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REVIEW

The use of biomarkers to assess the health of aquatic ecosystems in Brazil: a review

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Abstract Organisms in polluted environments are typically exposed to a complex mixture of chemical contaminants. The great concern about the health of aquatic ecosystems has led to the increased use of biomarkers over the past years. The aim of this work was to review the papers published from 2000 to 2015, which used biomarkers to assess the health of aquatic ecosystems in Brazil. A research resulted in 99 eligible papers. More than 80% of studies were conducted in the states of São Paulo and Rio Grande do Sul. Approximately 63% of studies used fish as bioindicator, whereas the micronucleus test and biochemical analyses were the most used biomarkers. A multibiomarker approach was used by 60.6% of studies, while 39.4% used one single biomarker. Furthermore, 68% were field studies and more than 75% of these used control animals sampled at reference sites. A relationship between the biomarker responses and pollution was reported by 87% of studies; however, 43.4% of studies analyzed only one sampling period, limiting comparisons and comprehension about possible seasonal variations. This review evidenced some weak points in studies using biomarkers in Brazil, especially related to the lack of studies in two important biomes (the Pantanal and the Amazon Rainforest) and experimental designs (small sample size, sampling in one single period, use of one single biomarker). Thus, future studies should consider mainly the use of multiple biomarkers, greater sample size, seasonal sampling and water physicochemical parameters to better diagnose the health of aquatic ecosystems.

Keywords Biomarkers · Environmental pollution · Toxicology assessment · Water quality

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Introduction

Pollution of the aquatic ecosystems is considered a serious and growing problem. Increasing amount of industrial, agricultural and urban pollutants discharged into the aquatic environment have led to various deleterious effects on aquatic organisms and also on human health (McGlashan and Hughies 2001). In Brazil, surface water quality is monitored only by the means of physicochemical and microbiological (coliforms) parameters, according to limits established by the Brazilian National Environment Council (CONAMA) (Brasil 2005). The CONAMA Resolution 357/2005 provides maximum values for certain substances in surface waters, but does not provide specific information on the use of ecotoxicological tests for assessing water quality. Furthermore, physicochemical analyses provide only information about the nature of the contaminants and their concentrations in the environment, and they cannot predict bioavailability or potential effects on biota (Seriani et al. 2015). On the other hand, ecotoxicological approaches represent a useful indicator of water quality (Gonzalez et al. 1993; Araújo et al. 2014), because they reflect the real conditions of interaction by synergy and/or antagonism among the contaminants and the effects on the organisms (Azevedo et al. 2013; Fuzinatto et al. 2013).

A biomarker can be defined as a quantitative measure of changes in molecular or cellular components, processes, structures and functions related to exposure to environmental chemicals (Depledge et al. 1995; He et al. 2012; Fasulo et al. 2013). Classes of biomarkers have been proposed according to the extent that they reflect exposure to environmental stressors, or adverse health effects from contaminant exposures (WHO 1993; van der Oost et al. 2003; Viarengo et al. 2007; Hook et al. 2014). Biliary fluorescent aromatic compounds, vitellogenin, cytochrome P4501A mRNA or protein, hepatic ethoxyresorufin-*O*-deethylase (EROD) and metallothioneins (MT) are examples of biomarkers of exposure. They show an early response to contaminants and are typically specific to a particular class of contaminants (Broeg et al. 2005). Biomarkers of effect are related to measurable biochemical, physiological or other alterations within tissues or body fluids of an organism that can be recognized as associated with an established or possible health impairment or disease. Heat shock proteins (HSP70 or HSP90), markers of oxidative stress [superoxide dismutase (SOD), glutathione, catalase (CAT), lipid peroxidation (LPO)], condition indices (condition factor, hepatosomatic index, gonadal index), histopathology evaluation, DNA damage and acetylcholinesterase (AChE) (which indicates both exposure and effects) are examples of biomarkers of biological effects (Hook et al. 2014). Yet, another class of biomarkers is described as "biomarkers that integrate chemical exposure and biological effects". They include AChE and also genomic approaches (Hook 2010). In general, some biomarkers allow the specific identification of exposure to a class of xenobiotics or alterations of physiological function, but the majority of biomarker applications monitor a general response to disturbance (Trapp et al. 2014). Nevertheless, it is important to note that many non-pollution factors may interfere with biomarker responses. These "confounding" factors include the organisms' health, sex, age, nutritional status, metabolic activity, migratory behavior, reproductive and development status, and population density, as well as factors like season, ambient temperature, heterogeneity of the environmental pollution and so forth (van der Oost et al. 2003).

