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Biomarkers: Their Vital Role in Understanding the Complexity of Marine Pollution Monitoring

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ABSTRACT

The monitoring of marine pollution plays a pivotal role in our efforts to comprehend and mitigate its detrimental effects on marine ecosystems. Biomarkers, quantifiable biological indicators of exposure or effect, have emerged as indispensable tools in gauging the magnitude of pollution. This review delves into the multifaceted challenges associated with the use of biomarkers in marine pollution monitoring, including species-specific responses, sensitivity and specificity concerns, extraneous factors, species variability, an exclusive focus on single stressors, and limited knowledge. Discerning between pollution-induced impacts and natural fluctuations is made challenging by the inherent natural variability and confounding factors. Differences in sensitivity and specificity add complexity to the precision of pollution detection. Species-specific variations and the presence of multiple contaminants add layers of complexity to biomarker examination. Additionally, the lack of standardized operating procedures and insufficient information hinder the widespread adoption of biomarkers. Limited studies on specific stressors restrict our comprehensive understanding of pollution effects particularly in the Philippines. Further research, collaborative efforts, and the establishment of standardized protocols are imperative to enhance the precision and efficacy of biomarker-based monitoring in marine environments.

INTRODUCTION

The demand for more sensitive indicators of sublethal ecological consequences of marine pollution led to the development of biomarkers (Bickham et al., 2000; Hook et al., 2014). Biochemical, cellular, physiological, or behavioral alterations that can be assessed in tissue or bodily fluid samples, or at the level of whole organisms, to provide evidence of exposure to and/or effects from one or more pollutants is one meaning of the word "biomarker" (Depledge, 1994; Smit et al., 2009; Hook et al., 2014).

Organisms engage in a multitude of molecular, cellular, biochemical, and physiological processes, each of which holds potential as a biomarker when subject to alteration. Yet, biomarkers are frequently categorized into three distinct groups: susceptibility, exposure, and effect (Chambers et al., 2002). It is essential to acknowledge, however, that the observed change in a biomarker may not always directly relate to the specific mechanism of action of the toxicant, and it may not necessarily reflect the severity of adverse effects at the organism or population level (Chambers et al., 2002; Hampel et al., 2016). Biomarkers of susceptibility play a crucial role in environmental toxicology by indicating an organism’s inherent sensitivity to pollutants or stressors, as it reflects an organism’s predisposition to adverse effects. These biomarkers are often associated with genetic or physiological factors that influence an organism’s response to environmental stressors. Likewise, the biomarkers of exposure function as indicators of an organism's exposure to a toxicant or other stressor, often serving as cost-effective alternatives to chemical tests or enabling the quantification of the impacts of short-lived substances. They provide both quantitative and qualitative assessments of exposure to a diverse array of molecules (Hook et al., 2014). The extent of change in biomarkers can be directly associated with the severity of adverse effects, as impact biomarkers are intricately linked to the toxicant's underlying mechanism of action (Chambers et al., 2002). Consequently, biomarkers of effect can illuminate qualitative aspects of hazard identification by confirming the presence of danger and shedding light on potential mechanisms of action. The degree

of specificity of these biomarkers to the stressor and their ability to elucidate higher-order effects determine their capacity to offer insights into both the causative factors of the hazard and its ecological repercussions (e.g., imposex in gastropods as a biomarker of organotin exposure, reproductive impairment, and population- level consequences) (Matthiessen, 2000; Hook et al., 2014). In contrast, biomarkers of susceptibility, unlike biomarkers of effect or exposure, signal an acceleration in the progression along the dose-effect continuum. As a result, susceptibility biomarkers can be instrumental in characterizing variability and defining uncertainty factors (Schlenk,1999; Hagger et al., 2006), aiding in our understanding of how different individuals or populations respond to stressors and contributing to risk assessment processes.

