Fish Antioxidant Enzyme Activity as Protein-Level Biomarkers of Ecological Stress in Tropical Eutrophic Wetlands: A Review

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ABSTRACT

Eutrophication-related stressors, including low dissolved oxygen, nutrient enrichment, and harmful algal blooms, negatively affect the health and function of organisms, populations, and ecosystems, resulting in ecological stress. Aquatic life struggles to withstand such irregular and abrupt disruption to ecosystem homeostasis, leading to alteration in ecosystem metabolism(e.g., simplified food webs and shortened food chains), reduced nutrient uptake and utilization efficiency, hyperphosphorylation-induced cellular damage, and increased pathogen virulence. As a first line of defense against eutrophication stress, fish release antioxidant enzymes (AOEs) such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), peroxidase (POD), acetylcholinesterase (AChE), and others from various body organs. These enzymes react with reactive oxygen species (ROS) produced during stress and convert them into less harmful compounds. Most studies indicate that SOD is the primary enzyme secreted in response to ammonia and nitrate toxicity in eutrophic water bodies. The liver is the main organ responsible for AOE release followed by the gills and brain. Maximum AOE activity is typically observed at 48–96 h of exposure to ammonia, nitrate, or microcystin contamination. Thus, species-specific AOE release mechanisms can reflect the nature and intensity of stressor impact and may serve as biomarkers of ecological stress in tropical eutrophic wetlands. These eco-remediation tools can help mitigate the effects of eutrophication and promote healthier aquatic environments for species to thrive and develop.

Keywords: Antioxidant enzymes, Eutrophication stress, Reactive oxygen species, Tropical wetland

INTRODUCTION

Ecological stress occurs when extremes of abiotic factors (beyond permissible limits) negatively affect the health and functioning of an organism, population and/or ecosystem (Nawaz *et al*., 2023). Natural or anthropogenic stressors can directly (e.g., decreased oxygen and increased reactive oxygen species)or indirectly (e.g., stress-induced decline in prey species) impact ecosystems, often through multi-interactive and synergistic pathways (Bănăduc *et al*., 2024). Compared to other ecosystems, abiotic stress in aquatic ecosystems is more harmful, due to the random discharge of inorganic and organic waste from industries, agriculture, municipal, and domestic sources, as well as the effects of global warming (Bashir *et al*., 2020). It becomes increasingly difficult for aquatic life to withstand such rapid and random disruptions of ecosystem homeostasis, ultimately leading to eutrophication symptoms such as depleted dissolved oxygen, nutrient enrichment, harmful algal blooms, and fish kill (Zeng *et al*., 2016).

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Eutrophic conditions represent a system with a perfect combination of abiotic stressors capable of causing deep environmental perturbation but offering poor prospects for ecosystem recovery. This situation prevents a clear-cut categorization of the stress status of a particular ecosystem, whether it is poorly, moderately, or severely stressed cannot be stated clearly (Birk *et al*., 2020). Therefore, it is most pertinent to consider a species-specific response to these eutrophication-induced abiotic stressors as a biomarker of stress status (El-SiKaily and Shabaka, 2024). Thus, to overcome eutrophication stress, fish release various types of antioxidant enzymes (AOEs) such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), peroxidase (POD), acetylcholinesterase (AChE), lactate dehydrogenase (LDH), and others (Abhijith *et al*., 2016; Hu *et al*., 2019; Jin *et al*., 2024; Monier *et al*., 2025) from different body organs to defend themselves from the abiotic stressor (Wang *et al*., 2016), which could serve as a meaningful biomarker (Teh *et al*., 1997). Quantitative and qualitative assay of antioxidant enzymes (Khan *et al*., 2016; Faheem and Lone, 2018), when compared with suitable controls, determine the fish health status due to pollution (including eutrophication) and toxicity stress in their tissues. Because it is difficult to quantify the impact of multiple stressors of eutrophication on biodiversity, physiology, and ecosystem functioning (Figure 1), species-specific impact quantification in terms of their ecological interactions can untangle the complex responses due to physical, chemical, and biological stressor more easily (Figure 2) (Sanon *et al*., 2020).

Therefore, the present review evaluates the interactive ecological roleof fish antioxidant enzymes as protein-level biomarkers of variable eutrophication stressors, with the potential to inform both preventive and remediation measures.

