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Combined Thermal and Chemical Stress in Freshwater Food Fishes: Evaluating the Link between Histopathological Responses and Nutritional Quality

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ABSTRACT:

Freshwater food fishes experience temperature fluctuations in the water as well as chemical pollution, however, the correlation between organ damage and edible tissue composition is unclear. This review discusses reported histopathological responses, muscle composition and contaminant levels and experimental evidence for thermal–chemical interactions. From a non-systematic web search and source checking 25 publications were found, of which 24 contained core experiments and one was a link to an already found experiment. Fourteen experiments reported organ histology and ten reported nutrient-related or contaminant endpoints in edible muscle. Gill and liver responses were most commonly reported and warming did not always exacerbate lesions. Damage to the intestine was reported in cases of exposure to the polyamide microplastic and damage of the muscles in cases of exposure to ammonia and warming. Studies on muscle chemistry reported phospholipid fatty acid remodelling and contamination levels from various contaminants, with no apparent concurrent histological assessment of the inspection evidence. Three experiments reported significant temperature × chemical terms for quantitative endpoints; treatment was confounded with housing in two, and no formal histological interaction test was identified in the inspected material. Most core experiments were assessed through abstracts or repository summaries, so unreported endpoints cannot be assumed to have been unmeasured. Nutrient delivery per edible portion, contaminant speciation and paired recovery of structure and composition could not be established. The review therefore differentiates evidence of fish injury, membrane acclimation and contaminant accumulation from sign of changed human nutritional rate. The best test of these relationships would be repeatedly performed factorial studies in which reliable histology would be accompanied by absolute quantities of muscle nutrients and chemically appropriate quantitative measurements of contaminants.

1 INTRODUCTION AND REVIEW QUESTIONS

1.1 Environmental and nutritional problem

Climate warming and chemical contamination increasingly act on the same freshwater systems (Noyes et al., 2009). Water temperature can alter how contaminants move, how fast fish take them up and transform them, and how toxic they become (Noyes et al., 2009; Sokolova & Lannig, 2008). In laboratory exposures of carp, Arctic charr and tilapia, warmer water raised tissue cadmium burdens (Castaldo et al., 2021; Martyniuk et al., 2025; Karayakar et al., 2022). All three are food fishes (Table 1). Fish provide protein, energy, and long-chain n-3 fatty acids and subsequent FAO/WHO expert consultations have considered the benefits against contaminants, mainly dioxins and methylmercury (FAO/WHO, 2011; FAO & WHO, 2024).

Additionally, temperature affects composition by pathways that do not necessarily involve tissue damage: temperature (acclimation) remodelled the fatty acid composition of the brook trout muscle; growth rate influenced muscle mercury concentration in brook trout (Fadhlaoui & Couture, 2016; Rutledge et al., 2025). Combined stress appears to alter human diet, so it must be measured edible-tissue composition, rather than fish health. All temperatures refer to the water temperature of the live fish and not processing or cooking temperature.

1.2 Unresolved connection

Some previous syntheses have applied some of these methods to parts of this problem. A recent narrative review analysed the experimental combination of warming with pharmaceuticals in fish (Lourenço et al., 2025) and another recent review assessed organ damage in fish using histopathological, biochemical and imaging method (Jerald et al., 2024), and a previous review suggested a reduction in energy metabolism as the mechanism for the temperature–metal interaction in aquatic ectotherms (Sokolova & Lannig, 2008). None of the abstracts presented by the judges questioned if there is a correlation between nutritional composition of edible tissue and organ injury.

This connection can't be identified on the basis of lesion listing or oxidative markers. Much published fish histopathology fails credibility criteria, so a lesion list is weak evidence even of tissue injury (Wolf & Maack, 2017; Wolf, 2026). Oxidative markers may also respond differently: Malondialdehyde levels were lower in the poorest condition group of yellow perch (Fadhlaoui & Couture, 2016). Another problem arises from the assumption of synergy; synergy and antagonism are only defined in relation to an explicit null model and scale, and scales are defined differently in different disciplines (Piggott et al., 2015; Orr et al., 2020).

1.3 Review questions and contribution

We pose three questions regarding combined thermal and chemical exposure to freshwater food fishes. Is the combination exposure making the tissue responses change or the relative vulnerability of organs change? Are there histological responses and edible-tissue composition measurements taken together and have these measured components changed together? Are the structures and/or compositions restored after the end of the exposure? In parallel to these questions, we evaluate published statements of interactions with explicit reference models.

The most significant part of the support is the suggestion that structural loss and edible-tissue chemistry are associated, not that all damaged fish essentially yield less nutritious food (Figure 1). A change in composition of fish which is histologically normal in all tissues sampled would be equally informative. The confirmation map classifies stated endpoint coverage from established endpoint pairing and from measurements whose status is still indistinct (Figure 4).

2 REVIEW METHODS

2.1 Literature retrieval

This is a critical review which includes a documented search, not a systematic review or an exhaustive evidence census. The search log from the original search contains 92 query strings that were run through a general web search service on 28th and 29th October 2026 with 48, 31 and 13 in each round (Supplementary Table S2). Searches also included freshwater fish and temperature with chemical exposures, muscle composition, histology, protein. fatty acids, and contaminant burden. Later rounds included the addition of plastics, nitrogenous compounds, intestinal and muscle histology and pharmaceuticals. Unsystematic scan of reference lists and partial scanning of tables in a review of warming and pharmaceuticals. During revision, targeted source verification revealed a previously unclassified paraquat–temperature experiment in Nile tilapia (Figueiredo-Fernandes et al., 2006); other queries regarding the same experiment are kept separately as Supplementary Table S2. These databases (Web of Science, CAB Abstracts Scopus, and ASFA) were not searched directly. The evolving retrieval and incomplete full-text access mean that coverage counts designate the reviewed evidence and do not establish the lack of other appropriate experiments.

2.2 Eligibility and classification

Core experiments exposed a freshwater food fish to contrasting water temperatures together with an environmental chemical or particulate contaminant and reported organ histology, a nutrient-related muscle endpoint or a contaminant burden in edible tissue. Exposure through sediment or feed, contaminated water was eligible. Therapeutic drug-administration and withdrawal trials were outside the environmental-exposure scope; pharmaceutical exposures designed as environmental toxicology

experiments remained potentially eligible. This distinction was made explicit during revision, rather than treated as a prespecified protocol rule. Classification decisions are recorded in Supplementary Table S1. Feeding interventions without separable thermal and chemical contrasts were retained as supporting evidence (Kumar et al., 2020b, 2021, 2024b). This included the core counts and also some contextual counts – namely ornamental/non-food species, thermal-only studies, and experiments without eligible endpoints. There were two records that were not resolved (Reinert et al., 1974; Rutledge et al., 2025). This revised set includes 24 core experiments (C01 to C24) and one related publication, for a total of 25 publications. Fourteen experiments reported histology (C01 – C13 and C24) and ten reported edible-muscle chemistry (C14 – C23). To the study of Grasset et al. (2016) with another 20 °C group, which was explicitly designated by Fadhlaoui and Couture (2016), the remaining fish were not counted as a new core experiment as there was no resolution of the relationship between the study and eligible endpoints.

2.3 Extraction and appraisal

The species and life stage extracted, thermal/chemical treatment, tissues extracted, water chemistry, exposure measurement, diet, replication, experimental groups, and assays and sampling times (Supplementary Table S3) were all covered. In full text, eight core reports were available, and sixteen through abstracts or summaries in the repository. Full text is not the same thing as all outcomes being extracted. If a measurement was not present in an abstract, and was not reported in the inspected source, then it was not coded as confirmed absent. Checks for bibliographic data and records in the publishers and PubMed and records in the repository were conducted and kept apart from the records of the scientific claims. The original search and extraction were performed without independent duplicate assessment; targeted re-extraction during revision corrected identified discrepancies but did not constitute a second independent review. No pooled effect sizes were calculated.

Appraisal considered factorial completeness, measured exposure, the independent unit of replication, histopathology methods, analytical reporting basis and internal consistency. Histology assessment documented diagnostic definitions, blinding, scoring, fixation and the number of fish where available, informed by guidance on interpretive pitfalls (Wolf et al., 2015; Wolf, 2026). Interaction appraisal separated the reported statistical result from the support provided by independently replicated treatment units (Piggott et al., 2015; Orr et al., 2020). Endpoint status was distinguished as reported measured, explicitly not measured where verifiable, or unreported/uncertain in the inspected material. Counts of zero in the evidence map mean no confirmed paired report was identified; they do not establish that unreported measurements were never made.

3 COMPARATIVE BIOLOGICAL FOUNDATIONS

3.1 Thermal niche and metabolic scope

The experiments span very dissimilar thermal niches, so the similar nominal contrast executes dissimilar physiological loads. Contrasts ranged from 6 vs. 16 °C in Arctic charr to 9 vs. 28 °C in yellow perch (Martyniuk et al., 2025; Fadhlaoui & Couture, 2016). Mozambique tilapia were compared at 25 and 32 °C, whereas striped catfish were held at a single elevated temperature of 34 °C (Ribeiro et al., 2024; Kumar et al., 2020b). The distance from the growth optimum is a problem within a species. Nile tilapia produced the best growth at 28 °C compared to four temperatures ranging from 20 to 32 °C in the absence of cadmium in the water (Abdel-Tawwab & Wafeek, 2014). At 18 °C and 35 °C, Tambaqui exhibited tissue architecture changes, while at 29 °C, it was normal (Rojas et al., 2013). The responses at market size remain largely untested as most experiments have been conducted using juveniles and one using adult males (Castaldo et al., 2021; Martyniuk et al., 2025; Ribeiro et al., 2024). Sex must be considered as well: in the Nile tilapia, sex-dependent biochemical and reproductive responses were observed as well as liver histopathology in the experiment using paraquat at 17 and 27 °C (Figueiredo-Fernandes et al., 2006). The evidence cannot therefore be qualified to be generalizable across sex or reproductive state.

Thermal history also varied: fish were ramped by 1–2 °C per day or held for at minimum two weeks at the assessment temperature (Fadhlaoui & Couture, 2016; Martyniuk et al., 2025; Castaldo et al., 2021). In carp, warming raised standard and maximum metabolic rates, but no thermal coefficient estimate was identified in the inspected core reports; Q10 is the factor by which a rate changes over 10 °C (Pillet et al., 2021). Oxygen- and capacity-limited thermal tolerance, which ties thermal limits to the match between metabolic oxygen demand and supply, is treated here as a context-dependent hypothesis (Ern et al., 2016; Brijs et al., 2015). Upper thermal limits did not depend on oxygen supply in red drum, lumpfish or European perch (Ern et al., 2016; Brijs et al., 2015). Across the fishes tested so far, hyperoxia raised thermal limits in about half (McArley et al., 2022).

