that degenerate primer strategies can be implemented to support broader taxonomic coverage, providing a practical template for extending ammonia panels beyond a single model species (Agrawal et al. 2024). For ammonia stress specifically, portability has been expected to be greatest for conserved gill excretion modules (Rh and acid–base transport machinery) and for core metabolic remodeling programs, while immune and endocrine readouts have been anticipated to require stronger species- and context-specific calibration (Parvathy et al. 2023; Zimmer 2024). To make the candidate marker sets more explicit for translational use, Fig. 2 summarizes a tissue-stratified panel framework for ammonia-stress biomonitoring. The gill panel is positioned as the primary exposure-diagnostic module and includes Rh glycoproteins and associated acid–base/ionoregulatory genes, whereas liver, kidney, and immune-organ panels are positioned mainly for severity grading and consequence mapping. The figure also distinguishes marker modules expected to show relatively higher cross-species portability, such as Rh-mediated ammonia transport, boundary-layer acidification, and core nitrogen-metabolism responses, from modules that require stronger species-, life-stage-, and water-chemistry-specific calibration, such as epithelial inflammation, immune activation, neuroendocrine signaling, and reproductive-axis markers. Accordingly, the proposed panels should be interpreted as candidate modules for ortholog-aware assay development and validation rather than as fixed universal gene lists with invariant directionality across all aquaculture species.

Noninvasive and minimally invasive sampling routes

Translation of ammonia transcriptional biomarkers to routine monitoring has been facilitated when sampling burden has been reduced. Nonlethal sampling has been positioned as a preferred approach for transcriptomics in wild fishes and in conservation-sensitive settings, and practical guidance has been consolidated for repeated sampling designs that link molecular state to later performance outcomes (Jeffries et al. 2021). Minimally invasive gill biopsy has been used extensively in salmonid biomarker programs, including

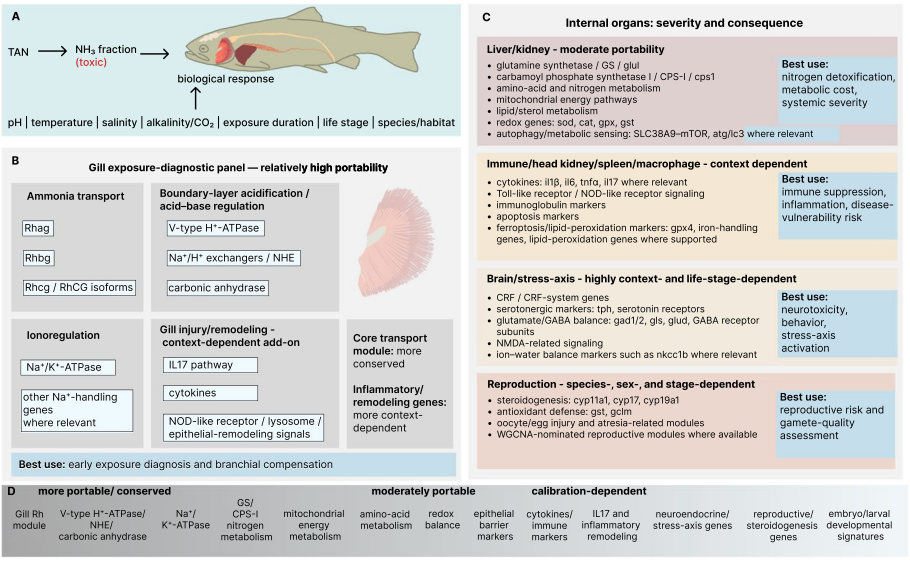

Fig. 2 Tissue-stratified translational biomarker panels for ammonia-stress monitoring in fish. The schematic summarizes candidate transcriptional modules that can be prioritized for assay development across tissues. The gill is positioned as the primary exposure-diagnostic tissue because branchial ammonia transport and acid–base regulation are directly linked to ammonia excretion. The most portable gill candidates include Rh glycoproteins, V-type H^+-ATPase, Na^+/H^+ exchangers, carbonic anhydrase, and Na^+/K^+-ATPase, whereas epithelial inflammation and remodeling markers provide complementary but less ammonia-specific information. Internal organs are positioned mainly for severity grading and consequence mapping. Liver and kidney panels emphasize glutamine synthetase, carbamoyl phosphate synthetase I, amino-acid/nitrogen metabolism, mitochondrial energy pathways, redox balance, and autophagy/metabolic-sensing modules. Immune tissues capture cytokine, pattern-recognition, apoptosis, and ferroptosis-associated responses. Brain and reproductive tissues provide additional information on neuroendocrine disruption, neurotransmission, steroidogenesis, and gamete vulnerability, but these markers are expected to require stronger species-, sex-, and life-stage-specific calibration. The portability gradient indicates that gill transport/acid–base modules and core nitrogen-metabolism markers are expected to be more broadly transferable, whereas immune, neuroendocrine, reproductive, and developmental markers should be validated within the relevant freshwater/marine, cold-/warm-water, life-stage, and water-chemistry context

high-throughput qPCR implementations that permit multi-gene panel measurement from small tissue samples, thereby offering an immediately transferable sampling architecture for ammonia panel deployment in aquaculture (Akbarzadeh et al. 2024; Houde et al. 2019). Epidermal mucus has also been proposed as a nonlethal transcript matrix, and mucus-focused transcriptomic approaches have been reviewed as feasible for physiological surveillance, particularly where repeated sampling is required (Jeffries et al. 2021).

