Pollution Biomarkers for Marine Biodiversity

Subjects: Biodiversity Conservation

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Marine biodiversity is threatened by several anthropogenic pressures. Pollution deriving from the discharge of chemical contaminants in the sea represents one of the main threats to the marine environment, influencing the health of organisms, their ability to recover their homeostatic status, and in turn endangering biodiversity. Molecular and cellular responses to chemical pollutants, known as biomarkers, are effect-based methodologies useful for detecting exposure and for assessing the effects of pollutants on biota in environmental monitoring. Pollution biomarkers can be useful tools for monitoring and assessment of pollution threats to marine biodiversity, both in the environmental quality monitoring of protected areas and the assessment of the health status of species at risk.

Keywords: biomarkers ; marine protected area ; endangered species ; biodiversity ; biomonitoring ; pollution

1. Introduction

Chemical pollution derived from the discharge of chemical contaminants in the sea, from both point and non-point pollution sources, represents one of the main threats to the marine environments and their resources and services and remains a great environmental challenge [1][2]. Shipping is a source of pollutants through accidental spillages, operational discharges, and antifouling paint leaching; mariculture accounts for medicinal product, biocide, and food additive release; offshore activities produce drill cuttings and hydrocarbon release; dredging of sediment and dumping at sea contribute to water column contaminant level increase [3]. Moreover, the release of chemical contaminants into the sea from land-based activities, such as urban wastewater discharge, industrial and agricultural activities, mining, and runoff from coastal areas, contributes dramatically to the contamination of the seas. Several of them are contaminants of emerging concern (CECs), which include a wide array of anthropogenic chemicals that have no regulatory standards yet [4][5].

Chemicals absorbed by the organisms through the gills, the gastrointestinal tract, and the tegument can interact with biological macromolecules, producing several toxicological effects at the cellular and molecular levels. This includes enzyme inhibition, alterations of transport properties, alteration of the functioning of membrane and intracellular receptors, alterations of intracellular signaling pathways, oxidative stress, and DNA damage [6][7][8][9][10]. These primary effects at the molecular and cellular levels can produce integrated toxicity effects over time, including impairment of organ and systems functioning such as neurotoxic effects, immunological responses, hepatotoxicity, behavioral changes, reproductive and developmental alterations, endocrine disruption, and genotoxicity [2]. For example, some persistent marine pollutants can exacerbate the adverse effects of certain pesticides as well as other persistent organic pollutants (POPs) in marine organisms [11].

Healthy oceans are among the main objectives of the EU by 2030. To reach these goals, water quality monitoring and assessment assume a fundamental role. The study of the molecular, cellular, and physiological alterations in the organism in relation to the exposure to chemical pollutants has contributed to developing several markers (biomarkers) of exposure and toxicological responses to chemical pollutants [12][13]. The application of the biomarker approach in marine environment monitoring and assessment, integrated into the physicochemical analysis of the environmental matrices, has greatly increased in recent years. This is mainly due to the fact that the assessment of the entity of the organism exposure to pollutants in a certain environment and the extent of the suffered toxicological effects is of fundamental importance for decision making related to habitat and species protection, ecosystem services provision, adoption of remediation procedures, or impacted area monitoring [14].

Recently, the application of the biomarker approach in biomonitoring is considered with great interest in the field of biodiversity conservations. Considering that chemical pollution is recognized as one of the major pressures driving biodiversity reduction loss worldwide [15], the study of the responses of the organisms to the anthropogenic alterations of the environment that may cause or contribute to population decline can support biodiversity conservation strategies. Biomarkers have been recently applied to several research areas of the biodiversity conservation field, including environmental quality monitoring of protected areas and the assessment of the health status of species at risk.

2. Pollution Biomarkers in Biomonitoring of Marine Protected Areas

Marine protected areas (MPAs) represent important tools in marine biodiversity conservation. They are increasingly being instituted worldwide to reduce the decline of biodiversity and conserve ecosystem function [16][17]. MPAs perform three key functions in modern conservation: conservation of marine biodiversity, preservation of productivity, and contribution to economic and social welfare. They involve the protective management of natural areas of seas, oceans, and estuaries according to specific protection objectives, such as habitats, biodiversity, and ecological processes conservation, species protection, and resources preservation.