3.2 Normal architecture and sampling

A lesion means something only against reference tissue from the same species, organ region and conditions. In tambaqui, gill, liver and kidney architecture was normal at 29 °C (Rojas et al., 2013). In rainbow trout, warming to 20 °C changed gill morphometry without histopathological abnormality (Lahnsteiner, 2024). Sampling was often partly stated. One study described both paraformaldehyde and Bouin fixation (Hu et al., 2024), whereas another used 10% buffered formalin, 3 μm sections and haematoxylin and eosin (Ribeiro et al., 2024). Fish histopathology can often lead to misdiagnosis of tissue artefacts or normal anatomical structures as lesions (Wolf et al., 2015). A survey of 100 gill toxicology papers found only 11 that were legitimate histopathology studies (Wolf, 2026). For the sample taken from the dorsal region for histology and/or chemistry, no reports indicated the fibre type, but only the dorsal region (Martyniuk et al., 2025; Abd-elaziz et al., 2026).

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3.3 Exposure kinetics and susceptibility

Warming may alter toxicity through changes in uptake and biotransformation, changes in enzyme activity (biotransformation), and changes in physiological sensitivity (Noyes et al., 2009; Sokolova & Lannig, 2008). These routes need to be kept apart. Pharmaceuticals: Warming might increase in-body exposure at the same time external exposure concentrations remain unchanged (Lourenço et al., 2025). Measured burdens show the same for cadmium in carp and Arctic charr (Castaldo et al., 2021; Martyniuk et al., 2025). Measured water concentrations can depart from nominal values; waterborne cadmium averaged 1.81 μg/L against a nominal 3 μg/L in the charr study (Martyniuk et al., 2025). Cadmium elimination in tilapia also increased with temperature (Karayakar et al., 2022). In carp, the authors attributed temperature effects on accumulation mainly to metabolism rather than to metal speciation (Castaldo et al., 2021). If the total ammonia nitrogen concentration in water is fixed at 10 mg/L and the pH is fixed at 8, for ammonia however, the speciation depends on the temperature: At 28 °C the calculated un-ionized ammonia is 0.68 mg/L, while it is 0.91 mg/L at 32 °C (Abd-elaziz et al., 2026). A greater toxic dose may thus be a part of any ammonia–temperature effect, rather than a biological interaction. Increased uptake was observed for warming, with striated catfish uptake less at 33 than at 27 °C – possibly due to a higher reliance on air-breathing (Ha et al., 2019). Reported water chemistry was unevenly reported. In the copper study of tilapia, the dissolved oxygen was not measured but rather "near saturation" and pH ranged between 7.1 and 7.6 (Ribeiro et al., 2024). Warming alone does not permit to infer oxygen limitation. The routes of internal exposure, chemical speciation, tissue sensitivity and acclimation are separated in Figure 1.

4 ORGAN-SPECIFIC TISSUE RESPONSES

4.1 Gills and exchange surfaces

Gill lesions are the most frequently reported tissue response to combined exposure in the included set, reported in 12 of the 14 histology experiments (Table 2). Lamellar fusion, epithelial hyperplasia and hypertrophy were described in Amur minnow exposed to hexavalent chromium and in striped catfish exposed to arsenic (Hu et al., 2024; Kumar et al., 2019). Vascular changes, including telangiectasis, aneurysm, congestion and hyperaemia, were reported in tambaqui, Mozambique tilapia and Amur minnow (Salazar-Lugo et al., 2011; Ribeiro et al., 2024; Hu et al., 2024). In Nile tilapia given a 24 h heat challenge after a week of ammonia exposure, telangiectasis and congestion were more pronounced than after either stressor alone, and lymphocytic infiltration appeared (Esam et al., 2022). In striped catfish, warming alone was accompanied by lamellar deformation and necrosis, and warming combined with ammonia by filament oedema and lamellar sloughing (Abd-elaziz et al., 2026). Older acute studies in common carp and Japanese eel described gill changes that became more apparent with rising temperature, pH, ammonia concentration and exposure time (Yang & Chun, 1986; Kim & Yang, 1996), although in eels high pH alone produced lamellar oedema and, at pH 9.0, epithelial lifting (Kim & Yang, 1996). In red tilapia held for 56 days in a 3 × 3 factorial of ammonia and temperature, moderate filament degeneration, focal autolysis and congestion were reported across all treatments, without an ammonia-free group (Saaed et al., 2021).

Warming did not consistently intensify gill lesions. At the highest copper concentration, aneurysm scores in Mozambique tilapia were higher at 25 than at 32 °C (Ribeiro et al., 2024), and carp exposed for 7 days to a copper–cadmium–zinc mixture showed no significant gill histopathology at 10 or 20 °C (Pillet et al., 2021). Several of these changes are nonspecific. Epithelial lifting, for example, is a recognised processing artefact, and much published gill histopathology fails credibility criteria (Wolf et al.,

2015; Wolf, 2026). A gill lesion also does not establish impaired gas exchange. Copper-exposed tilapia gasped at the surface at 32 °C, but neither water oxygen nor oxygen uptake was measured (Ribeiro et al., 2024), and the one experiment that measured aerobic scope found it raised by warming and accompanied by no significant gill damage (Pillet et al., 2021). We therefore treat gill lesions as reported structural responses whose diagnostic and functional significance requires corroboration. Figure 2 sets the reported alterations against reference architecture.

4.2 Liver and metabolic allocation

Liver findings range from vacuolation to necrosis and inflammation. Hepatocyte vacuolation increased with chromium concentration in Amur minnow and, in Mozambique tilapia, was most evident at 14 days at the lower copper concentration and at 25 °C (Hu et al., 2024; Ribeiro et al., 2024). In common carp, liver vacuolation became heavier with rising temperature and pH under ammonia, and the authors attributed it to oedema (Yang & Chun, 1986). The tilapia and minnow studies used haematoxylin and eosin staining without identifying vacuole contents, so vacuolation is reported here as a nonspecific change rather than as glycogen depletion or lipid accumulation. Necrosis, nuclear pyknosis, inflammation and bile stasis were reported in tambaqui, striped catfish and heat-challenged, ammonia-exposed Nile tilapia (Salazar-Lugo et al., 2011; Kumar et al., 2019; Esam et al., 2022). Liver and spleen damage of accumulated NH3 and/or NO2-induced largemouth bass were not significantly different between 34 °C and 28 °C, but were generally more severe at 34 °C, because they were not deliberately dosed but rather exposed to accumulated ammonia and nitrite (Zhang et al., 2025). Abd-elaziz et al. (2026) observed the acinar necrosis and loss of cell boundaries in the hepatopancreas of striated catfish at 32 °C and ammonia, as well as the depletion of glycogen (no specific stain). The Nile tilapia also exhibited vacuolation, necrosis and accumulation of macrophage aggregates and eosinophilic granular cells in the liver at 17 and 27 °C when exposed to paraquat (Figueiredo-Fernandes et al., 2006). The abstract outlines liver histology coverage, but does not give a quantitative estimate of the liver temperature × paraquat lesion.

Although the liver links between detoxification costs and deposition of nutrients in muscle has been proposed, there was no inspected evidence to test this link directly. It did not fit the place of organ indices. In tilapia, the hepatosomatic index increased with the highest dose of copper, decreased approximately threefold with temperature in unexposed yellow perch, and decreased with temperature and cadmium (interaction not significant) in Arctic charr (Ribeiro et al., 2024; Fadhlaoui & Couture, 2016; Martyniuk et al., 2025). These are indices of organ mass and stored reserves and not lesions. Likewise, hepatic results are not a substitute for muscle burdens since liver had higher concentrations of cadmium in Nile tilapia compared to muscle (Abdel-Tawwab & Wafeek, 2014; Karayakar et al., 2022).

4.3 Intestine and nutrient handling

The intestine may link ingestion of composition of edible tissues with digestion and absorption. In one of the experiments, Nile tilapia were maintained for 15 days under conditions of 30, 33 and 36 °C with or without the presence of the microplastics (10 mg/L) in the water, which was associated with the intestinal histological analysis (Hasan et al., 2023). Its abstract explicitly describes epithelial breakage, enterocyte vacuolization and shortened villi, alongside gill hyperplasia, deformation of the pillar system, epithelial necrosis, epithelial lifting and telangiectasia (Hasan et al., 2023). The amount of damage and ingestion of particles increased with temperature. The scoring method, blinding and independent replication-interaction model could not be reproduced from the inspected abstracts and so direct injury to the thermal rather than higher ingestion of particles cannot be distinguished. Intestinal congestion was observed at post mortem in striped catfish, however, no information was provided concerning the intestinal histology (Abd-elaziz et al., 2026).

There is indirect evidence that links the state of the intestine with the deposition of nutrients. Feeding rates were non-standard (from ad libitum commercial pellets to an imposed ration between 0.2% and 2% of biomass) and were little reported (Martyniuk et al., 2025; Castaldo et al., 2021). In all the temperatures tested, cadmium-exposed Nile tilapia fed less and increased less with the lowest growth at 32 °C (Abdel-Tawwab & Wafeek, 2014), and striped catfish at 32 °C under ammonia did not feed at all (Abd-elaziz et al., 2026). These might both contribute to decreased nutrient deposition, but were not separated by digestion or absorption measurements, since these were not developed in conjunction with intestinal histology of the inspected material.

4.4 Edible skeletal muscle

Skeletal muscle is the tissue people eat, and one experiment in the included set examined its histology. Striped catfish were held at 28 or 32 °C, with or without a nominal 10 mg/L total ammonia nitrogen, for 28 days (Abd-elaziz et al., 2026). The muscle fibres of 32 °C controls and of both ammonia groups were devoid of their normal fibre pattern and presented with condensed myofibrils and Zenker's necrosis with inflammatory infiltrate, while muscle of 28 °C controls was normal (Abd-elaziz et al.,

2026). Ammonia-exposed fish also had gross necrosis at the caudal peduncle, but histological samples came from the dorsal region, and fish at 32 °C without ammonia had no gross lesions. Findings were descriptive, without a reported scoring system or blinding, and without an unambiguous count assigned to histology, and ammonia was not measured (Abd-elaziz et al., 2026). Because fibre artefacts are frequently misread as lesions in fish (Wolf et al., 2015), the report establishes that muscle histology has been examined under combined exposure but does not yet estimate how often or how severely muscle is injured.

