Biomarkers in Aquatic Organisms: A Critical Review for Ecotoxicological Assessment and Environmental Monitoring

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Abstract

Biomarkers have become indispensable tools in aquatic ecotoxicology for evaluating the biological effects of environmental contaminants and supporting ecosystem health assessments. They provide measurable biological responses at the molecular, biochemical, cellular, physiological, and organismal levels, enabling the early detection of pollutant exposure and toxic effects before irreversible ecological damage occurs. This review critically synthesizes current advances in the application of biomarkers for ecotoxicological assessment, highlighting the major categories of biomarkers, including biomarkers of exposure, effect, and susceptibility, and their applications across diverse aquatic organisms, from plankton and benthic invertebrates to fish and marine mammals. Particular attention is given to the integration of multi-biomarker approaches with emerging omics technologies, which offer enhanced sensitivity and a more comprehensive understanding of contaminant-induced biological responses. The review also examines the practical applications of biomarkers in environmental monitoring, ecological risk assessment, pollution management, and biodiversity conservation, while discussing current limitations related to standardization, data interpretation, and regulatory implementation. Overall, biomarkers represent powerful early warning tools that significantly improve environmental monitoring programs and support evidence-based decision-making for sustainable aquatic ecosystem management.

Keywords: Biomarkers; Aquatic organisms; Ecotoxicology; Environmental monitoring; Omics technologies; Oxidative stress

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Introduction

Aquatic ecosystems are ultimate sinks for a multitude of pollutants, including heavy metals, pesticides, industrial chemicals, pharmaceuticals, and plastics (El-Sikaily and Shabaka, 2024). Monitoring chemical concentrations alone is insufficient for predicting ecological consequences, as this approach fails to account for bioavailability, synergistic effects, and the actual health of resident organisms (Hook *et al*., 2014). Consequently, biological monitoring, which assesses the responses of living organisms to environmental stressors, has become a cornerstone of modern ecotoxicology. Within this paradigm, biomarkers have emerged as indispensable diagnostic tools.

Biomarkers are defined as measurable variations in cellular, biochemical, physiological, or morphological components within an organism, induced by anthropogenic stressors (Hook *et al*., 2014; El-Sikaily and Shabaka, 2024). These changes occur at lower levels of biological organization, often serving as early warning signals before disturbances manifest at the population or community level over longer timeframes (Hagger *et al*., 2006). For instance, the induction of specific proteins or the inhibition of key enzymes can signal exposure to pollutants long before mortality or reproductive failure is observed.

The strategic importance of biomarkers is reflected in major environmental legislation. Frameworks such as the European Union's Water Framework Directive and Marine Strategy Framework Directive, which aim to achieve good ecological status, advocate for an integrative analysis of water quality by incorporating various biological elements (Polkane *et al*., 2020). In this context, biomarkers provide critical information on the health and stress response of individual organisms, bridging the gap between chemical presence and biological effect (Chahouri *et al*., 2022).

A reliable biomarker for environmental assessment must fulfil specific criteria: it should be easily measurable, sensitive, cost effective, and exhibit a clear dose response relationship with the stressor (Lomartire *et al*., 2021). Biomarkers are broadly categorized based on their indication of exposure to contaminants, the effects of such exposure, or an organism's inherent susceptibility (Schlenk, 1999). Commonly applied biomarkers include metallothionein for metal exposure (Lacorn *et al*., 2001), cytochrome P450 induction for organic pollutants like polycyclic aromatic hydrocarbons (Van der Oost *et al*., 2003), acetylcholinesterase inhibition for organophosphate pesticides (Lionetto *et al*., 2001), and various indices of oxidative stress and genotoxicity for assessing cellular damage (Bolognesi and Cirillo, 2014).

The selection of appropriate sentinel species is equally critical. Ideal bioindicators, such as mussels (*Mytilus* spp.), clams, and certain fish species like zebrafish (*Danio rerio*), are chosen for their wide distribution, ecological

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relevance, sedentary nature, and known response mechanisms to pollutants (Berthet, 2015; Sendra *et al*., 2021). Their use allows for the spatial and temporal tracking of contamination.