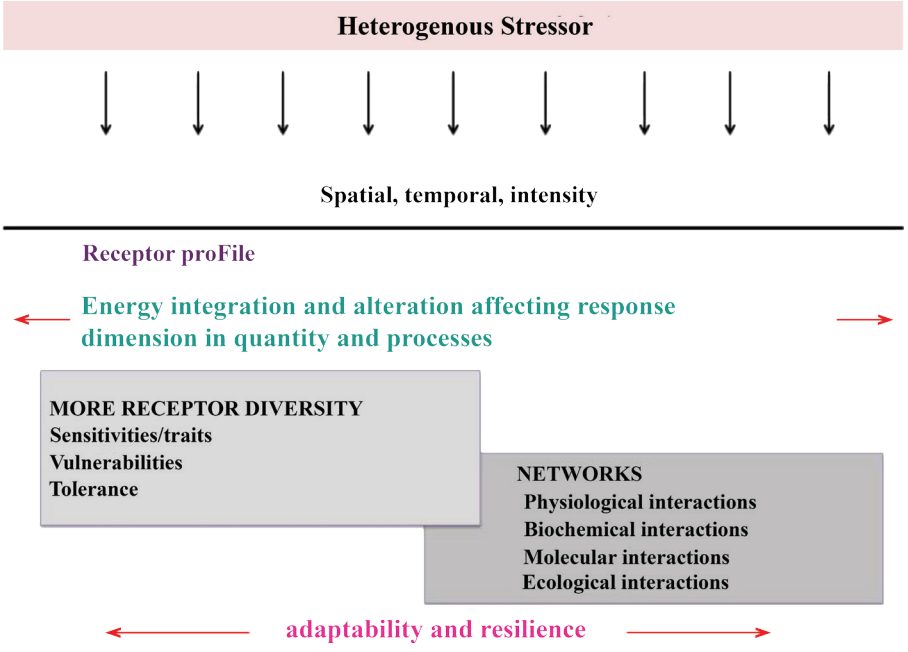

Figure 1. Conceptual model illustrating the impact of multiple stressors on freshwater biota, highlighting how receptor diversity (traits, sensitivities, vulnerabilities, tolerance) and interactive network (physiological, biochemical, molecular, and ecological) influence biological adaptability and resilience (adapted from Karageorgis *et al*., 2005).

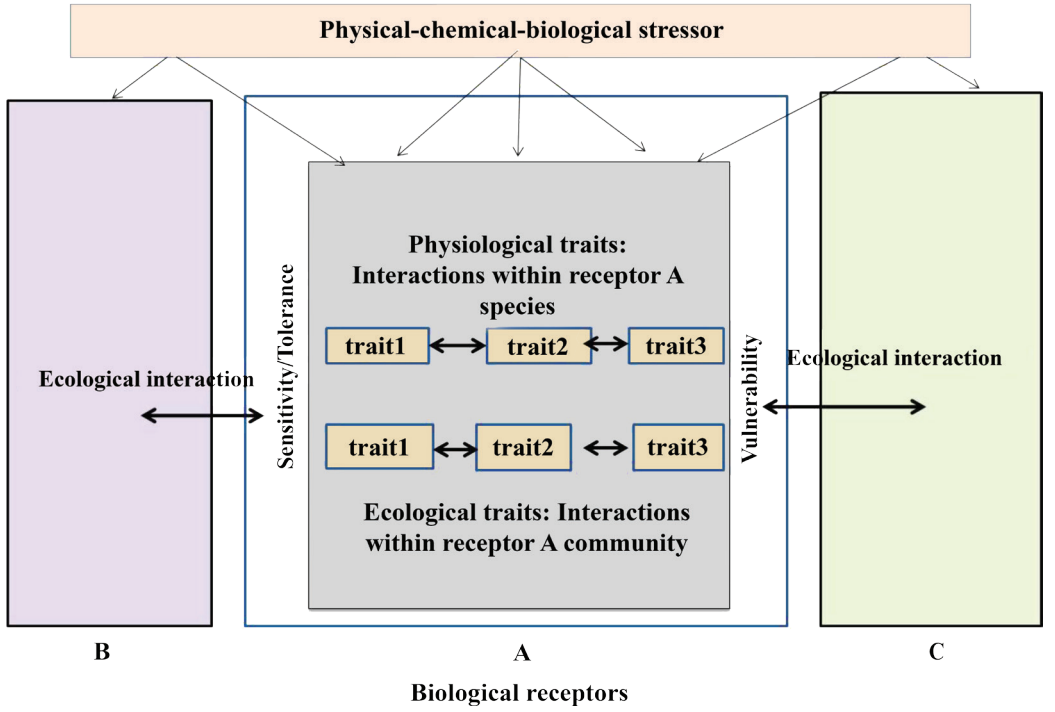

Figure 2. Ecological interactions between diverse receptors play a significant role in mediating responses to physical, chemical,and biological stressors (adapted from Segner *et al*., 2014).

REVIEW METHODS

Different secondary data were retrieved from manuscripts published in peer-reviewed journals relevant to the review topic. Various academic search engines were used to access literature for exhaustive (500 papers), representative, and priority-based coverage (Paré and Kitsiou, 2017). The main thrust areas of data collection included sources of eutrophication, such as the harmful impacts of urbanization, abiotic and biotic ecological stress, and first-line enzyme mediated defense responses of fish in eutrophic wetland ecosystems.

After evaluating the suitability of the collected review materials, potential study findings were identified. This was followed by a screening process based on the relevance of each source to the review objectives. The inclusion and exclusion of literature were conducted using predetermined criteria to ensure data suitability. To enhance objectivity and minimize errors, approximately 50 papers were finalized, focusing primarily on the qualitative and quantitative responses of fish antioxidant enzyme (AOE) activity to varying concentrations of eutrophication parameters.

A wide range of alterations in abiotic factors due to eutrophication was critically assessed to evaluate ecological stress across different levels of biological organization. A comprehensive synthesis of recent and past experimental studies and reviews was conducted. The functional attributes of antioxidant stress mechanisms in various freshwater fishes were then used to develop an interaction model linking ecological stress, AOE secretion levels, and fish species exposed.

This interaction model was designed to categorize stress status and interpret AOE activity accordingly. Based on this framework, the entire review was categorized into the sub-topics to systematically address the objectives outlined for the study (Figure 3).