The chemistry stream measured muscle phospholipid fatty acids; muscle histology data were obtained from a separate ammonia experiment (Tables 1 and 4). There was no inspected report which reported that organ histology and eligible edible-muscle chemistry were measured together. Most of the core reports were examined only by abstract and the status of unreported measurements cannot be determined. While muscle fibre injury is a prerequisite for the shift in muscle composition or pollutant concentrations, membrane acclimation, feeding and growth can both be important factors in changing the composition of the muscles or pollutant concentration without this injury (Fadhlaoui & Couture, 2016; Rutledge et al., 2025). These tendencies should be verified by pair measurements, and not assumed from separate groups or experiments.

4.5 Supporting tissues and within-study vulnerability

The findings of the kidneys are significant here primarily when there is a change in relative organ vulnerability in a single experiment. The severity of lesions caused by exposure to paraquat in tambaqui was ranked as liver>kidney>gills overall, with kidney lesion being the most severe at 18 °C (Salazar-Lugo et al., 2011). As a companion thermal-only study, changes were observed at both 18 °C and 35 °C, with normal architecture seen at 29 °C (Rojas et al., 2013), though it is not known if the paraquat experiment had temperature-only controls. At 20 °C, tubular epithelium remained intact at a pH of 6.5 and started to break down at a pH of 8.0 and an ammonia concentration of 30 mg/L, suggesting that pH and ammonia were as important as temperature in causing renal changes in carp (Yang & Chun, 1986). Red tilapia kidneys showed interstitial oedema, congestion and focal inflammation in all ammonia–temperature combinations (Saaed et al., 2021). Striped catfish showed tubular necrosis in all groups except the 28 °C controls, described as more severe at 32 °C under ammonia but not graded (Abd-elaziz et al., 2026). Because organs were scored with unrelated systems in different studies, we do not rank organ vulnerability across experiments. Gonadal histology was also examined in the paraquat–temperature experiment in Nile tilapia, including altered oocyte-stage proportions at 27 °C (Figueiredo-Fernandes et al., 2006). These findings support attention to sex and reproductive state as potential modifiers; they are not evidence of altered edible-muscle nutrition.

5 CROSS-ORGAN RESPONSES AND INTERACTIONS

5.1 Cross-organ concordance

If organ responses were coordinated, lesions in one organ should predict lesions or functional change in another. Several experiments reported lesions in more than one organ within the same groups: gill and liver changes appeared together in tambaqui, Amur minnow, striped catfish and heat-challenged Nile tilapia (Salazar-Lugo et al., 2011; Hu et al., 2024; Kumar et al., 2019; Esam et al., 2022), and gill, hepatopancreas, kidney and muscle changes in striped catfish exposed to warming and ammonia (Abd-elaziz et al., 2026). Co-occurrence at group level is nevertheless weak evidence of coordination. No inspected account established that these studies tested whether organ scores were associated within individual fish, recorded which organ changed first or measured a signal passing between organs, so the included evidence does not support claims of organ crosstalk.

Where histology was measured and found little damage, other levels of organisation still changed. In carp, warming raised standard and maximum metabolic rates and haematocrit while the gills showed no significant lesions (Pillet et al., 2021), and in the linked yellow perch cohort, transcriptional responses to temperature and metals differed from metabolic responses (Grasset et al., 2016). These reports show that physiological responses and reported structural alterations need not have the same apparent magnitude, and concordance should be tested in individual fish rather than inferred from shared treatment groups.

5.2 Interaction evidence

Interaction is deviation from a stated reference model, on a stated response scale (Piggott et al., 2015; Orr et al., 2020). In an additive model for a continuous response, the mean for the combination is the mean for the thermal response plus the mean for the chemical response, minus the mean for the control. If a combined group is not greater than either single-stressor group, then the group shows no interaction (Figure 3). If the response is adverse, a supported positive departure can be described as synergy under that specified model, but cannot be interchanged across endpoints or scales without support. Additivity is not necessarily determined by a non-significant interaction term. The basic binary-factor test is limited to four groups (no dosages

required), independent treatment replications and the estimate of the interaction effect with uncertainty; dosage information is required to further characterize the effect of dosage.

For the three core experiments, the temperature × chemical terms were significant in the inspected evidence for quantitative endpoints (Table 3). The two-way analyses conducted for haematology and biochemical markers among the striped catfish showed significant interaction p-values for red blood cells, haemoglobin, haematocrit and white blood cells, indicating that these factors were influenced by both the feed and age of the fish (Abd-elaziz et al., 2026) (Tables 1 and 3). These are not tests of histological lesions. The experiment used three aquaria per group, but the analytical unit was not specified; un-ionized ammonia also differed with temperature, and one ammonia concentration cannot establish a toxicity threshold. In yellow perch, interaction p-values were 0.0001 for polyunsaturated and 0.0023 for n-3 phospholipid fatty acid proportions after Cd- and Ni-exposed fish were combined into a metal variable (Fadhlaoui & Couture, 2016). Reported allocation involved one aquarium per original exposure condition, which resulted in limited treatment level inferences. For Arctic charr, the reported interaction was with cadmium in muscles, aquaria were nested within one recirculating unit per treatment and fish analysed as independent observations (Martynyuk et al., 2025). Warm unexposed fish had the highest muscle cadmium, complicating an exposure-driven interpretation. The same article described a synergistic hepatosomatic-index response despite a tabulated interaction p-value of 0.8906.

The inspected sources did not yield to a formal interaction test in the form of a histology test. Abd-elaziz et al. (2026) and Esam et al. (2022) described lesions in striated catfish and heat challenged tilapia qualitatively. The possible synergistic effect was not tested in Amur minnow because each temperature was analysed individually with one-way tests (Hu et al., 2024). Interaction terms were not reported and each treatment was placed in one aquarium in Mozambique tilapia (Ribeiro et al., 2024). It was reported that damage increased when exposed to polyamide microplastic with temperature, but the inspected abstract does not include any model, and in the tilapia species, the authors reported that the internal dose of the plastic increased with the temperature, thereby causing damage in the exposed experimental group (Hasan et al., 2023). There was no ammonia-free group in the red tilapia factorial, which meant that any interaction reported can be estimated only between the ammonia concentration (Saaed et al., 2021). The median lethal concentrations of arsenic and zinc nanoparticles decreased slightly at 34 °C, however, without confidence intervals or mortality alone at the various temperatures, these changes are not considered interactions (Kumar et al., 2019; Kumar et al., 2020a). However, lesions described in the abstract of the paraquat–temperature tilapia do not constitute a formal histological interaction (Figueiredo-Fernandes et al., 2006).

5.3 Competing mechanisms

Warming and metals are believed to interact via both decreased energy metabolism and changes in biotransformation, which would lead to diverted energy for maintenance and detoxification from that available to growth and tissue deposition (Sokolova & Lannig, 2008; Noyes et al., 2009). The experiments help implement some of this sequence, but do not split the routes. The growth loss can be attributed to reduced food intake and not to reallocation of growth because cadmium-exposed tilapia ate less (Abdel-Tawwab & Wafeek, 2014). For brook trout, it seemed that temperature would have the greatest effect on muscle mercury, predominantly via growth (Rutledge et al., 2025). For yellow perch, nickel decreased malondialdehyde in muscles at both temperatures, and the warm, nickel-exposed group exhibited the lowest condition, which the authors suggested was due to antioxidant defences (Fadhlaoui & Couture, 2016) paying too heavy a price for their overcompensation. There was no evidence of an energy budget separating feeding from allocation in the inspected core evidence.

There are other explanations at different levels. Membrane acclimation altered muscle phospholipids in a temperature-dependent way that metal exposure modified (Fadhlaoui & Couture, 2016), and metal mixtures up-regulated gill metallothionein in carp (Castaldo et al., 2021). For ammonia, temperature-dependent speciation offers a chemical rather than physiological explanation (Abd-elaziz et al., 2026), and for microplastics, greater ingestion at higher temperature offers an exposure-based one (Hasan et al., 2023). Oxygen limitation remains a candidate whose role varies among species (Ern et al., 2016; Brijs et al., 2015; McArley et al., 2022). These explanations should be tested against one another rather than assumed.

5.4 Context dependence

Responses differed by chemical, tissue and thermal range. Warming increased cadmium but not copper or zinc accumulation in carp (Castaldo et al., 2021), and in tilapia liver and kidney mercury rose with temperature whereas flesh mercury did not (Sunardi et al., 2017). Muscle nitrite was higher at 20 than at 14 °C in carp, whereas striped catfish took up less nitrite at 33 than

at 27 °C (Kroupová et al., 2006; Ha et al., 2019). Non-significant interaction terms, such as that for perch n-6 fatty acids ($p = 0.44$; Fadhlaoui & Couture, 2016), should not be read as evidence of additivity without an interval estimate.

6 NUTRITIONAL QUALITY AND HUMAN RELEVANCE

6.1 Protein quantity and quality

No inspected core report established measurement of proximate muscle protein or amino acid composition (Table 4). In yellow perch, soluble protein was measured in muscle homogenates by the Bradford assay, but as the denominator for enzyme activities, not as a nutritional endpoint (Fadhlaoui & Couture, 2016). The gap matters because the experiments repeatedly altered growth. Cadmium reduced growth and feed intake in Nile tilapia at all temperatures, with the poorest growth at 32 °C (Abdel-Tawwab & Wafeek, 2014). For brook trout, growth was reduced by warming, and the growth rate was a contributing factor to the concentration of muscle mercury (Rutledge et al., 2025). The proximate composition of farmed fish is largely determined by growth rate, ration and body size, and the amounts of various components recorded in the muscle reports that were inspected were not determined by these factors. Proximate composition was measured in the diets, but not the fish, of the selenium and riboflavin trial with striped catfish (Kumar et al. 2020b). Protein percentages in the future should be stated on a basis of moisture content on a measured basis, as the percentage of protein may go up when water and/or lipid decrease. Currently, there is no evidence for protein quality change, only growth change.

6.2 Lipid fractions and oxidative stability

This is the only core report associated with combined-exposure muscle lipid data—this report is related to membrane lipids instead of stored lipid, and refers to yellow perch. Total lipid was extracted from the muscle tissue and divided into neutral and polar fractions, with only the polar fraction (phospholipids) being analysed for fatty acids (Fadhlaoui & Couture 2016). The results were reported as per cent of total fatty acids, rather than in absolute amounts or total lipid content (Fadhlaoui & Couture, 2016). Among unexposed controls, perch held at 9 °C had proportionally more monounsaturated and polyunsaturated phospholipid fatty acids than perch at 28 °C (Fadhlaoui & Couture, 2016). Cadmium and nickel altered this pattern: at 9 °C, metal exposure lowered polyunsaturated, especially n-6, and raised saturated fatty acids, whereas at 28 °C polyunsaturated proportions exceeded those of cold fish (Fadhlaoui & Couture, 2016). The authors inferred desaturase and elongase activity from product-to-precursor ratios rather than measuring the enzymes (Fadhlaoui & Couture, 2016).