Biomarkers, regardless of whether they indicate susceptibility, exposure or effect, can provide valuable insights into toxicant exposure or effects. However, not all types of biomarkers are suitable for monitoring marine pollution (Hook et al., 2014; Hampel et al., 2016). Specific criteria have been established in selecting the most appropriate biomarkers (Hagger et al., 2006; Mayer et al., 2018). It is crucial to comprehend the variability in biomarker responses influenced by various biological and physical factors. Additionally, a biomarker should hold biological significance, meaning it should be connected to critical biological processes and exhibit changes that can be meaningfully interpreted (Mayer et al., 2018). This review endeavors to explore the challenges associated with the utilization of biomarkers in the assessment of marine pollution focusing on species-specific responses, sensitivity and specificity concerns, extraneous factors, species variability, exclusive focus on single stressors, and limited knowledge particularly in the Philippines (Table 1).

Table 1. Challenges associated with the use of biomarkers in marine pollution monitoring

Associated challenges	Biomarker	Stressor/s	Organism/s	Author/s
Species-specific responses	DNA strand breakage and the formation of DNA adducts	B[a]P and PAH	Green-lipped mussels (*Perna viridis*)	Xu et al., 1999; Ching et al., 2001; Siu et al., 2003; Siu et al., 2004a, b
Sensitivity and specificity	Aminolevulinic acid dehydratase (ALAD)	Lead	Sydney Rock Oyster (*Saccostrea glomerata*)	Thompson et al., 2011
	GST/GSH	B[a]P and PAH	Green-lipped mussel (*P.viridis)*	Cheung et al., 2001; Cheung et al., 2002; Cheung et al., 2004
	Ttriiodothyronine, estrogen, and testosterone serum levels	Hypoxia	Carp (*Cyprinus carpio)*	Wu et al., 2003
Extraneous factors	Oxidative stress	Temperature	European seabass (*Dicentrarchuslabrax), Marbled rock crab (Pachygrapsusmarmoratus), Common littoral crab (Carcinusmaenas), Marbled swimming crab (Liocarcinusmarmoreus)*	Vinagre et al., 2012; Mieiro et al., 2011; Madeira et al., 2014
	Oxidative stress	Season	Grey mullet (*Mugil cephalus*), Ccommon littoral crab (C. maenas)	Padmini et al., 2009; Dissanayake et al., 2011
	Oxidative stress	Salinity	Coastal crab species (*Callinectes danae*/and *Callinectes ornatus), Common littoral crab (C. maenas)*	Freire et al., 2011; Rodrigues et al., 2012

	Oxidative stress	Season and latitude	Mangrove oyster (*Cassostrea rhizosphere)*	Zanette et al., 2006
	Transcriptional responses	Chemicals	European ell (*Anguilla anguilla)*	Regoli et al., 2011
Variability between species	AChE and GST	Season and different body regions	Diverse range of aquatic insects, crab, and annelid species	Berra et al., 2004; Olsen et al., 2001; Bhavan and Geraldine, 2001; Timofeyev, 2006; Bhavan and Geraldine, 2001
	AChE	Trichlorfon	*Giant freshwater prawn (Macrobrachiumrosenbergii)*	Chang et al., 2006
Single stressor focus	Enzyme activities	Season	*Blue mussel (Mytilus edulis)*, Baltic clam (*Macomabalthica)*	Leinio and Lehtonen, 2005
	AChE, LDH and GST	Temperature, salinity and handling stress	Estuarine brown shrimp (*Crangoncrangon)*	Menezes et al., 2006
	Enzymatic expression	Salinity and temperature	Calanoid copepod (*Eurytemoraaffinis)*	Cailleaud et al., 2007
Limited knowledge	-	-	-	Lu et al., 2016; Ding et al., 2018; Qiao et al., 2019; Solomando et al., 2021

Species-specific responses

Studies utilizing molecular or biochemical biomarkers typically exhibit lower ecological significance but are often characterized by higher repeatability and reliability. Quantifying a chemical's effect on aspects such as DNA integrity or the activity of a specific enzyme may be straightforward. However, predicting the potential impacts of the chemical on an organism (or population and community) can prove more intricate. Furthermore, organisms possess the ability to withstand environmental stressors and repair damage, which means that exposure to a toxic substance does not invariably lead to adverse biological consequences (Lam, 2009; Sokolova, 2021).