This review article aims to provide a comprehensive study on the aquatic organisms and biomarker parameters utilized in ecotoxicological research. It will elaborate on the different classes and types of biomarkers, their mechanistic basis, and their application in monitoring major pollutant groups. Furthermore, it will discuss the application of biomarkers in specific marine organisms, their role in conservation and ecosystem health assessment, and the challenges and future directions in the field, particularly the integration of omics technologies into biomarker based environmental monitoring.

Classes and types of biomarkers in aquatic ecotoxicology

Biomarkers are systematically categorized to reflect their specific role in indicating an organism's interaction with environmental stressors. This classification is primarily based on whether the measured response signifies exposure to a contaminant, a resultant biological effect, or an inherent susceptibility of the organism Table 1.

Biomarkers of exposure

Biomarkers of exposure are early, often reversible, cellular changes that confirm an organism has encountered a specific class of pollutant. A primary example is metallothionein (MT), a metal binding protein whose synthesis increases in response to elevated concentrations of metals like cadmium and mercury. Measuring MT levels, particularly in the liver of bivalves and fish, has proven effective for determining exposure to trace metals (Lacorn *et al*., 2001; Lionetto *et al*., 2001). Research on Coho salmon indicates that MT mRNA levels in the olfactory system can be an even more sensitive indicator than in the liver, highlighting tissue specific responses (Espinoza *et al*., 2012; Williams and Gallagher, 2013). For organic pollutants, cytochrome P450 (CYP) enzymes, especially CYP1A, are pivotal biomarkers. These enzymes are involved in Phase I biotransformation and are induced by contaminants like polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). The catalytic activity of CYP1A, often measured as ethoxyresorufin O deethylase (EROD) activity, is commonly monitored in fish liver from polluted coastal areas (Van der Oost *et al*., 2003; Sen *et al*., 2010). Another critical exposure biomarker is vitellogenin, an egg yolk precursor protein normally synthesized in female fish. Its detection in the blood of male or juvenile fish is a definitive indicator of exposure to estrogenic endocrine disrupting chemicals (Houtman *et al*., 2007; Tran *et al*., 2019). Similarly, the inhibition of the enzyme acetylcholinesterase (AChE) in fish and invertebrates serves as a specific biomarker for exposure to organophosphorus and carbamate pesticides (Klumpp *et al*., 2002; Lionetto *et al*., 2001).

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Table 1: Overview of major biomarker classes, specific parameters, and their primary applications in aquatic ecotoxicology.

Biomarker cass	Specific biomarker parameter	Primary indication / pollutant class	Common sentinel organisms
Exposure Biomarkers	Metallothionein (MT) induction (protein/mRNA)	Exposure to heavy metals (Cd, Hg, Cu, Zn)	Fish (e.g., mullet, salmon), Bivalves (e.g., Mytilus spp.)
	Cytochrome P450 1A (CYP1A)/EROD activity	Exposure to PAHs, PCBs, dioxins (Ah-receptor agonists)	Fish (e.g., sole, cod), Marine invertebrates
	Vitellogenin (Vtg) induction (protein/mRNA)	Exposure to estrogenic endocrine disrupting chemicals	Fish (male/juvenile), Molluscs
	Biliary Fluorescent Aromatic Compounds (FACs)	Recent exposure to and metabolism of PAHs	Fish
	Acetylcholinesterase (AChE) inhibition	Exposure to organophosphate & carbamate pesticides	Fish, Crustaceans, Some bivalves
Effect Biomarkers	Antioxidant enzymes (SOD, CAT, GPx, GST)	Oxidative stress (response to metals, organics, MPs)	All major groups (Fish, Bivalves, Zooplankton)
	Lipid peroxidation (e.g., MDA levels)	Oxidative damage to cell membranes	Fish, Bivalves
	DNA damage (Comet assay, Micronucleus, 8-OHdG)	Genotoxicity (clastogenic/aneugenic effects)	Fish, Bivalves, Marine mammals
	Lysosomal Membrane Stability (LMS)	General cellular injury & health status	Bivalves, Other invertebrates
	Histopathological alterations (gills, liver, gonads)	Tissue-level damage and disease state	Fish, Bivalves
Biomarkers of Susceptibility	Aryl Hydrocarbon Receptor (AhR) genotype/activity	Inherent sensitivity to dioxin-like compounds	Various species (population-level differences)
	Flavin-containing Monooxygenase (FMO) presence	Detoxification capacity for specific pesticides (e.g., thioethers)	Species-specific (e.g., present in trout, absent in catfish)
Integrated & Novel Approaches	Transcriptomic/Genomic profiles	System-wide response, MOA identification, stressor fingerprinting	Model species (Zebrafish, Daphnia), Sentinel fish
	Metabolomic/Toxicometabolomic profiles	Functional metabolic disruption, early sublethal effects	Zebrafish, Molluscs
	Multi-Biomarker Response Indices (e.g., IBR)	Integrated health assessment of populations/sites	Bivalves, Fish (caged or wild)