The use of biomarkers with the purpose of biomonitoring natural aquatic systems by the use of bioindicator species is necessary to efficiently measure the degree of exposure in aquatic organisms to chemical contaminants (Sureda et al. 2011). Biomonitoring or biological monitoring can be defined as the systematic use of biological responses to evaluate changes in the environment (Cairns and van der Schalie 1980). In this context, biomarkers are increasingly worldwide-recognized tools for the assessment of pollution impacts, and some are already incorporated in environmental monitoring programs in other countries (Viarengo et al. 2007), although their systematic and large scale application is rare (Trapp et al. 2014). In this context, considering the increased contamination of water resources and the potential risk to biodiversity conservation and human health, as well as the importance of the use of biomarkers to complement the water physicochemical analysis, this study aims to review the use of biomarkers in the assessment of aquatic ecosystems health in the last 16 years in Brazil.

Data survey

The papers were searched using the databases Science Direct and Scientific Electronic Library Online (SCIELO). The keywords used on the search were biomarker, biomonitoring, water pollution and Brazil. The criteria for the selection of papers included original articles and short communications published between January 2000 and July 2015. Studies should be performed in field and/or laboratory (with native or commercially acquired organisms) and use at least one type of biomarker in any organism exclusively aquatic (including larvae) to assess the water quality of natural environments, such as rivers, lakes, wetlands, streams and sea in Brazil. The exclusion criteria were studies related to the assessment of water quality from environments altered by constructions of reservoirs and dams, as well as studies using semiaquatic organisms (biphasic life cycle).

A qualitative analysis was conducted considering the bioindicator organism, type of study (field, in laboratory or with caged organisms), type of biomarkers, use of control or reference site, number of sampling sites, number of collections, sample size, combination of biomarkers, physicochemical parameters analyses and the response of the bioindicator to the environmental contamination.

Published studies

Considering the criteria aforementioned, 99 papers published in national and international scientific journals were selected (Table 1).

Regarding the number of publications along the years, less than five papers were published per year from 2000 to 2005. A peak of publications was reached in 2007 (13 papers) and 6–11 papers were published per year from 2008 to 2015 (Fig. 1). These data show that scientific publication in the field of aquatic ecotoxicology is stable and without tendency of increase in Brazil.

A higher number of studies carried out in Southeastern and Southern regions were observed, corresponding to more than 80% of total studies (Table 2). Most studies were carried out in the states of São Paulo, in Southeastern region (26%) and Rio Grande do Sul, in Southern region (21%), probably because some of the most polluted rivers in Brazil are located in these states, such as Sinos River, Gravataí River and Caí River, in Rio Grande do Sul; and Tietê River and Paraíba do Sul River, in São Paulo (Hupffer et al. 2013). In addition, these states present well-structured universities and research centers, and also receive more funding for this type of research. It is relevant to note the lack of studies published about the Midwestern region (one single study) and Northern region (three studies) despite the existence of two important biomes of Brazil in these regions—the Pantanal and Amazon Rainforest, respectively. According to Carvalho-Neta and Abreu-Silva (2010) and Montes et al. (2010), the lack of studies using biomarkers as predictors of aquatic health in these regions, and also in the Northeast, indicates the need of biomonitoring studies that might estimate the potential effects suffered by native species.

Bioindicator organisms

Various organisms have been considered as bioindicators of environmental quality. In Brazil, 62.6% were carried out exclusively with fish, 35.4% used other organisms, such as bivalves, plants and gastropods, and 3% used two types of bioindicators simultaneously (Table 3). Studies using biomarkers in larvae were not found.

Fish have been considered suitable organisms for biomonitoring studies as they are sensible to changes in the aquatic environment. Their biological responses change, even at low levels of pollution (Linde-Arias et al. 2008b; Souza et al. 2013). Fish can be found virtually everywhere in the aquatic environment and they play a major ecological role in aquatic food webs as carriers of energy from lower to higher trophic levels (van der Oost et al. 2003). Thus, genetic, biochemical, behavioral and morphological responses represent useful biomarkers in environmental biomonitoring (Pesce et al. 2008; Ballesteros et al. 2009). In addition, the high percentage of studies carried out using fish as bioindicator can also be explained by the simple fish sampling techniques.

