

# Bioindicators of Marine Contaminations at the Frontier of Environmental Monitoring and Environmental Genomics



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## Abstract

Oceans provide major resources for rapidly increasing population worldwide. Ocean sustainability thus constitutes a major issue for human health, as well as economic and ecological perspectives. Indicators of oceanic contamination have been selected in order to identify, but also further prevent impact of human activities on marine ecosystems. However while some bioindicating species appear consensual, others are typically representatives of restricted marine areas and/or restricted range of marine contaminants. We here attempt a review of marine species used as bioindicators/ biomarkers of major human activities focusing on the requirement to integrate modern metagenomics approaches of marine ecosystems in order to define consensual, pertinent, ubiquitous bioindicators of marine health.

**Keywords:** Biomarkers; Bioindicators; Oceans; Ecotoxicology; Omics Sciences; Epigenetics

## Introduction

Coastal Areas support increasing population worldwide, for whom marine ecosystems constitute either directly or indirectly, principal economic resources. For instance, over two billion people worldwide rely on seafood consumption and sea products for their diet [1]. Alternatively, the ocean appears as a promising reservoir for novel pharmaceuticals [2], but simultaneously, novel energetic and mining resources [3-5]. However, oceanic ecosystems are today suffering from past but also novel, rapidly diversifying modern human activities. Indeed, this common reservoir suffers from environmental pressure exerted by humans on the marine ecosystems itself, such as of shore petroleum production, sea transport or fishing [6] but also, more recently exploitation of deep sea metallic nodules [5], marine aquaculture [7], rocket launching activities and installation of offshore wind mills fields, but also, indirectly from the exploitation of nearby terrestrial ecosystems by tourism, agriculture and industry, (including mining) [8-10]. Even exponentially increasing marine aquaculture (that is today

expected to supplement natural stocks of seafood and which relies on availability of uncontaminated water), actively impacts marine ecosystems, through the release from marine farms, of antimicrobials, food supplements, nutrients, and disease controlling substances [7] (in final EMIDA MOLTRAQ project's report). While most contaminations are concentrated in coastal zones, mainly affecting pelagic and benthic food webs of the continental shelf [11], long-range transport of contaminants through large distances has been described in the literature [12]. The sustainability of these marine resources and their derivative activities thus appears today as a major common preoccupation worldwide [1,13].

Pollutants affecting marine ecosystems include a wide range of synthetic organic chemicals, (Substances of particular concern are chlorobiphenyls, chlorinated dioxins, pesticides and some industrial solvents); diverse heavy metals and alloys with principal focus on mercury or chromium VI; but also, alternatively, toxins and pathogenic species; pharmaceuticals

and personal care products; plastic materials; and more recently, genetically modified organisms [14]. Contaminants tend to accumulate through marine food webs, biomagnified in a greater extent than through their continental counterparts due, among other, to their higher complexity and compartment level. Their final accumulation in fish tissues is of major public concern [15,16]. Indeed, since the widely discussed Minamata case [17], end-level human consumption of contaminated marine products evolves novel interrogations, while reported impacts on human health increases and diversifies. Effects on human health include today, among other, neurologic disorders, endocrine-disrupting functions, developmental problems [18] but also, human reproduction, neurobehavioral development, liver function, birth weight, immune response, and tumorigenesis [19]. Additionally, in given cases, microorganisms have been reported that are able to degrade, sometimes only partially, given molecules through complex pathways, sometimes liberating metabolic intermediates with higher toxicity than the originally released environmental contaminant [20]. Unfortunately, association with deleterious effects of specific compounds is very difficult and follow up of this extending diversity and complexity of environmental contaminants, requires integrated approaches.