Waterborne environmental RNA has recently expanded the noninvasive horizon (Lofeu and Ahi 2026). Messenger RNA has been demonstrated to be detectable from fish tank water, and methodological steps and constraints for eRNA mRNA typing have been described, supporting feasibility of gene-expression readouts without direct tissue collection (Ahi and Schenekar 2025; Tsuri et al. 2021). Although this review focuses on mRNA biomarkers, future eRNA applications may also incorporate noncoding, structural, or modification-associated RNA layers once their stability and biological interpretation are better resolved (Ahi and Schenekar 2025; Ahi and Singh 2025). Proof-of-concept toxicant studies have further shown that exposure-linked transcriptional signals can be recovered from

Table 5 Roadmap for developing cross-tissue and noninvasive transcriptional biomarker panels of ammonia stress in fish

Step	What is optimized	Typical outputs	Key risks addressed for ammonia applications	Ref
1. Curate comparable datasets	Harmonized exposure descriptors (TAN vs NH_3), pH/temperature (± salinity), timing, tissue labels	Structured evidence matrix for synthesis	Misclassification of "ammonia severity" when NH_3 fraction is not reconstructable	(Emerson et al. 1975; Zimmer 2024)
2. Build tissue-first signatures	Gill transport/acid–base modules; liver metabolic rewiring; immune-organ consequence signatures	Tissue-anchored gene sets and pathway summaries	Overweighting generic stress genes without mechanistic anchoring to ammonia handling	(Zimmer 2024)
3. Infer co-expression modules	Module structure within each tissue and across tissues	Modules, eigengenes, hub genes suitable for panel reduction	Non-replicating DEG lists; unstable single-gene choices under compensation	(Langfelder and Horvath 2008; Shankar et al. 2021)
4. Train and validate classifiers	Minimal gene sets with performance under confounders	Compact qPCR panels + classification model	Overfitting; failure under realistic confounders (temperature/pH, oxygen, handling)	(Akbarzadeh et al. 2024; Houde et al. 2019)
5. Port panels across species	Ortholog mapping and primer design rules	Cross-species assays or conserved modules	Paralogy and annotation gaps in teleosts	(Agrawal et al. 2024)
6. Translate to nonlethal surveillance	Gill biopsy/mucus sampling; water eRNA feasibility tests	Field-ready sampling plan; exploratory eRNA targets	RNA decay, shedding bias, microbial background RNA	(Ahi and Khorshid 2025; Jeffries et al. 2021; Khorshid et al. 2026; Tsuri et al. 2021; Zou et al. 2025)

environmental RNA in controlled fish systems, indicating that physiologically informative targets can be captured from surrounding water under appropriate sampling and analytical conditions (Hiki et al. 2023). More recently, aquarium-water eRNA sequencing has been shown to capture accumulative fish stress responses over short sampling windows, strengthening the practical case for water-based transcript monitoring as methods mature (Miyata et al. 2025; Papakostas et al. 2025). Method-focused syntheses have nevertheless emphasized that RNA decay kinetics, shedding bias among tissues, filtration and extraction choices, and background microbial RNA remain decisive constraints for quantitative interpretation and field deployment (Costea et al. 2026; Zou et al. 2025). Current evidence indicates that tissue-biased fish mRNAs can be recovered from water (Wood et al. 2020), but it remains unclear whether transcripts derived from different matrices, such as gill mucus, skin mucus, intestinal shedding, or gametes, follow comparable degradation trajectories once released. For ammonia applications, this means that candidate eRNA targets should be evaluated not only for tissue specificity and exposure responsiveness but also for matrix-dependent shedding, particle or mucus association, target-transcript stability, microbial/RNase degradation, and water-chemistry effects before quantitative field interpretation is attempted. Accordingly, near-term ammonia applications have been most defensibly framed as tiered: tissue-derived panels have been used to define the target signature first, after which detectability and stability in mucus or water eRNA have been tested empirically for a restricted subset of high-signal transcripts. A translational workflow has therefore been outlined in which (i) tissue-first ammonia signatures are defined for gill and liver, (ii) co-expression modules and hub genes are used to reduce signatures to compact panels, (iii) classifiers are trained and stress-tested under realistic confounders (especially pH and temperature effects on NH_3 fraction), and (iv) deployment is pursued through gill biopsy, mucus, and finally eRNA where stability and sensitivity are demonstrated. A roadmap consistent with this logic is summarized in Table 5.

Conclusions

Ammonia-stress transcription in fish has been supported by a coherent physiological foundation in which first-contact exchange epithelia and metabolically integrative organs have been co-implicated, but diagnostic interpretation has remained highly contingent on exposure chemistry and timing. Accordingly, the most defensible biomarker candidates have been those embedded in ammonia handling itself—particularly Rh-mediated transport and acid–base coupling at the gill—while downstream immune, oxidative, and cell-death signals have been treated as informative but less specific unless evaluated as coordinated panels rather than isolated genes. For field- and farm-facing deployment, several priorities have been indicated. First, ammonia reporting has been required to be biologically interpretable, meaning that TAN has been paired with pH and temperature (and salinity where relevant) at the sampling resolution used for transcript collection, so that NH_3 fraction and gradient context have been reconstructable across studies. Second, tissue choice has been treated as part of assay design: gill signatures have been positioned for exposure diagnosis, whereas liver and immune tissues have been positioned for severity grading and consequence mapping. Third, validation has been expected to be performed explicitly against key confounders typical of aquaculture and field conditions (temperature, oxygen, handling density, and nutritional state), because robustness has not been demonstrated by single-stressor trials alone. Finally, nonlethal monitoring has been positioned as an attainable near-term goal via