Table 1 Summary of studies using biomarkers to assess the health of aquatic ecosystems in Brazil, in chronological order

	Study	Bioindicator	Species
1	Bainy et al. (2000)	Bivalve	*Perna perna*
2	Schulz and Martins-Junior (2001)	Fish	*Astyanax fasciatus*
3	Torres et al. (2002)	Bivalve	*Mytella guyanensis*
4	Ventura et al. (2002)	Fish	*Orthopristis ruber*
5	Andrade et al. (2004)	Fish	*Mugil* sp. and *Netuma* sp.
6	Geracitano et al. (2004)	Worm	*Laeonereis acuta*
7	Parente et al. (2004)	Fish	*Oreochromis niloticus*
8	Ranzani-Paiva and Silva-Souza (2004)	Fish	*Mugil platanus*
9	Alberto et al. (2005)	Fish	*Astyanax fasciatus*
10	Prá et al. (2005)	Planaria	*Girardia schubarti*
11	Amado et al. (2006a)	Fish	*Micropogonias furnieri*
12	Amado et al. (2006b)	Fish	*Paralichthys orbignyanus*
13	Silva et al. (2006)	Fish	Multispecies
14	Souza and Fontanetti (2006)	Fish	*Oreochromis niloticus*
15	Tortelli et al. (2006)	Fish	*Micropogonias furnieri* and *Cathorops spixii*
16	Villela et al. (2006)	Bivalve	*Limnoperna fortunei*
17	Zanette et al. (2006)	Bivalve	*Crassostrea rhizophorae*
18	Camargo and Martinez (2007)	Fish	*Prochilodus lineatus*
19	Domingos et al. (2007)	Bivalve	*Crassostrea rhizophorae*
20	Fernandez et al. (2007)	Gastropod	*Stramonita haemastoma* and *Thais rústica*
21	Ferreira-Cravo et al. (2007)	Worms	*Laeonereis acuta*
22	Francioni et al. (2007)	Bivalve	*Perna perna*
23	Junior et al. (2007)	Plant	*Allium cepa*
24	Lemos et al. (2007)	Fish	*Pimephales promelas*
25	Lüchmann et al. (2007)	Shrimp	*Farfantepenaeus brasiliensis*
26	Lupi et al. (2007)	Fish	*Oreochromis niloticus*
27	Oliveira et al. (2007)	Fish	Multispecies
28	Pereira et al. (2007)	Bivalve	*Perna perna*
29	Silva and Martinez (2007)	Fish	*Astyanax altiparanae*
30	Villela et al. (2007)	Bivalve	*Limnoperna fortunei*
31	David et al. (2008)	Bivalve	*Mytella falcata*
32	Lemos et al. (2008)	Fish	*Astyanax jacuhiensis*
33	Linde-Arias et al. (2008a)	Fish	*Oreochromis niloticus*
34	Linde-Arias et al. (2008b)	Fish	*Oreochromis niloticus*
35	Medeiros et al. (2008a)	Bivalve	*Crassostrea gigas*
36	Medeiros et al. (2008b)	Bivalve	*Crassostrea gigas*
37	Miranda et al. (2008)	Fish	*Hoplias malabaricus*
38	Parente et al. (2008)	Fish	*Oreochromis niloticus* and *Geophagus brasiliensis*
39	Ruas et al. (2008)	Fish	*Oreochromis niloticus, Tilapia rendalli* and *Geophagus brasiliensis*
40	Zanette et al. (2008)	Bivalve	*Crassostrea rhizophorae* and *Crassostrea gigas*
41	Barberio et al. (2009)	Plant	*Allium cepa*
42	Cardoso et al. (2009)	Fish	*Trichiurus lepturus*
43	Galindo and Moreira (2009)	Fish	*Bathygobius soporator*
44	Katsumiti et al. (2009)	Fish	*Cathorops spixii*
45	Kirschbaum et al. (2009)	Fish	*Centropomus parallelus*
46	Barbosa et al. (2010)	Fish and plant	*Oreochromis niloticus* and *Allium cepa*