### Discussion

Environmental monitoring requires rapid, efficient and cost-effective methods for detecting pollutants at risk to accumulate in marine food webs and to impact human health. However, toxicity level for a given compound is not restricted to the

chemical property of a substance or to its concentration, but rather relies on its bioavailability which is highly dependent on environmental conditions [21]. For instance, levels of clay particles, dissolved or particulate carbonates, silicates, sulfides and organic matters are acting as complexing factors for most metals but also given organic contaminants. Additionally, metal toxicity is highly related to their redox level [22], the latter being itself influenced, among other, by microbial metabolism of metals, that may be used for given microbial groups, as alternative electron receptors in anaerobic respiration processes. Among available methods, the use of bioindicators and biomarkers of marine contamination is an interesting tool to assess deleterious effects of environmental contaminants in marine ecosystems, as it is clearly correlated with levels of bioavailable contaminants. Thus, several inventories of marine biotest methods have already been compiled and their interest reviewed [23-26]. Indeed, both marine microflora, (among others foraminifers [27,28], diatoms [29], dinoflagellate cyst [30] but also macrofauna have been mined for relevant bioindicator species and used in biomonitoring of environmental contaminations [31]: Among macrofaunal organisms, literature reports communities and individual species of copepods [32,33], bivalves [34,35], echinoderms [36,37], sponges [38,39], anemones [40], crustaceans [41,42], insects [43], fishes [44,45] and even birds [46]. Some examples are summarized in Table 1 that illustrates the extreme diversity of bioindicator organisms and the lack of consensus at international scale.

**Table 1:** Illustration of the diversity of bioindicators used for environmental monitoring in marine areas.

Species	Environment	Contaminant	Reference
Bacteria <i>Vibrio fischeri</i> <i>Vibrio sp.</i>	<i>in vitro</i> Mediterranean lagoon	wide range of chemicals pathogens	[47,48]
Phytoplankton	Taipei		[49]
Green algae <i>Ulva lactuca</i> <i>Ulva rigida</i> Brown algae <i>Lobophora variegata</i>	Hong Kong  tropical waters	metals Hg, Cu, Cd, Zn, Ni, Cr  Cd, Co, Cr, Ni, Zn	[49-51]
Foraminifera	marine, benthic	heavy metals	[27,28,52]
Sponges <i>Spongia officinalis</i> <i>Haliclona tenuiramosa</i>	Indian Gulf	Polychlorobiphenyl Fe, Mn, Ni, Cu, As, Co, Cd	[53,54]
Cnidaria <i>Aurelia aurita</i> (ephyra) <i>Anemona viridis</i> <i>Actinia equina</i> <i>Nematostella vectensis</i>	water column sessile sessile estuarine sediments	SDS, Cd(NO <sub>3</sub> ) <sub>2</sub> Cd, Zn Cd, Zn CdCl <sub>2</sub>	[55,56]
Annelida <i>Arenicola marina</i> <i>Hediste diversicolor</i>	sediment, coasts, ports	PAHs, Pb, Pharmaceuticals pharmaceuticals	[57,58]

