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Review

A brief review of the use of biomarkers in Mexico's aquatic ecosystems pollution assessment: 2001-2017

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ABSTRACT. The present work reviews the different biomarkers and organisms that have been used to assess pollution in aquatic ecosystems of Mexico in the last 16 years. Ninety-three publications were reviewed; they showed that 70 species, most of them native (70%), have been used for this purpose. Fish have been the most commonly used group, but other non-conventional organisms have also been used. Biomarkers of oxidative stress such as catalase and superoxide dismutase activity, as well as cellular lipid peroxidation, were the most widely used and versatile. Those used less frequently included Acetylcholinesterase, Ethoxyresorufin-O-deethylase or Metallothionein. The omic approach was used for Cytochrome P450, Vitellogenin and heat shock proteins. Sixty-two percent of the species were used only on one occasion during the period studied here, while 13% were used more than twice. *Girardinichthys viviparrus* and *Goodea atripinnis* were the most frequently used species due to their regional endemism, but their use was restricted to the center of the country. Forty-four percent of the studies evaluated the data from at least two weather stations, and only 10% of the studies monitored pollution levels during more than two seasonal cycles. In Mexico, traditional and omic biomarkers are commonly used by researchers; however, further investigation is needed to determine which species and biomarkers should be used for each region and particular situation. It requires a joint effort between research centers and public funding agencies for the development of regional and national monitoring networks.

Keywords: water quality, biomarkers, chemical pollution, Mexican waters.

INTRODUCTION

The chemical contamination of aquatic ecosystems is a growing problem since these ecosystems are the destination of most of the pollutants derived from industrial, agricultural and domestic activities (Sarukhán *et al*., 2012; Amiard-Triquet, 2015; WWAP, 2015). This problem is expected to worsen as a direct consequence of population growth, industrialization and the expansion of urban sprawl (Halder & Islam, 2015), as well as the deficiencies of current public sanitation programs and wastewater treatment systems (mainly in developing countries) (Schwarzenbach *et al*., 2010).

The contamination of aquatic ecosystems is a complex, evolving and wide-ranging problem, with direct and indirect ecological, economic and social repercussions (Fleeger *et al*., 2003; Persson *et al*., 2010). This problem has created the need to develop and implement strategies that guarantee the protection and sustainable use of aquatic resources (MEA, 2005). At the global level, this concern is reflected in the creation of national and international agreements and organizations (both governmental and non-governmental) (Burger, 2006).

Mexico faces great responsibilities regarding the conservation and management of its aquatic ecosystems; it has signed international agreements and treaties

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on the matter and has implemented laws, norms and national policies that promote the conservation of its aquatic ecosystems. It has also instituted monitoring programs such as the National Network of Water Quality Monitoring (RNM) (Comisión Nacional del Agua, 2015).

Environmental monitoring programs are a crucial tool to meet the commitments acquired in international treaties and conventions (such as the Minamata agreement, recently ratified by several countries of the European Union and by Mexico in 2015), and to guarantee the success of natural resource management programs. For several decades, aquatic monitoring programs and networks have worked in other countries, keeping track of changes in water quality (physical, chemical and microbiological), the presence and concentration of persistent toxic substances (metals, pesticides, etc.) in various environmental matrices (water, sediments, biota), and applying biotic indices based on diverse species (plants, mollusks and macroinvertebrates, among others) to determine the health status of the monitored ecosystems (Dixon & Chiswell, 1996; Markert *et al.*, 2003; Li *et al.*, 2010; Borja *et al.*, 2015).

The use of indicator species (bioindicators) made it possible to evaluate the biological response to the presence of contaminants (known and unknown) in different ecosystems. The implementation of tools such as Indices of Biotic Integrity (IBI), for example, provides information on the medium and long-term effect of contaminants at the higher levels of biological organization (populations and/or communities) (De la Lanza-Espino & Hernández-Pulido, 2014; Schmitter-Soto, 2014).

The need to identify earlier the effect of pollutants stimulated the use of early-response biomarkers. Even though they have been widely used by researchers for several decades to assess pollution in aquatic ecosystems, the use of biomarkers in monitoring programs is relatively new (Hook *et al.*, 2014; Trapp *et al.*, 2014). In recent decades, the U.S.A. and some countries of the European Union have incorporated the use of biomarkers into national monitoring programs (Collier *et al.*, 2012; Wernersson *et al.*, 2015). However, in other countries biomarkers are still little used in national monitoring programs (Trapp *et al.*, 2014). In Mexico, biomarkers have been little used in large-scale monitoring programs (temporal and spatial), but Mexican researchers have been using them to assess the contamination of aquatic ecosystems. The objective of this work was to conduct a review of the different biomarkers and organisms that have been used to evaluate the pollution of aquatic ecosystems in Mexico in the last 16 years (2001-2017).

Selection of bibliographic material

Different academic databases and search engines (Elsevier-Scopus, SCIELO, CONRICyT, and Google Scholar) were queried for scientific publications on the use of biomarkers to assess the pollution of aquatic ecosystems in Mexico (including studies that evaluated environmental samples). The search queries included combinations of keywords such as Mexico, ecosystems, aquatic, lagoon, estuary, bay, lake, river, wetland, pollution, pollutant, COPs, heavy metals, PCBs, PAHs, biomonitor, biomarker, genotoxic, histopathological, oxidative stress, cytotoxic, aquatic organisms, fish, bivalves, clams, crustaceans, aquatic birds, rotifers, and cladocerans, among others, both in Spanish and in English. The search range was from 2001 to 2017. The following criteria were used for selecting articles, and book chapters: 1) studies conducted in Mexican aquatic ecosystems, 2) field or laboratory studies (in the case of laboratory studies, those using water and/or sediment as exposure matrix, excluding air), and 3) studies on the use of biomarkers of effect and/or exposure, at the individual and/or sub-individual level.

Selected studies

Ninety-three publications were selected based on the above criteria. The chosen studies provide a representative picture of the field even though the total number of related studies conducted in Mexico during the studied period is higher (Dalzochio *et al.*, 2016). The number of associated publications per year in the period 2001-2017 had an upward trend, with an average of 5.5 papers per year (Fig. 1). The growing number of publications may be related to the increase in spending on science and technology by Mexico, which went from 0.31% of Gross Domestic Product (GDP) in 2000 to 0.55% of GDP in 2015. This type of research is financed mostly by federal and state funds through the National Council of Science and Technology (CONACyT). Although it has made some progress, Mexico is still one of the of OECD countries that invest less in science, given that the average spending on science of the member countries of the OECD was about 2.5% of GDP in 2015 (UNESCO, 2015; World Bank, 2018).