Environmental pollutants can exert damage on an animal's genetic integrity by causing both DNA strand breakage and the formation of DNA adducts. In a recent field investigation, green-lipped mussels (*Perna viridis*) were relocated from a pristine site to several polluted locations, and an assessment of DNA adducts in the gill tissues was conducted using a 32P-post labeling technique. The findings revealed a positive correlation between tissue B[a]P and total PAH concentrations with adduct levels (Xu et al., 1999). Subsequently, green-lipped mussels were subjected to B[a]P concentrations that mimic environmental conditions within a laboratory setting. The results showed that after 3-6 days of exposure, increasing B[a]P concentrations resulted in elevated DNA adduct levels. However, this dose-related increase pattern ceased to be discernible after 12 days (Ching et al., 2001), implying the presence of an efficient repair system.These results raise questions regarding the suitability of utilizing DNA adduct levels to monitor genotoxicants in coastal environments. The type and extent of reactions appear closely tied to both exposure levels and the timing of biomarker assessment, and in such scenarios, observed increases in biomarker responses are likely reflective of exposure. Conversely, a perceived lack of response could stem from either a dearth of inducers (e.g., insufficiently high quantities of genotoxic chemicals) or the effectiveness of a proficient repair system (Ching et al., 2001). When the DNA strands incur damage, whether singly or doubly, improper or incomplete repairs may ensue,

potentially detrimentally impacting the organism's overall health. The degree of DNA strand breakage has been employed as a biomarker to monitor specific environmental pollutants (Dos Santos et al., 2022).

Following one day of exposure, a significant increase in strand breakage was observed in laboratory-treated green-lipped mussels exposed to B[a]P at concentrations of 0.3 and 3mg L^{-1}. For the 0.3mg L^{-1} treatment group, DNA strand break levels peaked on Day 3, whereas the 3mg L^{-1} treatment group peaked on Day 6. These levels remained notably elevated and statistically different from the control values throughout this period. Subsequently, there was a gradual decline in the number of strand breaks, with both treatment groups returning to control levels after 12 days. Intriguingly, mussels exposed to 30mg L^{-1} B[a]P exhibited no increase in DNA strand breaks during the initial 12 days of exposure. However, a substantial rise in strand breaks occurred from Days 12 to 24 (Ching et al., 2001). These findings, in conjunction with the earlier data on DNA adducts, underscore the need for a more comprehensive understanding of the intricate interplay between exposure levels and duration to utilize DNA adduct levels and DNA strand effectively breaks in *P. viridis* as biomarkers for monitoring genotoxicants in the marine environment (Siu et al., 2003).

Siu et al. (2004a) investigated the proportion of micronucleus generation and the relative amounts of DNA strand breaks (measured using an alkaline comet test) in the hemoglobin of mussels that had been exposed to B[a]P for 12 days. The comet assay results showed that the DNA repair system was threshold-dependent and that increased strand break fraction generally occurred with increasing B[a]P concentration (Siu et al., 2004a). Additionally, the prolonged onset and recovery of the DNA damage response in *P. viridis* hemoglobin compared to patterns observed in mussel hepatopancreas suggest a tissue-specific nature of DNA modification in response to B[a]P exposure. Additionally, observing how frequently mussel hemocytes form micronuclei revealed both time- and dose-response connections during exposure (Siu et al., 2004b). Furthermore, the strong correlation between the level of DNA strand

breakage and the level of micronucleus induction showed a possible cause-and-effect relationship between the two damage types. The aforementioned laboratory-based results indicate that DNA strand breaks and the development of micronuclei in mussel hemoglobin may be employed as indicators of genotoxicant exposure (Lam, 2009).

The interplay between exposure levels and durations is required before specific biomarkers can be employed as reliable monitoring tools in the field (Lionetto et al., 2021; Kadim and Risjani, 2022). The vital difference between a biomarker response and a long-lasting biological consequence is highlighted by the fact that some organisms have efficient repair systems. False negatives can become more problematic because many organisms can repair tissue damage and modify biological responses (Lam, 2009).