Biomarkers of effect

Biomarkers of effect evaluate the toxicological impacts and health impairments resulting from pollutant exposure. These biomarkers are strongly associated with potential adverse outcomes. A major category involves biomarkers of oxidative stress, which

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occurs when the production of reactive oxygen species (ROS) overwhelms an organism's antioxidant defences. This imbalance can lead to damage of biomolecules, measured as lipid peroxidation (LPOX), protein oxidation, or DNA damage such as 8 hydroxy 2' deoxyguanosine (8 OHdG) (Gallego Ríos *et al*., 2021; Rahman *et al*., 2023). Key antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione S transferase (GST) are frequently assayed as effect biomarkers, as their activities change in response to contaminant induced oxidative stress (Desai *et al*., 2023). Genotoxicity biomarkers are another crucial class of effect biomarkers. These assess damage to the genetic material and include measures of DNA strand breaks (e.g., via the Comet assay), micronucleus formation, and the formation of DNA adducts (Harvey *et al*., 1999; Bolognesi and Cirillo, 2014). Lysosomal membrane stability (LMS) is a well-established cellular biomarker of effect. Destabilization of these organelles indicates cellular injury and has been ecologically linked to reduced growth, reproduction, and health in organisms like mussels and oysters (Ringwood *et al*., 2004; Edge *et al*., 2012). Histopathological alterations in tissues, such as gill lesions or hepatic abnormalities, also serve as powerful morphological biomarkers of effect, directly linking exposure to tissue level damage (Stentiford *et al*., 2014).

Biomarkers of susceptibility

Biomarkers of susceptibility reflect the inherent, often genetic, characteristics of an organism that influence its sensitivity to pollutants. These markers explain why different species or populations exhibit varying responses to the same contaminant exposure. For instance, the aryl hydrocarbon receptor (AhR) is a key protein that binds to dioxin like compounds and initiates a toxic response, including the induction of CYP1A. Genetic variation in the AhR pathway can determine an organism's susceptibility to these contaminants (Kawajiri and Fujii Kuriyama, 2017). Another example is the enzyme flavin containing monooxygenase (FMO), which activates certain thioether pesticides. The absence of this enzyme family in many freshwater fish, compared to its presence in species like rainbow trout, makes the latter more susceptible to the toxic effects of these pesticides (El Sikaily and Shabaka, 2024). These inherent differences underscore that susceptibility is a critical factor in ecological risk assessment.

Types of biomarkers according to biological level

Biomarker responses can be measured at different levels of biological organization, providing a hierarchical view of toxic insult. Biochemical/Molecular Biomarkers: This includes the measurement of enzymes (e.g., AChE, GST, EROD), stress proteins (e.g., heat shock proteins), hormones (e.g., vitellogenin, cortisol), and the expression of specific

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genes (e.g., MT, CYP1A). Transcriptomic analyses now allow for the simultaneous measurement of thousands of genes, providing a broad, integrative profile of exposure and effect (Hook *et al*., 2014; Porto *et al*., 2023). Cellular Biomarkers: These assess the integrity and function of cells. Examples include LMS, neutral red retention assays, and measurements of cell viability or metabolic activity (e.g., MTT assay) (Domouhtsidou *et al*., 2004; Barmshuri *et al*., 2023). Physiological/Organismal Biomarkers: These reflect the integrated health of the whole organism. They can include growth rates, reproductive output (fecundity), scope for growth, metabolic rate, and behavioural responses such as changes in swimming performance or feeding (Connon *et al*., 2009; Brosset *et al*., 2021).

Histopathological Biomarkers: The microscopic examination of tissues for lesions, necrosis, inflammation, or neoplasia provides direct evidence of toxin induced damage at the organ level and is a strong link between cellular change and organismal health (Pinto *et al*., 2010). The integrated application of biomarkers across these classes and types, known as a multi biomarker approach, offers a powerful and holistic strategy for diagnosing the health of aquatic organisms and the ecosystems they inhabit (Aguilera *et al*., 2019; Lionetto *et al*., 2001).