<p>Bivalves  <i>Mytilus edulis</i>  <i>Mytilus galloprovincialis</i>  <i>Crassostrea gigas</i>  <i>Crassostrea virginica</i>  <i>Crassostrea corteziensis</i>  <i>Mytella strigata</i>  <i>Pinctada radiata</i>  <i>Venus verrucosa</i></p>	<p>coast       Arabian Gulf Mauritania</p>	<p>Cd, Hg  Cu, Cr, Ni, Zn, Fe, Mn, 137Cs  Zn&gt;Fe,Cu&gt;Mn&gt;Ni&gt;Pb&gt;Cr&gt;Cd    Zn&gt;Fe,Cu&gt;Mn&gt;Ni&gt;Pb&gt;Cr&gt;Cd  metals  Cd, Cu, Fe, Mn, Zn   Hg, Cu, Cd, Zn, Ni, Cr</p>	<p>[35,50,59-68]</p>
<p><i>Macoma balthica</i>  <i>Tapes philipinarum</i>  <i>Perna viridis</i>  <i>Anadara granosa</i>  <i>Soletellina acuminata</i>  Gastropoda  <i>Trivia monacha</i>  <i>Trivia arctica</i>  <i>Hinia reticulata</i>  <i>Nucella lapillus</i>  <i>Donax trunculus</i></p>	<p>estuaries   Hong Kong Malaysia Mangroves   North Atlantic North Atlantic Mauritania</p>	<p>PCB  As, Cd, Cr, Cu, Pb, Se, Zn  PAH    Tributyltin  Tributyltin  Tributyltin  Tributyltin  Cd, Cu, Fe, Mn, Zn</p>	
<p>Amphipoda  <i>Corophium volutator</i>  <i>Echinogammarus pirloti</i>  <i>Gammarus locusta</i>  <i>Gammarus zaddachi</i>  <i>Gammarus salinus</i>  Branchiopoda  <i>Artemia salina</i>  Ostracoda  <i>Cypris sp.</i>  <i>Cyprideis torosa</i>  <i>Leptocythere psammophila</i>  Decapoda  <i>Palaemon elegans</i>  <i>Litopenaeus vannemei</i>  Cirripedia  <i>Balanus amphitrite</i>  <i>Fistulobalanus dentivarians</i>  <i>Elminis modestus</i></p>	<p>Mediterranean coast, marine and estuarine sediments       in vitro</p>	<p>Cu, Zn, Cd  Zn, Cu, Cd    CuSO<sub>4</sub>, PbSO<sub>4</sub>, ZnSO<sub>4</sub>, NiSO<sub>4</sub>   Cu, Zn, Cr, Pb, Al, Si, Fe, Ca, Mg, Na, Mn, Ba, Sr, S, Cl, oil, industrial waste water   Zn, Cu, Cd  Zn&gt;Mn&gt;Cu&gt;Pb&gt;Ni&gt;Cr&gt;Cd   Zn, Cu, Cd</p>	<p>[60,69-76]</p>
<p>Insecta  <i>Halobates micans</i></p>	<p>surface of open ocean, Atlantic</p>	<p>Cd</p>	<p>[43]</p>
<p>Echinodermata  <i>Paracentrotus lividus</i>  <i>(premature)</i></p>	<p>Iberian coast</p>	<p>Cu, Cd, Pb, Hg  Fe, Zn</p>	<p>[36]</p>
<p>Fish  <i>Thunnus thynnus</i>  <i>Katsuwonus</i></p>	<p><b>offshore, Japanese coastal waters, wetland</b></p>	<p>Organochlorines  Polybrominate biphenyl ethers</p>	<p>[45]</p>

<p><i>pelamis</i>  <i>Oreochromis niloticus</i>  <i>Mullus barbatus</i>  <i>Serranus hepatus</i>  <i>Serranus cabrilla</i>  <i>Zoarcetes viviparus</i></p>	<p>Mediterranean  Mediterranean  Mediterranean</p>	<p>Zn, Cu, Cr, Mn  PAH, PCB  PAH, PCB  PAH, PCB  As, Hg, Pb, Ti</p>	<p>[57-59]</p>
<p>Birds  <i>Sterna sp.</i>  <i>Larus sp.</i>  <i>Fulmarus glacialis</i></p>	<p>estuaries, oceans    North Pacific</p>	<p>Pb, Hg, Cd  Pb, Hg, Cd  plastic pollution</p>	<p>[46,77,78]</p>

Further, bioindicators may be classified into two groups: Biomarkers on one hand, and sentinel species on the other [79,80], biomarkers being generally defined on taxa considered as sentinel organisms:

A. Distinctively, biomarkers of marine contamination require (bio) chemical analysis, such as the concentration of a given tracer or pollutant in a given tissue or measure of a given biological marker in it [26], using a wide range of chemical, molecular, but also physiological approaches (among other: biochemical assays, enzyme linked immunosorbent assays (ELISA), spectrophotometric, fluorometric measurement, differential pulsed polarography, liquid chromatography, atomic absorption spectrometry and more recently transcriptomics and metabolomics). Classical biomarkers include for instance, cytochrome P4501A activity, DNA integrity, acetylcholinesterase activity or metallothionein induction. Biomarkers assess, at an infra-organism level (i.e; they address a tissue, a cell type etc...), a physiologic, genetic, molecular, or morphologic response [80]. Biomarkers may be considered as anticipative as they can enlighten a toxic effect earlier than a lethal effect observed at a population level, and affecting whole organisms

B. Sentinel species [81] trace the occurrence level of selected species at the population level. Choice of sentinel species is based on previously demonstrated correlation between the contamination level of a given pollutant and the species occurrence and or behaviour.