Sensitivity and specifity

Toxic stress can elicit distinct responses from various biomarkers, each characterized by varying levels of specificity. Certain biomarkers exhibit exceptional specificity, responding exclusively to one or a limited number of substances. For example, lead directly inhibits the enzyme aminolevulinic acid dehydratase (ALAD) (Thompson et al., 2011), and the sensitivity of ALAD is so high that findings from such investigations may obviate the need for chemical analysis of the environment. However, most biomarkers, if not all, demonstrate lower specificity and react to environmental stress in a broader context (Hagger et al., 2006; Lam, 2009). For instance, the mixed function oxygenase (MFO) system, despite its widespread use and apparent success, could present challenges due to its activation in response to a wide range of xenobiotic and natural substances, making it challenging to assess MFO activity in field samples, particularly in locations lacking identifiable point sources (Lam, 2009).

Numerous organisms have been studied to assess the suitability of various antioxidant parameters, including glutathione S transferase (GST), superoxide

dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), NADPH DT-diaphorase (DT-d), glutathione (GSH), and lipid peroxidation (LPO), as biomarkers. In the case of the green-lipped mussel, *Perna viridis* (L.), results indicated that rising tissue concentrations of polycyclic aromatic hydrocarbons (PAHs) predominantly induced alterations in most antioxidant measures, except for gill SOD, DT-d, and LPO (Cheung et al., 2001). Among these measures, the one most closely associated with tissue PAH concentrations was the oxyradical scavenger GSH. In the context of organochlorines, only one of the enzymatic antioxidants, GPx, displayed a substantial response to tissue PCBs (Cheung et al., 2002). This same study also revealed a correlation between GST/GSH and chlorinated compounds. However, the levels of chlorinated pesticides in mussel tissues were linked to oxidative stress, as determined by thiobarbituric acid reactive compounds, while the tissue concentrations of chlorinated hydrocarbons and other enzymatic antioxidants examined did not exhibit significant correlations (Cheung et al., 2002).

In a subsequent study, Cheung et al. (2004) exposed *P. viridis* to two different concentrations of benzo[a]pyrene (B[a]P) (0.3mg L^{-1} and 3mg L^{-1}) and two concentrations of Aroclor 1254 (0.5mg L^{-1} and 5mg L^{-1}). Additionally, the pollutants were used in combinations, specifically, 0.3mg L^{-1} B[a]P with 0.5mg L^{-1}Aroclor 1254 and 3mg L^{-1} B[a]P with 5mg L^{-1}Aroclor 1254. Over 18 days, the researchers monitored the GST, SOD, CAT, GPx, GR, GSH, and LPO levels in both the mussel gill and hepatopancreas. Notably, Aroclor 1254 concentration was positively associated with CAT and GSH levels in gill tissue. Furthermore, the body burden of Aroclor 1254 exhibited a significant correlation with hepatic GST and SOD activity. Positive correlations were observed between B[a]P concentration and the levels of LPO, GR, and GPx in the gill and hepatopancreas, as well as hepatic GST. In conjunction with previous studies, these findings suggest that certain antioxidative biomarkers hold the potential as early warning indicators for these responses (Lam, 2009).

Moreover, food availability, water temperature fluctuations, and reproductive activity can interfere with biomarker responses (Niyogi et al., 2001). This complexity arises from the fact that a specific biomarker response may be induced by factors unrelated to pollutant exposure, rendering the interpretation of results more challenging (Sheehan et al., 1999). As Wu et al. (2003) demonstrated, in the case of carp (*Cyprinus carpio*) subjected to prolonged hypoxia, notable reductions in triiodothyronine, estrogen, and testosterone serum levels were observed. These hormonal alterations were linked to diminished spawning success, sperm motility, fertilization success, hatching rate, larval survival, and delayed gonadal development in both male and female carp. It is worth noting that these effects could also be induced by naturally occurring endocrine-disrupting substances. Consequently, even when a specific agent can unequivocally elicit a particular biomarker response under precisely controlled laboratory conditions, observing the same response in organisms exposed to that agent in a field setting does not definitively confirm its presence. This is because other chemicals or factors capable of provoking the observed response(s) may also be at play, leading to an increased risk of false positives. This significant constraint imposes limitations on the efficacy of various biomarkers as indicators for specific chemicals (Lam, 2009).