gill biopsy and mucus sampling, while environmental RNA has been treated as a plausible medium-term extension once target stability, shedding bias, and decay kinetics have been quantified for ammonia-relevant transcripts under realistic water chemistry. From an implementation perspective, standard chemical water testing for TAN and calculated NH_3 should remain the fastest and most cost-effective first-line approach for routine aquaculture monitoring, whereas targeted RT-qPCR panels are better positioned as scalable complementary assays that report the biological response of fish, support severity grading, and improve interpretation under chronic or confounded exposure scenarios.

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Declarations

Competing interests The authors declare no competing interests.

References

Agrawal I, Lee AQ, Gong Z (2024) Identifying universal fish biomarker genes in response to PCB126 exposure by comparative transcriptomic analyses. Curr Issues Mol Biol 46:7862–7876. https://doi.org/10.3390/CIMB46080466/S1

Ahi EP (2025) eRNA-min: minimum information standard for environmental rNA reporting. https://doi.org/10.20944/PREPRINTS202512.1765.V1

Ahi EP, Khorshid M (2025) Potentials of RNA biosensors in developmental biology. Dev Biol 526:173–188. https://doi.org/10.1016/J.YDBIO.2025.07.011

Ahi EP, Schenekar T (2025) The promise of environmental RNA research beyond mRNA. Mol Ecol 34:e17787. https://doi.org/10.1111/MEC.17787

Ahi EP, Singh P (2025) Emerging Orchestrator of Ecological Adaptation: m6A Regulation of Post-Transcriptional Mechanisms. Mol Ecol 34(15):e17545. https://doi.org/10.1111/MEC.17545

Ahi EP, Lindeza AS, Miettinen A, Primmer CR (2025) Transcriptional responses to changing environments: insights from salmonids. Rev Fish Biol Fish 2025:1–26. https://doi.org/10.1007/S11160-025-09928-9

Akbarzadeh A, Ming TJ, Schulze AD, Kaukinen KH, Li S, Günther OP, Houde ALS, Miller KM (2024) Developing molecular classifiers to detect environmental stressors, smolt stages and morbidity in coho salmon, *Oncorhynchus kisutch*. Sci Total Environ 951:175626. https://doi.org/10.1016/J.SCITOTENV.2024.175626

Anderson PM, Broderius MA, Fong KC, Tsui KNT, Chew SF, Ip YK (2002) Glutamine synthetase expression in liver, muscle, stomach and intestine of *Bostrichthys sinensis* in response to exposure to a high exogenous ammonia concentration. J Exp Biol 205:2053–2065. https://doi.org/10.1242/JEB.205.14.2053

Arksey H, O'Malley L (2005) Scoping studies: towards a methodological framework. Int J Soc Res Methodol 8:19–32. https://doi.org/10.1080/1364557032000119616

Batley GE, Simpson SL (2009) Development of guidelines for ammonia in estuarine and marine water systems. Mar Pollut Bull 58:1472–1476. https://doi.org/10.1016/J.MARPOLBUL.2009.06.005

Bobe J (2015) Egg quality in fish: present and future challenges. Anim Front 5:66–72. https://doi.org/10.2527/AF.2015-0010

Bower CE, Bidwell JP (1978) Ionization of ammonia in seawater: effects of temperature, pH, and salinity. J Fish Res Board Can 35:1012–1016. https://doi.org/10.1139/F78-165

Braun MH, Steele SL, Perry SF (2009) The responses of zebrafish (Danio rerio) to high external ammonia and urea transporter inhibition: nitrogen excretion and expression of rhesus glycoproteins and urea transporter proteins. J Exp Biol 212:3846–3856. https://doi.org/10.1242/JEB.034157

Chen XL, Zhang B, Chng YR, Ong JLY, Chew SF, Wong WP, Lam SH, Nakada T, Ip YK (2017) Ammonia exposure affects the mRNA and protein expression levels of certain Rhesus glycoproteins in the gills of climbing perch. J Exp Biol 220:2916–2931. https://doi.org/10.1242/JEB.157123/262611/AM/AMMONIA-EXPOSURE-AFFECTS-THE-MRNA-AND-PROTEIN

Cheng CH, Yang FF, Liao SA, Miao YT, Ye CX, Wang AL (2015) Effect of acute ammonia exposure on expression of GH/IGF axis genes GHR1, GHR2 and IGF-1 in pufferfish (Takifugu obscurus). Fish Physiol Biochem 41:495–507. https://doi.org/10.1007/S10695-015-0025-1/METRICS

Chivite-Alcalde M, Culbert BM, Larson-Hossack S, Míguez JM, Bernier NJ (2025) Regulation of the neuroendocrine stress axis in response to ammonia exposure in rainbow trout: pharmacological and transcriptional evidence implicating serotonin and multiple hypophysiotropic peptides. Gen Comp Endocrinol 371:114773. https://doi.org/10.1016/J.YGCEN.2025.114773

Costea BA, Boushehri YS, Ahi EP (2026) Targeted environmental RNA profiling through RNase-guided depletion and enrichment. Preprints https://doi.org/10.20944/PREPRINTS202605.1744.V1