Table 1 continued

	Study	Bioindicator	Species
47	Carvalho-Neta and Abreu-Silva (2010)	Fish	*Sciades herzbergii*
48	Egito et al. (2010)	Fish	*Crenicichla menezesi*
49	Franco et al. (2010)	Fish	*Oreochromis niloticus*
50	Montes et al. (2010)	Fish	*Brachyplatystoma rousseauxii*
51	Rechenmacher et al. (2010)	Rat	Wistar rats
52	Rocha et al. (2010)	Fish	*Brachyplatystoma rousseauxii*
53	Sáenz et al. (2010)	Bivalve	*Perna perna*
54	Santos et al. (2010)	Fish	*Mugil curema*
55	Scalon et al. (2010)	Fish	*Hyphessobrycon luetkenii*
56	Seriani et al. (2010)	Fish	*Micropogonias furnieri*
57	Flores-Lopes and Thomaz (2011)	Fish	*Astyanax fasciatus* and *Cyanocharax alburnus*
58	Lemos et al. (2011)	Cell culture	Human linphocytes
59	Nunes et al. (2011)	Cell culture and plant	Chinese hamster lung fibroblasts and *Allium cepa*
60	Pereira et al. (2011)	Bivalve	*Perna perna*
61	Souza-Bastos and Freire (2011)	Fish	*Atherinella brasiliensis*
62	Toste et al. (2011)	Gastropod	*Stramonita haemastoma*
63	Anzolin et al. (2012)	Fish	*Trichechus manatus*
64	Azevedo et al. (2012a)	Fish	*Cathorops spixii*
65	Azevedo et al. (2012b)	Fish	*Cathorops spixii*
66	Carvalho et al. (2012)	Fish	*Oreochromis niloticus*
67	Hauser-Davis et al. (2012a)	Fish	*Mugil Liza*
68	Hauser-Davis et al. (2012b)	Fish	*Oreochromis niloticus, Mugil liza and Gephafus brasiliensis*
69	Nascimento et al. (2012)	Fish	*Oligosarcus hepsetus, Hypostomus auroguttatus* and *Geophagus brasiliensis*
70	Oliveira et al. (2012)	Plant	*Allium cepa* and *Eichhornia crassipes*
71	Rola et al. (2012)	Bivalve	*Mytilus edulis*
72	Seriani et al. (2012)	Fish	*Oreochromis niloticus*
73	Souza et al. (2012)	Bivalve	*Crassostrea gigas*
74	Azevedo et al. (2013)	Fish	*Cathorops spixii*
75	Bastos et al. (2013)	Fish	*Mugil* sp.
76	Davanso et al. (2013)	Crab	*Goniopsis cruentata*
77	Fuzinatto et al. (2013)	Fish	*Oreochromis niloticus*
78	Melo et al. (2013)	Fish	Multispecies
79	Ribeiro et al. (2013)	Fish	*Atherinella brasiliensis*
80	Seriani et al. (2013)	Fish	*Centropomus parallelus*
81	Sousa et al. (2013)	Fish	*Sciades herzbergii* and *Bagre bagre*
82	Souza et al. (2013)	Fish	*Centropomus parallelus*
83	Venancio et al. (2013)	Reptile	*Phrynops geoffroanus*
84	Batista et al. (2014)	Fish	*Astyanax bimaculatus*
85	Castro et al. (2014)	Fish	*Hoplias malabaricus*
86	Costa et al. (2014)	Plant	*Tradescantia pallida* var. *purpúrea*
87	Factori et al. (2014)	Plant	*Landoltia punctata*
88	Osório et al. (2014)	Fish	*Geophagus brasiliensis*
89	Pereira et al. (2014)	Bivalve	*Crassostrea rhizophorae* and *Perna perna*
90	Procópio et al. (2014)	Fish	*Prochilodus argenteus*
91	Vieira et al. (2014)	Fish	*Astyanax altiparanae*
92	Barrilli et al. (2015)	Fish	*Astyanax paranae, Phalloceros harpagos* and *Poecilia reticulata*