Extraneous factors

According to Vinagre et al. (2012), temperature significantly impacts the biomarkers for oxidative stress in the muscle of European seabass (*Dicentrarchus labrax*) over an extended time at various temperatures. Mieiro et al. (2011) found the same result after examining the brain of *D. labrax*. Padmini et al. (2009) discovered that the season significantly influenced the oxidative stress indicators in the liver of the grey mullet (*Mugil cephalus*). Similar findings were made by experiments using crabs and other tissues such as the season-affected oxidative stress biomarkers in the hemolymph of common littoral crab, *Carcinus maenas* (Dissanayake et al., 2011). In numerous additional studies, salinity significantly impacted how crab tissues responded

to oxidative stress. Salinity affected oxidative stress biomarkers in the hemolymph, hepatopancreas, muscle, and gills of two coastal crab species, *Callinectes danae* and *Callinectes ornatus*, according to Freire et al. (2011). A similar finding was reached by Rodrigues et al. (2012) after examining the muscle and digestive glands of *C. maenas*. Madeira et al. (2014) also evaluated the concentrations of oxidative stress indicators in the hemolymph of the crabs, *Pachygrapsus marmoratus*, *C. maenas*, and *Liocarcinus marmoreus* at rising temperatures. In addition, Zanette et al. (2006) reported that the season and latitude significantly influenced the levels of oxidative stress indicators in the gills of a bivalve, the mangrove oyster, *Cassostrea rhizosphere*. The primary factor influencing this seasonal and latitudinal effect was temperature.

These studies demonstrate that different tissues are impacted by temperature, which is an essential variable in the oxidative stress response of fish, crabs, and bivalves. Therefore, it is essential to consider temperature when monitoring the surroundings carefully (Schmidt et al., 2013). It can be inferred that when using oxidative stress biomarkers in research in the wild, seasonal and latitudinal effects are to be anticipated. Another significant result of this investigation is the unresponsive gills of rock goby (*Gobius paganellus*). Despite having high constitutive levels of each biomarker examined, organelles exhibit no response to rising temperatures for lipid peroxidation or SOD, two of the most often examined biomarkers in environmental evaluation. Since temperature will not be a confounding factor in their response to oxidative stress, tissues that are not temperature-responsive may be particularly relevant as pollution indicators. Regoli et al. (2011) discovered that European ell (*Anguilla anguilla*) gills exposed to chemical pollution showed susceptible transcriptional responses for phase I and phase II genes, compared to the liver. However, they revealed little catalytic changes and were not particularly reactive in terms of oxidative metabolism, suggesting their utility to reveal exposure to chemical pollution more than its effects.

The temperature niches of each species are significant in the oxidative stress response, according to physiological investigations at the subcellular level of coastal animals exposed to the CTMax experience (Madeira et al., 2013, 2014). Rock goby (*G. paganellus*) lives in the intertidal, a region with erratic temperatures. Since the gills are one of the tissues in closer contact with the environment, having high levels of oxidative stress biomarkers in a constitutive state indicates both ongoing damages, as demonstrated by lipid peroxidation, as well as ongoing antioxidant mechanisms in operation, given that the enzymes under investigation are involved in the antioxidant response. This could be a tactic for coping with the highly fluctuating heat conditions. The scientists concluded that the activity of oxidative stress indicators rises with temperature until it reaches a peak, after which it falls off, and that this peak temperature is based on the species and the thermal niche it inhabits (Schmidt et al., 2013).

According to the research conducted by Madeira et al. (2014), some intertidal organisms, including crabs, maintain many constitutive heat shock proteins that can be employed whenever necessary for cellular repair. Dong et al. (2008) discovered similar behavior in intertidal limpets and labeled it a "preparative defense" tactic. It is speculated that oxidative stress defense mechanisms are similar in susceptible tissues, such as fish gills. In the future, research should aim to elucidate the response of different tissues and organisms to the seasonal and spatial variations in oxidative stress caused by environmental factors. This should be considered while choosing the species and target tissues to evaluate the environmental quality. Investigating variations in reactions across age ranges, stages of development, and gender is also essential (Vinagre et al., 2014).