Pollution has been recognized as one of the main menaces to MPAs [18][19]. As recently reviewed by Abessa et al. [20], a great number of MPAs show some signs of chemical pollution. Several MPAs are located near sources of pollution, such as industrial activities, harbors, agricultural farms, urban areas, and sewage outfalls. Contaminants may be introduced from adjacent areas [21], and marine currents can transport pollutants over long distances from pollution sources. Pollution should be considered a critical aspect in the creation and management of an MPA, which should require the identification and the assessment of the extent of the pollution pressure on the native species and ecosystems [22]. Pollution conditions are unknown in most MPAs worldwide, and even when some information is available, it is often inadequate to assess the threats to biodiversity or to address further actions [20]. In this context, there is an urgent need for adequate diagnostic tools useful for monitoring and assessment of the health status of the organisms in MPAs. The biomarker approach can meet this need since it allows to detect exposure and biological effects that are occurring in the organisms due to the presence of chemical substances in the environmental matrices.

The review of the literature produced in the past 20 years on the biomarker approach application in MPA biomonitoring all over the world is summarized in **Table 1**. The criterion for the inclusion of a paper in this review was represented by the fact that the work concerned the study of at least an MPA and included the experimental collection of specimens and the analysis of molecular and cellular biomarkers. The research was carried out on Scopus and Web of Science employing “marine protected areas*”, or “marine reserve*” or “marine sanctuary*” or “marine park*” and “biomarker*” as search terms. A total of 22 studies were included in the analysis.

Table 1. Literature produced in the past 20 years on the application of pollution biomarkers in marine protected area biomonitoring all over the world. The criterion for the inclusion of a paper was represented by the fact that the work concerned the study of at least a marine protected area and included the experimental collection of specimens and the analysis of molecular and cellular biomarkers. The research was carried out on Scopus and Web of Science employing “marine protected areas*”, or “marine reserve*” or “marine sanctuary*” or “marine park*” and “biomarker*” as search terms.

Protected Areas	Bioindicator Species	Bioindicator Class	Endpoint	Biomarkers Analyzed	Ref.
Europe					
Egadi Islands Marine Protected Area (Italy)	***Coris julis, Patella caerulea, Paracentrotus lividus***	**Osteichthyes Gastropoda Echinoidea**	**Detoxification of organic pollutants**	**EthoxyresorufinO-deethylase, BaPMO, NADH ferry red, and NADH cyt c**	[23]
Tremiti Islands Marine Protected Area (Italy)	***Paracentrotus lividus***	**Echinoidea**	**Coelomocytes alterations**	**Coelomocytes subpopulations ratio, heat-shock protein 70**	[24]
National Park of La Maddalena Arcipelago (Italy)	***Mytilus galloprovincialis***	**Bivalvia**	**Lysosomal alterations**	**Lysosomal membrane stability, lipofuscin content, neutral lipid contents, lysosomal structural changes**	[25]
Capo Peloro Natural Reserve (Italy)	***Atherina boyeri***	**Osteichthyes**	**Detoxification of organic pollutants, neurotoxicity, genotoxicity**	**Acetylcholinesterase, benzo(a)pyrene-monooxygenase, polycyclic aromatic hydrocarbons metabolites in bile, erythrocytic nuclear abnormalities assay**	[26]