These results describe membrane remodelling. Phospholipids are the structural lipids of cell membranes and are rich in long-chain n-3 fatty acids, whereas triacylglycerols in the neutral fraction are the main energy store and, in fattier muscle, carry most of the lipid (Tocher, 2003). The neutral fraction was not analysed. Phospholipid composition is therefore not irrelevant to nutrition, particularly in lean muscle with little storage lipid, but percentages within one fraction cannot be converted into EPA or DHA mass per edible portion. That conversion needs total lipid, lipid class proportions, fatty acids quantified per gram of wet tissue, measured moisture and a defined sampling site. In a thermal-only catfish study, warming reduced muscle n-3 polyunsaturated fatty acids and DHA, but the abstract does not give the reporting basis (Dalvi et al., 2025).

Oxidation endpoints call for the same caution. In perch, malondialdehyde was measured in muscle stored at −80 °C; temperature did not affect it, despite the greater polyunsaturation of cold-acclimated phospholipids, and nickel lowered it at both temperatures (Fadhlaoui & Couture, 2016). Malondialdehyde indicates oxidative status; it does not quantify nutrient loss.

6.3 Micronutrients and contaminant burdens

Micronutrient data under combined exposure come only from one feeding intervention without single-stressor groups: selenium nanoparticles and riboflavin increased muscle vitamin C in striped catfish exposed to arsenic at 34 °C (Kumar et al., 2020b). Contaminant data are more plentiful. The Arctic charr study reported temperature-dependent muscle cadmium, but its highest muscle concentration occurred in warm unexposed fish and treatment was confounded with recirculating unit (Martyniuk et al., 2025). Muscle cadmium was undetectable in carp at 10 °C and measurable in tilapia only at 2 mg/L and 30 °C (Castaldo et al., 2021; Karayakar et al., 2022). In tilapia, muscle carried less cadmium than liver or kidney (Abdel-Tawwab & Wafeek, 2014; Karayakar et al., 2022), so liver concentrations cannot substitute for muscle measurements. Tilapia flesh mercury did not rise with temperature, whereas in carp fed mercury, slow heating accelerated muscle accumulation (Sunardi et al., 2017; Smirnov et al., 2025). Nitrite, a common aquaculture contaminant, also reached muscle. In carp exposed for 48 h, muscle nitrite was about twice as high at 20 as at 14 °C (1.5 versus 0.76 mmol/kg) (Kroupová et al., 2006). Striped catfish showed higher muscle nitrite concentration reaching 0.2 mM within 1 day and decreasing to 0.05 mM after 7 days of continuous exposure (Ha

et al., 2019). The reports inspected were non-conclusive with respect to the chemical form of mercury, thus the fraction of methylmercury remains unknown, and no muscle speciation of arsenic was determined (Table 4). There is no core report to report on microplastic measurement in muscle, for the polyamide study particles were counted as ingested particles per fish (Hasan et al., 2023).

6.4 Paired evidence and compositional interpretation

The revised core set includes fourteen experiments that include histology and ten experiments that include edible-muscle chemistry, but there are no confirmed experiments that include both (Table 1; Figure 4). This is a finding about reporting and retrieval, rather than proof that pairing has never been performed. Cross-study comparisons cannot supply individual-level associations. Carp gill histology and muscle cadmium came from separate 7-day and 27-day exposures (Pillet et al., 2021; Castaldo et al., 2021). Striped-catfish muscle histology and nitrite chemistry likewise came from different chemicals and thermal regimes (Abd-elaziz et al., 2026; Ha et al., 2019). These comparisons illustrate the limitations of combining studies and are not an exhaustive inventory of same-species comparisons.

Some experiments already show how pairing could be done. In Arctic charr, muscle cadmium was related to biometric and physiological markers in individual fish (Martyniuk et al., 2025). In brook trout, individual tags allowed growth rank to be related to muscle mercury (Rutledge et al., 2025). Neither inspected account established concurrent histology. Three levels of evidence should be kept distinct. Pairing means measuring both outcomes in the same fish; association means testing whether they covary after accounting for tanks; mediation additionally requires evidence that injury lies on the pathway to compositional change. Concordance would appear as higher lesion scores in fish with larger compositional change. Dissociation would appear as compositional change in fish whose examined tissues are normal. Membrane acclimation and growth dilution are plausible routes to dissociation, but neither was confirmed as tested against histology in the inspected evidence (Fadhlaoui & Couture, 2016; Rutledge et al., 2025).

The reporting basis limits even the unpaired evidence. Muscle cadmium was reported on a dry-weight basis without moisture in charr and carp, and carp samples were pooled from two fish (Martyniuk et al., 2025; Castaldo et al., 2021). Fatty acids were reported only as percentages of the phospholipid fraction (Fadhlaoui & Couture, 2016).

6.5 Dietary interpretation

Dietary relevance is judged from the amounts of nutrients and contaminants in edible portions, notably long-chain n-3 fatty acids set against methylmercury and dioxins (FAO/WHO, 2011; FAO & WHO, 2024). No inspected core report supplied both nutrient and contaminant data sufficient for a portion-based assessment. The charr study compared dry-weight muscle cadmium with a food guideline without stating whether the two shared a basis (Martyniuk et al., 2025); its reported exceedances should be recalculated on a wet-weight basis. Cooking effects and frequency of consumption were not determined in the inspected core reports, nor was bioaccessibility determined. There are separate verdicts on changes in contaminants and nutrients: An increase in muscle cadmium is a food safety change and not a nutrient change, and is reported here as a contaminant burden, and not as a nutrient deterioration. One bounded statement supported by the evidence is that, under laboratory exposure, warming can increase the amount of cadmium and nitrite in edible muscle. There is no evidence in the inspected material to show whether combined stress decreases the nutritional value of an edible part.

7 PERSISTENCE, RECOVERY AND DIETARY MODIFIERS

7.1 Recovery trajectories

No recovery can be assumed when an endpoint is measured during continuous exposure. None of the inspected core accounts had a recovery design complying with both histology and edible-muscle composition following chemical removal, temperature restoration or both. The evidence that is available is primarily for contaminant kinetics. The depuration efficiencies of Nile tilapia were higher with an increase in the duration of depuration and temperature, and the depuration data for muscle were not separated in the inspected abstract (Karayakar et al., 2022). The authors suggested that the higher cadmium level was more likely due to more efficient depuration under warm water conditions (20 °C) than under cold water conditions (10 °C) for copper and zinc, not to the fact that depuration was used to explain the higher burden (Castaldo et al., 2021). In striped catfish, muscle nitrite declined during continued exposure, which concerns metabolism or clearance during exposure rather than recovery after removal (Ha et al., 2019).

Histological timing only for change, through exposures. Liver vacuolation was most pronounced at 14 days and liver hyalinization was most pronounced at 28 days for tilapia (Ribeiro et al., 2024). The sequence refers to group means of the different fish sampled destructively at each time, and not to individual progressions (Ribeiro et al., 2024). It is not known if structural, functional and nutritional responses recover at different time scales; this would require stressor removal, repeated sampling and tank-level replication.

7.2 Dietary modification

There was no evidence of feeding interventions for the wild caught striped catfish. Only for the farmed striated catfish (Kumar et al., 2020b; Kumar et al., 2021; Kumar et al., 2024b). In the two selenium trials, arsenic and 34 °C were always applied together (Kumar et al., 2020b; Kumar et al., 2021). The manganese trial combined arsenic, ammonia and high temperature and had no temperature-only group (Kumar et al., 2024b). Relative to the control diet under combined stress, selenium nanoparticles with riboflavin lowered lipid peroxidation, cortisol and heat shock protein 70 (Kumar et al., 2020b). Selenium nanoparticles with 0.2–0.4% EPA plus DHA reduced stress markers, whereas 0.6% raised cortisol (Kumar et al., 2021). Dietary manganese reduced tissue arsenic (Kumar et al., 2024b). Muscle arsenic results in the selenium–riboflavin trial conflict between its table and its abstract (Kumar et al., 2020b). Without single-stressor groups, these trials cannot show which stressor a diet modified. The diets were fed during exposure, so the results concern prevention rather than recovery, and none demonstrates a benefit to people who eat the fish.

8 EVIDENCE GAPS AND EXPERIMENTAL PRIORITIES

8.1 Directly paired evidence and confidence

A main shortage is the lack of paired histology and edible muscle chemistry in the detected evidence. There is some uncertainty about the apparent separations due to the fact that 16 out of the 24 core reports were evaluated on the basis of abstracts or repository summaries (Tables 1 and 3). Of the three experiments that had significant interaction terms reported, two of them are influenced by housing confounding. The reliability of the diagnosis should not be interpreted with the aim of statistical significance, due to known interpretive difficulties in fish histopathology (Wolf & Maack, 2017; Wolf, 2026). Targeted verification recovered a previously unclassified eligible report, demonstrating that the search cannot support an exhaustive claim of absence.

Within the inspected core reports, intestinal and skeletal-muscle histology were each represented by one identified experiment, without a verified lesion scoring system (Table 2). Whole-muscle EPA and DHA mass, proximate protein, amino acids, moisture-corrected nutrient quantities and muscle methylmercury or arsenic speciation were not established (Table 4). Most thermal treatments were constant; a sequential heat challenge and heating ramps were also represented (Esam et al., 2022; Smirnov et al., 2025). These reporting gaps identify priorities for retrieval and experimentation, but an omitted abstract detail must not be treated as proof of an unmeasured endpoint.

We rank these gaps by how directly they block the review question. Same-fish pairing comes first, because without it concordance and dissociation cannot be distinguished. Scored, blinded intestinal and muscle histology come second, because these tissues sit closest to nutrient acquisition and to the food itself. Whole-muscle nutrient mass and contaminant speciation come third, because any dietary interpretation depends on them.

8.2 Minimum integrated experiment

The most informative experiment would pair histology and edible-muscle chemistry within an independently replicated factorial design. Four groups would be reference, elevated temp, chemical exposure and chemical exposure + elevated temp. Concentrations would be additional and indicate dose dependence. Exposure units are tanks or recirculating systems to which independent treatments are applied, but not multiple aquaria in the same system to which a single treatment is applied. (Martyniuk et al., 2025). Replication should follow a power or precision analysis using the expected variation among independent units and the smallest biologically relevant interaction. No universal number of tanks guarantees an adequate design. An independent repeat would strengthen generalizability, particularly across species, thermal histories or laboratories.