Additionally, all biomarkers showed seasonal fluctuations. After the ring spawning event, it was observed that the glutathione S-transferase activity exceeded that observed during other seasons. Unlike the earlier seasonal pattern, Power and Sheehan (1996) reported no seasonal variations in GST protein activity within the

digestive gland. Their study involved collecting mussels from a population in Cork Harbour, in southern Ireland. Numerous hydrological and environmental factors influence a distance exceeding 200 miles from Lettermullan to Cork Harbour. Different populations of Adriatic mussels exhibit spatial variations in biomarker responses, as observed by Bocchetti and Regoli (2006). Bebianno et al. (2007) hypothesized that variations in GST activity in mussels between the South Portuguese coast and the French Mediterranean coast could be attributed to changes in seawater temperature. Their study indicated a correlation between decreasing salt levels and declining GST activity. Given that Cork Harbour's salinity is lower than the study site's, seasonal fluctuations in the digestive gland of Cork Harbour mussels may not have been present. Additionally, a significant positive relationship between GST activity and the mussel gonad development stage was reported in another study, suggesting the role of the reproductive stage as an endogenous factor (Schmidt et al., 2013).

Variability between species

Enzyme-specific functions differ between taxonomic groups. Berra et al. (2004) collected a diverse range of aquatic insects, a crab, and an annelid species from the Taro and Tocino Rivers in northern Italy. They discovered considerable variations in AChE and GST-specific activity between taxa. They noted seasonal fluctuations in AChE activity were evident in most taxa, occurring three to five times per season, whereas GST exhibited less frequent fluctuations. Freshwater clams of *Corbicula fluminea* were collected from Sanguinet Lake and Dronne River in southwest France. Vidal et al. (2002) noted that GST exhibited fewer seasonal fluctuations compared to other biomarkers such as catalase, propionyl cholinesterase, nicotinamide adenine dinucleotide (NADH)-cytochrome c reductase, and lipids. On the other hand, Olsen et al. (2001) investigated the natural variability of AChE and GST in fourth-instar larvae of the midge, *Chironomus riparius*, across 13 uncontaminated sites. They observed nearly a twofold difference in the activity of both biomarkers across many loci in

southeast England, supporting the idea that biomarker levels vary across different spatial scales.

Invertebrates often exhibit diverse response biomarkers influenced by age, reproductive status, tissue type, and various physiological conditions. Additionally, natural seasonal and spatial variations further contribute to the complexity of these responses. Nevertheless, predicting how these physiological conditions may differ across different species poses a formidable challenge. For instance, an organism's size, which is closely linked to its age, may or may not exert an influence on the levels of certain biomarkers, depending on the specific species under investigation (Guilhermino et al., 1996; Robillard et al., 2003; Printes and Callaghan, 2003; Timofeyev, 2006). Similar to enzyme activity, distinct patterns based on the body region under examination are not readily discernible among different species. Notably, in the case of amphipods like *Pallasea cancellous*, *Eulimnogammarus verrucose*, and *Gammarus lacustris*, consistent GST activity has been reported across body sections, including the head, thorax, abdomen, urosome, pereopods, antennae) (Timofeyev, 2006).

In contrast, in *Macrobrachium malcolmsonii*, notable disparities in GST activity were observed between the hepatopancreas and gills (Bhavan and Geraldine, 2001). Notably, investigations have revealed that choosing tissue for examination can significantly impact cholinesterase activity. This enzyme activity was more pronounced in *M. malcolmsonii*, particularly in the mantle, than in other bodily tissues (Mora et al., 1999). Similarly, distinct variations in cholinesterase activity were identified in various tissues, including the brain, hepatopancreas, gills, and muscles (Bhavan and Geraldine, 2001). Lastly, Chang et al. (2006) demonstrated that exposure to Trichlorfon in *Macrobrachium rosenbergii* resulted in reduced AChE in the hepatopancreas and hemolymph, while no such effect was observed in muscle tissue. These findings underscore the importance of conducting preliminary research on the specific test species before employing them in pesticide risk assessment procedures (Domingues et al., 2010).

Furthermore, cadmium contamination in North Bais Bay, Negros Oriental, Philippines was investigated (Ho and Bantoto-Kinamot,2021) using three brown algae species: *Sargassum* sp., *Padina* sp., and *Turbinaria* sp. Results show significant variability in cadmium concentration between species, with *Sargassum* sp. exhibiting the highest levels (2.14 to 4.45 mg kg^{-1}), followed by *Padina* sp. (2.2 to 3.4 mg kg^{-1}) and *Turbinaria* sp. (2.36 to 2.76 mg kg^{-1}). This variability underscores the importance of considering specific responses of different species to heavy metal pollution when using algae as bioindicators or for phytoremediation purposes.