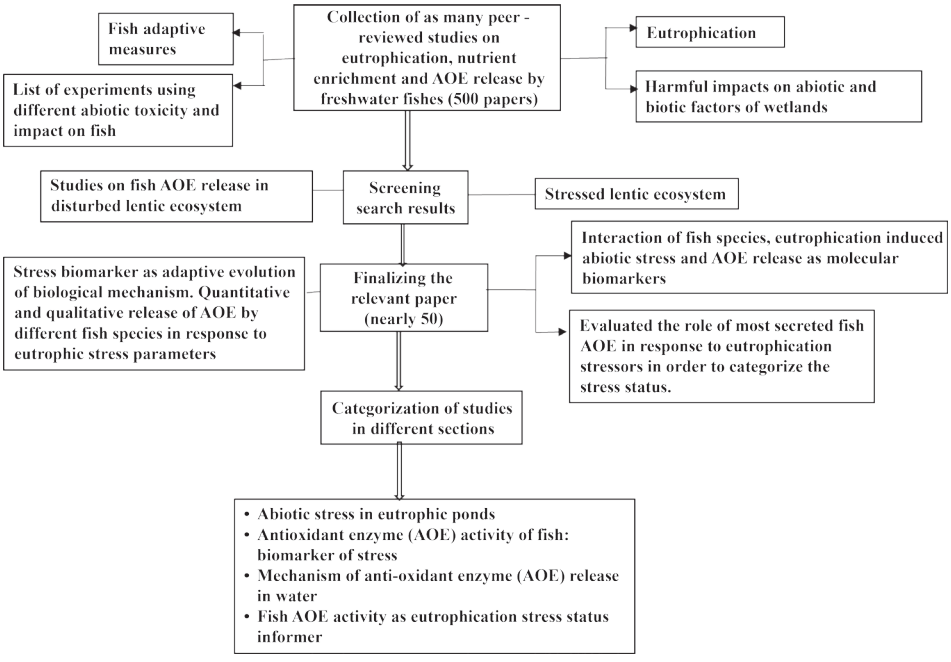

Figure 3. Workflow diagram of the present study outlining the methodological approach.

RESULTS AND DISCUSSION

Abiotic stress in eutrophic ponds

Nutrient enrichment

Anthropogenic pressures have consistently intensified the scale and rate of eutrophication by introducing limiting nutrients such as nitrogen and phosphorus into aquatic ecosystems through both point and non-point sources (Schindler, 2012; Paul *et al*., 2021). Phosphorus-rich sources include fertilizers, untreated sewage, detergents, and industrial waste discharge (Khan and Mohammad, 2014) generating 10.8 gN and 2.2 gP, respectively of average nitrogen and phosphorous per individual (Witek-Krowiak *et al*., 2022).

Half of the nitrogen applied to crops is lost to groundwater, making agriculture a significant contributor to nitrate contamination of freshwater systems, in contrast to phosphate enrichment. In comparison, Wastewater Treatment Plants (WTPs), livestock manure, and urban runoff are major sources of phosphorus input into aquatic ecosystems (Figure 4). Additionally, livestock farming plays a major role in agricultural eutrophication.

This nutrient stress triggered a feedback loop between macrophyte community and water transparency (Dubey and Dutta, 2020), disrupts metabolic balance (Cross *et al*., 2022), and alters nutrient uptake and utilization. The primary consequences include changes in algal biomass quantity and quality, as along with dissolved oxygen depletion. These disturbances affect the balance between dominant and redundant species in aquatic communities (Clark *et al*., 2013).

High harmful algal biomass

Blooms of *Ankisrtodes musfalcatus, Chlorococcum humicola, Melosira granulate* and *Monoraphidium arcuatum* (Ray *et al*., 2021), as well as cyanobacteria such as *Microcystis aeruginosa* and *Anabaena flos-aquae*, release toxic substances known as cyanotoxins, including cyclicpeptides, alkaloids, and lipopolysaccharides, into the water. These toxins induce stress in aquatic organisms by inhibiting protein phosphatases,

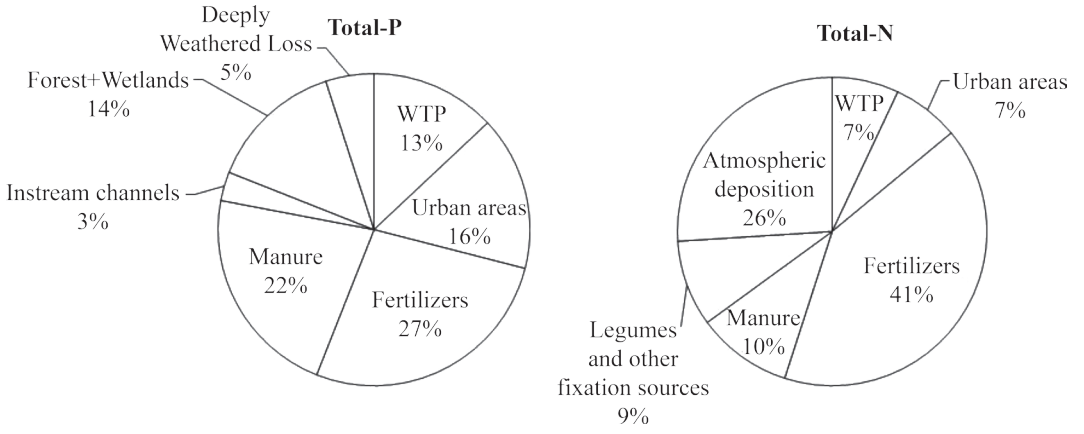

Figure 4. Proportions of different sources of phosphorus and nitrogen loading in wetlands, compiledfrom various literature sources.

leading to hyperphosphorylation and cellular damage (Wiltsie *et al*., 2018). Microcystins also affect fish by impairing immune responses, altering haematological parameters and liver function, disrupting osmoregulation, and damaging reproductive health (Pham and Utsumi, 2018; Banerjee *et al*., 2021). Consequently, only tolerant species are able to survive, resulting in reduced biodiversity and a shift toward simplified food webs and shorter food chains compared to non-eutrophic systems (Zhu *et al*., 2020).