Use of reference organisms as biomarkers

In general, bioindicator organisms can be defined as whole organism systems with one or more easily detectable endpoints (for example, viability, metabolism, behavior, genetic damage, etc.) that respond to disturbances in their environment (Butterworth *et al.*, 2001), and which due to their ecological characteristics (Páez-Osuna & Osuna-Martínez, 2011; Berthet, 2012; González-Zuarth & Villarino, 2014) can be used as indicators of the ecological status of the ecosystems in

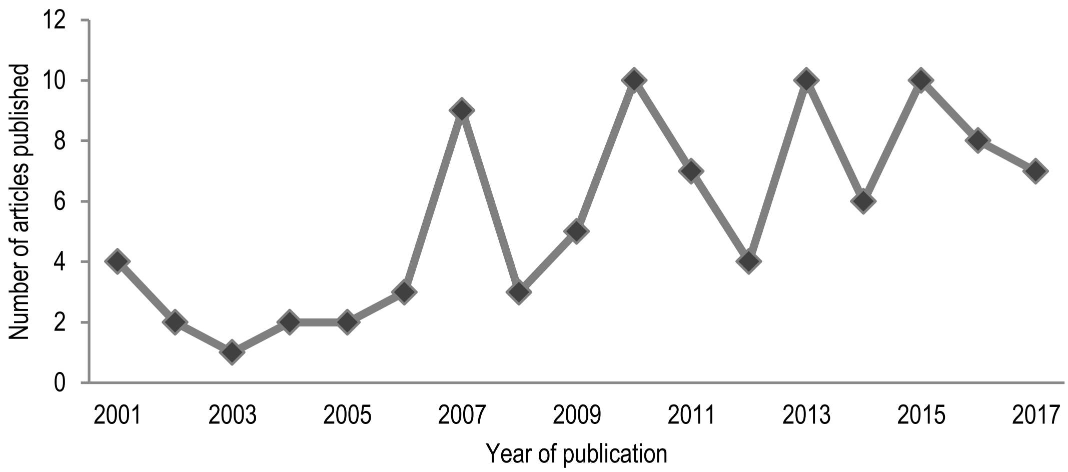

Figure 1. Number of published articles on the use of biomarkers to assess pollution in aquatic ecosystems in Mexico (2001-2017).

in which they inhabit. The use of bioindicators for the assessment of pollution allows for a more realistic analysis of the effect of various pollutants in different ecosystems, at the population, community and ecosystem level. Moreover, the use of the biomarker approach based on reference species provides information on the molecular, cellular, physiological and individual levels of an ecosystem (Van-der Oost *et al.*, 2003). In this regard, ecotoxicology can serve to evaluate the early effect of various pollutants through the use of a series of tools (molecular tests, bioassays, transplantation of organisms, etc.) that include not only ecologically relevant species, but also model organisms, transgenic organisms and even cell lines (human and animal) (Zhang *et al.*, 2013; Lee *et al.*, 2015). Currently, a large number of organisms (both wild and model) are used as bioindicators in pollution assessment studies in aquatic ecosystems; the most commonly used are fish (marine and freshwater), bivalves (marine), cladocerans, rotifers, macro-crustaceans, plants, and birds, among others (Zhou *et al.*, 2008; Li *et al.*, 2010; Minier *et al.*, 2015; Colin *et al.*, 2016).

Table 1 shows the variety of species that have been used in Mexico in biomarker studies to assess the pollution of aquatic ecosystems. The review of the data indicates that, in the last 16 years, close to 70 species, belonging to 19 taxa, have been used in this manner, fish being the most commonly used. Other groups of interest are cladocerans and bivalves. Non-conventional organisms such as turtles, sharks, crocodiles, and corals have also been used (Fig. 2).

Regarding the frequency with which the different species have been used, 62% of the species identified in the present study had only a single record between 2001-2017; 25% of the species had two records, and the remaining 13% were used in three or more studies. It is worth noting that of the nearly 70 species used, 50 are native to Mexico and were used in 62% of the studies; 8 can be considered as model species that are widely used in laboratories in different countries and were used in 25% of the total number of studies reviewed here. Another seven species can be considered an exotic species that have been introduced to Mexico (ornamental or aquaculture species) and were used in 8% of the studies. The remaining species were not identified (5%). The species that were most frequently used during the period under review are shown (Fig. 3).

The above data shows the great variety of native species that have been used to assess the pollution of aquatic ecosystems in Mexico. It is an indication of the efforts made to diversify the organisms used as bioindicators. Thus, improving the accuracy of the biological response by using the regions' native organism, or by comparing the response of different organisms at different trophic levels, is especially important for countries such as Mexico, which has a great variety of bioclimates. Moreover, the data shows that most native species were used only in one or two studies during the entire period under review, which makes it difficult to compare different experiences with the same species under different environmental conditions.

In contrast, model species such as *Daphnia magna* and *Cyprinus carpio* have been frequently used (Fig. 2). These organisms have been validated in many countries; however, since they are not representative of the ecosystems studied, their response cannot be considered entirely realistic. To make valid interpretations of the effects of pollution in a given ecosystem, researchers should use local species as bioindicators. In

Table 1. Basic information of the reviewed studies on the use of biomarkers to assess pollution in aquatic ecosystems in Mexico: 2001-2017. The column titled Origin refers to the origin of the organisms used, classifying them into the following categories. Model, refers to model organisms or organisms widely used in toxicological or ecotoxicological studies around the world; Native, refers to organisms that are native to Mexico; N/A: not applicable; N/E: not specified; Exotic, refers to organisms that are exotic to Mexico according to González *et al*. (2014). *Cortez-Gómez *et al*. (2018) was reviewed in 2017 when it was still in press.