C. Additionally one may distinguish the toxicological approaches performed under laboratory conditions (rather relevant for biomarkers) from environmental impact studies performed in the field that are often based on numeration of sentinel species.

### Microbial Bioindicators

Historically, defined microbial species were used as bioindicators of water quality to assess risks of microbiological contamination by pathogens and guidelines were for long defined in the three water-related areas (drinking water, wastewater but also (marine) recreational water) by measuring indicator bacteria [82,83]. Further, bacterial indicators were

then derived, among which *Vibrio* species, to monitor microbial status of marine environments. For instance, comparative health status and level of contamination by terrestrial sewages of three laguna ecosystems of the French Mediterranean coast were monitored through the search for *Vibrio* species that were here used as bioindicators of risks of environmental contamination by pathogenic bacteria [84]. Similarly, number of microbial bioindicator species were defined to monitor ranges of environmental pollutants in the water column, at the water-sediment interface or within the sediment. Indeed, as microbes constitute key actors of the end loop of most biogeochemical cycles ( i.e. carbon , nitrogen phosphorous cycles etc..), their role is vital for the health of the aquatic ecosystem and modification of their population or activity can indeed anticipate further impacts noticeable only lately on food webs. Microbial indicators were thus generalized to assess environmental changes. For instance, Benthic diatoms, among numerous others, have been used as markers of marine eutrophication in coastal ecosystems [85].

Beyond microbial natural species, microbes present the advantage to evolve rather rapidly to adapt to adverse condition, to degrade novel compounds [20] and to be genetically modifiable through mutagenesis and recombinant DNA technology. These, advantages were thus used for the development of recombined biosensors for numerous environmental contaminants (both metallic and organic) and number of bioluminescent biosensors especially have been constructed [25,86-88].

Finally today, as molecular tools and their associated computing methods develop, comparative diversity analysis of marine microbial communities constitutes as a whole, a promising indicator of impacts of human activities on marine ecosystems. Diversity loss can indeed for long be easily monitored using whole community DNA-based molecular approaches. Originally, Denaturation Gradient Gel Electrophoresis (DGGE) of community amplified 16S or 18S sequences [89] and derived DNA pattern analysis tools (based on Random Amplification of 16S-23S Intergenic Spacers (RISA), Single Strand Conformation Polymorphism of community amplified 16S rDNA sequences (SSCP) and all derivative methods) appeared useful tools for ecological monitoring. More recently, metabarcoding (<https://www.embl.de/tara-oceans/start/>) [90,91], further, marine

ecological (meta)genomics which have been defined as the application of genomic sciences to attempt to understand the structure and function of marine ecosystems [90], and its derivative, comparative metagenomics have started to emerge in marine ecotoxicological approaches to assess environmental impacts [92]. As Marine ecological (meta) genomics evolves, associated computer based analysis of such data could evolve rarefaction curves that would indeed be informative of impacts at an ecosystemic scale. Further, the -omic based analysis of the metabolic behaviours of microbial communities and identification of mechanisms that microbes use to respond to environmental changes and to adapt to man-made pressures may be used for environmental monitoring purposes: Microbial biodiversity at itself starts to be used to evolve response-specific functional indices tentatively integrating evolution of complex interactions between microbial communities. These were based on species ecological preferenda and autoecology, especially in order to allow the discernment of the stressing factor involved in the ecosystem perturbation [85]. Such indexes are informative as they attempt to integrate the evolution of the microbial community as a whole and combined to modern omics, should open novel environmental monitoring area