Edgar R, Domrachev M, Lash AE (2002) Gene expression omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 30:207–210. https://doi.org/10.1093/NAR/30.1.207

Edwards TM, Puglis HJ, Kent DB, Durán JL, Bradshaw LM, Farag AM (2024) Ammonia and aquatic ecosystems – a review of global sources, biogeochemical cycling, and effects on fish. Sci Total Environ 907:167911. https://doi.org/10.1016/J.SCITOTENV.2023.167911

El-Greisy ZAEB, Elgamal AEE, Ahmed NAM (2016) Effect of prolonged ammonia toxicity on fertilized eggs, hatchability and size of newly hatched larvae of Nile tilapia, Oreochromis niloticus. Egypt J Aquat Res 42:215–222. https://doi.org/10.1016/J.EJAR.2016.04.001

Emerson K, Russo RC, Lund RE, Thurston RV (1975) Aqueous ammonia equilibrium calculations: effect of pH and temperature. J Fish Res Board Can 32:2379–2383. https://doi.org/10.1139/F75-274

Feldman B, Tuchman M, Caldovic L (2014) A zebrafish model of hyperammonemia. Mol Genet Metab 113:142–147. https://doi.org/10.1016/J.YMGME.2014.07.001

François-Étienne S, Nicolas L, Eric N, Jaqueline C, Pierre-Luc M, Sidki B, Aleicia H, Danilo B, Luis VA, Nicolas D (2023) Important role of endogenous microbial symbionts of fish gills in the challenging but highly biodiverse Amazonian blackwaters. Nature Communications. 14(1):3903. https://doi.org/10.1038/s41467-023-39461-x

Gan L, Yao L, Zhang M, He K, Zhang H, Lin Y, Qin C, Shao J, Jiang H, Wen M (2022) Ammonia triggers ferroptosis in macrophages of yellow catfish *Pelteobagrus fulvidraco*. Aquac Res 53:568–575. https://doi.org/10.1111/ARE.15600

Guo H, Chen S, Ouyang K, Kuang Y, Yang H, Wang Y, Tang R, Zhang X, Li D, Li L (2022) Evaluation of ammonia nitrogen exposure in immune defenses present on spleen and head-kidney of Wuchang bream (Megalobrama amblycephala). Int J Mol Sci 23:3129. https://doi.org/10.3390/IJMS23063129/S1

He K, Luo X, Wen M, Wang C, Qin C, Shao J, Gan L, Dong R, Jiang H (2021) Effect of acute ammonia toxicity on inflammation, oxidative stress and apoptosis in head kidney macrophage of Pelteobagrus fulvidraco and the alleviation of curcumin. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 248:109098. https://doi.org/10.1016/J.CBPC.2021.109098

He K, Long X, Jiang H, Qin C (2024) The differential impact of iron on ferroptosis, oxidative stress, and inflammatory reaction in head-kidney macrophages of yellow catfish (*Pelteobagrus fulvidraco*) with and without ammonia stress. Dev Comp Immunol 157:105184. https://doi.org/10.1016/J.DCI.2024.105184

He Y, Ouyang K, Yang H, Wang L, Wang X, Li D, Li L (2024) The impact of ammonia and microcystin-LR on neurobehavior and glutamate/gamma-aminobutyric acid balance in female zebrafish (*Danio rerio*): ROS and inflammation as key pathways. Sci Total Environ 920:170914. https://doi.org/10.1016/J.SCITOTENV.2024.170914

Hiki K, Yamagishi T, Yamamoto H (2023) Environmental RNA as a noninvasive tool for assessing toxic effects in fish: a proof-of-concept study using japanese medaka exposed to pyrene. Environ Sci

Technol 57:12654–12662. https://doi.org/10.1021/ACS.EST.3C03737/SUPPL_FILE/ES3C03737_SI_001.XLSX

Houde AL, Akbarzadeh A, Günther OP, Li S, Patterson DA, Farrell AP, Hinch SG, Miller KM (2019) Salmonid gene expression biomarkers indicative of physiological responses to changes in salinity and temperature, but not dissolved oxygen. Journal of Experimental Biology. 222(13):jeb198036. https://doi.org/10.1242/JEB.198036/259723/AM/SALMONID-GENE-EXPRESSION-BIOMARKERS-INDICATIVE-OF

Huang M, Shang ZH, Wu MX, Zhang LJ, Zhang YL (2022) Regulation of Rhesus glycoprotein-related genes in large-scale loach Paramisgurnus dabryanus during ammonia loading. Ecotoxicol Environ Saf 244:114077. https://doi.org/10.1016/J.ECOENV.2022.114077

Hung CYC, Tsui KNT, Wilson JM, Nawata CM, Wood CM, Wright PA (2007) Rhesus glycoprotein gene expression in the mangrove killifish *Kryptolebias marmoratus* exposed to elevated environmental ammonia levels and air. J Exp Biol 210:2419–2429. https://doi.org/10.1242/JEB.002568

Ip YK, Chew SF (2010) Ammonia production, excretion, toxicity, and defense in fish: a review. Frontiers in physiology. 1:134. https://doi.org/10.3389/FPHYS.2010.00134/XML