Table 1 continued

	Study	Bioindicator	Species
93	Bueno-Krawczyk et al. (2015)	Fish	*Astyanax bifasciatus*
94	Cruz et al. (2015)	Fish	*Oreochromis niloticus*
95	Gomes et al. (2015)	Plant	*Allium cepa*
96	Maceda et al. (2015)	Fish and plant	*Astyanax altiparanae* and *Allim cepa*
97	Prado et al. (2015)	Fish	*Achirus lineatus*
98	Seriani et al. (2015)	Fish	*Oreochromis niloticus*
99	Zanette et al. (2015)	Barnacles	*Balanus improvisus*

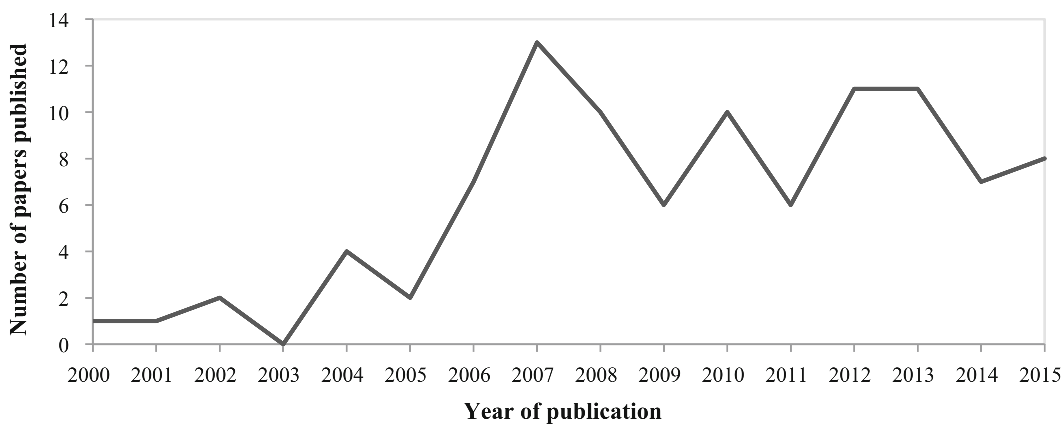

Fig. 1 Number of papers published in journals between January 2000 and July 2015 regarding the use of biomarkers to assess the water quality in Brazil

Table 2 Studies using biomarkers to assess the health of aquatic ecosystems in Brazilian regions

Region	Number of papers	%
Southeast	42	42.4
South	42	42.4
Northeast	9	9.1
North	3	3.0
More than one region[a]	2	2.0
Midwest	1	1.0
Total	99	99.9

[a] Studies with sampling sites located in different Brazilian regions

Table 3 Bioindicator organisms of studies using biomarkers to assess the health of aquatic ecosystems in Brazil

Organism	Number of studies	%
Fish	62	62.6
Bivalves	17	17.2
Plants	6	6.1
Others	11	11.1
More than one type of bioindicator	3	3.0
Total	99	100

Bivalves were the second group most common (17.2%). Bivalves present a wide geographic distribution, availability in different types of aquatic environments, possibility of breeding in aquacultures and are adequate for studies with caged organisms (Viarengo et al. 2007). Other bioindicator organisms, such as crabs, shrimps, worms and planarians were occasionally used. Additionally, two studies applied cell cultures of mammals' fibroblasts (V79 line) and human lymphocytes to assess water quality. Cell cultures are a useful tool in environmental evaluation, being an alternative methodology due to its easy manipulation and sensibility when exposed to physical and chemical agents; in addition, it presents good reproducibility (Rogero et al. 2003; Zegura et al. 2009). Despite the wide use in other countries (Leme and Marin-Morales 2009), the biomarkers analysis in plants to assess water quality is still unusual in Brazil since only six studies were found in the present review.

Types of studies

The studies using biomarkers for the evaluation of aquatic ecosystems can present different approaches. They can be field studies (organisms are sampled in situ), laboratory studies (water samples are collected in an area of interest and transported to the laboratory, where bioassays of exposure are conducted) and with caged organisms (animals are exposed in cages in the study area for a period of time). Considering studies carried out in Brazil, 68% were field studies, 19% were laboratory studies, 10% were with caged organisms and 3% combined laboratory and caged animals.