Applications of biomarkers in monitoring specific pollutants

The utility of biomarkers is most evident in their targeted application for detecting and assessing the biological impact of major classes of aquatic pollutants. By linking specific molecular and cellular responses to contaminant exposure, biomarkers provide a functional assessment of pollution that transcends mere chemical quantification.

Organic pollutants: PAHs, PCBs, and pesticides

Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are persistent, bio accumulative organic contaminants of significant concern. A well-established biomarker for exposure to these Ah receptor agonists is the induction of the cytochrome P450 1A (CYP1A) enzyme system, frequently measured as ethoxyresorufin O deethylase (EROD) activity (Van der Oost *et al*., 2003). CYP1A induction in fish liver has been widely used to map exposure gradients following oil spills and in industrially impacted areas (Huggett *et al*., 2003; Martinez-Gomez *et al*., 2009). Furthermore, the presence of fluorescent aromatic compounds (FACs) in fish bile serves as a direct biomarker of recent PAH uptake and metabolism (Aas *et al*., 2000; Johnson *et al*., 2008b). The toxicity of PAHs is often mediated through the formation of reactive metabolites that cause oxidative stress and bind to DNA, creating adducts. Consequently, biomarkers of oxidative stress, such as increased activities of glutathione S transferase (GST) and glutathione peroxidase (GPx), along

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with measurements of DNA adducts, are critical for assessing the biological effects of PAH exposure (Myers *et al*., 2003; Santana *et al*., 2018).

For organophosphorus and carbamate pesticides, the primary biomarker is the inhibition of acetylcholinesterase (AChE) in neural and neuromuscular junctions. This inhibition is a rare biomarker that clearly indicates both exposure and a direct neurotoxic effect, as it disrupts cholinergic neurotransmission, potentially leading to impaired behaviour and survival (Fulton and Key, 2001; Laetz *et al*., 2009). AChE inhibition has been documented in fish and invertebrates from agricultural runoff areas and is considered a factor in the poor recovery of some salmonid populations (Klumpp *et al*., 2002). Endocrine disrupting effects of certain pesticides and industrial chemicals are monitored using the vitellogenin biomarker, where its induction in male fish signals exposure to estrogenic compounds (Folmar *et al*., 1996; Jobling *et al*., 1998).

Heavy metals and trace elements

Metals such as cadmium, mercury, lead, and copper are non-degradable and exert toxicity through mechanisms including oxidative stress and enzyme inhibition. The quintessential exposure biomarker for metals is metallothionein (MT). This cysteine rich, low molecular weight protein binds and sequesters intracellular metals, playing a central role in detoxification and homeostasis (Amiard *et al*., 2006). MT induction in tissues like the liver and gills of fish and bivalves strongly correlates with bioavailable metal concentrations in the environment (Chesman *et al*., 2007; Oliveira *et al*., 2010). The toxic effects of metals are frequently assessed through biomarkers of oxidative stress. Metal exposure can generate reactive oxygen species, leading to a cascade of antioxidant responses. The activities of enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione related enzymes (GST, GPx, GR), as well as levels of reduced glutathione (GSH) and markers of lipid peroxidation (e.g., malondialdehyde), are commonly measured to evaluate metal induced oxidative damage (Farombi *et al*., 2007; Taylor and Maher, 2010). For instance, fish from metal contaminated sites often show altered antioxidant enzyme profiles and increased lipid peroxidation in sensitive tissues (Otto and Moon, 1996). Lysosomal membrane stability has also proven to be a sensitive cellular biomarker for metal toxicity, often showing destabilization that correlates with tissue metal burdens and overall health impairment in invertebrates (Domouhtsidou *et al*., 2004; Moore *et al*., 2013).