Ip YK, Hou Z, Chen XL, Ong JLY, Chng YR, Ching B, Hiong KC, Chew SF (2013) High brain ammonia tolerance and down-regulation of Na+:K+:2Cl- cotransporter 1b mRNA and protein expression in the brain of the swamp eel, Monopterus albus, exposed to environmental ammonia or terrestrial conditions. PLoS ONE 8:e69512. https://doi.org/10.1371/JOURNAL.PONE.0069512

Jeffries KM, Teffer A, Michaleski S, Bernier NJ, Heath DD, Miller KM (2021) The use of non-lethal sampling for transcriptomics to assess the physiological status of wild fishes. Comparative Biochemistry and Physiology Part B: Biochemistry and Mol Biol 256:110629. https://doi.org/10.1016/J.CBPB.2021.110629

Jia R, Liu BL, Han C, Huang B, Lei JL (2017) Effects of ammonia exposure on stress and immune response in juvenile turbot (Scophthalmus maximus). Aquac Res 48:3149–3162. https://doi.org/10.1111/ARE.13145

Kais B, Schneider KE, Keiter S, Henn K, Ackermann C, Braunbeck T (2013) DMSO modifies the permeability of the zebrafish (Danio rerio) chorion-Implications for the fish embryo test (FET). Aquat Toxicol 140–141:229–238. https://doi.org/10.1016/J.AQUATOX.2013.05.022

Katz K, Shutov O, Lapoint R, Kimelman M, Rodney Brister J, O'Sullivan C (2022) The sequence read archive: a decade more of explosive growth. Nucleic Acids Res 50:D387–D390. https://doi.org/10.1093/NAR/GKAB1053

Kermen F, Franco LM, Wyatt C, Yaksi E (2013) Neural circuits mediating olfactory-driven behavior in fish. Front Neural Circuits 7:62. https://doi.org/10.3389/FNCIR.2013.00062

Khorshid M, Khatibani ZA, Ahi EP (2026) Building field-ready environmental RNA panels. Preprints https://doi.org/10.20944/PREPRINTS202605.0141.V1

Langfelder P, Horvath S (2008) WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9:1–13. https://doi.org/10.1186/1471-2105-9-559

Levac D, Colquhoun H, O'brien KK (2010) Scoping studies: advancing the methodology. Implementation science. 5(1):69. https://doi.org/10.1186/1748-5908-5-69/TABLES/3

Li L, Gao F, Jian Y, Wang X, Wang X, Pan L, Guo W, Liu D, Hu F (2020) Transcriptomic analysis of liver tissue in fat greenling (Hexagrammos otakii) exposed to elevated ambient ammonia. Front Mar Sci 7:535250. https://doi.org/10.3389/FMARS.2020.00418/BIBTEX

Li X, Wang S, Zhang M, Yu Y, Li M (2022) Glutamine synthetase (GS) deficiency can affect ammonia tolerance of yellow catfish *Pelteobagrus fulvidraco*. Fish Shellfish Immunol 126:104–112. https://doi.org/10.1016/J.FSI.2022.05.038

Li X, Wang S, Zhang M, Li M (2024) The SLC38A9–mTOR axis is involved in autophagy in the juvenile yellow catfish (*Pelteobagrus fulvidraco*) under ammonia stress. Environ Pollut 343:123211. https://doi.org/10.1016/J.ENVPOL.2023.123211

Lin K, Zhu Y, Zhang Y, Lin H (2019) Determination of ammonia nitrogen in natural waters: recent advances and applications. Trends Environ Anal Chem 24:e00073. https://doi.org/10.1016/J.TEAC.2019.E00073

Lin LY, Horng JL, Cheng CA, Chang CY, Cherng BW, Liu ST, Chou MY (2022) Sublethal ammonia induces alterations of emotions, cognition, and social behaviors in zebrafish (Danio rerio). Ecotoxicol Environ Saf 244:114058. https://doi.org/10.1016/J.ECOENV.2022.114058

Lindholm-Lehto P (2023) Water quality monitoring in recirculating aquaculture systems. Aquac Fish Fish 3:113–131. https://doi.org/10.1002/AFF2.102

Liu S, Luo L, Zuo F, Huang X, Zhong L, Liu S, Geng Y, Ou Y, Chen D, Cai W, Deng Y (2023) Ammonia nitrogen stress damages the intestinal mucosal barrier of yellow catfish (Pelteobagrus fulvidraco)

and induces intestinal inflammation. Front Physiol 14:1279051. https://doi.org/10.3389/FPHYS.2023.1279051/BIBTEX

Lofeu L, Ahi EP (2026) Biomonitoring 3.0: from taxa lists to interaction-ready, time-resolved ecosystem monitoring. Preprints https://doi.org/10.20944/PREPRINTS202603.2242.V1

Lundberg SM, Allen PG, Lee S-I (2017) A unified approach to interpreting model predictions. NeurIPS 30. https://doi.org/10.48550/arXiv.1705.07874

Ma H, Martin K, Dixon D, Hernandez AG, Weber GM (2019) Transcriptome analysis of egg viability in rainbow trout. Oncorhynchus mykiss. BMC genomics. 20(1):319. https://doi.org/10.1186/S12864-019-5690-5/TABLES/4

Mes W, Lücker S, Jetten MS, Siepel H, Gorissen M, van Kessel MA (2024) Gill-associated ammonia oxidizers are widespread in teleost fish. Microbiology Spectrum. 12(11):e00295-24. https://doi.org/10.1128/SPECTRUM.00295-24