### Molluscs

Molluscs species are interesting bioindicators considering their ability to filter large volumes of sea water and thus to accumulate trace contaminants. As sessile species presenting increased longevity, they constitute interesting bioindicators in long term impact studies in given habitats [93,94]. Among molluscs, mussels and oysters have been particularly used as bioindicators in many countries for marine pollution monitoring [95,96]. According to physico-chemical properties of pollutants (especially their solubility in sea water and complexing affinity to organics or minerals), either filtrating species such as the blue mussel (*Mytilus edulis*), [95,96] or conversely, scavengers such as the Manila clam (*Ruditapes philippinarum*) are used [97,98]. However, for the latter, accumulation of a number of anthropogenic compounds in clam's tissues suggests that these species may present mechanisms that allow them to cope with the toxic effects of contaminants and thus question their use as bioindicators.

Alternatively, some authors have used animal behaviour to estimate effects of a range of contaminants in various marine conditions. For instance Redmond et al. [99] used mussels (*Mytilus edulis*) valve opening and shell movements to assess toxicity of dispersed crude oil (DCO); further, changes in patterns of movement and social interaction in the gilthead seabream, *Sparus aurata*, were linked to several biomarkers following exposure to phenanthrene, a common PAH in petroleum products [100]. (Additional examples of use animal behaviour or social interactions as bioindicator can be found in [101,102].

### Fishes

In Europe, the EC Water Framework Directive (WFD), requires from its member states to ensure, among others, a satisfactory ecological and chemical status of their coastal and marine waters which is defined on a basis of an a priority list of hazardous chemicals and substances with associated standard values based on concentrations found in certain marine organisms, and notably in fishes. Length and cost of chemical determinations lack of anticipation of potential pollution risks by other substances have been underlined and stress tests and bioindicators of fish health have been evolved for a range of species. Fish species used as bioindicators include, among others,; *Thunnus thynnus*, *Katsuwonus pelamis*, *Oreochromis niloticus*, *Mullus barbatus*, *Serranus hepatus*, *Serranus cabrilla*, *Zoarces viviparus* [45,103-105](Table 1). Biomarkers have also been derived from a set of fish species to assess various marine contaminants [105]. However, most classic ecotoxicologic test species are currently reconsidered due to the lack of genomic sequences that could allow development of cheap and rapid PCR based ecotoxicological kits based on long known tissue specific responses of target species.

### Definition and main properties of performant bioindicators

Conversely to terrestrial conditions for which consensual model exists that use for instance rats as reference species for toxicity assessments, no consensus has yet been reached for marine biomonitoring and often, marine species have been used in biomonitoring independently of what should constitute the basic properties of a bioindicator [48] that we have to remain here:

Indeed, to be relevant and reliable, bioindicators, have to present given characteristics:

- A. They should appear/ or disappear or react concomitantly with the contaminant itself, and behave in a quantitative manner (i.e; the measured bioindicator population level or intensity of the biomarker response have to be proportional to the bioavailable contaminant)
- B. Anticipative as they require to be particularly sensitive to toxic compounds. Resistant species do not constitute proper bioindicators or sources for biomarkers.
- C. Integrative as they may collect and cumulate over a period of time the impact of ranges of diverse environmental contaminants but also their potential interactions
- D. Able to distinguish impacts from xenobiotic compounds from natural ecological stresses
- E. They are required to present a wide geographical range. Site specific species are not suitable as they do not allow inter site comparisons. For instance The clam *Ruditapes*

*decussatus* and the mussel *Mytilus edulis* that both present a worldwide distribution constitute interesting bioindicators. However they do not occur in all marine environments

- F. They are easy to sample and to measure
- G. Cost efficient as they aim at avoiding numerous instant measures of potentially toxic substances
- H. For legal purposes they need to have been previously referenced as suitable for ecological impact studies