Complex exposure dynamics to pollutants and resulting biological responses found in the field are seldom replicated in laboratory studies (Crane et al. 2007), thus effects of pollution might be under or overestimated. Field studies comparing impacted and reference areas enable an evaluation of the health condition of organisms in their own environment, although organisms can move and potentially avoid contaminant hot spots in the field (Ward et al. 2013). In addition, it is not always possible to determine with precision the causal agent of any given alteration (Alberto et al. 2005). In this context, laboratory studies are extremely necessary to investigate the potential of an organism to be used as a bioindicator in field studies, and also enable a better understanding of chemical modes of toxicity. However, limitations of laboratory studies include the difficulty in incorporating native species into laboratory tests, problems with size and number of organisms that can be held in the laboratory and the inability of reproducing complex behaviors, such as spawning aggregation or migration (Hook et al. 2014). Moreover, less than 17% of laboratory studies were conducted with replicate. On the other hand, studies with caged organisms are more realistic than experiments conducted in laboratory in environmental assessment and present the advantage of using organisms with a known life history (Crane et al. 2007).

Reference areas and laboratory controls

Usually, tap and mineral water (for fish and bivalves assays) or distilled water (for plant assays) is used for the control group in laboratory experiments, while samplings in reference areas (areas under minor anthropogenic impact) are conducted in the field studies. However, finding clean areas can be difficult, and then, researchers choose to use controls in laboratory with animals acquired at breeding facilities and maintained in tap water, or do not use any type of control. For biomarker analysis, the use of negative control is important, although it is also possible to monitor temporal variations of a biomarker response in only one sampling site. In Brazil, taking into account only field studies and studies with caged animals, more than 75% used controls animals sampled at reference areas, whereas others used animals kept under laboratory conditions or did not use any type of control. Considering studies which reported sampling at reference sites, 27.1% found altered biomarker responses in organisms from these areas, evidencing the difficulty in finding clean areas under minor anthropic influence, and thus complicating the comparison of biomarker results.

Biomarkers used in Brazil

Various types of biomarkers have been used to assess the effects of exposure to pollutants in water, including morphometric indexes, histopathological alterations and molecular analyses. In Brazil, the biomarkers most frequently used were the micronucleus test, the biotransformation enzyme GST, the antioxidant enzyme CAT and histopathological analyses (Table 4). Other biomarkers not listed in Table 4 include the evaluation of physiological and hematological parameters, HSP70, vitellogenin, EROD, glutathione peroxidase (GPx), hepatic CYP1A and gene expression.

The DNA damage was assessed mainly by the comet assay (or single cell gel electrophoresis) and the micronucleus test. These techniques are sensitive, rapid and extensively used as genotoxic biomarkers (Zapata et al. 2016). The micronucleus test is one of the biomarkers most widely used for in situ monitoring of genotoxic pollution (Al-Sabti and Metcalfe 1995; Bolognesi et al. 2006; Udroiu 2006). This technique is based on the quantification of whole or fragmented chromosomes that are not incorporated into the main nucleus during mitosis (Al-Sabti and Metcalfe 1995). The comet assay is also an indicator of genotoxicity and an effective biomarker for detecting DNA strand breaks, cross-links and alkali labile sites in aquatic organisms (Tice et al. 2000; Frenzilli et al. 2009).

Xenobiotic metabolism is the central detoxification process that occurs in all the organisms. Phase I enzymes are involved in the first stage of detoxification of xenobiotics compounds and implicates in enzymatic transformation of a chemically modifying lipid soluble toxin into water-soluble toxin. Most of the transformation reactions in this phase include a broad family of enzymes, cytochrome P450s (Lardone et al. 2010). Cytochrome P450s are monooxygenases responsible by a set of functions for controlling homeostasis, including the metabolism of drugs and other xenobiotics (McDonnell and Dang 2013). Phase II enzymes are involved in the second stage of the detoxification process related to enzymatic conjugation. The enzymes of this phase (as GST) modify phase I products into more water-soluble and less toxic forms (Hassan et al. 2015). Antioxidant enzymes, such as CAT and SOD, are considered biomarkers of oxidative damage. Contaminant-stimulated “reactive oxygen species” (ROS) production and resulting oxidative damage may be a mechanism of toxicity in aquatic organisms exposed to a variety of pollutants (Livingstone 2001; Azevedo et al. 2013).