Miyata K, Inoue Y, Yamane M, Honda H (2025) Fish environmental RNA sequencing sensitively captures accumulative stress responses through short-term aquarium sampling. Sci Total Environ 959:178182. https://doi.org/10.1016/J.SCITOTENV.2024.178182

Mwaura JG, Wekesa C, Ogutu PA, Okoth P (2023) Whole transcriptome analysis of differentially expressed genes in cultured nile tilapia (*O. niloticus*) subjected to chronic stress reveals signaling pathways associated with depressed growth. Genes 14(4):795. https://doi.org/10.3390/GENES14040795

Nakada T, Westhoff CM, Kato A, Hirose S (2007) Ammonia secretion from fish gill depends on a set of Rh glycoproteins. FASEB J 21:1067–1074. https://doi.org/10.1096/FJ.06-6834COM

Nawata CM, Hung CCY, Tsui TKN, Wilson JM, Wright PA, Wood CM (2007) Ammonia excretion in rainbow trout (Oncorhynchus mykiss): evidence for Rh glycoprotein and H+-ATPase involvement. Physiol Genomics 31:463–474. https://doi.org/10.1152/PHYSIOLGENOMICS.00061.2007/ASSET/IMAGES/LARGE/ZH701507313200015.JPEG

Ortega VA, Renner KJ, Bernier NJ (2005) Appetite-suppressing effects of ammonia exposure in rainbow trout associated with regional and temporal activation of brain monoaminergic and CRF systems. J Exp Biol 208:1855–1866. https://doi.org/10.1242/JEB.01577

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. https://doi.org/10.1136/BMJ.N71

Papakostas S, Schenekar T, Ahi EP (2025) Translational biodiversity beyond genomics: toward systemic action. https://doi.org/10.32942/X23644

Park TJ, Lee JH, Lee MS, Park CH, Lee CH, Moon SD, Chung J, Cui R, An YJ, Yeom DH, Lee SH, Lee JK, Zoh KD (2018) Development of water quality criteria of ammonia for protecting aquatic life in freshwater using species sensitivity distribution method. Sci Total Environ 634:934–940. https://doi.org/10.1016/J.SCITOTENV.2018.04.018

Parvathy AJ, Das BC, Jifiriya MJ, Varghese T, Pillai D, Rejish Kumar VJ (2023) Ammonia induced toxico-physiological responses in fish and management interventions. Rev Aquac 15:452–479. https://doi.org/10.1111/RAQ.12730

Pashay Ahi E (2025) Fish evo-devo: moving toward species-specific and knowledge-based interactome. J Exp Zool B Mol Dev Evol. https://doi.org/10.1002/JEZ.B.23287

Pelka KE, Henn K, Keck A, Sapel B, Braunbeck T (2017) Size does matter - determination of the critical molecular size for the uptake of chemicals across the chorion of zebrafish (*Danio rerio*) embryos. Aquat Toxicol 185:1–10. https://doi.org/10.1016/J.AQUATOX.2016.12.015

Peters MDJ, Marnie C, Tricco AC, Pollock D, Munn Z, Alexander L, McInerney P, Godfrey CM, Khalil H (2020) Updated methodological guidance for the conduct of scoping reviews. JBI Evid Synth 18:2119–2126. https://doi.org/10.11124/JBIES-20-00167

Peters MD, Marnie C, Colquhoun H, Garritty CM, Hempel S, Horsley T, Langlois EV, Lillie E, O'Brien KK, Tunçalp Ö, Wilson MG (2021) Scoping reviews: reinforcing and advancing the methodology and application. Systematic reviews. 10(1):263. https://doi.org/10.1186/S13643-021-01821-3/PEER-REVIEW

Probst J, Kölker S, Okun JG, Kumar A, Gursky E, Posset R, Hoffmann GF, Peravali R, Zielonka M (2020) Chronic hyperammonemia causes a hypoglutamatergic and hyperGABAergic metabolic state associated with neurobehavioral abnormalities in zebrafish larvae. Exp Neurol 331:113330. https://doi.org/10.1016/J.EXPNEUROL.2020.113330

Qi Q, Zhang C, Xu R, Lv C, Xue Y, Wang P, Li Z (2024) Chen M (2024) High Ammonia Nitrogen-Induced Reproductive Toxicity in Goldfish (Carassius auratus) Mature Ovary. Aquaculture Research. 1:9577902. https://doi.org/10.1155/2024/9577902

Qi Q, Zhang C, Wu W, Zhou Q, Lv C, Sun X, Yang F (2025) Ammonia stress induces transcriptional expression changes in the mature eggs of the Acipenser baerii. Animals 15:3122. https://doi.org/10.3390/ANI15213122/S1

Randall DJ, Tsui TKN (2002) Ammonia toxicity in fish. Mar Pollut Bull 45:17–23. https://doi.org/10.1016/S0025-326X(02)00227-8

Rethlefsen ML, Kirtley S, Waffenschmidt S, Ayala AP, Moher D, Page MJ, Koffel JB (2021) PRISMA-S: an extension to the PRISMA statement for reporting literature searches in systematic reviews. Systematic reviews.10(1):39. https://doi.org/10.1186/S13643-020-01542-Z/PEER-REVIEW

Shang ZH, Huang M, Wu MX, Mi D, You K, Zhang YL (2021) Transcriptomic analyses of the acute aerial and ammonia stress response in the gill and liver of large-scale loach (Paramisgurnus dabryanus). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 250:109185. https://doi.org/10.1016/J.CBPC.2021.109185