Throughout, water and tissue should be used to measure exposure. In the case of ammonia, un-ionized ammonia should be reported by each temperature, along with total ammonia nitrogen, pH and temperature. When recording ammonia, total ammonia nitrogen, pH and temperature should be recorded, and the un-ionized ammonia should be reported by each temperature (Abd-elaziz et al., 2026). For metals, measured dissolved concentrations and total tissue burdens are appropriate

(Castaldo et al., 2021; Martyniuk et al., 2025). Mercury studies should measure methylmercury as well as total mercury in muscle, because the risk–benefit assessment of fish consumption is made for methylmercury (FAO/WHO, 2011; FAO & WHO, 2024). Arsenic studies should speciate arsenic in muscle rather than report the total, as the included trial did (Kumar et al., 2020b). Microplastic studies should count particles in muscle as well as ingested items, of which only the latter were reported (Hasan et al., 2023), and nitrite studies should report muscle nitrite over time, since it can fall during continued exposure (Kroupová et al., 2006; Ha et al., 2019). Each sampled fish should have a unique specimen identifier linking its tissues and analytical results. Individual tags are useful when growth or repeated observations must be linked over time, as in the brook trout study (Rutledge et al., 2025).

From each identified fish, gill, liver, intestine and white muscle would be scored blind on a defined index, as two included studies did for gill or organ indices (Hu et al., 2024; Ribeiro et al., 2024). Adjacent muscle would be analysed for moisture, total lipid, lipid classes, fatty acids per gram of wet tissue, protein and amino acids. Feed intake, growth, dissolved oxygen, pH and diet composition should be recorded, because intake and growth alone can alter composition and contaminant concentration (Abdel-Tawwab & Wafeek, 2014; Rutledge et al., 2025). The analysis would include uncertainty around interactions and also involve using a model suitable for the endpoint taking into account fish within independent tanks or systems (Piggott et al., 2015; Orr et al., 2020). In a given group, scores of lesioned fish would correlate with composition of the same fish. A recovery arm with stressor elimination would test whether composition and structure improve on different timescales, and claims of mediation would moreover need temporal ordering or investigational manipulation of the anticipated pathway.

8.3 Harmonization and reuse

The location, the type of the muscle, the diet and feeding, and exposure and internal burden should be reported in studies. Any lesion index should be accompanied by a description of the lesions, raw scores, numbers of fish used for the analysis and representative images (Wolf et al., 2015; Wolf, 2026). Nutrient results should report units, wet or dry basis, moisture measured and uncertainty. The individual-level data with tank identifiers enable pairing and reanalysis at a later time, as illustrated by the brook trout dataset (Rutledge et al., 2025). The uncertainty of an interaction should be included in the model name in a statistical report, along with the scale and unit of replication of the interaction (Piggott et al., 2015; Orr et al., 2020). These recommendations are based on gaps identified here but not a validated reporting standard.

9 CONCLUSIONS

Combined thermal and chemical contact was linked with reported organ changes in several freshwater food fishes, though warming did not constantly intensify lesions. Gill and liver outcomes control the inspected histology reports. Intestinal damage under polyamide exposure and expressive muscle necrosis under ammonia exposure prove the relevance of tissues close to nutrient achievement and the edible product, while leaving diagnostic frequency, reliability and functional consequences incompletely resolved.

No inspected core report confirmed concurrent organ histology and edible-muscle compositional or contaminant measurements. This reporting gap limits any inference that tissue injury predicts changes in nutritional value. Phospholipid remodelling concerns membrane composition, and contaminant accumulation concerns food safety; neither alone establishes a reduction in nutrients delivered per edible portion. Abstract-only access prevents an exhaustive claim that paired measurements have never been made.

Reported statistical interactions require endpoint-specific interpretation and independent treatment replication. Three experiments provided significant interaction terms for quantitative outcomes in the inspected evidence, but housing confounding limits inference in two. No formal histological interaction test or integrated recovery assessment was identified in the inspected material.

The evidence provisions biological concern and targeted investigation, rather than consumption advice. A broader database search and full-text retrieval should test the apparent coverage gaps. The clearest investigational advance would be a factorial exposure design that links trustworthy organ histology to absolute muscle nutrient quantities, contaminant speciation where relevant, feed intake and growth in the same fish, followed by a recovery phase.

Data availability

The search log, classification decisions, extraction records and figure source counts are provided in the supplementary material. This review did not generate new experimental animal data.

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Conflicts of Interest

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Table 1. Included experiments: species, thermal regimes, chemical exposures, and experimental designs

C01–C13 and C24 reported histology; C14–C23 reported eligible edible-muscle chemistry. No inspected report confirmed both domains. Twenty-four core experiments and one linked publication give 25 publications; linked and supporting records are classified in Supplementary Table S1. FT, full text inspected (8 core reports); Abs, abstract or repository summary (16). NR means not reported in the inspected material and does not establish absence. Exposure concentrations are nominal unless stated otherwise.

ID	Study	Species and stage	Thermal contrast and duration	Chemical exposure	Groups and replication	Tissues and endpoints	Access
C01	Salazar-Lugo et al. (2011)	Colossoma macropomum (tambaqui), juvenile	18, 29 and 35 °C; 21 d	Paraquat 10 mg/L; measured concentration NR	Single-stressor groups and tank number NR	Gill, liver, kidney histology	Abs
C02	Ribeiro et al. (2024)	Oreochromis mossambicus, adult males	25 vs 32 °C; 28 d	CuSO4 1.1 and 3.6 mg/L nominal; Cu measured	Full 2 × 3; one ~175 L aquarium per treatment	Gill, liver histology (grade × extent, described as blinded)	FT
C03	Hu et al. (2024)	Phoxinus lagowskii (Amur minnow)	18 vs 25 °C; 30 d	Cr(VI) 0–10 mg/L nominal	Full 2 × 5; 3 tanks × 12 fish per group	Gill, liver, kidney histology (organ indices)	FT
C04	Kumar et al. (2019)	Pangasianodon hypophthalmus (striped catfish), 6.25 g	34 °C vs ambient; 96 h	As(III), acute range 25–30 mg/L	Acute LC50 design; temperature-only group NR	Liver, gill histology	Abs

C05	Kumar et al. (2020a)	P. hypophthalmus	34 °C vs ambient; 96 h	Zn nanoparticles, acute range 16–30 mg/L	Acute LC50 design; temperature-only group NR	Liver, gill histology	Abs
C06	Pillet et al. (2021)	Cyprinus carpio (common carp), juvenile	10 vs 20 °C; 7 d	Cu–Cd–Zn mixture	Exposed and control at each temperature; sampled days 1, 3 and 7	Gill histology; metabolic rates, aerobic scope	Abs
C07	Yang & Chun (1986)	C. carpio, young of the year, 5.96 g	20, 25 and 30 °C × pH 6.5, 7.0 and 8.0; 24–72 h	Ammonia 10, 20 and 30 mg/L; ammonia-free group NR	Multifactor acute design; replication NR	Gill, liver, kidney histology	Abs
C08	Kim & Yang (1996)	Anguilla japonica (Japanese eel), 40 g	27 vs 32 °C × pH 7.5–9.0; 24–120 h	Total ammonia 0, 10, 20 and 30 mg/L	Factorial with pH; replication NR	Gill histology	Abs
C09	Saaed et al. (2021)	Oreochromis sp. (red tilapia), 11.1 g	24, 28 and 32 °C; 56 d	Total ammonia 1.5, 3.0 and 4.5 mg/L; ammonia-free group NR; water salinity NR	3 × 3 factorial; two replicates per treatment	Gill, kidney histology; growth, haematology, biochemistry	Abs
C10	Esam et al. (2022)	Oreochromis niloticus (Nile tilapia)	27–28 °C, or 32 °C for 24 h after 7 d of ammonia (sequential)	5 mg/L total ammonia nitrogen	Four groups reported in triplicate; 180 fish total; independence and analytical unit require confirmation; 24 h heat after ammonia exposure	Gill, liver histology; gene expression	FT
C11	Zhang et al. (2025)	Micropterus salmoides (largemouth bass), juvenile	28 vs 34 °C; 14 d	Ammonia and nitrite accumulated from waste in a closed system, not dosed	Second experiment without accumulation as thermal reference; exposure confounded with sampling day	Liver, spleen histology	Abs

C12	Abd-elaziz et al. (2026)	P. hypophthalmus, 18 g	28 vs 32 °C; 28 d	10 mg/L total ammonia nitrogen nominal, not measured; calculated NH3 0.68 and 0.91 mg/L	Full 2 × 2; 3 × 90 L aquaria × 10 fish per group; 6 fish sampled per group	Gill, hepatopancreas, kidney, dorsal muscle histology; haematology, cortisol, glucose, liver enzymes, oxidative markers	FT
C13	Hasan et al. (2023)	O. niloticus	30, 33 and 36 °C; 15 d	Virgin polyamide microplastics 10 mg/L (500 μm–4 mm)	Each temperature with and without polyamide; replication NR	Gill, intestine histology; ingested particles; haematology, erythrocyte abnormalities	Abs
C14	Fadhlaoui & Couture (2016); linked: Grasset et al. (2016)	Perca flavescens (yellow perch)	Acclimated at 20 °C, ramped 2 °C/d to 9 or 28 °C; 7 weeks	Cd 3.98 and Ni 605.9 μg/L measured	Six 40 L aquaria, one per condition; n = 8 analysed; remaining fish used in the linked publication	Muscle phospholipid fatty acids; muscle MDA; soluble protein as enzyme denominator	FT
C15	Castaldo et al. (2021)	C. carpio, ~8 months	10 vs 20 °C; 27 d	Cu, Zn, Cd at 10% of 96 h LC50; measured	Full 2 × 2; 3 control and 3 exposed 150 L tanks per temperature	Muscle Cd, Cu, Zn; gill metallothionein	FT
C16	Martyniuk et al. (2025)	Salvelinus alpinus (Arctic charr), juvenile	6 vs 16 °C; 8 weeks	Cd 1.81 μg/L measured (3 μg/L nominal)	Full 2 × 2; three 75 L aquaria per treatment sharing one of four recirculating units; fish analysed as units	Dorsal muscle Cd; hepatosomatic index; condition	FT
C17	Abdel-Tawwab & Wafeek (2014)	O. niloticus, 26 g	20, 24, 28 and 32 °C; 8 weeks	Cd 0 or 0.5 mg/L	4 × 2; tank number NR	Muscle and organ Cd; growth, feed intake	Abs
C18	Karayakar et al. (2022)	O. niloticus	20, 25 and 30 °C; uptake then elimination	Cd 1 or 2 mg/L	3 × 2 with elimination phase	Muscle and organ Cd	Abs
C19	Ihunwo & Ibezim-	O. niloticus, juvenile	Heaters of 60, 100 and 200 W;	Contaminated sediment	Mesocosm; low survival under heating	Muscle Cd, Cr, Ni, Pb	Abs