Anoxia

Algal blooms-induced rapid bacterial decomposition reduces dissolved oxygen levels, a common outcome of eutrophication. Aquatic animals exposed to dissolved oxygen (DO) concentrations below optimal thresholds suffer from acute stress, including reduced feeding, stunted growth, increased disease susceptibility, and eventual mortality (Abdel-Tawwab *et al*., 2019). Additionally, pathogenicity may increase due to microbial genomic alterations and the emergence of antibiotic-resistant genes (Ahmad *et al*., 2024). Most fish species show signs of distress and may die when DO levels fall below 2–4 $mg \cdot L^{-1}$ (Lushchak and Bagnyukova, 2006), although some hardy species such as Nile tilapia (1.39–2.92 $mg \cdot L^{-1}$ at the incipient stage) and catfish, can tolerate lower oxygen levels (Abdel-Tawwab *et al*., 2015).

Hydrogen ion concentration (pH)

Phytoplankton blooms utilize the majority of available carbon dioxide during peak phases of photosynthesis, driving pH levelsabove10. Beyond the optimal range of 6.5–8.5, fish can become physiologically stressed. In water with a pH<6.5, fish growth is inhibited, reproduction is halted, and fry may die. Mortality is almost certain at pH<4.0 or >11.0 (Stevens *et al*., 2010). Both low and high pH enhance the production of ROSs, leading to oxidative stress that damages DNA, proteins, and lipids, ultimately causing developmental harm (Aranda-Rivera *et al*., 2022). Significant impacts have also been observed on calcium ion availability and cellular signalling. Genetic alterations include *Hox* gene expression, epigenetic modification, and DNA methylation patterns (Madesh *et al*., 2024).

Biochemical and chemical oxygen demand (BOD and COD) stress

The ideal pond BOD values ranged between 24 and 64 $mg \cdot L^{-1}$, with the highest and lowest values recorded during summer and post-monsoon seasons, respectively (Lkr *et al*., 2020). Rapid decomposition of organic matter by saprophytic bacteria in eutrophic systems increases biological oxygen demand (BOD) and depletes almost the entire available dissolved oxygen (Reynolds, 1992), causing anoxic stress to the existing aquatic organisms (Bhateriaand Jain, 2016).

Light and temperature

In addition to industrial and domestic runoff, global climate change contributes to increased nutrient enrichment of water bodies, thereby accelerating eutrophication. Algal growth improves under light intensity of approximately 4,000 lux (Satthong *et al*., 2019) and higher temperature (around 30 °C). Many shallow lakes experience a loss of submerged macrophytes as eutrophication progresses, primarily due to reduced light penetration triggered by dense algal blooms (Tu *et al*., 2015), which can extend several metres and absorb almost the entire light (Amorin and Moura, 2021). Increased temperature due to altered precipitation pattern triggers increased nutrient loadings in freshwater habitats, creating environmental conditions of enhanced cyanobacterial blooms (Rodgers, 2021).

Reactive oxygen species (ROS)

There is increased production of ROS in response to eutrophication conditions in degraded wetlands (Jin *et al*., 2025). The overproduction of ROS disrupts the typical equilibrium of $O_2^{\bullet-}$, $^{\bullet}OH$, and H_2O_2 in the intracellular milieu, resulting in increased oxidative damage and ultimately leading to cell death (Zainab *et al*., 2021) of aquatic organisms. A cell is considered to be in a condition of "oxidative stress" when the levels of ROS exceed the defensive systems and cause protein oxidation, lipid peroxidation, damage to nucleic acids, enzyme inhibition, and activation of pathways associated with programmed cell death, culminating in cell death.

Antioxidant enzyme (AOE) activity of fish: Biomarker of stress

To resist this eutrophication-triggered extreme change in aquatic ecological function and structure, organisms have developed certain mechanisms by manipulating either their metabolic pathways, adaptive evolution, or phenotypic plasticity (Norin and Metcalfe, 2019). Fish antioxidant enzymatic and non-enzymatic systems (Hu *et al*., 2019) present in the tissues help to maintain cellular redox homeostasis by removing ROS species. Increased ROS levels trigger the biological system to develop a first-line defence mechanism by modulating the activities of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione related enzymes (GPx, GR etc.) (Jomova *et al*., 2024). Evolutionary evidence indicates the development of sophisticated antioxidant defence systems in fishes, although their underlying activating mechanisms and variation among fish species are yet to be understood. This is very important for accessing the health of the derelict and eutrophic aquatic systems in order to develop mitigation strategies (Kumari *et al*., 2014; Bakiu *et al*., 2024).