Species	Biomonitor	Origin	Reference	
Limnodrilus hoffmeisteri	Oligochaeta	Model	Flores-Tena & Martínez-Tabche, (2001)	[1]
Limnodrilus hoffmeisteri	Oligochaeta	Model	Martínez-Tabche *et al*. (2001)	[2]
Lecane quadridentata	Rotifers	Model	Rico-Martínez *et al*. (2001)	[3]
Daphnia magna	Cladocerans	Model		
Daphnia pulex	Cladocerans	Native		
Simocephalus vetulus	Cladocerans	Native		
Daphnia magna	Cladocerans	Native	Villegas-Navarro *et al*. (2001)	[4]
No identificado	Fishes	Native	Favari *et al*. (2002)	[5]
Stagnicola sp.	Gastropods	Native	Martínez-Tabche *et al*. (2002)	[6]
Xiphophorus hellerii	Fishes	Exotic	Favari *et al*. (2003)	[7]
Xiphophorus hellerii	Fishes	Exotic	López -López *et al*. (2003)	[8]
Goodea atripinnis	Fishes	Native		
Ameca splendens	Fishes	Native		
Ariopsis assimilis	Fishes	Native	Noreña-Barroso *et al*. (2004)	[9]
Danio rerio	Fishes	Model	Báez-Ramírez & García-Prieto (2005)	[10]
Dendrocygna autumnalis	Birds	Native	Rendón-Von Osten *et al*. (2005)	[11]
Oreochromis niloticus	Fishes	Exotic	Gold-Bouchot *et al*. (2006)	[12]
Girardinichthys viviparus	Fishes	Native	López-López *et al*. (2006)	[13]
Gambusia yucatana	Fishes	Netive	Rendón-Von Osten *et al*. (2006)	[14]
Crassostrea virginica	Bivalves	Native	Gold-Bouchot *et al*. (2007)	[15]
Sorghum bicolor	Plants	Model	López-Hernández *et al*. (2007)	[16]
Daphnia pulex	Cladocerans	Native	Sánchez-Meza *et al*. (2007)	[17]
Lactuca sativa	Plants	Model		
Lecane quadridentata	Rotifers	Model	Santos-Medrano *et al*. (2007)	[18]
Daphnia magna	Cladocerans	Model		
Ameca splendens	Fishes	Native	Tejeda-Vera *et al*. (2007)	[19]
Goodea atripinnis	Fishes	Native		
Xenotoca melanosoma	Fishes	Native	Torres-Bugarin *et al*. (2007)	[20]
Oreochromis aureus	Fishes	Exotic		
Chirostoma consocium	Fishes	Native		
Chirostoma lucius	Fishes	Native		
Lepomis macrochirus	Fishes	Native		
Alloophorus robustus	Fishes	Native		
Zoogoneticus quitzeoensis	Fishes	Native		
Chapalychthys encaustus	Fishes	Native		
Poeciliopsis infans	Fishes	Native		
Goodea atripinnis	Fishes	Native		
Chelonia mydas agassizii	Turtles	Native	Valdivia *et al*. (2007)	[21]
Girardinichthys viviparus	Fishes	Native	Vega-López *et al*. (2007)	[22]
Carcinogenic cells MCF-7	Humans	N/A		
Ariopsis felis	Fishes	Native	Zapata-Pérez *et al*. (2007)	[23]
Haemulon plumieri	Fishes	Native	Alpuche-Gual & Gold-Bouchot (2008)	[24]
Simocephalus mixtus	Cladocerans	Native	Martínez-Jerónimo *et al*. (2008)	[25]
Daphnia magna	Cladocerans	Model		
Girardinichthys viviparus	Fishes	Native	Vega-López *et al*. (2008)	[26]
Megapitaria squalida	Bivalves	Native	Cantú-Medellín *et al*. (2009)	[27]
Crassostrea virginica	Bivalves	Native	Guzmán- Garcia *et al*. (2009)	[28]
Pocillopora capitata	Corals	Native	Liñán-Cabello *et al*. (2009)	[29]
Goodea atripinnis	Fishes	Native	Reinoso-Silva *et al*. (2014)	[30]

Continuation

Species	Biomonitor	Origin	Reference	
Girardinichthys viviparus	Fishes	Native	Vega-López *et al*. (2009)	[31]
Crassostrea corteziensis	Bivalves	Native	Bernal-Hernández *et al*. (2010)	[32]
Megapitaria squalida	Bivalves	Native	Escobedo-Fregoso *et al*. (2010)	[33]
Ariopsis felis	Fishes	Native	González-Mille *et al*. (2010)	[34]
Centropous parallelus	Fishes	Native		
Oreochromis sp.	Fishes	Exotic		
Mugil cephalus	Fishes	Native		
Cyprinus carpio	Fishes	Model	Galar-Martínez *et al*. (2010)	[35]
Ankistrodesmus falcatus	Plankton	Model	López-López *et al*. (2010)	[36]
Hyallela azteca	Amphipod	Native		
Ambystoma mexicanum	Tritons	Native		
Chelonia mydas	Turtles	Native	Richardson *et al*. (2010)	[37]
Caretta caretta	Turtles	Native		
Lepidochelys olivacea	Turtles	Native		
Nassarius vibex	Gastropods	Native	Rodríguez-Romero (2010)	[38]
Daphnia magna	Cladocerans	Model	Salazar-Coria *et al*. (2010)	[39]
Panagrellus redivivus	Nematode	Model		
Vibrio fischeri	Bacteria	Model		
Salmo trutta	Fishes	Exotic		
Mugil curema	Fishes	Native	Ríos-Sicarios *et al*. (2010)	[40]
Lecane quadridentata	Rotifers	Model	Torres-Guzmán *et al*. (2010)	[41]
Daphnia magna	Cladocerans	Model		
Pandion haliaetus	Birds	Native	Rivera-Rodríguez & Rodriguez-Estrella (2011)	[42]
Echinolittorina ziczac	Birds	Native		
Cerithium lutosum	Birds	Native		
Goodea atripinnis	Fishes	Native	Arévalo-Hernández *et al*. (2011)	[43]
Chelonia mydas	Turtles	Native	Labrada-Martagón *et al*. (2011)	[44]
Goodea atripinnis	Fishes	Native	López-López *et al*. (2011)	[45]
Danio rerio	Fishes	Model	Rodríguez-Fuentes *et al*. (2011)	[46]
Astyanax aeneus	Fishes	Native	Trujillo-Jimenez *et al*. (2011)	[47]
Chirostoma riojai	Fishes	Native	Vega-López *et al*. (2011)	[48]
Crassostrea virginica	Bivalves	Native	Aguilar *et al*. (2012)	[49]
Prionace glauca	Sharks	Native	Barrera-Garcia *et al*. (2012)	[50]
Cupleidae spp. (embryo)	Fishes	Native	Jaward *et al*. (2012)	[51]
Goodea atripinnis	Fishes	Native	Ruiz-Picos & López-López (2012)	[52]
Prionace glauca	Sharks	Native	Barrera-Garcia *et al*. (2013)	[53]
Crassostrea corteziensis	Bivalves	Native	Girón-Pérez *et al*. (2013)	[54]
Rhinella marina	Anurans	Exotic	González-Mille *et al*. (2013)	[55]
Rhinella marina	Anurans	Exotic	Ilizaliturri-Hernández *et al*. (2013)	[56]
Vicia faba	Plants	Model	Juárez-Santa Cruz *et al*. (2013)	[57]
Goodea gracilis	Fishes	Native	Olivares-Rubio *et al*. (2013)	[58]
Cyprinus carpio	Fishes	Model	San juan- Reyes *et al*. (2013)	[59]
Astyanax aeneus	Fishes	Native	Trujillo-Jiménez *et al*. (2013)	[60]
Not identified	Fitoplankton	N/E	Vega-López *et al*. (2013)	[61]
Isurus oxyrinchus	Sharks	Native	Vélez-Alvez *et al*. (2013)	[62]
Lactuca sativa	Plants	Model	Rodríguez-Romero *et al*. (2014)	[63]
Chirostoma jordani	Fishes	Native	Dzul-Caamal *et al*. (2014)	[64]
Cyprinus carpio	Fishes	Model	García-Nieto *et al*. (2014)	[65]
Cyprinus carpio	Fishes	Model	González-González *et al*. (2014)	[66]
Daphnia magna	Cladocerans	Model	Mejía-Saavedra *et al*. (2014)	[67]
Crassostrea gigas	Bivalves	Exotic	Vázquez-Boucard *et al*. (2014)	[68]
Ankistrodesmus falcatus	Fitoplankton	Model	Abeja-Pineda *et al*. (2015)	[69]
Humans	Humans	N/A	Alvares-Moya & Reynoso-Silva (2015)	[70]
Crocodylus moreletii	Crocodiles	Native	Buenfil-Rojas *et al*. (2015)	[71]
Plicopurpura pansa	Gastropods	Native	Domínguez-Ojeda *et al*. (2015)	[72]