Protected Areas	Bioindicator Species	Bioindicator Class	Endpoint	Biomarkers Analyzed	Ref.
The Pelagos Sanctuary (International Sanctuary for the Protection of Mediterranean Marine Mammals) (Italy, France)	*Meganyctiphanes norvegica*	Malacostraca	Detoxification of organic pollutants, neurotoxicity, response to xenoestrogens	Cytochrome P450, BaPMO activity, NADPH cytochromec reductase, NADH-ferricyanide reductase, esterases, porphyrins, vitellogenin, zona radiata proteins, acetylcholinesterase	[27]
	Stenella coeruleoalba	Mammalia	Detoxification of organic pollutants, oxidative stress	Cytochrome P4501A, cytochrome P4502B, catalase	[28]
	Balaenoptera physalus	Mammalia	Detoxification of organic chemical pollutants, oxidative stress	Cytochrome P4501A, cytochrome P4502B, lipoperoxidation	[29]
	Balaenoptera physalus, Physeter macrocephalus	Mammalia	Metal excretion	Metals in the fecal material	[30]
North America					
Florida Keys National Marine Sanctuary (U.S.A.)	*Montastraea annularis*	Anthozoa	Oxidative stress, stress protein multidrug resistance induction	Superoxide dismutase, glutathione peroxidase, glutathione-s-transferase, heat-shock proteins, metabolic condition, multixenobiotic resistance proteins	[31]
Veracruz Coral Reef System National Park (Mexico)	*Haemulon Aurolineatum, Ocyurus chrysurus*	Osteichthyes	Detoxification of organic chemical pollutants, response to xenoestrogens	Cytochrome P4501A, vitellogenin, glutathione-S-transferase, PAH metabolites in fish bile	[32]
Natural protected area of Laguna Madre in the Gulf of Mexico (Mexico)	*Chione elevata*	Bivalvia	Neurotoxic effects, oxidative stress, metabolic alterations	Acetylcholinesterase, butyrylcholinesterase, carboxylesterase, alkaline phosphatase, glutathione s-transferase, oxygen radical absorbance capacity	[33]
South America					
Morrocoy National Park (Venezuela)	*Siderastrea sidereal*	Anthozoa	Detoxification of organic chemical pollutants, oxidative stress	Cytochrome P450 I, cytochrome P450 II, NADPH reductase, glutathione *S*-transferase, catalase, superoxide dismutase	[34]
Parque Nacional Archipielago Los Roques (Venezuela)	*Siderastrea sidereal*	Anthozoa	Detoxification of organic chemical pollutants, oxidative stress	Cytochrome P450 I, cytochrome P450 II, NADPH reductase, glutathione *S*-transferase, catalase, superoxide dismutase	[34]
Fernando de Noronha Archipelago protected area (Brazil)	*Amphistegina lessonii*	Foraminifera	Oxidative stress, metal detoxification	Antioxidant capacity against peroxyl radicals, lipid peroxidation, protein carbonylation, metallothionein-like proteins	[35]
Paranaguá Bay protected areas (Brazil)	*Atherinella brasiliensis*	Osteichthyes	Neurotoxicity, detoxification of organic chemical pollutants, oxidative stress	Cholinesterase, ethoxyresorufinO-deethylase, glutathione S-transferase, catalase	[36]

Protected Areas	Bioindicator Species	Bioindicator Class	Endpoint	Biomarkers Analyzed	Ref.
Cananéia–Iguape–Peruíbe Environmental Protected Area (Brazil)	*Cathorops spixii*	Osteichthyes	Detoxification of organic pollutants, oxidative stress, genotoxicity, metal detoxification	Glutathione S-transferase, glutathione peroxidase, GSH levels, lipid peroxidation, DNA strand breaks, metallothonein	[37]
	Cathorops spixii	Osteichthyes	Genotoxicity	Comet assay, micronucleus test (MN), and nuclear abnormalities test (NA) in peripheral blood	[38]
Natural Protected Area San Antonio Bay (Argentina)	*Neohelice granulata*	Malacostraca	Detoxification of organic pollutants, oxidative stress, metal detoxification	Catalase, lipid radical content, lipid peroxidation, α-tocopherol, catalase, glutathione-S-transferases, metallothioneins	[39]
Cananéia–Iguape–Peruíbe Protected Area (Brazil)	*Callinectes danae*	Malacostraca	Genotoxicity, detoxification of organic pollutants, oxidative stress, metal detoxification, neurotoxicity	Glutathione S-transferase, glutathione peroxidase, intracellular glutathione, acetylcholinesterase, lipid peroxidation, metallothionein, DNA strand breaks	[40]
Estuarine Lagoon Complex of Iguape–Cananéia (Brazil)	*Gobioides broussonnetii*	Osteichthyes	Oxidative stress, genotoxicity, metal detoxification, histopathological alterations	Superoxide dismutase, catalase, glutathione peroxidase activity, glutathione S-transferase, glutathione, metallothionein, lipoperoxidation, micronuclei, histological alterations	[41]
Australia					
Great Barrier Reef (Australia)	*Plectropomus leopardus*	Osteichthyes	Detoxification of organic chemical pollutants, neurotoxicity	EROD, cholinesterase	[42]
	Acropora millepora	Anthozoa	Oxidative stress	Genetic loci involved in environmental stress tolerance and antioxidant capacity	[43]

The scientific interest in the use of pollution biomarkers in MPA biomonitoring has grown in the past decade, as indicated by the recent increment in the number of publications produced in this field: two papers were found in the period 2001–2005, three papers in the period 2006–2010, five papers in the period 2011–2015, and nine papers in the period 2016–2020.

As shown in **Figure 1**, the most investigated responses are represented by biomarkers of organic chemical pollutant detoxification (including cytochrome P540 family) and antioxidant and oxidative-stress-related biomarkers (such as antioxidant enzyme activity, lipid peroxidation, antioxidants depletion), followed by neurotoxicity (inhibition of the activity of acetylcholinesterase), genotoxicity (DNA damage assessed by comet assay, micronuclei, and erythrocyte nuclear abnormalities), metal detoxification biomarkers (induction of metallothionein), cytological/histological alterations, xenoestrogen exposure biomarker (vitellogenin), lysosomal alterations, heat-shock proteins, multidrug resistance induction, porphyrins, and biomarkers of pollutant excretion.