Mechanism of antioxidant enzyme (AOE) release in water

Superoxide dismutase (SOD)

This enzyme alternately catalyzes the dismutation of the superoxide (O_2^-) radical into two less harmful species: molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), by either donating or removing an electron from the superoxide molecules it encounters (Tahri *et al*., 2016). The resulting products are far less damaging than superoxides. This dismutation catalysed by SOD canbe expressed as Cu, Zn-SOD, with the following half-reactions (Equation 1.1–1.3) (Bragadóttir, 2001).

$$Cu^{2+}SOD + O_2^- \rightarrow Cu^{+}SOD + O_2 \quad \text{Equation 1.1}$$

$$Cu^{+}SOD + O_2^- + 2H^+ \rightarrow Cu^{2+}SOD + H_2O_2 \quad \text{Equation 1.2}$$

$$2\,O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad \text{Equation 1.3}$$

Three major families of SOD are categorized based on protein folding and the type of metal cofactor, including Cu/Zn-, Fe-, Mn- and Ni-containing SODs (Tahri *et al*., 2016).

Catalase (CAT)

Catalase (CAT) is a tetrameric, metal-containing enzyme that rapidly reacts with H_2O_2 to producewater and molecular oxygen. It can also react with H donors (such as methanol, ethanol, formic acid, or phenols) to exhibitperoxidatic activity in a redox reaction (Equation 2).

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Equation 2

Hydrogen peroxide is not highly reactive with most of the significant biological molecules; however, it serves as a precursor for more reactive oxidants such as hydroxyl radicals (·OH) (Bragadóttir, 2001). Therefore, the concurrent activation of SOD and CAT is commonly observed response to oxidative stress (Figure 5), often assisted by the action of GPx (Peixoto *et al*., 2013).

Glutathione peroxidase (GSH-px)

Glutathione Peroxidase (GSH-px) functions as an additional propagation inhibitor within the aqueous phase of fish muscle, mitochondria and the cytosol of skeletal muscle cells. The presence of selenium in the enzyme structure enables the reduction of lipid peroxides (LOOH) using reduced glutathione (GSH) as an electron donor (Equation 3.1 and 3.2). This reaction produces oxidized glutathione disulphide (GSSG), which is subsequently reduced back to GSH by glutathione reductase(GR)using NADPH as a cofactor.

$$2GSH + H_2O_2 \rightarrow 2H_2O + GSSG$$

Equation 3.1

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$$

Equation 3.2

Although GPx shares H_2O_2 as a substrate with CAT, it alone can react effectively with lipid and other organic hydroperoxides, serving asthe major source of protection against low levels of oxidant stress (Krishnamurthy and Wadhwani, 2012).

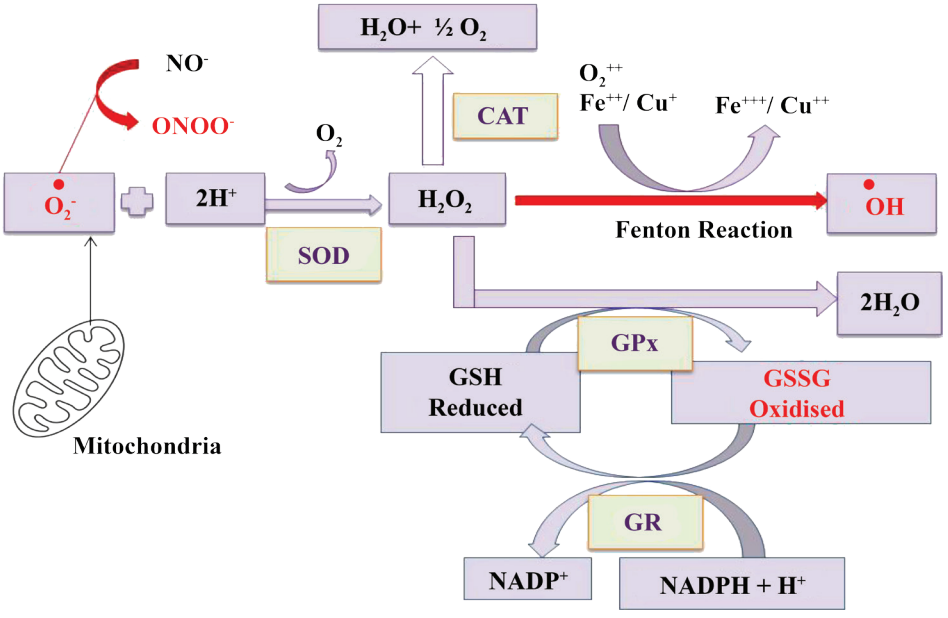

Figure 5. Synchronizedroles of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the cellular antioxidant defense system (adapted from Brunetti, 2017).

Fish AOE activity as an eutrophication stress status informer

Experimental studies on various fish pecies exposed to abiotic stressors have shown that antioxidant enzyme responses are multi-dimensional and influenced by habitat, life stages, and environmental conditions (Table 1). Peak enzyme activities varied across species and stressors, indicating differences intolerance potentials. Therefore, the linkage between these multi-dimensional aspects of AOE release supports the potential use of AOE activity as an indicator of eutrophication stress status in various degraded and eutrophic wetlands.

To highlight the major findings, it was observed that the minimum exposure time required for most of the studied AOE secretion was 24 h, while maximum exposure durations varied:96 h for SOD, POD, and GST; 48 h for CAT; and 84 h for GPx (Figure 6). The liver was identified as the main organ responsible for the maximum release of SOD (37%), CAT (35%), POD (29%), GR (47%), GPx (43%), and GST (38%), according to the reviewed literature. The gill was the second most significant organ, with LDH (31%), GSH (33%), and ACP (50%) being secreted at the highest levels from this tissue (Figure 7). However, 46% of the literature reported the brain as the organ primarily responsible for releasing only one enzyme: AChE.