Continuation

Species	Biomonitor	Origin	Reference	
Cyprinus carpio	Fishes	Model	Morachis-Valdez *et al*. (2015)	[73]
Cyprinus carpio	Fishes	Model	Neri-Cruz *et al*. (2015)	[74]
Girardnichthys viviparus	Fishes	Native	Olivares-Rubio *et al*. (2015)	[75]
Goodea atripinnis	Fishes	Native	Ruiz-Picos *et al*. (2015)	[76]
Cyprinus carpio	Fishes	Model	San juan- Reyes *et al*. (2015)	[77]
Selenastrum capricornutum	Fitoplankton	Model	Sobrino-Figueroa *et al*. (2015)	[78]
Daphnia magna	Cladocerans	Model		
Crocodylus moreletii	Crocodiles	Native	Dzul-Caamal *et al*. (2016)	[79]
Girardinichthys viviparus	Fishes	Native	Dzul-Caamal *et al*. (2016)	[80]
Fulica americana	Birds	Native	López-Islas *et al*. (2016)	[81]
Chirostoma jordani	Fishes	Native	López-López *et al*. (2016)	[82]
Ambystoma mexicanum	Tritons	Native		
Hyalella azteca	Amphipod	Native	Novoa-Luna *et al*. (2016)	[83]
Cyprinus carpio	Fishes	Model	Olvera-Néstor *et al*. (2016)	[84]
Ambystoma mexicanum	Tritons	Native	Ortiz-Ordoñez *et al*. (2016)	[85]
Crassostrea corteziensis	Bivalves	Native	Toledo-Ibarra *et al*. (2016)	[86]
Crassostrea sp.	Bivalves	N/E	Bautista-Covarrubias *et al*. (2017)	[87]
Lepidochelys olivacea	Turtles	Native	Cortez-Gómez *et al*. (2018)*	[88]
Rhincodon typus	Fishes	Nativo	Fossi *et al*. (2017)	[89]
Haemulon aurolineatum	Fishes	Native	Gold-Bouchot *et al*. (2017)	[90]
Ocyurus chrysurus	Fishes	Exotic		
Daphnia magna	Cladocerans	Model	Guerrero-Jiménez *et al*. (2017)	[91]
Lecane quadridentata	Rotifers	Model		
Fulica americana	Birds	Native	López-Islas *et al*. (2017)	[92]
Cyprinus carpio	Fishes	Model	Pérez-Coyotl *et al*. (2017)	[93]

Mexico, species such as *Goodea atripinnis* and *Girardinichthys viviparus* have been frequently used to assess pollution in aquatic ecosystems, mainly in the center of the country. Other species such as *Crassostrea virginica* and *C. corteziensis* have been used in the Mexican northwestern Pacific coast; in addition to bivalves (for example *Crassostrea* and *Megapitaria*), they have proven to be very useful in this environment due to their excellent qualities as bioindicators (Páez-Osuna & Osuna-Martínez, 2011). Other organisms considered validated in Mexico, include *Gambusia yucatana* (Rendón-Von Osten, 2015), cladocerans (Mendoza-Cantú *et al*., 2013) and rotifers (Rico-Martínez *et al*., 2013). However, these organisms have been little used to evaluate the effect of pollution on environmental samples (*in situ* or *ex-situ*). Probably be because these and other species used as bioindicators are endemic to specific regions of the country, as is the case of *Goodea atripinnis* and *Girardinichthys viviparus*, a consequence of the climatic and orographic diversity of Mexico (Dzul-Caamal *et al*., 2012).

Use of biomarkers

Biomarkers provide information on the early effects of exposure to environmental pollutants at organism or sub-organism levels, allowing researchers to detect and quantify these effects during their first manifestations, facilitating the implementation of a rapid preventive and/or restorative response in impacted ecosystems (Amiard-Triquet & Berthet, 2015). Biomarkers can be defined as biochemical, cellular, physiological or behavioral variations that can be measured in fluid or tissue samples at the whole organism level and which provide evidence of exposure to and/or effect of one or more pollutants (Van der Oost *et al*., 2003).

For several decades, biomarkers have been widely used by researchers to assess the effect of environmental pollution and have recently been integrated into monitoring programs in some countries (Collier *et al*., 2012; Wernersson *et al*., 2015). Biomarkers can be classified into effect biomarkers, exposure biomarkers and susceptibility biomarkers (Van der Oost *et al*., 2003), or into defense biomarkers, damage biomarkers, energy biomarkers and behavioral biomarkers (Amiard-Triquet & Berthet, 2015). It is well known that biomarkers can be influenced by variation factors (Forbes *et al*., 2006) that are both intrinsic and extrinsic to the test organism (Amiard-Triquet & Berthet, 2015). However, several biomarkers have been validated, both in laboratory and field tests. Thus, they can be used successfully, with due precautions (Forbes *et al*., 2006), and thanks to their specificity (see Rendón-

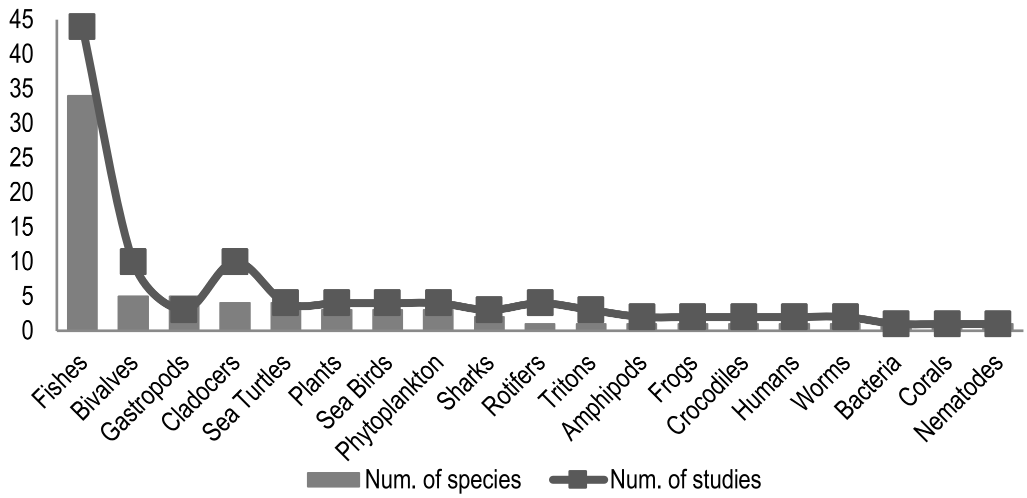

Figure 2. The number of species of each group of organisms used as bioindicators, and the number of studies in which they have been used. The total number of studies differs from the total number of articles because some studies used more than one species.