Histopathological analyses represent useful tools for environmental diagnosis and monitoring. This type of analysis provides a method for the detection of morphological alterations in multiple organs (Johnson et al. 1993), as gills and liver. The analysis of gills of fish and bivalves has been widely used because changes in this organ may lead to the impairment of several functions, including gas exchange, ion regulation and excretion of metabolites (Cruz et al. 2015). In addition to these characteristics, the low cost justifies the use of histopathological analyses in ecotoxicological studies.

The morphometric indexes were used in 18.2% of studies, mostly in fish species. The condition factor of the whole body (calculated using the weight and length) provides information on potential pollution impacts. Although this parameter is not very sensitive and may be affected by non-pollutant factors, such as season,

Table 4 Main biomarkers used in the assessment of health of aquatic ecosystems in Brazil

	Number of studies	%
Micronucleus test	31	31.3
Glutathione-S-transferase (GST)	28	28.3
Catalase (CAT)	26	26.3
Histopathological analyses	23	23.2
Comet assay	18	18.2
Morphometric index	18	18.2
Acetylcholinesterase/cholinesterase activity (AChE)	16	16.1
Lipoperoxidation analysis (LPO)	14	14.1
Metallothionein (MT)	9	9.1
Superoxide dismutase (SOD)	9	9.1
Others[a]	52	52.5

[a] Sum of studies which used other biomarkers

disease and nutritional level, it is used as an initial screening biomarker to indicate exposure and effects or to provide information on energy reserves (Mayer et al. 1992; Linde-Arias et al. 2008b). Its low cost, ease and rapidity makes it a valuable tool to assess preliminary effects of pollutants in fish (van der Oost et al. 2003).

The AChE enzyme occurs in cholinergic synapses and motor end plates, and is responsible for the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid. Inhibition of AChE has been associated with the mechanism of toxic action of organophosphates and carbamates insecticides (Galgani and Bocquene 1990; Payne et al. 1996; Valbonesi et al. 2003; Andreescu and Marty 2006). However, some studies assessing metal and polycyclic aromatic hydrocarbons (PAHs) exposure have also evidenced the inhibition of this enzyme (Zinkl et al. 1991; Akaishi et al. 2004; Richetti et al. 2011).

Other biomarkers analyzed include MTs and LPO. MTs are low molecular weight proteins, high cysteine content, and good heat stability that can be used as biomarkers (Langston et al. 1998). They consist of thiol groups (sulfur–hydrogen) that bind to metals, preventing oxidative stress to the organism. MT induction as a response to metal exposure is well documented in many species and is known to play a role in the detoxification to toxic metals (Amiard et al. 2006). LPO is a consequence of the decomposition of polyunsaturated fatty acid peroxides of membrane lipids, producing a complex mixture of hydroperoxides and secondary products of oxidation, as malondialdehyde (MDA) (Banerjee et al. 1999; Akhgari et al. 2003). LPO can be enhanced by exposure to xenobiotics and some trace metal in ionic form, leading to cellular damage (Viarengo et al. 1990; Montine et al. 2004; Filipak Neto et al. 2008).

Genomics is an emerging approach in biomarkers assessment. Genomics deals with the analysis of the complete genome to understand the function of single genes. On the other hand, functional genomics is based on the analysis of gene expression (transcriptomics) and comprehensive proteins/metalloproteins analysis (proteomics/metallomics) (González-Fernández et al. 2008). New approaches in functional genomics and bioinformatics can help discriminate individual chemicals, or group of chemicals among complex mixtures that may contribute to adverse biological effects (Hook et al. 2014). Furthermore, environmental metabolomics is an emerging field referred to the application of metabolomics to characterize the interactions of living organisms with their environment (García-Sevillano et al. 2015). In Brazil, these approaches are still rare since gene expression was assessed only in 3% of studies.

Multibiomarker approach (combination of two or more biomarkers) allows a better understanding of stress responses due to pollutants exposure (Domingos et al. 2007). This approach may provide results that can be complementary and help in cases when a single biomarker response is affected by non-pollutant factors. Most Brazilian studies used this approach, while 39.4% were carried out using a single biomarker (Table 5).