The summarized information derived from Table1 clearly reflects an interactive mechanism among environmental stressors, fish species, and the levels of AOE activity released under specific regulating environmental conditions. The doses and types of experimental stressors simulate the conditions found in degraded, contaminated, or eutrophic wetlands. Since SOD has been identified as the most prominently secreted AOE, it has been used to develop a representative molecular biomarker, indication its potential as an indicator of eutrophication stress in freshwater fishes (Figure 8).

The stress impact of ammonia on *Lates calcarifer* is high, even at a low concentration of 15 mg·L^{-1}, as evidenced by the elevated secretion of SOD as a first line of defence. In contrast, *Ctenopharyngodon idella* exhibited a strong

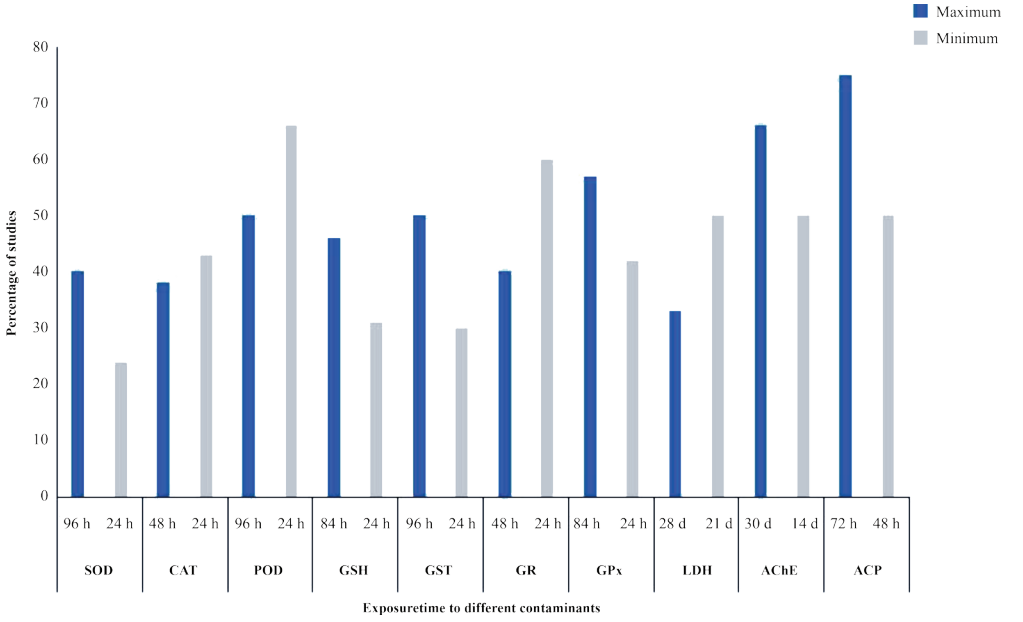

Figure 6. Maximum and minimum activities of different anti-oxidant enzymes at different hours of exposure based on percentage of studies.

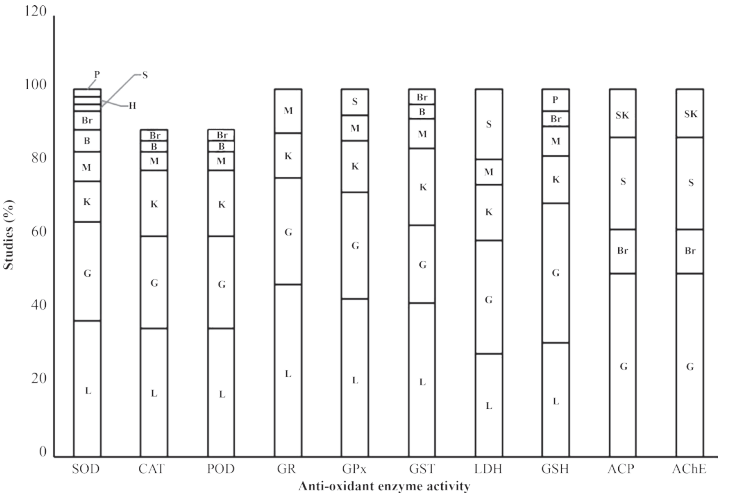

Figure 7. Maximum AOE secretion by different fish organs based on the percentage of studies.
Note: L = Liver; G = Gill; K = Kidney; M = Muscle; B = Body Tissue; Br = Brain; S = Serum; H = Heart; P = Plasma; Sk = Skin.