Emerging contaminants: Microplastics, nanoplastics, and PFAS

The ecological threat posed by microplastics (MPs) and nano plastics is an active area of biomarker research. Exposure to MPs can induce oxidative stress, reflected in the modulation of antioxidant enzymes such as SOD, CAT, and GST in organisms like mussels, crustaceans, and zebrafish (Ribeiro *et*

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al., 2017; Prokić *et al*., 2019). Studies show that the size, concentration, and polymer type of MPs influence the biomarker response, with smaller particles often inducing stronger oxidative effects (Jeong *et al*., 2016; Lei *et al*., 2018). Furthermore, MPs can act as vectors for adsorbed hydrophobic pollutants like PAHs, potentially leading to combined toxic effects, though the evidence for synergistic biomarker responses remains complex (Oliveira *et al*., 2013; Hanslik *et al*., 2022). Neurotoxicity is also a concern, with AChE inhibition reported in some species exposed to MPs (Oliveira *et al*., 2013). Per and polyfluoroalkyl substances (PFAS) are persistent synthetic chemicals. Their toxicity in aquatic organisms is linked to biomarkers of oxidative stress and immunotoxicity. For example, exposure to perfluorooctanesulfonic acid (PFOS) in crayfish led to decreased antioxidant enzyme activities (CAT, GPx) in hepatopancreas and gill tissues, increased total antioxidant status in hemolymph, and histopathological damage, indicating multi organ stress (Belek *et al*., 2022). Biomarker studies on PFAS in fish and molluscs are contributing to an understanding of their sublethal toxicity mechanisms (Hayman *et al*., 2021; Fabbri *et al*., 2014).

Integrated application for pollution assessment

The power of biomarkers is magnified when applied in an integrated, multi biomarker framework. This approach is essential for monitoring complex field scenarios where organisms face mixtures of pollutants. For example, studies have successfully used suites of biomarkers including EROD (for PAHs), MT (for metals), AChE (for pesticides), and oxidative stress parameters to discriminate pollution gradients, identify major contributing stressors, and assess the overall health status of sentinel species like mussels and fish in contaminated estuaries and coastal areas (Broeg *et al*., 2005; Dagnino *et al*., 2007; Marigomez *et al*., 2013). Such integrated indices provide a more robust and ecologically relevant diagnosis of environmental degradation than any single biomarker alone.

Biomarker applications in specific aquatic organisms

The effectiveness of a biomarker is intrinsically linked to the organism in which it is measured. Selecting appropriate sentinel or model species is therefore a critical step in ecotoxicological monitoring. Ideal species are characterized by their ecological relevance, wide distribution, ease of collection, well understood biology, and sensitivity to pollutants, providing a representative reflection of environmental health (Berthet, 2015).

Planktonic organisms

Phytoplankton and zooplankton form the base of aquatic food webs and are highly responsive to environmental change. In phytoplankton, lipid biomarkers from diatoms and dinoflagellates serve as reliable indicators of eutrophication, nutrient dynamics, and temperature

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shifts (Ding *et al*., 2019; Cao *et al*., 2023). For toxicity assessment, chlorophyll *a* fluorescence is a sensitive biomarker for evaluating the inhibitory effects of heavy metals and herbicides on photosynthetic efficiency (Suresh *et al*., 2014; Carafa *et al*., 2021). Among zooplankton, the water flea Daphnia spp. is a cornerstone model organism in ecotoxicology and regulatory testing. Its short parthenogenetic life cycle, genomic resources, and central role in freshwater food chains make it invaluable for linking molecular biomarkers to population level effects (Shaw *et al*., 2008; Altshuler *et al*., 2011). Biomarker studies on Daphnia have advanced the understanding of chemical modes of action and contributed to environmental risk assessment frameworks (Parish *et al*., 2020). Similarly, marine rotifers (e.g., *Brachionus* spp.) and copepods are used in biomarker-based studies to assess oxidative stress and genotoxicity from emerging pollutants like brominated flame retardants and microplastics (Jeong *et al*., 2016; Cao *et al*., 2023).

Benthic invertebrates: nussels and clams

Bivalve molluscs, particularly mussels (*Mytilus* spp.) and clams (*Ruditapes* spp., *Venerupis* spp.), are among the most widely used sentinel species globally due to their filter feeding habit, sessile nature, and capacity to bioaccumulate contaminants. The Mussel Watch program exemplifies their application in large scale monitoring. A multi biomarker approach is routinely applied to these organisms, including assays for lysosomal membrane stability (LMS), metallothionein (MT) induction, antioxidant enzyme activities (SOD, CAT, GST, GPx), acetylcholinesterase (AChE) inhibition, and genotoxicity (e.g., Comet assay, micronucleus test) (Hagger *et al*., 2008). Studies on mussels have demonstrated the utility of these biomarkers in detecting gradients of metal and organic pollution (Lionetto *et al*., 2001; Domouhtsidou *et al*., 2004). For example, the biomarker response in Mytilus galloprovincialis has been used to assess the health of coastal ecosystems and the effectiveness of remediation efforts (Serafim *et al*., 2011; Marigomez *et al*., 2013). Clams like Ruditapes philippinarum are similarly employed in integrated biomarker indices to evaluate water quality and metal toxicity (Aouini *et al*., 2018; Ji *et al*., 2019).