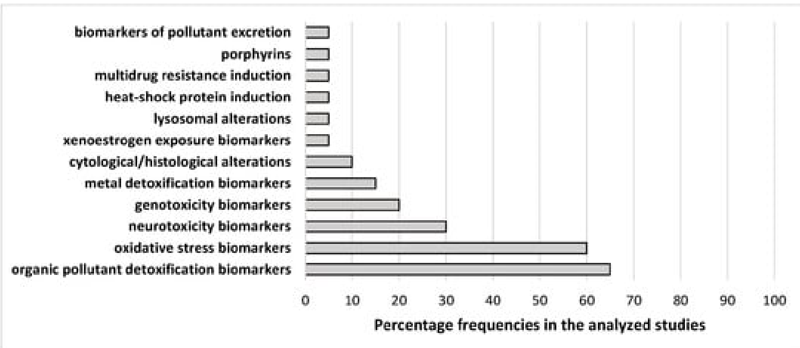

Figure 1. Percentage frequencies of different biomarkers in the studies on MPA pollution biomonitoring selected for this review, as reported in **Table 1**.

As observed in **Table 1**, fish are widely utilized as bioindicator species for biomarker analysis in MPAs, thanks to their high sensitivity to chemical pollutant exposure which triggers their antioxidant and biotransformation systems [44][45][46]. They were used for MPA biomonitoring in 40% of the analyzed studies. Other bioindicator organisms investigated for biomarker responses in MPAs are represented by bivalve mollusks and crabs. Bivalve mollusks are extensively used as bioindicator organisms thanks to their wide distribution, sessile and filter-feeding nature, and tolerance to a wide range of pollutants. Their biological responses to pollutant exposure are extensively used as biomarkers in coastal and marine environmental monitoring and assessment [47][48][44][49][50]. Crabs are important components of coastal ecosystems and represent good bioindicator organisms for the biomarker approach application since they can accumulate contaminants absorbed from water and food in their tissues developing detectable molecular and cellular responses to pollution exposure and effects [51][52]. Corals have been employed for biomarker application in water quality assessment in coral reef environments [31][34][43]. In one study foraminifera were also successfully utilized for reef water quality assessment [35].

In the case of the only pelagic MPA in the Mediterranean Sea, the Pelagos Sanctuary (International Sanctuary for the Protection of Mediterranean Marine Mammals, Corsican-Ligurian Provencal Basin, Northern-Western Mediterranean Sea), biomarkers have been successfully applied to marine mammals, utilizing a nondestructive approach on animal biopsies and fecal material analysis [28][29][30]. In addition, Fossi et al. [27] explored the possibility to measure biomarker responses in zooplanktonic euphausiids for the assessment of the environmental quality of the Pelagos Sanctuary.

All the studies analyzed underline the usefulness of the biomarker approach responsiveness to environmental pollutants exposure as a tool for monitoring the environmental quality of MPAs, detecting eventual threats from anthropogenic pressures, and assessing the effectiveness of the adopted measures in MPAs to preserve the quality of the marine senvironment.

For example, in the Estuarine Lagoon Complex of Iguape–Cananéia (Brazil), the authors complemented the chemical analysis of sediments (metals, polycyclic aromatic hydrocarbons, pharmaceuticals, and personal care products were determined) with the analysis of alterations in antioxidant, biotransformation, histopathological, and genotoxic biomarkers in the fish *Gobioides broussonnetii* allowing to detect the contribution of anthropogenic activities to contaminant inputs and compromising of the conditions of fish in the area [41].

In the Cananéia–Iguape–Peruíbe Environmental Protected Area (Brazil), the use of a multimarker approach (including glutathione S-transferase, glutathione peroxidase, GSH levels, lipid peroxidation, metallothionein, and genotoxicity biomarkers, such as DNA strand breaks, comet assay, micronucleus test, and nuclear abnormalities) in the fish *Cathorops spixii,* paralleled by the analysis of metal body burden and polycyclic aromatic hydrocarbons in bile, allowed for the identification of both seasonal and spatial variations in pollution sources [37][38].

Moreover, Caliani et al. [26], who studied biomarkers in the key fish species *Atherina boyeri* of Capo Peloro lakes in Sicily (Italy), confirmed that a biomarker-based approach (including acetylcholinesterase, benzo(a)pyrene-monooxygenase, polycyclic aromatic hydrocarbons metabolites in bile, and erythrocytic nuclear abnormalities) can be useful for monitoring seasonal and spatial variations in pollution sources impacting MPAs according to variations in anthropogenic activities in the surrounding areas [26].