Single stressor focus

Marine organisms are significantly affected by a multitude of environmental conditions, including physicochemical changes, exposure to environmental toxins, natural environmental changes, and following of daily or seasonal environmental variations. However, many prior investigations (Leinio and Lehtonen, 2005; Menezes et al., 2006; Cailleaud et al., 2007) have focused on the effects of isolated environmental components, such as temperature, salinity and pollution, on specific biochemical markers. In particular, Marteja and Modina (2021) assessed the effects of fenobucarb pesticide exposure on hepatic and splenic melanomacrophage centers in Nile tilapia. Five groups of fish were exposed to fenobucarb for varying periods. Results show significant changes only in splenic melanomacrophage centers, with increased numbers observed with longer exposure. Additionally, size and cover increased significantly after 28 days. Hemosiderin and lipofuscin pigment changes at 28 days suggest tissue damage. The study confirms the potential of melanomacrophage centers, particularly in the spleen, as sensitive biomarkers for fenobucarb exposure.

In reality, aquatic organisms often contend with the complex interplay of various environmental stressors. For instance, in the Mekong River Delta (MRD) of Vietnam, water temperatures in shrimp ponds fluctuate between 23.9 to 34°C, exhibiting variations throughout the day and the changing seasons, influenced by factors like air temperature, water depth, pond design, and water management (Phuong,

2005). Similarly, MRD salinity levels surge during the dry season, ranging from 21.5 to 25.0ppt, but drop to 15.0ppt during periods of substantial rainfall (Phuong, 2005). The adaptability of aquatic organisms may be compromised when subjected to suboptimal temperatures or inappropriate salinity levels, and these conditions can interact with toxins in antagonistic, additive, or synergistic ways. Consequently, contemporary toxicological research underscores the necessity of exploring the intricate interactions between multiple environmental components (Tu et al., 2012).

Limited knowledge

Low-level biomarkers, characterized by their higher repeatability and predictability, serve as valuable indicators of contaminant presence, offering early warnings. However, their capacity to forecast significant biological impacts remains constrained. As a result, scientists are divided in their approach, opting to employ biomarkers spanning molecular, biochemical, subcellular, and physiological levels (Lomartire et al., 2021). Conversely, biomarkers operating at elevated levels tend to hold greater ecological significance, albeit with slower responsiveness and increased detection challenges (Lam, 2009).

Many biomarkers in current use exhibit a lower degree of specificity, typically responding to various forms of environmental stress. The precise understanding of the dose-response relationship between a biomarker and the specific pollutant under investigation is crucial for selecting the appropriate biomarker (McCarty et al., 2002). Given that it is not feasible to deduce cause-and-effect relationships at the population, community, or ecosystem levels based solely on primary toxicology test data, validation and site-specific confirmation are imperative (Lomartire et al., 2021). It is important to note that not all biomarkers are universally applicable, as they may serve distinct purposes or enable the identification of specific stressors. Furthermore, more ecotoxicological techniques have been developed to assess the impact of priority pollutants such as metals, PAHs, and PCBs compared to emerging contaminants (Martinez-Haro et al., 2015). The availability of ecotoxicological tools varies

depending on the stressors under investigation. To prevent potential confounding of tool responses with environmental factors such as food availability, water temperature (Niyogi et al., 2001), and reproductive activity (Canesi et al., 1991), additional research is essential for the development and validation of ecotoxicological tools intended for use as early-warning systems of emerging pollutants.

The utilization of biological markers is further limited by bioremediation and species adaptation. Certain species can repair the damage inflicted by chemical agents, potentially leading to an underestimation of contamination levels or yielding false negative results (Lam, 2009; Wu et al., 2005). An illustrative example is *P. viridis*, where the concentration of B[a]P in hepatic DNA increased to a peak following exposure but returned to control levels after approximately three weeks of continuous chemical exposure (Ching et al., 2001). Similarly, the estuary fish *Fundulus heteroclitus* has demonstrated an ability to adapt to polychlorinated biphenyls (PCBs) (Nacci et al., 2010). This case demonstrates that pollution alone does not guarantee that an organism will effectively function as an environmental stressor detector (Lomartire et al., 2021).