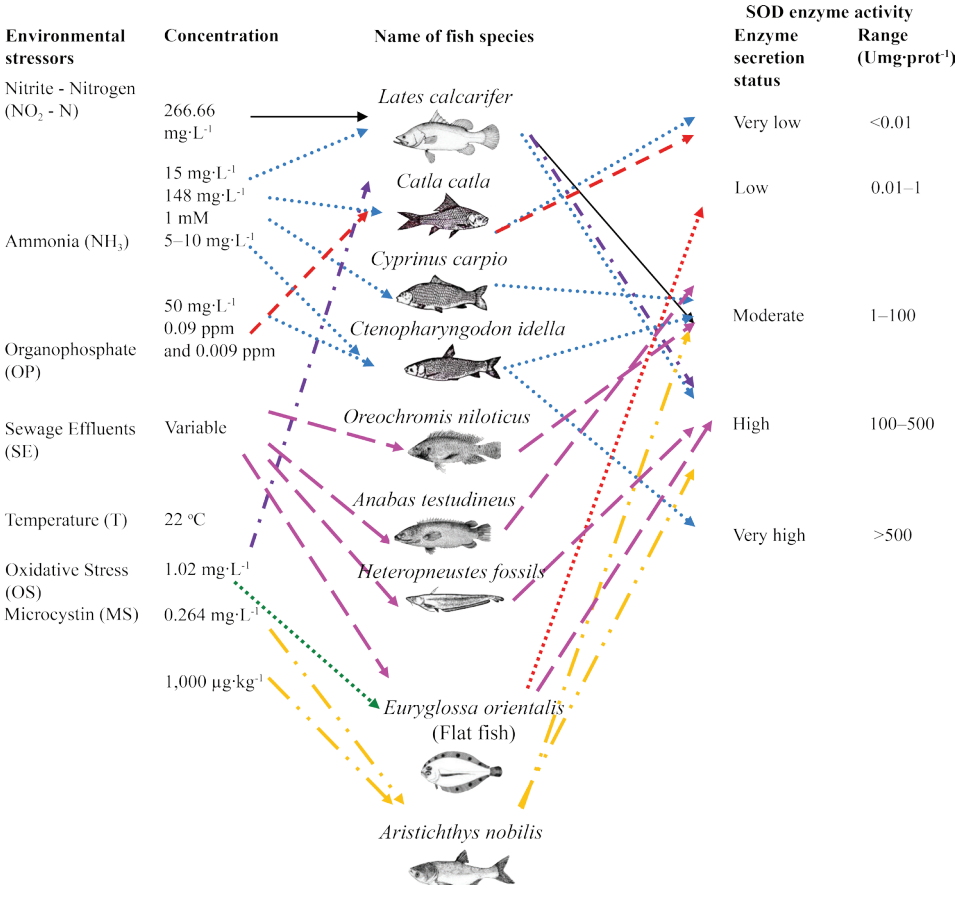

Figure 8. Amount of SOD secretion invariousfish species in response to varying types and concentrations of environmental stressors related to eutrophication.
Note: Different stressor actions are depicted by the following colours:
NO_2-N ⟶, NH_3 ⋯▸, OP – –▸, SE —▸, T —▸, OS – – –▸, MS —·▸

Table 1. Concentration of anti-oxidant enzyme released by fishes in response to different stressors in freshwatersystems.

Name of Fish Species	Stressor ($mg \cdot L^{-1}$)	Organ	Concentration of antioxidant enzyme/Peak·h^{-1}											References
			SOD	POD	CAT	GSH	ACP	AChE	LDH	ALP	GPx	GR	GST	
Lates calcarifer	NO_2–266.6	Skin, Gill,	25^a/48	-	-	-	150^a/48	-	-	-	-	-	-	Hu *et al.* (2019)
	NO_2–399.9	Body Tissue		5^a/48	35^a/96	$1,800^a$/48	-	-	-	-	-	-	-	Liu *et al.* (2018);
	NH_4–15		180^a/72	18^a/24	35^a/96	320^a/24	-	-	-	-	-	-	-	Zhou *et.al.*
	NH_4–20		-	-	-	-	180^a/72	-	-	-	-	-	-	(2018)
Catla catla (C)	NO_2–10.4	Brain	-	-	-	-	0.52^c; M	48^c; C	5^b; M	0.5^c; R	-	-	-	Das *et al.*
Labeo rohita (R)	(C, R, M)	Liver					-	48^c; M	7.5^b; M	-	-	-	-	(2004)
Cirrhinus mrigala (M)		Kidney					-	-	4.9^b; M	-	-	-	-	
Catla catla		Gill					0.7^c; R	-	2.0^b; M	0.45^c; R	-	-	-	
		Serum					135^d; R	-	0.10^b;C	11.5^d; M	-	-	-	
	NH_4–148	Serum	4.35^f	-	6.87^f	-	-	-	-	-	5.65^f	-	-	Senthilkumar
		Liver	7.85^f	-	9.56^f	-	-	-	-	-	7.89^f	-	-	and
		Kidney	6.75^f	-	8.45^f	-	-	-	-	-	6.12^f	-	-	Sivasubramania
		Gill	5.90^f	-	8.12^f	-	-	-	-	-	5.11^f		-	(2018)
Dicentrarchus labrax	NH_3–20	Brain	45^a/48	-	180^h/84	-	-	-	-	-	33^e/84	2.8^e/240	-	Sinha *et al.*
		Liver	35^a/48	-	225^h/180	-	-	-	-	-	66^e/180	1.8^e/180	-	(2015)
		Gill	18^a/180	-	70^h/84	-	-	-	-	-	22^e/12	1.3^e/180	-	
		Muscle	20^a/240	-	135^h/240	-	-	-	-	-	40^e/84	1.5^e/84	-	
		Kidney	11.2^a/84	-	60^h/12	-	-	-	-	-	35^e/240	1.48^e/240	-	
Cyprinus carpio	(NO_2 + NO_3 + P) –	Body Tissue	49.4%	-	82.6%	62.8%	-	-	-	-	-	-	-	Karadag *et al.*
	9 NH_4–1mM	Liver	43^a/84	-	330^h/84	$1,100^g$/84	-	-	-	-	30^e/48	1.3^e/24	-	(2014)
		Gill	19^a/84	-	75^h/84	700^g/3	-	-	-	-	23^e/48	1.2^e/24	-	Sinha *et al.*
														(2014)

Table 1. Cont.