In 87.8% of studies, the authors reported a relationship between the biomarker responses and pollution in the sampling areas; however, such relation is subjective in most studies given the lack of well-designed experiments or statistical support. Furthermore, the level of environmental contamination was superficially diagnosed, since 51.5% of studies reported only the analysis of physicochemical parameters that can be obtained with portable apparatus (water temperature, salinity, pH or dissolved oxygen). The concentrations of at least one metal were assessed in 17.2% of studies, and one single study assessed the presence of PAHs in water. Pesticides, hormones and other emergent contaminants were not assessed. Most studies provided data on the possible type of pollution (oil spills, domestic sewage discharges, agricultural runoffs and industrial effluents) only based on previous studies, and/or local observations.

Table 5 Number of biomarkers used by studies to assess the health of aquatic ecosystems in Brazil

Number of biomarkers	Number of studies	%
One	39	39.4
Two	21	21.2
Three	11	11.1
Four or more	15	27.8
Total	99	100

Experimental designs

In addition to the use of a multibiomarker approach, the assessment of more than one sampling site in different sampling periods (seasonal variation) may help in the interpretation of biomarker responses. The number of sampling sites that were assessed in Brazil is shown in Table 6. Most studies were carried out in two or three sampling sites; however, 9.1% of studies analyzed one single sampling site. This is problematic because it precludes comparisons of the biomarker responses between organisms from different areas and does not provide significant information on the impacted area. Moreover, ~43% of studies were carried out in one single sampling period. Therefore, comparisons between periods were not performed, and consequently, these studies do not provide data on possible seasonal variations in biomarker responses and the real contamination scenario.

An important aspect in studies related to biomarker responses is the sample size—number of individuals analyzed per site in each sampling period or exposure experiment. Small sample size may lead to inconclusive results. As sample sizes increase, their variability tends to decrease, leading to a better hypothesis testing, a higher statistical power and smaller confidence intervals (Cochran 1977). However, sometimes it is not possible to maintain a certain sample size along the experimental design, especially in field studies. Table 7 shows the sample sizes in studies with biomarkers in the assessment of aquatic ecosystems in Brazil. Studies regarding biomarker responses with less than five organisms in at least one site or sampling period corresponded to 19.1%. Information on sample size was not provided or was not clear in 7.1% of studies, since only the total sample size (sum of organisms from all sampling sites studied and/or sampling periods) was reported.

Conclusions

In this review, we provide information on the use of biomarkers to assess the health of aquatic ecosystems in Brazil, in the last 16 years. In general, the approaches used in Brazilian studies did not differ from other countries. However, some shortcomings were observed. The data analyses points towards a limited use of this approach in the country (basically restricted to two regions), a great variety of organisms used as bioindicators (regional biodiversity) and different sampling patterns. A great number of studies were conducted using fish as bioindicator organisms, therefore, the analysis of others organisms should be stimulated. Furthermore, most studies used biomarkers which are easy, fast and cheap to assess, whereas biomarkers which require more funding and/or more sophisticated equipments were rare. Differently from other countries, water physico-chemical analyses are still poor and fail in providing information to establish relations between biomarker responses and contaminants. In general, a good relationship between biomarker responses and environmental pollution has been observed by the authors. However, experimental designs with multiple biomarkers, greater sample size, long-term biomonitoring and knowledge about organisms' ecological aspects may enable a better data interpretation on the environmental quality, as well as the interference of non-polluting factors in the biomarker responses. Additionally, new approaches in the genomics field may be a promising tool to better understand the impacts of sublethal concentrations of pollutants on living organisms, as well as to provide information on pollution-induced genetic changes in organisms' tolerance.

Table 6 Number of sampling sites of studies using biomarkers to assess the health of aquatic ecosystems in Brazil

Number of sampling sites	Number of studies	%
One	9	9.1
Two	31	31.3
Three	21	21.2
Four	18	18.2
Five	9	9.1
Six or more	11	11.1
Total	99	100

Table 7 Sample size (number of organisms analyzed per sampling site or exposure experiment) of studies using biomarkers to assess the health of aquatic ecosystems in Brazil

Sample size	Number of studies	%
Less than 5	19	19.1
6–10	41	41.4
11–20	16	16.2
21–49	9	9.1
50 or more	6	6.1
Not applied	1	1.0
Not informed/not clear	7	7.1
Total	99	100

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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