	Ezeani (2022)		temperatures NR				
C20	Sunardi et al. (2017)	O. niloticus	Elevated vs ambient	Hg, chemical form NR	Accumulation and elimination phases	Flesh Hg	Abs
C21	Smirnov et al. (2025)	C. carpio, juvenile	Monthly heating tests (~8 °C/h; 1 °C/d)	Dietary Hg 0.02 vs 0.27 mg/kg; 5 months	Groups and tanks NR	Muscle Hg; thermal tolerance	Abs
C22	Kroupová et al. (2006)	C. carpio, two years old	14 vs 20 °C; 48 h	Nitrite 1.45 mmol/L	Full 2 × 2; 30 fish per group, 7 sampled; tanks NR	Muscle nitrite	FT
C23	Ha et al. (2019)	P. hypophthalmus	27 vs 33 °C; up to 7 d	Nitrite 0.8 mM	Exposed and control groups at each temperature	White muscle nitrite	Abs
C24	Figueiredo-Fernandes et al. (2006)	O. niloticus; males and females	17 vs 27 °C; duration NR in abstract	Paraquat; low concentration, exact dose NR in abstract	Exposed and control comparisons at both temperatures; tank replication NR	Liver and gonadal histology; hepatic EROD; sex-dependent responses	Abs

Table 2. Reported organ-specific histopathological responses and diagnostic limitations

Fourteen core experiments reported histology: gill 12, liver or hepatopancreas 10, kidney 5, and spleen, intestine, skeletal muscle and gonads one each. Organ categories overlap. Coverage counts indicate reported examination, not diagnostic certainty or comparable severity. An unmentioned organ in an abstract remains of uncertain measurement status. IDs refer to Table 1.

Organ	**Reported alteration (harmonised category)**	**Experiments and key observation**	**Assessment method**	**Functional evidence**	**Interpretation and alternative explanations**
Gill	Hyperplasia, hypertrophy, lamellar fusion, epithelial lifting, sloughing (proliferative or degenerative change)	C03; C04; C12: filament oedema and sloughing at 32 °C with ammonia, lamellar deformation at 32 °C alone; C02: lifting and fusion not significant	Organ index (C03); grade × extent (C02); descriptive (C04, C12)	Not established alongside lesions in inspected source	Epithelial lifting is a recognised artefact (Wolf et al., 2015); of 100 gill toxicology papers, 11 had credible histopathology (Wolf, 2026)
Gill	Telangiectasis, aneurysm, congestion,	C01; C03; C04; C10: more pronounced with ammonia plus heat than either alone; C02: aneurysm	As above; C10 descriptive	C02: surface gasping at 32 °C, oxygen uptake not measured	Direction in C02 runs against the authors' claim that warming amplified copper

	hyperaemia (vascular change)	higher at 25 than 32 °C at 3.6 mg/L Cu			effects; C02 had one aquarium per treatment
Gill	Changes increasing with temperature, pH, ammonia and time; moderate degeneration, autolysis, congestion	C07; C08: high pH alone caused lamellar oedema and lifting; C09: changes in all ammonia–temperature combinations	Descriptive	Not established in inspected source	Un-ionized ammonia rises with temperature and pH, so dose and temperature are confounded; C09 lacks an ammonia-free group
Gill	Hyperplasia, epithelial necrosis, deformed pillar system, epithelial lifting and telangiectasia	C13	Lesions named in abstract; scoring and blinding not established	Ingested particles counted; haematology measured	Greater particle ingestion at higher temperature may explain part of the effect
Gill	No significant lesion	C06	Not reported in abstract	Aerobic scope and metabolic rates measured	Short exposure at environmentally relevant metal concentrations
Liver	Vacuolation (nonspecific vacuolar change)	C02; C03; C07: heavier with rising temperature and pH, attributed to oedema; C24: vacuolation under paraquat	Haematoxylin and eosin only	No glycogen or lipid stains	Storage, fasting, oedema or fixation can produce the same appearance
Liver	Hyalinization (cytoplasmic hyaline change)	C02: peak at 3.6 mg/L Cu and 32 °C, day 28	0–5 scale	Not established in inspected source	Discussion contradicts results; single aquarium per treatment
Liver	Necrosis, pyknosis, inflammation, congestion, bile stasis (degenerative and inflammatory change)	C01; C03; C04; C05; C10; C11: more pronounced at 34 °C; C12: acinar necrosis at 32 °C and under ammonia; C24: necrosis, macrophage aggregates and eosinophilic granular cells	Descriptive or organ index	C03 enzymes not linked to lesion scores; C12 glycogen depletion asserted without a glycogen stain	Diagnostic alternatives and confounding require study-specific assessment; abstract-only access cannot exclude infection, fasting or handling effects; C11 exposure confounded with sampling day
Kidney	Tubular necrosis or breakdown, interstitial oedema, inflammation, haemorrhage	C01: highest relative damage at 18 °C; C03; C07: tubular breakdown driven by pH and ammonia; C09: in all treatments; C12: all groups except 28 °C controls	Organ index (C03); descriptive (others)	Not established in inspected source	Used for within-study shifts in vulnerability (Section 4.5); C03 results text contradicts its kidney index

Spleen	Pathological change, more pronounced at 34 °C	C11	Not reported in abstract	Not established in inspected source	Ammonia and nitrite accumulated from waste rather than being dosed
Intestine	Epithelial breakage, enterocyte vacuolization and shortened villi	C13; C12 noted a congested intestine at necropsy without sectioning	Lesions named in C13 abstract; scoring, blinding and replication not established	Particle ingestion counted; digestion and absorption not measured	Direct thermal injury cannot be separated from greater particle ingestion at higher temperature
Skeletal muscle	Loss of fibre pattern, condensed myofibrils, Zenker's necrosis with inflammatory infiltrate	C12: 32 °C controls and both ammonia groups; normal at 28 °C	Descriptive; scoring and blinding unreported; histology-specific sample count unclear	Muscle chemistry not measured	Fibre artefacts not excluded; dorsal site sampled, gross lesions caudal; no gross lesions at 32 °C alone
Gonads	Altered oocyte-stage proportions	C24: reproductive and liver outcomes examined in males and females at 17 and 27 °C; ovarian stage distribution altered under paraquat at 27 °C	Histology and oocyte-stage proportions; methods incompletely available in abstract	Reproductive endpoints; not edible-muscle nutrient measurements	Sex and reproductive state may modify responses; no nutritional inference follows from gonadal change alone

Table 3. Reported thermal–chemical interactions: statistical evidence and experimental limitations

Rows describe study–endpoint contrasts rather than independent experiments. C12, C14 and C16 reported significant quantitative interaction terms in the inspected evidence; this is not an exhaustive count of all possible analyses. Statistical reporting and design support are appraised separately. A non-significant term does not establish additivity, and an unreported term is not evidence of no interaction (Piggott et al., 2015; Orr et al., 2020). MDA, malondialdehyde; LC50, median lethal concentration.

Experiment and endpoint	**Groups present**	**Analysis and scale**	**Reported result**	**Authors' interpretation**	**Statistical report**	**Design support for treatment-level inference**
C12: haematology, cortisol, glucose, liver enzymes, MDA, antioxidant enzymes	Full 2 × 2 with one ammonia concentration	Two-way ANOVA	Selected interaction p: RBC 0.002; Hb <0.0001; Hct 0.032; WBC 0.013; hepatic MDA <0.0001; CAT 0.042 (source Tables 1 and	Synergistic; warming lowers the ammonia threshold	Reported statistical interaction	Limited: three aquaria per group but statistical unit not stated; ammonia not measured; un-ionized fraction higher at 32 °C; one concentration

			3). Other stress and liver-enzyme interactions reported significant.			cannot test a threshold
C12: gill, hepatopancreas, kidney, muscle histology	As above	Descriptive	No test	Lesions most severe with ammonia at 32 °C	Not tested	As above
C14: muscle phospholipid % polyunsaturated and % n-3 fatty acids	2 temperatures × (control, metal); Cd and Ni pooled	Two-way ANOVA, additive on the percentage scale	Interaction p = 0.0001 and p = 0.0023	Metals altered the thermal response	Reported statistical interaction	Not supported: one aquarium per condition with fish as replicates; closed-sum percentage data
C14: % monounsaturated, % saturated, % n-6 fatty acids	As above	As above	p = 0.0498, 0.0504 and 0.4361	Not classified	MUFA p <0.05 as reported; SFA p >0.05; n-6 not significant. Proximity to 0.05 does not establish equivalence.	As above
C16: dorsal muscle Cd (dry weight)	Full 2 × 2	Two-way ANOVA	Interaction significant; p-value not given in the text	Warming increased Cd uptake	Reported statistical interaction	Limited: aquaria shared recirculating units with no tank or unit term; unexposed fish at 16 °C had the highest muscle Cd
C16: hepatosomatic index	Full 2 × 2	Two-way ANOVA	Interaction p = 0.8906	Described as synergistic	Not significant; the synergy claim is not supported by the tabulated test	As above
C15: tissue Cd	Full 2 × 2 × time	Three-way ANOVA	Interaction terms not reported	Higher accumulation at 20 °C	Interaction evidence not established from	Replicated tanks; samples pooled from two fish