Fish as bioindicators

Fish are pivotal in aquatic ecotoxicology due to their ecological and economic importance, their position at higher trophic levels, and their physiological complexity. They integrate exposure over time and space, making them excellent indicators of ecosystem health. The zebrafish (*Danio rerio*) is a preeminent model organism in toxicology. Its fully sequenced genome, transparent embryos, and well characterized development allow for the discovery and validation of biomarkers across all levels of biological organization, from gene expression to behavior (Sendra *et al*., 2021; Porto *et*

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al., 2023). It is extensively used to study the effects of heavy metals, nanomaterials, pharmaceuticals, and microplastics (Teles *et al*., 2016; Alak *et al*., 2024; Santos *et al*., 2024). Wild fish species serve as in situ sentinels. The induction of CYP1A (EROD activity) and bile FACs in fish like English sole and flounder is a classic biomarker approach for monitoring PAH contamination in urbanized estuaries (Myers *et al*., 2003; Johnson *et al*., 2008b). Metallothionein levels in species such as the golden grey mullet (*Liza aurata*) correlate strongly with ambient metal concentrations, providing a history of metal exposure (Oliveira *et al*., 2010). Furthermore, population level concerns, such as the decline of salmonids, have been investigated using biomarkers like AChE inhibition to link pesticide exposure to impaired survival and recruitment (Laetz *et al*., 2009; Scholz *et al*., 2011).

Marine mammals

Marine mammals, as long-lived top predators, are critical indicators of oceanic health and bio accumulative pollution. However, their protected status necessitates the use of non-destructive or minimally invasive biomarker techniques. Blubber and skin biopsies allow for the measurement of contaminant body burdens and associated biochemical responses. For example, cytochrome P450 enzymes and antioxidants like catalase have been measured in dolphin biopsies to assess exposure to planar halogenated aromatic hydrocarbons and PAHs (Fossi *et al*., 2013). Genotoxicity biomarkers, including micronucleus frequency and DNA adducts, have been applied to assess cancer risks in whale and sea lion populations exposed to high levels of environmental contaminants (Gauthier *et al*., 1999; Randhawa *et al*., 2015). Serum biomarkers such as cardiac troponin I are also being explored for diagnosing health conditions like cardiomyopathy in stranded or managed animals (Joblon *et al*., 2022). These biomarker applications in marine mammals are vital for conservation efforts and understanding the impacts of chronic pollution on vulnerable species. The strategic selection of these diverse aquatic organisms, combined with biomarker suites tailored to their biology and the pollutants of concern, creates a powerful, hierarchical monitoring network capable of diagnosing environmental stress from the molecular to the ecosystem level.

Challenges, future directions, and integration into regulatory frameworks

Despite their demonstrated utility in scientific research, the formal incorporation of biomarker-based approaches into standardized environmental monitoring and regulatory decision making presents significant challenges. Addressing these limitations while harnessing novel technological advancements is critical for the future evolution of aquatic ecotoxicology.

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Challenges in biomarker application and interpretation

A primary obstacle to the regulatory adoption of biomarkers is the influence of confounding natural variables. Biomarker responses can be significantly modulated by physiological factors such as age, sex, reproductive status, and nutritional condition, as well as environmental variables including temperature, salinity, and seasonality (Hylland *et al*., 2009; Padmini and Vijaya Geetha, 2009). For instance, metallothionein levels in bivalves can fluctuate with reproductive cycle and handling stress, not solely with metal exposure (Amiard *et al*., 2006). Similarly, the activity of antioxidant enzymes follows natural cycles, making it essential to establish robust baselines and employ appropriate reference conditions for accurate interpretation (Barrick *et al*., 2016).