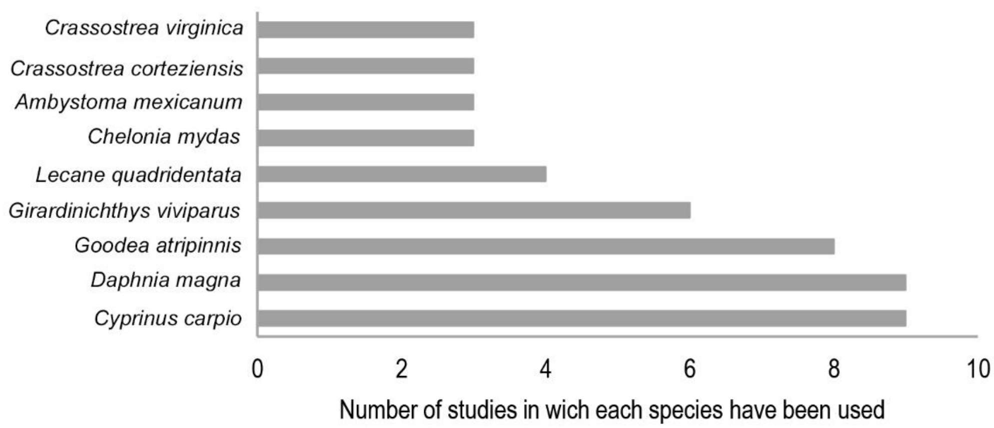

Figure 3. Most used species in biomarker studies in Mexico between 2001 and 2017. The total number of studies differs from the total number of articles because some studies used more than one species.

Von Osten, 2005 and Hook *et al*., 2014 for an in-depth discussion of the biomarkers specificity most commonly used to evaluate aquatic contamination), to determine the presence and effects of various pollutants (metals, PAHs, pesticides and estrogenic compounds, mainly). Which is why several of these biomarkers are well recommended for regulatory applications and monitoring programs (Roméo & Giambérini, 2012).

The selection of suitable biomarkers for use in ecotoxicological studies depends on several factors, including the type of pollutant to be evaluated, the reference species, or even technical and budgetary factors (Rendón-Von Osten, 2005). The present work found that a great variety of biomarkers have been used in Mexico to evaluate the effect of pollution in the aquatic ecosystems. The biomarkers that have been used, the associated organisms, and the different pollution scenarios in which they have been used are shown (Table 2). In this Table we shown the complex pollution conditions that can be found in aquatic ecosystems in Mexico; it also shows that the most commonly used biomarkers, which were associated with a wide variety of organisms, are non-specific and of the rapid response type, such as biomarkers of oxidative stress, which include CAT, SOD, and TBARS, among others. This strategy is recommended for complex pollution scenarios because these biomarkers respond to a wide variety of pollutants and mixtures of contaminants. These characteristics make this type of biomarker a versatile and relatively cheap tool that can be suitable for a first assessment of the effect of pollution on aquatic ecosystems in complex pollution scenarios.

Specific biomarkers such as δ-ALAD, which is specific for lead (Wernersson *et al*., 2015), and semi-specific biomarkers such as Vitellogenin, AChE, EROD and MT's, among others, were also used during the period under review. These biomarkers can be used as evidence of the presence of a group of pollutants (heavy metals, PAHs, organophosphates, pharmaceuticals) when no previous evidence has been found, or to correlate the level of response to a given concentration of the pollutant when its presence is already known. In Mexico, specific and non-specific biomarkers have been used simultaneously in different scenarios.

Some researchers recommend the use of biomarkers in association with the so-called omic sciences (mainly genomics, transcriptomics, proteomics, and metabolomics) (Martyniuk & Simmons, 2016). These new approaches offer a number of advantages that allow an in-depth analysis of the effect of pollutants on biological systems, which can be used to find new and better biomarkers, and to shorten the time to implement preventive and/or restorative actions in the affected areas. However, these tools also have certain drawbacks that may limit their application in monitoring programs; for example, they are relatively expensive and require an in-depth knowledge of bioinformatics, as well as access to omic databases, which currently have information only about a limited number of wild aquatic species (González & Pierron, 2015). In Mexico, omic approaches have been used (although very little) in association with some biomarkers, such as VTG and CYP1A1, and oxidative stress biomarkers such as SOD, GST, and HSP70 (Table 2).

One of the advantages of the use of omic tools in ecotoxicological studies is the fact that they allow to extract biological material in a non-destructive way (through the extraction of biopsies and/or fluid samples), which can reduce the pressure on the populations studied (*e.g*., protected species) and improve the bioethical standards of *ex-situ* tests. In most of the studies reviewed here, the biological material was obtained using destructive techniques; however, non-destructive techniques have been used to obtain skin biopsies from fish (Fossi *et al*., 2017) and crocodiles (Dzul-Caamal *et al*., 2016), blood samples from turtles (Labrada-Martagón *et al*., 2011) and skin mucus from fish (Dzul-Caamal *et al*., 2016), among others.

The present work also showed that the reviewed studies used biomarkers under three main approaches, which can be classified as follows:

a) Baseline studies: This type of studies aims to evaluate a biomarker's behavior within a reference species under certain environmental conditions (scenarios with known or unknown contamination). In general, these studies compare the behavior of a biomarker between sexes, sizes, reproductive stages or organs; when several species are used, the behavior of the biomarker is compared between them. Thus, this type of studies can be used to study how the behavior of different biomarkers vary in species that had not been previously considered as bioindicators and can help identify species with potential to be used as such. These studies constituted 20% of the total number of studies reviewed here.

b) Studies of the association between pollutants and effects. This type of research aims to correlate the response of a biomarker with the concentration of one or several contaminants of interest. For example, these studies can be used to identify which biomarkers are more sensitive to certain pollution conditions, and this can serve to validate their use in pollution assessment studies. This type of approach was used in 30% of the studies reviewed here.

c) Characterization studies. This type of studies aims to characterize the study areas based on the response of the biomarkers used and can be used to find sites of interest or pollution hot spots. In general, a single reference species is used in large or multiple study areas, for two or more sampling campaigns; frequently an already known species and validated biomarkers are used. This type of approach was used in 50% of the studies reviewed here. Most studies with this approach evaluated only a single area and carried out only one sampling campaign in a single annual cycle. However, some of these studies involved two or more sampling campaigns during an annual cycle (such as spring, summer or rainy and dry seasons); these cases are already considered monitoring studies.

Use of biomarkers to monitor aquatic pollution in Mexico

It is a fact that monitoring programs are one of the most important tools for the protection of aquatic ecosystems and for ensuring rational use of the resources of these ecosystems, as well as for complying with the commitments acquired in international treaties and agreements. In past decades, aquatic pollution monitoring programs focused on measuring physical and chemical variables, while biological variables were only occasionally taken into account (Lam, 2009). Currently, this approach is used by many countries. In Mexico, the RNM monitors water quality from a physical, chemical and bacteriological point of view (CNA, 2015); however, this type of approach only pro-

Table 2. Biomarkers used to evaluate aquatic contamination in Mexico: 2001-2017. The numbers in square brackets refer to the numerical assignment made in Table 1.