Shankar P, McClure RS, Waters KM, Tanguay RL (2021) Gene co-expression network analysis in zebrafish reveals chemical class specific modules. BMC genomics. 22(1):658. https://doi.org/10.1186/S12864-021-07940-4/TABLES/5

Shi S, Sun X, Zhang C, Lv C, Liu Y, Du J, Qi Q (2025) Transcriptome analysis of the effect of acute ammonia stress on Pseudobagrus ussuriensis liver tissue. Fishes. 10(1):17. https://doi.org/10.3390/FISHES10010017

Sinha AK, Liew HJ, Nawata CM, Blust R, Wood CM, De Boeck G (2013) Modulation of Rh glycoproteins, ammonia excretion and Na+ fluxes in three freshwater teleosts when exposed chronically to high environmental ammonia. J Exp Biol 216:2917–2930. https://doi.org/10.1242/JEB.084574/257650/AM/MODULATION-OF-RH-GLYCOPROTEINS-AMMONIA-EXCRETION

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 102:15545–15550. https://doi.org/10.1073/PNAS.0506580102/SUPPL_FILE/06580FIG7.JPG

Sun Y, Fu Z, Ma Z (2024) The effects of acute ammonia nitrogen stress on antioxidant ability, phosphatases, and related gene expression in the kidney of juvenile yellowfin tuna (Thunnus albacares). Journal of Marine Science and Engineering. 12(6):1009. https://doi.org/10.3390/JMSE12061009

Tang L, Bian M, Zhang P, Wang J (2024) Salinity mediates the damage caused by acute and chronic ammonia stress in largemouth bass (*Micropterus salmoides*). Sci Total Environ 957:177494. https://doi.org/10.1016/J.SCITOTENV.2024.177494

Tierney KB, Baldwin DH, Hara TJ, Ross PS, Scholz NL, Kennedy CJ (2010) Olfactory toxicity in fishes. Aquat Toxicol 96:2–26. https://doi.org/10.1016/J.AQUATOX.2009.09.019

Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, Moher D, Peters MDJ, Horsley T, Weeks L, Hempel S, Akl EA, Chang C, McGowan J, Stewart L, Hartling L, Aldcroft A, Wilson MG, Garritty C, Lewin S, Godfrey CM, MacDonald MT, Langlois EV, Soares-Weiser K, Moriarty J, Clifford T, Tunçalp Ö, Straus SE (2018) PRISMA extension for scoping reviews (PRISMA-ScR): checklist and explanation. Ann Intern Med 169:467–473. https://doi.org/10.7326/M18-0850/SUPPL_FILE/M18-0850_SUPPLEMENT.PDF

Tsuri K, Ikeda S, Hirohara T, Shimada Y, Minamoto T, Yamanaka H (2021) Messenger RNA typing of environmental RNA (eRNA): a case study on zebrafish tank water with perspectives for the future development of eRNA analysis on aquatic vertebrates. Environ DNA 3:14–21. https://doi.org/10.1002/EDN3.169

Wang S, Tsun ZY, Wolfson RL, Shen K, Wyant GA, Plovanich ME, Yuan ED, Jones TD, Chantranupong L, Comb W, Wang T (2015) Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1. Science. 347(6218):188–94. https://doi.org/10.1126/SCIENCE.1257132/SUPPL_FILE/1257132.WANG.SM.PDF

Wilkie MP, Pamenter ME, Duquette S, Dhiyebi H, Sangha N, Skelton G, Smith MD, Buck LT (2011) The relationship between NMDA receptor function and the high ammonia tolerance of anoxia-tolerant goldfish. J Exp Biol 214:4107–4120. https://doi.org/10.1242/JEB.057513

Wilkinson MD, Dumontier M, Aalbersberg IJ, Appleton G, Axton M, Baak A, Blomberg N, Boiten JW, da Silva Santos LB, Bourne PE, Bouwman J (2016) The FAIR Guiding Principles for scientific data management and stewardship. Scientific data. 3(1):1–9. https://doi.org/10.1038/sdata.2016.18

Williams TA, Bonham LA, Bernier NJ (2017) High environmental ammonia exposure has developmental-stage specific and long-term consequences on the cortisol stress response in zebrafish. Gen Comp Endocrinol 254:97–106. https://doi.org/10.1016/J.YGCEN.2017.09.024

Wilson LB, McClure RS, Waters KM, Simonich MT, Tanguay RL (2022) Concentration-response gene expression analysis in zebrafish reveals phenotypically-anchored transcriptional responses to retene. Front Toxicol 4:950503. https://doi.org/10.3389/FTOX.2022.950503/BIBTEX

Wood SA, Biessy L, Latchford JL, Zaiko A, von Ammon U, Audrezet F, Cristescu ME, Pochon X (2020) Release and degradation of environmental DNA and RNA in a marine system. Sci Total Environ 704:135314. https://doi.org/10.1016/J.SCITOTENV.2019.135314

Wright PA, Wood CM (2009) A new paradigm for ammonia excretion in aquatic animals: role of Rhesus(Rh) glycoproteins. J Exp Biol 212:2303–2312. https://doi.org/10.1242/JEB.023085