The inherent complexity of field exposures, where organisms face mixtures of pollutants and multiple non chemical stressors (e.g., hypoxia, temperature rise), further complicates the attribution of a specific biomarker response to a single causative agent. This lack of absolute specificity can be a drawback in regulatory contexts demanding clear causal links. Furthermore, the ecological relevance of many molecular and biochemical changes, while indicative of stress, must be more firmly linked to population level consequences such as reduced growth, reproduction, or survival to justify management actions (Forbes *et al*., 2006; Lam, 2009). Although linkages exist for biomarkers like AChE inhibition to survival in salmonids (Laetz *et al*., 2009), and lysosomal stability to reproductive output in bivalves (Ringwood *et al*., 2004), such connections require extensive validation.

The impact of global change

Climate change introduces an additional layer of complexity. Warming temperatures, ocean acidification, and altered hydrological cycles can modify the fate, bioavailability, and toxicity of pollutants, thereby influencing biomarker responses (Tilli and Mouneyrac, 2021). For example, elevated temperatures can exacerbate metal toxicity and oxidative stress in organisms like the bivalve Cerastoderma edule (Mesquita *et al*., 2023). Climate stressors may also alter the baseline physiology of organisms, potentially masking or amplifying contaminant induced biomarker signals. Future biomarker strategies must therefore account for these interacting global change factors to remain predictive under future environmental conditions.

Emerging technologies and novel biomarkers

Advanced omics technologies genomics, transcriptomics, proteomics, and metabolomics are revolutionizing biomarker discovery and application. These approaches enable the untargeted, system level characterization of biological responses, providing unprecedented insight into the

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mechanisms of toxicity and identifying sensitive, novel biomarkers.

Toxicometabolomics, for instance, analyses the full suite of small molecule metabolites in an organism, capturing the functional outcome of genomic and proteomic changes. It has been used to elucidate the metabolic disruptions caused by pesticides, metals, and diluted bitumen in zebrafish and other species, often revealing effects at lower exposure levels than traditional endpoints (Cappello *et al*., 2016; Fujita *et al*., 2021; Porto *et al*., 2023). Transcriptomic profiling via microarrays or RNA sequencing can generate specific gene expression signatures that discriminate between classes of contaminants and differentiate impacted from reference sites, acting as a "super biomarker" for exposure and effect (Hook, 2010; Sellin Jeffries *et al*., 2012; Van Straalen and Feder, 2012).

These technologies also facilitate the development of novel biomarker concepts. For example, the hydrogen isotope signature of lipid biomarkers is being explored as a tool to assess mixotrophy in marine microbes, offering insights into carbon cycling impacted by environmental change (Cormier *et al*., 2022). Furthermore, omics approaches are crucial for developing robust biomarkers within an adverse outcome pathway (AOP) framework, which seeks to logically link a molecular initiating event (e.g., binding to a receptor) through key biological responses to an adverse outcome relevant to risk assessment (Ankley *et al*., 2010).

Integration into regulatory and management frameworks

For biomarkers to transition from research tools to regulatory assets, structured frameworks for their evaluation and application are needed. Initiatives like the "Biomarkers and Biodiversity" project aim to inventory biomarkers, assess their limitations, and provide guidance for their use within directives like the EU Water Framework Directive (Milinkovitch *et al*., 2019).

A weight of evidence (WOE) approach, where biomarker data are integrated with lines of evidence from analytical chemistry, traditional toxicity testing, and ecological surveys, is widely advocated (Chapman *et al*., 2002; Hook *et al*., 2014). In this model, biomarkers serve as a critical line of evidence to confirm bioavailable exposure (exposure biomarkers) and diagnose sublethal stress (effects biomarkers), thereby clarifying ambiguities left by chemical data alone. They are particularly valuable for assessing the effectiveness of remediation actions and monitoring recovery, as demonstrated in studies following oil spills where biomarker levels returned to baseline post clean-up (Myers *et al*., 2000; Martinez-Gomez *et al*., 2009).

Ultimately, the successful integration of biomarkers into management relies on clear criteria: demonstrated dose response relationships, understanding of temporal dynamics and confounding factors, and, most importantly, established linkages to ecologically relevant outcomes on organism fitness and population sustainability (Huggett *et*

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al., 1992). As these criteria are increasingly met through targeted research and the power of omics, biomarkers are poised to become indispensable components of holistic, proactive, and effective aquatic ecosystem health assessment and conservation strategies.

Conflict of Interest

The authors declare no conflict of interest.

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