Biomarkers	Sources of contamination or pollutants associated with the study areas	Organisms used in the different studies
Acetylcholinesterase (AChE)	Agricultural and urban runoff, organochlorine and organophosphates pesticides, PAHs, PCBs, heavy metals	Fishes [5, 7, 8, 12, 13, 14, 19, 24, 64]; Bivalves [15, 32, 68, 86, 87]; Amphipods [36]; Birds [11]; Oligochaetes [2]; Gastropods [6]; Tritons [36]; Birds [11]; Phytoplankton [36]
Alanine aminotransferase (ALT)	Urban wastewater and agricultural runoff	Birds [92]
Alcohol dehydrogenase (ADH)	PCBs	Fishes [31]
Algal growth potential	Urban wastewater, agricultural runoff, heavy metals, PAHs	Phytoplankton [36, 61, 69]
Alkaline phosphatase (ALP)	Urban wastewater, agricultural runoff, heavy metals, PAHs	Fishes [8]
Apoptosis tunnel assay	Hospital and urban effluents, pharmaceutical products, heavy metals.	Fishes [77, 84, 93]
Bioluminescence inhibition	Refineries, aromatic hydrocarbon	Bacteria [39]
Carboxylesterases (CbE)	Benzo (a) pyrene and chlorpyrifos	Fishes [24]
Caspase-3 activity	Hospital and urban effluents, pharmaceutical products, heavy metals	Fishes [77, 84, 93]
Catalase activity (CAT)	Urban and industrial wastewater, agricultural runoff, heavy metals, PAHs, pharmaceutical products, organochlorine pesticides, halomethanes	Fishes [26, 35, 45, 48, 52, 58, 59, 66, 73, 74, 80, 82]; Bivalves [27, 54, 86]; Sharks [50, 53, 62]; Turtles [21, 44, 88]; Phytoplankton [61, 69]; Crocodiles [79]; Tritons [36, 82]; Corals [29]; Amphipods [83]
Condition index	Urban and industrial wastewater, agricultural runoff, heavy metals, halomethanes	Fishes [39,58, 60, 82]; Bivalves [49]; Birds [81,92];
Ethoxyresorufin-O-deethylase (EROD)	Urban and industrial wastewater, agricultural runoff, PCBs, PAHs, organochlorine pesticides	Fishes [12, 19, 31, 39, 47, 75]
Cytochrome P450-1A1(CYP1A1) (regard western-blotting and gene expression)	Urban and industrial wastewater, agricultural runoff, PCBs, PAHs, organochlorine pesticides, heavy metals, halomethanes	Fishes [12, 40, 46, 48, 58, 89, 90]; Turtles [37]; Crocodiles [79]
Embryo deformities	PAHs	Fishes [51]
Comet assay	Urban wastewater, organochlorine pesticides, pharmaceutical products, heavy metals, PAHs, PCBs	Fishes [10, 30, 34, 43, 65, 77, 93]; Anurans [55]; Bivalves [68]; Humans [70]
Epoxide hydrolase (EH1)	Urban wastewater, halomethanes	Fishes [75]
Esterases	Agricultural, urban and industrial runoff	Rotifers [3, 18]
Gamma-glutamyl transferase (GGT)	Urban and agricultural runoff, organochlorine and organophosphorus pesticides	Fishes [5, 8, 19]; Birds [92]
Glutathione S-transferase activity (GST) (regard gene expression)	Urban wastewater and agricultural runoff, heavy metals, PAHs, PCBs, Halomethanes, Organochlorine pesticides	Fishes [47, 48, 90]; Bivalves [27, 68, 86]; Sharks [62]; Turtles [21, 37, 88]; Phytoplankton [61]; Tritons [36]; Corals [29]
Glutathione peroxidase activity (GPx) (include gene expression)	Urban and industrial wastewater, agricultural runoff, heavy metals, PAHs, pharmaceutical products, organochlorine pesticides, halomethanes	Fishes [35, 45, 48, 52, 58, 59, 73, 82]; Bivalves [86]; Sharks [62]; Turtles [44]; Phytoplankton [61, 69]; Tritons [36, 82]; Corals [29]; Amphipods [83]
Glutathione reductase activity (GR) (regard gene expression)	Urban wastewater and agricultural runoff, heavy metals, PAHs	Sharks [50, 53, 62]; Turtles [88]; Fishes [80]; Crocodiles [79]
Heat shock protein (HSP70)	Urban and agricultural effluents	Fishes [40]
Histopathological lesions (HPL)	Urban and agricultural effluents, PAHS, PCBs, Heavy metals, organochlorine pesticides	Bivalves [15, 28]; Fishes [9, 40, 76]; Tritons [85]; Birds [82, 92]
Hydrogen peroxide (H_2O_2) content	Urban wastewater and agricultural runoff, PAHs, Heavy metals, halomethanes	Fishes [58, 80]; Bivalves [54]; Crocodiles [79]
Hydroperoxides (ROOH) content	Urban wastewater and agricultural runoff, PAHs, heavy metals, halomethanes, pharmaceutical products	Fishes [58, 59, 66, 73, 74]; Bivalves [54, 86]; Plankton [61]; Amphipods [83]