Wu Y, Xia Y, Hu A, Xiong G, Wu W, Shi L, Chen L, Guo X, Qiao Y, Liu C, Yin T, Wang L, Chen S (2024) Difference in muscle metabolism caused by metabolism disorder of rainbow trout liver exposed to ammonia stress. Sci Total Environ 924:171576. https://doi.org/10.1016/J.SCITOTENV.2024.171576

Wyant GA, Abu-Remaileh M, Wolfson RL, Chen WW, Freinkman E, Danai LV, Vander Heiden MG, Sabatini DM (2017) mTORC1 activator SLC38A9 is required to efflux essential amino acids from lysosomes and use protein as a nutrient. Cell 171:642-654.e12. https://doi.org/10.1016/J.CELL.2017.09.046/ASSET/2B9476AA-06BA-4193-8540-DC79BD26690C/MAIN.ASSETS/GR7.JPG

Xu Z, Cao J, Qin X, Qiu W, Mei J, Xie J (2021) Toxic effects on bioaccumulation, hematological parameters, oxidative stress, immune responses and tissue structure in fish exposed to ammonia nitrogen: a review. Animals. 11(11):3304. https://doi.org/10.3390/ANI11113304

Xu H, Zhang Z, Zhu H, Xu Q, Li S, Chen J (2025) Histological and transcriptomic profiling reveals metabolic and immune responses to ammonia stress in Scatophagus argus. Fishes. 10(8):412. https://doi.org/10.3390/FISHES10080412

Yuan D, Ahamed A, Burgin J, Cummins C, Devraj R, Gueye K, Gupta D, Gupta V, Haseeb M, Ihsan M, Ivanov E, Jayathilaka S, Kadhirvelu VB, Kumar M, Lathi A, Leinonen R, McKinnon J, Meszaros L, O'Cathail C, Ouma D, Paupério J, Pesant S, Rahman N, Rinck G, Selvakumar S, Suman S, Sunthornyotin Y, Ventouratou M, Vijayaraja S, Waheed Z, Woollard P, Zyoud A, Burdett T, Cochrane G (2024) The European Nucleotide Archive in 2023. Nucleic Acids Res 52:D92–D97. https://doi.org/10.1093/NAR/GKAD1067

Zarantoniello M, Bortoletti M, Olivotto I, Ratti S, Poltronieri C, Negrato E, Caberlotto S, Radaelli G, Bertotto D (2021) Salinity, temperature and ammonia acute stress response in seabream (Sparus aurata) juveniles: A multidisciplinary study. Animals. 11(1):97. https://doi.org/10.3390/ANI11010097

Zhang M, Wang S, Sun L, Gan L, Lin Y, Shao J, Jiang H, Li M (2022) Ammonia induces changes in carbamoyl phosphate synthetase I and its regulation of glutamine synthesis and urea cycle in yellow catfish *Pelteobagrus fulvidraco*. Fish Shellfish Immunol 120:242–251. https://doi.org/10.1016/J.FSI.2021.11.023

Zhao XF, Huang J, Li W, Wang SY, Liang LQ, Zhang LM, Liew HJ, Chang YM (2024) Rh proteins and H+ transporters involved in ammonia excretion in Amur Ide (Leuciscus waleckii) under high alkali exposure. Ecotoxicol Environ Saf 273:116160. https://doi.org/10.1016/J.ECOENV.2024.116160

Zhong L, Liu S, Zuo FY, Geng Y, Ouyang P, Chen D, Yang S, Zheng W, Xiong Y, Cai W, Huang X (2023) The IL17 signaling pathway: a potential signaling pathway mediating gill hyperplasia and inflammation under ammonia nitrogen stress was identified by multi-omics analysis. Sci Total Environ 867:161581. https://doi.org/10.1016/J.SCITOTENV.2023.161581

Zhou J, Mogollón JM, van Bodegom PM, Beusen AHW, Scherer L (2024) Global regionalized characterization factors for phosphorus and nitrogen impacts on freshwater fish biodiversity. Sci Total Environ 912:169108. https://doi.org/10.1016/J.SCITOTENV.2023.169108

Zhu ZX, Jiang DL, Li BJ, Qin H, Meng ZN, Lin HR, Xia JH (2019) Differential transcriptomic and metabolomic responses in the liver of Nile tilapia (Oreochromis niloticus) exposed to acute ammonia. Mar Biotechnol 21:488–502. https://doi.org/10.1007/S10126-019-09897-8/FIGURES/6

Zimmer AM (2024) Ammonia excretion by the fish gill: discoveries and ideas that shaped our current understanding. Journal of Comparative Physiology B. 194(5):697–715. https://doi.org/10.1007/S00360-024-01561-5

Zimmer AM, Perry SF (2020) The Rhesus glycoprotein Rhcgb is expendable for ammonia excretion and Na+ uptake in zebrafish (Danio rerio). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 247:110722. https://doi.org/10.1016/J.CBPA.2020.110722

Zimmer AM, Nawata CM, Wood CM (2010) Physiological and molecular analysis of the interactive effects of feeding and high environmental ammonia on branchial ammonia excretion and Na+ uptake in freshwater rainbow trout. J. Comp Physiol B Biochem Syst Environ Physiol 180:1191–1204. https://doi.org/10.1007/S00360-010-0488-4/METRICS

Zou N, Wang S, Qiu W, Kong W, Wang G, Wang S (2025) Environmental RNA as a transformative tool for aquatic ecosystem health assessment: progress and challenges. Ecol Indic 180:114328. https://doi.org/10.1016/J.ECOLIND.2025.114328