### Revisiting the biomarker and bioindicator concepts in the light of modern -omic sciences

While pre-millennium ecotoxicological studies concentrated on the description of biomarkers and bioindicators, basing their choice on preliminary often biased knowledge, modern -omics enlighten the requirement to revisit previous concepts, while extending the description of biological diversity far beyond the known isolable, cultivable, and identifiable species, and extending the list of potential genes and function potentially used as biomarkers. Conversely, long used consensual biomarkers/bioindicators have lost interest while failing to provide available complete and full annotated genomic sequences. This is the case of *Mytilus edulis* for which a group of researchers interested in the use of bivalves as a research model for environmental and biomedical purposes, lately decided to join efforts to produce and assemble sequences from Sanger and NSG methods to elucidate genomic sequence. The project was initiated in 2010 only (<http://www.openmytilusconsortium.org/>) and seems today still ongoing, as only the mitochondrial sequences and cDNA libraries of *M. edulis* [107] and *M. galloprovincialis* [108], respectively, seem available. Late sequence availability indeed reordered interest for previously consensual bioindicator species and their associated biomarker. For instance, availability of the fish *Danio rerio* genomic sequence [109] paved the way for the set-up of quantitative PCR based ecotoxicological tests [110]. Indeed, the genomic tools for ecotoxicogenomics have now been reviewed [111], Miracle & Ankley (2005) with a particular emphasis on fish testing that are emerging in this field, such as that of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure on zebrafish caudal fin regeneration. Soon sequence characteristics (such as sequence length of given marine species [112] appeared itself a base for environmental impact studies. Genomes may also be the base for further metabolomics approaches that now emerge as novel ways to assess impact of pollutants on the complete metabolism of species whose sequence is available –such as in the case of *Ruditapes philippinarum* [113].

Novel trends are also integrating epigenetics as novel tools to assess organismal response to environmental stressors [106,114]: indeed, Epigenetic mechanisms in an ecotoxicological context is a new concept and has not yet been considered to be integrated into current environmental regulatory practices [115]. Epigenetic biomarkers have been demonstrated in humans, mice

and zebrafish [116-118]. While some newer studies have focused on bivalves and other marine invertebrates, epigenetic responses appears as next-generation pollution biomonitoring [106,114]. Epigenetic techniques can provide the link from environmental stressor to detectable biomarker responses and ultimately the goal of linking these omic responses to physiological changes that can be tied to classical ecotoxicological endpoints.

Conversely, lack of available genomic sequences for classically used bioindicator species such as those species long used by the US EPA and other environmental regulatory agencies for marine toxicity studies including the mysid shrimp, *Americamysis bahia* [119], the sheepshead minnow, *Cyprinodon variegatus* [120] the inland silverside, *Menidia beryllina* [121], the sea urchin *Arbacia punctulata* [122], and the red macroalgae *Champia parvula* [123], clearly slow down the development of functional molecular biomarkers from these classic ecotoxicological workhorses. With the passing of legislation such as REACH in the EU, the use of whole organism toxicity studies will steadily decrease while the demand for non-lethal ecotoxicological studies will increase. Biomarker and bioindicator studies are excellent candidates to fill this gap. Although classical ecotoxicological endpoints (mortality, growth, and reproduction) are still used in regulatory decision making, we anticipate the use of biomarker and bioindicator information in regulatory frameworks becoming more practical and needed.

### Conclusion

Legal issues are solved for terrestrial ecosystems. However, consensual international definition of marine biomarkers and bioindicators remains under discussion [48,124]. Classic bioindicator species and their derived biomarkers remain thus often not fully consensual and vary from country to country requiring final common approval. Finally, Environmental Protection Agencies appear extremely slow in adapting new technologies into their policy making decisions and still relies on classical toxicological endpoints such as mortality, growth, and reproductive output. We here want to underline the necessity to revisit biomarkers in bioindicator species in the light of novel omics data. New techniques and technologies provide understanding in organismal omic response to stressors (chemical or environmental) and warrant more attention and integration into regulatory policies [93-124].

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