Continuation

Biomarkers	Sources of contamination or pollutants associated with the study areas	Organisms used in the different studies
Imposex	Port activity and agricultural areas	Gastropods [38, 72]
Germination index	Agricultural areas	Plants [63]
Root elongation index	Urban and agricultural areas, industrial effluents	Plants [16, 17, 63]
Gonadosomatic index (GSI)	Urban wastewater and agricultural runoff, industrial discharges	Fishes [60, 82]; Birds [81, 92]
Hepatosomatic index (HSI)	Urban wastewater and agricultural runoff, industrial discharges	Fishes [39, 58, 60, 82]; Birds [81, 92]
Ingestion rate	Industrial wastewater	Cladocerans [18]
Lactate dehydrogenase (LDH)	Diffuse pollution, hospital effluent	Fishes [47, 84]
Lethality	Urban and industrial wastewater, PAHs, PCBs, hormones,	Fishes [22, 26]; Rotifers [41, 91]; Nematodes [39]; Cladocerans [3, 4, 17, 25, 41, 67, 78, 91]
Lipid peroxidation (TBARS)	Urban and industrial wastewater, agricultural runoff, heavy metals, PAHs, organochlorine pesticides, Organophosphorus pesticides, Heavy metals, Pharmaceutical products, Halomethanes	Sharks [50, 53, 62]; Bivalves [27, 54, 86]; Crocodiles [79]; Fishes [5, 7, 8, 13, 19, 26, 28, 32, 35, 44, 45, 47, 48, 52, 58, 59, 60, 66, 73, 74, 80, 82]; Corals [29]; Turtles [21, 44]; Amphipods [36, 83]; Gastropods [6]; Tritons [36, 82, 85]; Phytoplankton [61, 69]; Birds [81, 92]
Metallothionein (MT's)	Urban wastewater and runoff, PAHs, PCBs, heavy metals, hormones	Fishes [75, 80]; Bivalves [15, 32, 33]; Crocodiles [71]
Mycosporine-like amino acids (MAAs)	Urban wastewater	Corals [29]
Na/K-ATPase	Urban and industrial wastewater	Fishes [45]
Neutral Red Retention Time (NRRT)	Organochlorine pesticides, PCBs, PAHs, heavy metals	Bivalves [15]
PAH bile metabolites	PAHs, PCBs, heavy metals	Fishes [12, 90]
Phospholipase A2	Agricultural, urban, and industrial runoff	Rotifers [3, 18]
Carbonyl radical (RC=O) content	Urban wastewater and agricultural runoff, PAHs, heavy metals, halomethanes, pharmaceutical products	Fishes [58, 59, 66, 73, 74, 80]; Crocodiles [79]; Phytoplankton [61]; Amphipods [83]; Sharks [62]
Superoxide dismutase activity (SOD)	Urban wastewater and agricultural runoff, heavy metals, PAHs, pharmaceutical Urban wastewater and agricultural runoff, heavy metals, PAHs, pharmaceutical products, Halomethanes	Fishes [26, 35, 45, 48 , 52, 58, 59, 66, 73, 74, 80, 82]; Phytoplankton [61, 69]; Sharks [50, 53, 62]; Crocodiles [79]; Turtles [21, 44]; Corals [29]; Tritons [36, 82]; Bivalves [86]; Amphipods [83]
Superoxide radical ($O_2 \bullet-$) content	Urban wastewater and agricultural runoff, PAHs, heavy metals	Fishes [58, 80]; Bivalves [54]; Sharks [50, 53, 62]; Turtles [21]; Crocodiles [79]; Corals [29]
Vitellogenin (VTG) (include gene expression)	Urban wastewater and agricultural runoff, PAHs, PCBs, organochlorine pesticides, hormones	Fishes [22, 23,46, 75, 80, 90]
δ-aminolevulinic acid dehydratase (δ-ALAD)	Urban areas and petrochemical industry	Anurans [56]
Micronucleus (MN)	Urban wastewater, agricultural runoff, organochlorine pesticides, PCBs, pharmaceutical products	Fishes [20, 34, 65, 77, 84, 93]; Plants [57]

vides information about the nature of the pollutants and their concentrations in the environment, but cannot predict their possible effects on the organisms that inhabit the affected ecosystems (Lam, 2009). It is currently accepted that the careful use of biomarkers may be the best tool to assess the early effects of aquatic pollution (Hook *et al*., 2014); however, biomarkers are not widely used in national monitoring programs. The European Union has incorporated the use of biomarkers into monitoring programs; for example, under the framework of the Marine Strategy Framework Directive (MSFD), a series of biomarkers (EROD, AChE, Vtg, MT's, PAH bile metabolites, ALAD, among others) have been used to monitor the effect of pollution on European coasts (Wernersson *et al*., 2015). Moreover, decades ago the United States implemented the use of biomarkers (histopathology, PAH bile metabolites and CYPA1 in benthic fish) to evaluate the effects of pollution (PAHs mainly) in lakes and rivers (Collier *et al*., 2012).

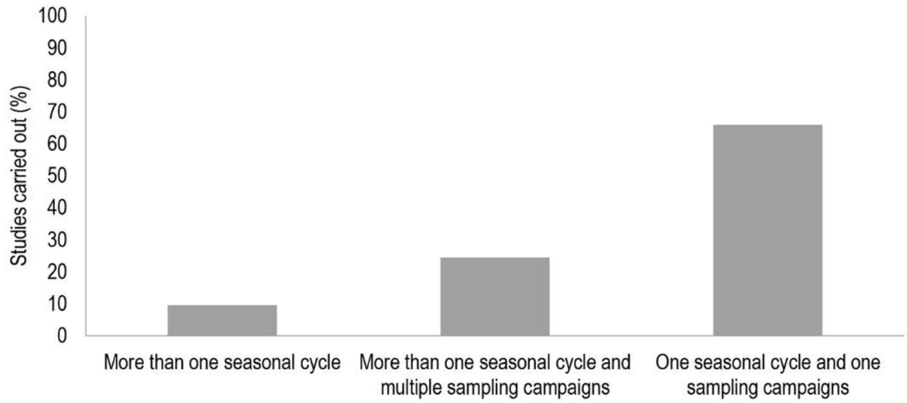

Figure 4. Temporal range of biomarker studies carried out in Mexico between 2001 and 2017.

In Mexico as in other countries, biomarkers are not used in national monitoring programs; however, as already mentioned, researchers have used biomarkers in monitoring studies. Close to 34% of the studies reviewed here evaluated the behavior of different biomarkers in two or more sampling campaigns (Fig. 4). Biomarkers can be affected by a series of sources of variation called confounding factors, which may be intrinsic (size, weight, age, sex, reproductive stage, etc.) or extrinsic (temperature, salinity, dissolved oxygen, pH and time of year) to the test organism (Amiard-Trique & Berthet, 2015).

Monitoring studies allow us to understand the patterns of variation within an annual cycle; for example, changes between spring, summer, autumn, and winter, or, in tropical zones, between the rainy season and the dry season. Moreover, because pollutants flow into aquatic ecosystems constantly, carrying out more than one sampling campaign allows understanding the relationship between variations in the effect of pollutants and these natural cycles, making it possible to identify the seasons in which organisms are more or less affected by pollutants.

For example, Toledo-Ibarra *et al.* (2016) and Bautista-Covarrubias *et al.* (2017) studied an estuary (Boca de Camichín Estuary) in northeastern Mexico, that is under the strong influence of agricultural areas during the dry and rainy seasons. Both studies used ACEh in bivalve gills (*Crassostrea*), and both studies found that ACEh decreases considerably from the dry to the rainy season because of the increase of pollutants during the rainy season, which inhibits the activity of ACEh in bivalve gills. As was corroborated by Toledo-Ibarra *et al.* (2016) who evaluated eight aquatic bodies under the influence of agricultural zones (Nayarit State, Mexico) and found the same pattern in all of them.

Monitoring studies not only evaluate the variation patterns within a single annual cycle but also seek to understand how the effects of pollution evolve; thus, it is recommended to extend the studies to more than one annual cycle (monitoring programs). As mentioned earlier, in Mexico, biomarker studies that evaluate more than one annual cycle are still scarce (Fig. 4). Although this review found that some study areas were assessed in more than one occasion during the review period, in most of these occasions different types of organisms and different biomarkers were used, and this makes it difficult to understand the evolution of the effects of pollution in those study areas. Only in very rare cases, one area was evaluated using the same group of organisms and biomarkers (*e.g.*, Vega-López *et al.*, 2007, 2008, 2009, 2011; Olivares-Rubio *et al*., 2013, Dzul-Caamal *et al*., 2014). Those studies analyzed a lake in the Valley of Mexico (Lake Zumpango) using fish (see Table 1 for species and Table 2 for biomarkers), showing how that ecosystem evolved.