Furthermore, only a few studies have investigated the effects of marine pollution on plasma and erythrocytes after prolonged exposure in laboratory settings (Lu et al., 2016; Ding et al., 2018; Qiao et al., 2019; Solomando et al., 2021). However, the predominant focus has been on examining the effects of microplastics (MPs) on the liver and gut. A practical approach for assessing the total oxidant and antioxidant defenses in the liver, plasma, and erythrocytes is assessing the balance between these defenses (Collard et al., 2017; Mattsson et al., 2017). Blood samples also make it possible to monitor research with minimally invasive sampling and without animal sacrifice; thus, exploring their potential as biomarkers is crucial. Many studies have been developed due to the worries regarding the severe effects of MP pollution on aquatic life. Critical knowledge gaps are the toxicological potential of long-term exposure to these pollutants and whether these effects are reversible following a

recovery period without exposure to MPs. Employing a juvenile *Clarias gariepinus* model, the longest known exposure duration to MPs lasted for 45 days, followed by a 30-day of depuration period (Iheanacho and Odo, 2020; Solomando et al., 2021).

In the Philippines, Bondoc et al. (2021) highlighted the scarcity of data regarding polychlorinated biphenyls (PCBs) contamination in cetaceans stranded along Philippine coasts. PCBs are persistent organic pollutants known to accumulate in the marine food chain, posing risks to cetaceans and potentially to human health. The analysis of PCBs in cetacean tissues is challenging due to lipid interferences, necessitating the development of a modified analytical method. This method, involving three clean up steps, demonstrated robustness in recovery and repeatability. Results revealed the presence of 38 PCB congeners in cetacean blubber samples, indicating widespread contamination. This observation underscores the significance of monitoring PCB levels in marine environments to safeguard cetacean populations and human health.

Magbanua et al. (2023) underscore the importance of assessing the condition of Philippine rivers, which face threats from changing land uses and deteriorating water quality. Although not common, river biomonitoring using benthic macroinvertebrates is recognized to be essential. In their study, multiple studies examining water quality across Mindanao, Visayas, and Luzon identified sensitive macroinvertebrate families indicative of various water quality classes. However, there remains to be a gap in assessing rivers intended for primary contact recreation. Consequently, the authors proposed developing a standardized macroinvertebrate-based biotic index to provide a comprehensive tool for effective ecosystem health assessment and management.

CONCLUSION

The utilization of biomarkers for monitoring marine pollution presents a critical need for enhancement. This imperative arises from the inherent complexities associated

with biomarker responses to pollutants, the intricate dynamics of natural fluctuations within marine ecosystems, the interference of multiple pollutants and confounding variables, the distinctive species-specific variations, gaps in our existing knowledge base, and the prevailing narrow focus on individual stressors. Addressing these multifaceted challenges necessitates a concerted effort involving sustained research endeavors, interdisciplinary collaboration, and formulating universally accepted standard protocols. By collectively committing to these initiatives, we can strive to significantly enhance the precision and efficacy of biomarker-based monitoring in the dynamic and sensitive context of marine environments.

Furthermore, it is essential to recognize that the successful evolution of biomarker-based monitoring in marine ecosystems will not only contribute to our ability to assess pollution impacts more accurately but also foster a deeper understanding of the intricate interplay between environmental stressors and biological responses. Therefore, prioritizing biomarker research is recommended to enhance the monitoring of marine pollution. This entails allocating resources toward transdisciplinary projects, establishing comprehensive observation programs, and leveraging advanced omics technology. Additionally, fostering collaboration, validating biomarkers, and conducting species-specific investigations are crucial. Lastly, raising public awareness, advocating for policy backing, and implementing capacity-building initiatives are essential steps. Refining biomarker applications can ultimately aid in more effective mitigation efforts and preserve our invaluable marine resources for future generations by shedding light on the complex web of interactions within these ecosystems.

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