					extracted results	
C02: liver hyalinization; gill aneurysm	Full 2 × 3	Three-way ANOVA implied	Interaction terms not reported	Warming amplified Cu effects	Claimed, test not reported; aneurysm means run the opposite way	Not supported: one aquarium per treatment
C03: gill, liver, kidney indices	Full 2 × 5	One-way ANOVA within each temperature	No interaction test	Possible synergistic effect	Not tested	Three tanks per group
C13: gill and intestinal histology; haematology	Three temperatures, each with and without polyamide	Not reported in abstract	Damage with polyamide increased with temperature	Warming increased ingestion and toxicity	Not determinable from the abstract	Replication not reported; exposure dose (ingestion) changed with temperature
C09: growth, haematology, biochemistry, gill and kidney histology	3 × 3 factorial of ammonia levels and temperature; no ammonia-free group	Not extracted (abstract only)	Not extracted	Interaction between ammonia and temperature affected health	Not determinable from the abstract	Two replicates per treatment; interaction estimable only among ammonia levels, not against unexposed fish
C10: gill and liver lesions; gene expression	Four groups; heat applied after ammonia	Statistical model not recorded in extraction	Opposing gene responses in single and combined groups; lesions descriptive	Combined exposure induced oxidative stress and inflammation	Not tested for lesions	Triplicate groups reported; independent allocation and analytical unit not established; sequential heat challenge
C07, C08: gill, liver, kidney histology	Multifactor acute designs with pH	Descriptive	No test	Changes increased with temperature, pH and ammonia	Not tested	pH and temperature co-determine un-ionized ammonia
C01: lesion pattern and organ ranking	Single-stressor groups not reported	Not reported	Not reported	Damage depended on temperature	Not determinable	Tank number not reported
C04, C05: 96 h LC50	Chemical alone vs chemical plus 34 °C	Comparison of two LC50 values	28.16 vs 26.88 mg/L (C04); 21.89 vs 19.74 mg/L (C05);	Synergistic (C05 title)	Not estimable without temperature-only mortality	Acute, lethal-range exposure

			confidence intervals not reported			
C11: liver and spleen pathology	Accumulation vs no-accumulation experiments	Not reported	Not reported	Combined effects of hyperthermia and ammonia or nitrite	Not estimable	Exposure endogenous and confounded with sampling day
C06: gill histology	2 × 2 × time	Not reported in abstract	No significant damage	Carp coped with the exposure	No interaction estimate	Replication not reported in abstract
C17: growth	4 × 2	Not reported in abstract	Not reported	Synergetic	Not determinable	Tank number not reported
Feeding interventions (Kumar et al., 2020b, 2021, 2024b)	No temperature-only or arsenic-only groups	Not applicable	Not applicable	Combined stress mitigated by diet	Not estimable	Design cannot separate the stressors
C24: liver and gonadal histology	17 and 27 °C; paraquat and control comparisons; both sexes	Histological changes and oocyte-stage comparisons described in abstract	No formal temperature × paraquat lesion test established in inspected abstract	Temperature and sex considered as modifiers	Not determinable from abstract	Tank replication and analytical unit NR in abstract

Table 4. Edible-muscle composition and contaminant burdens: findings, reporting basis, and dietary relevance

Core experiments are followed by explicitly labelled intervention and contextual records. No inspected core report provided absolute whole-muscle nutrient amounts per edible portion or confirmed pairing with histology. Muscle methylmercury, arsenic species and microplastic burdens were not established. NR, not reported in the inspected material; MDA, malondialdehyde. Lack of an abstract mention does not establish that an assay was not performed.

Study reference and ID	**Species and edible tissue**	**Exposure contrast**	**Endpoint category and analyte or lipid fraction**	**Main finding with units and reporting basis**	**Concurrent histological assessment**	**Evidence limitations**	**Nutritional or food-safety interpretation**
Fadhlaoui & Couture (2016), C14	Yellow perch; muscle, site and fibre type NR	9 vs 28 °C × Cd or Ni; 7 weeks	Nutrient-related: fatty acids of the polar (phospholipid) fraction only	Warm unexposed controls had proportionally fewer monounsaturated and polyunsaturate	Not confirmed in inspected report	One aquarium per condition; n = 8; neutral lipid and total lipid not analysed	Membrane remodelling; relevant to lipid quality but not convertible to EPA or DHA

				d fatty acids; metals lowered polyunsaturated (especially n-6) at 9 °C; % of total fatty acids in the polar fraction; moisture NR			mass per portion
Fadhlaoui & Couture (2016), C14	As above	As above	Oxidation: muscle MDA; soluble protein (Bradford) as enzyme denominator	Temperature did not affect MDA; Ni lowered MDA at both temperatures; protein per g wet weight	Not confirmed in inspected report	As above	MDA indicates oxidative status, not nutrient loss; Bradford protein is not proximate protein
Castaldo et al. (2021), C15	Common carp; muscle	10 vs 20 °C × Cu–Zn–Cd mixture; 27 d	Contaminant: total Cd, Cu, Zn	Muscle Cd below detection at 10 °C and measurable at 20 °C; Cu and Zn did not rise; dry weight, moisture NR	Not confirmed in inspected report	Samples pooled from two fish	Food safety: temperature-dependent muscle Cd
Martyniuk et al. (2025), C16	Arctic charr; dorsal muscle	6 vs 16 °C × Cd 1.81 µg/L; 8 weeks	Contaminant: total Cd	Reported temperature association and interaction; warm unexposed fish had highest muscle Cd; nine samples at 16 °C exceeded 0.05 mg/kg on the reported dry basis; moisture NR	Not confirmed in inspected report	Shared recirculating units; guideline comparison basis not stated	Potential food-safety relevance; causal exposure interpretation and wet-weight guideline comparison require resolution
Abdel-Tawwab & Wafeek (2014), C17	Nile tilapia; muscle among other organs	20–32 °C × Cd 0.5 mg/L; 8 weeks	Contaminant: total Cd	Muscle carried the lowest Cd load of the organs examined; basis NR	Not confirmed in inspected report	Abstract only; feed intake fell under Cd	Food safety; liver burdens cannot substitute for muscle

Karayakar et al. (2022), C18	Nile tilapia; muscle	20, 25, 30 °C × Cd 1 or 2 mg/L	Contaminant: total Cd	Muscle Cd measurable only at 2 mg/L and 30 °C; basis NR	Not confirmed in inspected report	Abstract only	Food safety at the highest temperature and dose
Ihunwo & Ibezim-Ezeani (2022), C19	Nile tilapia; muscle	Heater wattage gradient × contaminated sediment	Contaminant: total Cd, Cr, Ni, Pb	Bioaccumulation factors Pb > Cd > Cr > Ni; basis NR	Not confirmed in inspected report	Temperatures NR; low survival when heated	Associative only
Sunardi et al. (2017), C20	Nile tilapia; flesh	Elevated vs ambient × Hg	Contaminant: Hg, form NR	Flesh Hg did not rise with temperature, whereas liver and kidney Hg did; basis NR	Not confirmed in inspected report	Abstract only; methylmercury fraction unknown	Total Hg indicates exposure; methylmercury-specific risk needs speciation or justified assumptions
Smirnov et al. (2025), C21	Common carp; muscle	Heating tests × dietary Hg; 5 months	Contaminant: Hg, form NR	Up to 1.82 mg/kg; basis NR	Not confirmed in inspected report	Abstract only; groups and tanks NR	Slow heating associated with faster muscle Hg accumulation
Kroupová et al. (2006), C22	Common carp; muscle	14 vs 20 °C × nitrite; 48 h	Contaminant: nitrite	0.76 mmol/kg at 14 °C vs 1.5 mmol/kg at 20 °C; wet or dry basis not stated	Not confirmed in inspected report	Tanks NR	Food safety: warming raised muscle nitrite
Ha et al. (2019), C23	Striped catfish; white muscle	27 vs 33 °C × nitrite 0.8 mM; up to 7 d	Contaminant: nitrite	Peak ~0.2 mM at day 1, ~0.05 mM by day 7; uptake lower at 33 °C	Not confirmed in inspected report	Abstract only	Transient muscle burden during exposure
Supporting intervention — Kumar et al. (2020b), feeding intervention	Striped catfish; muscle	As(III) plus 34 °C, both always applied; Se nanoparticle and riboflavin diets; 90 d	Micronutrient: muscle Se and vitamin C; contaminant: total As	Supplemented diets raised muscle vitamin C; muscle As not significantly different in the article's table, but reduced in its abstract; basis not stated	Not confirmed in inspected report	No single-stressor groups; table and abstract conflict	Diet effect under combined stress only; Se reflects supplementation
Unresolved contextual record —	Brook trout; muscle	Temperature × ration	Contaminant: Hg, form NR	Growth rank related to muscle Hg in tagged fish; dry	Not confirmed in	Mercury source and chemical	Growth dilution as a route by which temperature

Rutledge et al. (2025), unresolved record				weight; values not extracted	inspected report	manipulation unclear	alters Hg concentration
Thermal-only context — Dalvi et al. (2025), thermal-only context	Horabagrus brachysoma; muscle	26–36 °C; 30 d; no chemical factor	Nutrient-related: lipid content and fatty acids	Warming reduced muscle n-3 polyunsaturated fatty acids and DHA; reporting basis not given in abstract	Not confirmed in inspected report	Abstract only	Thermal context for lipid change without contaminants