Some of the reviewed studies evaluated an area for more than one annual cycle, sometimes for a period equivalent to two annual cycles, although most of those studies limited their evaluation to one and half cycles, allowing for a broader understanding of the behavior (evolution) of the effects of pollutants on living organisms. The main studies that evaluated the impact of pollution for more than one annual cycle are shown (Table 3).

The results of this review show that the main strategy used by biomarker studies that assessed pollution in aquatic ecosystems during more than seasonal cycle has been to carry out in situ studies using native fish. This strategy has the advantage of being cheaper and more practical than laboratory studies using environmental matrices. The results also show that most studies used multiple biomarkers, which can validate each other or detect anomalies. Although in a limited way, these studies allow observing the trend followed by biomarkers from one cycle to another. The

Table 3. Monitoring studies covering more than one seasonal cycle carried out in Mexico: 2001-2017. In the seasonal cycle [season] column, reference is first made to the seasonal cycles to which the sampling campaigns extend, including complete or incomplete cycles, and the second refers to the stations in which the sampling was conducted. *R: refers to the numerical assignment made in Table 1 to the reviewed articles.

*R	Study	Study zone	Seasonal cycles [season]	Reference species	Biomarkers	General trend between seasonal cycles
[14]	*In situ*	Marentes stream, Las Piñas stream	2 [Dry; Rains; Dry; Rains]	Fishes (*Gambusia yucatana*)	AChE	AChE it shows a decrease from dry to dry in Las Piñas Stream and does not present an important variation in Marentes Stream.
[19]	*In situ*	De La Vega dam	2 [Rains; Dry; Rains]	Fishes (*Ameca splendens*)	GGTP AChE EROD TBARS	TBARS hepatica presents a very high decrease of rains to rains. GGTP hepatic and AChE in muscle do not show significant variation from rains to showers. EROD in the liver presents a large reduction in rainfall to rainfall.
			2 [Rains; Dry; Rains]	Fishes (*Goodea atripinnis*)	GGTP AChE EROD TBARS	TBARS hepatica presents a large decrease in rainfall to rainfall. GGTP hepatic presents an increase, while AChE in muscle does not show significant variation from rains to rains. EROD in the liver presents a large increase in rainfall to rainfall.
[28]	*In situ*	Mandinga lagoon	2 [Dry; Rains; Dry; Rains]	Bivalves (*Crassostrea virginica*)	Condition index (K)	K shows an increase from dry to dry and decreases from rains to rains
[47]	*In situ*	Chanpoton river	2 [Dry; Rains; Dry]	Fishes (*Astyanax aeneus*)	TBARS GST EROD LDH	EROD in the liver does not show a significant variation from dry to dry. GST presents a large increase from dry to dry. EROD presents a decrease from dry to dry. LDH presents a very high rise from dry to dry.
[40]	*In situ*	Urías lagoon, Teacapán lagoon	2 [Rains; Dry; Rains]	Fishes (*Mugil curema*)	CYP1A HSP70	Hepatic CYP1A and HSP70 tend to decrease in Urias lagoon from rains to rains, while in Teacapán lagoon. CYP1A and HSP70 tend to increase from rains to rains.
[60]	*In situ*	Chanpoton river	2 [Dry- Rains- Dry	Fishes (*Astyanax aeneus*)	TBARS GC IGS IHS	TBARS Hepatic does not show a significant variation from dry to dry. GC presents a slight increase from dry to dry. IGS and IHS do not show considerable variation from dry to dry.
[75]	*In situ*	Mayor lake and Menor lake	2 [Rains- Dry- Rains- Dry]	Fishes (*Girardinichthys viviparus*)	VTG MT's EROD EH1	VTG in the liver of males from Menor lake there is a great increase in rains. Hepatic EROD showed a large decrease from dry to dry in Lake Mayor for both sexes. EH1 in the liver showed a rise from dry to drain in Mayor lake for both genders. MTs hepatica presents a great increase of the first cycle to second for both sexes of Menor lake.
[82]	*In situ*	Yuriria lagoon	2 [Rains; Dry; Rains]	Fishes (*Chirostoma jordani*)	SOD GPx CAT TBARS IHS IGS	CAT, GPx, SOD they decrease slightly from rains to rains. TBARS decreases from rains to rains. IGS tends to increase rainfall to rain while IHS does not vary significantly during cycles.

combination of all these elements allows reaching much more robust conclusions about the status of the study areas.

It is clear that, in recent decades, biomarkers have gone from being a good alternative tool for assessing pollution in aquatic ecosystems to be a necessary instrument for guaranteeing the protection, preservation, and management of these ecosystems. It has become evident that no pollution monitoring programs should be carried out without them. Mexico has a great responsibility because it has a great wealth of aquatic ecosystems; for example, it has 142 wetlands considered RAMSAR sites, making it the signatory country with the second largest number of sites registered under this agreement (RAMSAR Convention, 2018). This fact obliges the country to develop and implement strategies that guarantee the protection of its aquatic ecosystems. The present review shows that, in Mexico, the use of biomarkers in national monitoring programs to assess aquatic pollution has not been fully imple-mented yet. However, as in other countries, researchers have been using these tools in the last decades. The present review also shows which biomarkers and species have been used to assess pollution in aquatic ecosystems in Mexico.

In Mexico, researchers have used both classical biomarkers and omic biomarkers, although the latter approach has been used only rarely. The alternative, non-lethal strategies have been used to obtain samples, such as biopsies or fluids, in accordance with the need to develop new biomarkers and strategies for using them in large-scale monitoring programs. Although omic sciences have allowed the development of what has been proposed, as the next generation of biomarkers for ecotoxicological evaluations, in Mexico, as in other countries, these tools are just beginning to be used by researchers. Due to the technical and financial needs involved in their application, it is unlikely that their use will become widespread in the coming years. There is still the need to find effective strategies that can be applied to the national context, which could be done more easily if the country's research centers worked in a coordinated way to find and standardize the largest number of common biomarkers that offer a good cost-benefit ratio. It is also necessary to increase efforts regarding the study of reference species; although researchers in Mexico have experimented with a large number of native species, the results of this review show that only a small number of species were used repeatedly during the study period.

Nevertheless, the review also shows that some native species have already been validated, even though their use is limited to specific regions due to the high endemism rates in the country. Thus, the task remains to continue to the study species and biomarkers that can be used in each region of the country to implement a country-wide monitoring network. In general, it is possible to conclude that, in Mexico, the use of biomarkers in the assessment of the effects of aquatic pollution is a practice well known by researchers; however, there are still important challenges to face, which make it difficult to spread their use in the country. We must not forget the great responsibility that falls on Mexico as the owner of a great wealth of aquatic ecosystems, which requires the commitment not only of research centers but also of the government and the society in general.

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