In the Paranaguá Bay (Brazil), the histopathological analysis of tissues of the fish *Atherinella brasiliensis* revealed a significant presence of severe pathological conditions in the liver and gills of the animals paralleled by biochemical alterations and DNA damage assessing the pollution threat to aquatic organisms coming from anthropogenic activities (urban, industrial, agricultural, and harbor activities) of the surrounding areas [36].

In the MPA of La Maddalena Archipelago (Italy), the biomarker approach using lysosomal biomarkers (lysosomal membrane stability, lipofuscin content, neutral lipid contents, lysosomal structural changes) in transplanted *Mytilus galloprovincialis* was coupled to the measure of trace metals in mussel tissues, the analysis of the presence of endocrine disruptors in the water column, and the in vitro cellular toxicity of POCIS (Polar Organic Chemical Integrative Sampler) extracts on mussel hemocytes measured by lysosomal membrane stability assay [25]. This integrated approach allowed assessment of the effects of anthropic stressors in the MPA and to evaluate the effectiveness of the adopted measures to preserve the quality of the marine environment.

In the Tamaulipas Laguna Madre, the Gulf of Mexico, the use of a wide-ranging battery of biochemical biomarkers (acetylcholinesterase, butyrylcholinesterase, carboxylesterase, alkaline phosphatase, glutathione s-transferase, oxygen radical absorbance capacity) analyzed on the clam *Chione elevata* as a sentinel organism was integrated into a stress index, the Integrated Biomarker Response (IBR), and complemented by the chemical analysis of metals, organochlorine pesticides, and hydrocarbons in the sediments [33]. The authors underlined the potential of the approach to be a useful tool for monitoring the health status of the sentinel organisms and, in turn, the environmental quality of the MPA.

In the Pelagos Sanctuary (Italy-French), Fossi et al. [28] for the first time provided the first complete evidence of the toxicological stress in cetaceans living in the Pelagos Sanctuary by applying an integrated approach based on the analysis of persistent chemicals combined with biochemical markers of exposure to planar halogenated aromatic hydrocarbons and polycyclic aromatic hydrocarbons (such as cytochrome P4501A, cytochrome P4502B) and the antioxidant enzyme catalase to anthropogenic contaminants in striped dolphin (*Stenella coeruleoalba*) skin biopsies. Moreover, through the biomarker approach, Fossi et al. [29] investigated the potential toxicological effects of microplastics and their related contaminants on free-ranging fin whale populations.

The biomarker approach has been applied to reef environments, typical of many shallow coastal areas in tropical regions, which are at increased risk due to several threats, including pollution. Antioxidant defenses and metal detoxification analysis in foraminifers [35], biotransformation and neurotoxicity biomarker in reef fish [42], and detoxification proteins, antioxidant defense, metabolic profile, and stress proteins in corals [31][43] have been applied for gaining a measurable impact on selected reef environments caused by anthropogenic contaminants.

These studies underline the importance and usefulness of the biomarker approach for assessing the quality of the habitat in MPAs, in the detection of any space and time variations in anthropogenic pressures on the area, and the effectiveness of the conservation policies. Some studies utilized an integrated approach complementing the biomarker analysis with the measurement of the pollutant residues in the tissues of the organisms. The importance of this approach in the continuous monitoring of the health status of the organisms living in protected areas appears evident from these works; however, considerable research efforts still need to be made to fill some gaps such as the lack of specific guidelines for the use of these tools in MPA monitoring and assessment and for their useful application to MPA management. Guidelines should address the criteria for the employment of key species as sentinel organisms for the multi-biomarker approach application in MPA biomonitoring and the choice of suitable biomarker responses in relation to the specific protection objectives of MPAs. Moreover, guidelines should indicate protocols for the validation of the biological responses used and even the standardization of a biomarker-based index for specifically assessing the ecotoxic risk.

3. Conclusions

In conclusion, the analyses and discussion of the recent literature suggest that pollution biomarkers have proved to be useful tools for monitoring and assessment of pollution threat to marine biodiversity, both in the environmental quality monitoring of protected areas and the assessment of the health status of species at risk.

However, great efforts should be still devoted to developing the research in this field. In particular, important issues that require further development concern (1) the development of new biomarkers specifically addressed to conservation purposes, also thanks to the development of omics technologies, (2) the extension of the study to a wider number of endangered species, and (3) their validation in the field and inclusion into organic guidelines that could support conservation policies and management.

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