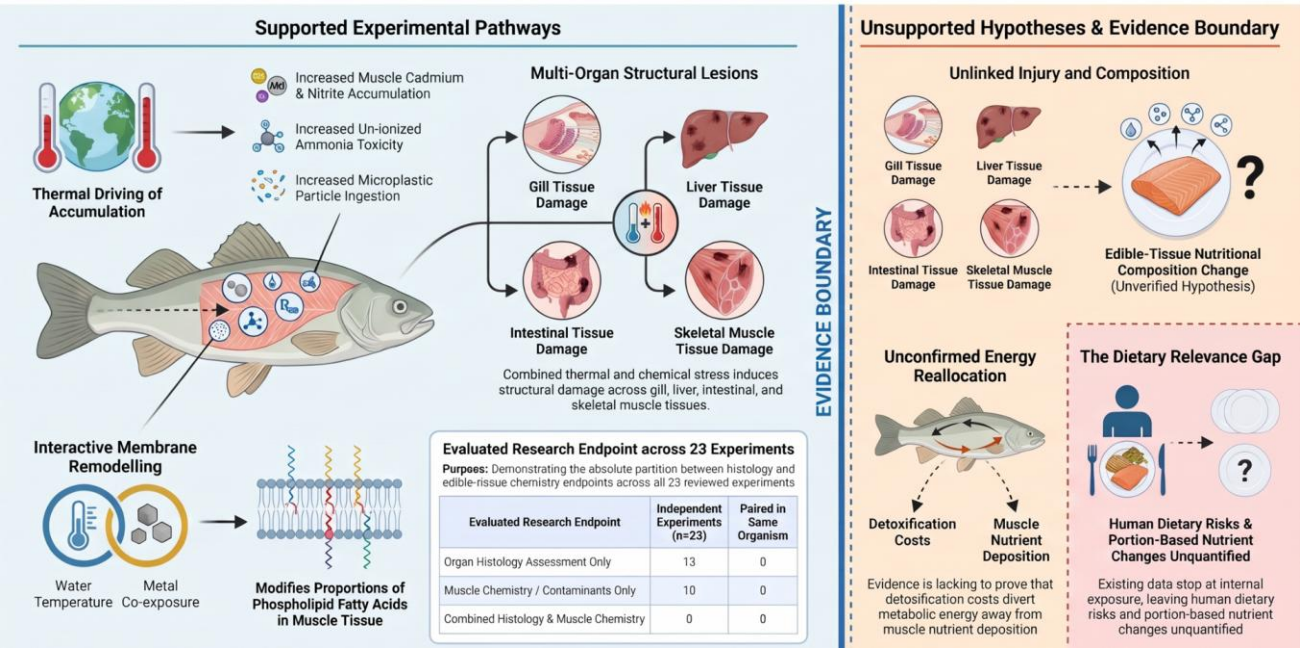


Figure 1. Conceptual framework for combined thermal and chemical exposure in freshwater food fishes. Solid Arrows (Experimental Evidence): Represent relationships empirically demonstrated in experimental studies: Warming leading to elevated cadmium and nitrite accumulation in edible white muscle. Interactive temperature × heavy metal exposure altering cell membrane phospholipid fatty acid composition. Thermal shifts altering chemical speciation, driving higher proportions of toxic un-ionized ammonia (NH_3). Elevated water temperatures increasing polyamide microplastic particle ingestion rates. Combined thermal and chemical stress inducing histopathological lesions in gill, liver, intestinal, and white muscle tissues. Dashed Arrows (Unsupported Hypotheses): Highlight mechanistic pathways lacking direct experimental verification within the same individual organisms: Direct causal link from organ/tissue injury to changes in edible-tissue nutritional composition. Edible-tissue compositional alterations occurring independently of structural tissue damage (e.g., via homeoviscous membrane acclimation or growth dilution). Energetic costs of metabolic detoxification diverting energy allocation away from muscle protein and lipid deposition. Evidence Boundary: Delineates internal physiological and histopathological responses from human dietary and food-safety relevance, which requires quantified edible-portion nutrient/contaminant loads and intake data.

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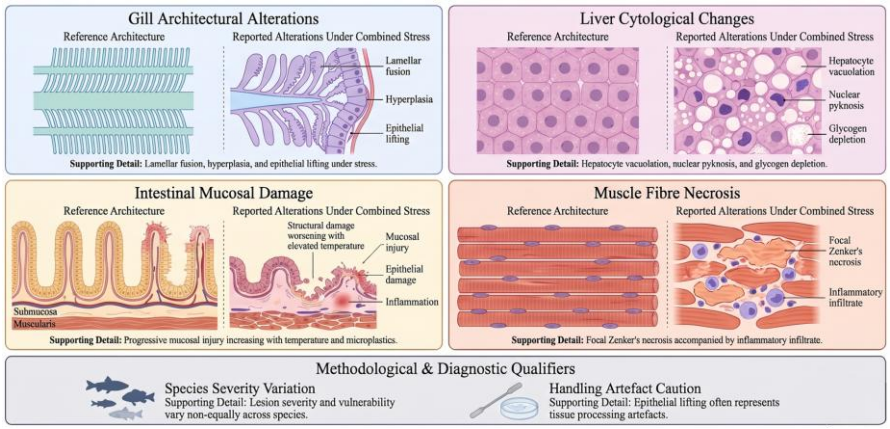


Figure 2. Schematic comparison of reference tissue architecture and histopathological alterations reported under combined thermal and chemical exposure in freshwater food fishes (not to scale). Gill Panel: Compares normal primary and secondary lamellar architecture against stress-induced alterations (e.g., lamellar fusion, epithelial hyperplasia, and vascular congestion). Epithelial lifting is explicitly highlighted as a potential sample-handling or fixative artefact rather than a definitive in vivo pathological response. Liver Panel: Depicts baseline hepatic parenchyma alongside multi-study experimental observations (Table 2) showing hepatocyte vacuolation, glycogen depletion, cellular swelling, and nuclear pyknosis under co-exposure. Intestinal Panel: Displays mucosal integrity vs. structural damage based on microplastic co-exposure evidence (C13), where overall tissue damage scaled positively with water temperature under polyamide ingestion (note: specific lesion categories and ordinal scoring were not defined in the source study). Skeletal Muscle Panel: Illustrates intact, packed muscle fibers versus focal muscle fiber necrosis accompanied by leucocytic inflammatory infiltrate, derived from descriptive histopathological reporting (C12). Shared descriptive terms across tissue panels do not denote equivalent pathological severity, a common physiological mechanism, or identical species-specific thresholds among freshwater teleosts.

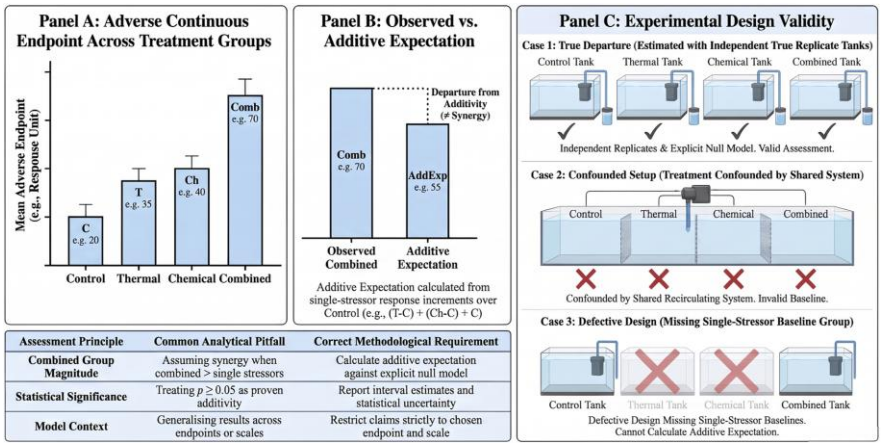


Figure 3. Hypothetical example of interaction assessment under a stated additive model (all values are illustrative). (A) Treatment Means: Comparison of continuous adverse endpoint means across baseline Control, Thermal stress, Chemical stress, and Combined co-exposure groups. (B) Observed vs. Additive Expectation: Direct comparison between the observed combined stressor mean and the calculated additive expectation derived from adding single-stressor response increments to the control baseline. (C) Statistical Departure vs. Design Validity: Evaluates statistical conclusions across three experimental design scenarios: *Independent Tank Replicates:* Valid departure from additivity backed by true, independently replicated experimental tanks. *Confounded System:* Apparent statistical departure where treatment effects are confounded with single tank housing or shared recirculating water infrastructure. *Incomplete Factorial:* Defective experimental structure lacking one or both single-stressor control groups. A combined group response that numerically exceeds both single-stressor responses does not automatically indicate non-additivity or synergistic interaction. A statistically non-significant interaction term ($p > 0.05$) fails to reject the null hypothesis but does not positively establish additivity. Interaction conclusions are context-dependent and apply strictly to the specific endpoint, measurement scale, and statistical model evaluated (Table 3) (Piggott et al., 2015; Orr et al., 2020).

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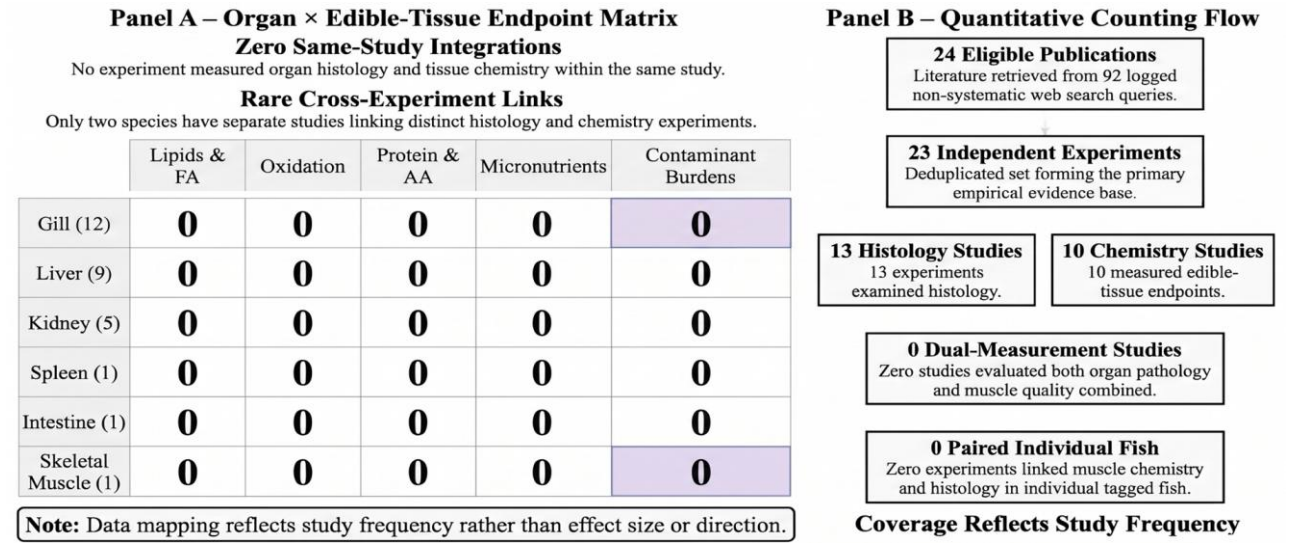


Figure 4. Coverage of evidence linking organ histology with edible-tissue composition under combined thermal and chemical exposure in the included study set. (A) Organ × Edible-Tissue Endpoint Matrix: Cross-tabulation of independent experimental studies evaluating organ-specific histology—Gill (12), Liver (9), Kidney (5), Spleen (1), Intestine (1), and Skeletal Muscle (1)—against edible-tissue compositional endpoints—Lipids/Fatty Acids (1), Lipid/Protein Oxidation (1), Protein/Amino Acids (0), Micronutrients (0), and Contaminant Burdens (9). Zero (0) experiments in the literature measured both organ histology and edible-tissue quality within the same study. Only two matrix cells contain linkages across separate experiments in the same species (Cyprinus carpio, C06 and C15; Pangasianodon hypophthalmus, C12 and C23). (B) Quantitative Evidence Flow: Sequential categorization tracking research allocation: 24 eligible publications yielding 23 independent experiments, comprising 13 measuring organ histology and 10 measuring edible-tissue quality. None (0) evaluated both domains, and zero (0) linked organ lesion severity directly to muscle tissue chemistry in individual fish. Coverage mapping represents research frequency rather than response magnitude or direction. Observed gaps in matrix coverage may also reflect non-systematic search boundaries (Supplementary Table S4).