Name of Fish Species	Stressor (mg·L^{-1})	Organ	Concentration of antioxidant enzyme/Peak·h^{-1}											References
			SOD	POD	CAT	GSH	ACP	AChE	LDH	ALP	GPx	GR	GST	
Ctenopharyngodon idella	NH_4–1.7 & 50	Gill Liver	45[a]/24	-	2.8[a]/48	-	-	-	-	-	-	-	-	Cao *et al.* (2021)
Oreochromis niloticus	NH_4–5 & 10	Liver	700[h]	-	350[h]	-	-	-	-	-	3[h]	1.8[h]	2.2[h]	Hegazi *et al.* (2010)
		Muscle	240[h]	-	180[h]	-	-	-	-	-	1.5[h]	3[h]	1.2[h]	
Oreochromis mossambicus	DO–1.02	Gill	0.12[i]	-	-	-	-	-	-	-	9.07[i]	-	-	Ahmed *et al.* (2016)
		Liver	0.15[i]	-	-	-	-	-	-		8.6[i]	-	-	
Hypopthalmichthys molitrix	Microcystei n–206 µg·g^{-1}	Body Tissue	-	-	-	9.5[j]	-	-	-	-	-	-	-	Bláha *et al.* (2004)
	M-Cys with NH_3–0.06, 0.264		80[a]/168	-	-	48[a]/72	-	-	-	-	-	-	-	Sun *et al.* (2011)
Aristicthys nobilis	M-Cys -1,000 µg·kg^{-1}		390[k]/48	-	8,000[k]/48	-	-	-	-	-	3.5[k]/48	0.9[k]/24	3[k]/24	Li *et al.* (2010)
Clarias gariepinus	M-Cys LR – (400 µg MC.LR·kg^{-1})	Liver	850[a]/28days	-	-	-	-	-	-	-	-	-	185[l]/28 days	Isibor (2017)
		Gill	33[a]/14days										3.4[l]/14 days	
		Muscle	4.1[a]/14days										-	
Eleutheronema tetradatylum	NH_3–10±0.4	Liver	1,020[a]/24		38[a]/24						68[a]/24			Jin *et al.* (2025)
Labeo rohita	NH_3–0.1		220[m]/48		54[m]/24						40[m]/48		100[m]/48	Parida and Sahoo (2023)

Note: [a] = U·mg^{-1} prot; [b] = OD min·g^{-1}; [c] = U·g^{-1}; [d] = U·L^{-1}; [e] = nmol Note: NADPH·min^{-1}·mg^{-1} protein; [f] = U·mL^{-1}; [g] = nmol·g^{-1}·wet wt^{-1}; h = µ mol H_2O_2·min^{-1}·mg^{-1} protein; [i] = U·mL·L^{-1}; [j] = µg·mg^{-1} protein; [k] = nkat·mg^{-1} p; [l] = nmol·min^{-1}·mg^{-1} protein; Units for peak hour are hours unless indicated; SOD = Super oxide dismutase; POD = Peroxidase; CAT = Catalase; GSH = Glutathione; ACP = Acid phosphatase; AChE = Acetylcholinesterase; LDH = Lactate dehydrogenase; ALP = Alkaline phosphatase; GPx = Glutathione peroxidase; GR = Glutathione reductase; GST = Glutathione S-transferase.

antioxidant response only when exposed to a higher ammonia dose (50 $mg \cdot L^{-1}$), suggesting that *L. calcarifer* possesses lower tolerance to ammonia-induced stress compared to this exotic freshwater species. Conversely, the indigenous freshwater Indian major carp (*Catla catla*) demonstrated remarkable resilience to high ammonia concentration (148 $mg \cdot L^{-1}$), as indicated by its very low SOD secretion (<0.01 $U \cdot mg^{-1} \cdot prot^{-1}$), relative to other fish species. Among other tolerant species, *Aristichthys nobilis* showed increased SOD secretion only when exposed to extremely high concentration of microcystin (1,000–2,000 $\mu g \cdot kg^{-1}$). Additionally, moderate SOD secretion levels (1–100 $U \cdot mg^{-1} \cdot prot^{-1}$) observed in other fishes in response to various eutrophication-related stressors reflect their capacity to mitigate oxidative stress through regulated AOE release. Therefore, the secretion patterns of antioxidant enzymes serve as useful indicators for assessing stress levels in fish and for classifying species into tolerant and less tolerant categories.

CONCLUSIONS

Nutrient enrichment isan emerging issue as a result of alterations in ecological integrity related to global warming in most wetlands. The role of fish AOE has the potential to provide the platform as a protein-level biomarker for this stress assessment. From the review, it can be concluded that due to the high enrichment of nutrients into waterbodies, tolerant fishes secrete some AOEs like SOD, CAT, GPx, etc., to overcome the stress condition, while some non-tolerant fishes cannot easily cope up with the harsh environment and ultimately may die, indicating a toxic effect on them. The differential release of fish AOEs, as demonstrated by the interaction model of SOD in response to random variations in nutrient enrichment, reveals a swift activation of fish defence mechanisms. Consequently, this review emphasizes the significance of fish antioxidant enzyme release in assessing the nutrient stress status of eutrophic wetlands that are trending towards more climate change-induced trophic state degradation in the future.

Further research in this area could facilitate the development of novel molecular biomarkers through advanced techniques of projections, emphasizing the importance of biological organization in bio-indication. The potential application of this function can serve as a sustainable mitigation strategy to tackle the combined impacts of climate change and nutrient enrichment on other affected species and their habitats in a eutrophic wetland.

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