Int Aquat Res (2020) 12:231–241
https://doi.org/10.22034/IAR.2020.1896881.1030

ORIGINAL RESEARCH

Mugil liza (Valenciennes, 1836) biomarker responses in a hypersaline lagoon in southeastern Brazil before and after a microalgal bloom

Manildo M Oliveira . Ana MF Ventura . Aline S Fraga . Maria Helena C Baeta Neves . Rafael F Dantas . Rachel A Hauser-Davis . Renato M Lopes . João B Salles . Frederico F Bastos . Vera Lúcia F Cunha Bastos . Jayme Cunha Bastos

Received: 14 April 2020 / Accepted: 04 September 2020 / Published online: 25 December 2020

Abstract Araruama lagoon is one of the largest permanent hypersaline lagoons in the world and has been impacted for twenty years by sewage dumping. In early 2005, a Prasinophyceae (*Pseudoscourfieldia* sp.) phytoplankton bloom occurred, leading to fish mortality during subsequent summers (2006 and 2007). In this context, the aim of the present study was to accompany this historical period through the use of biochemical biomarkers in mullet (*Mugil liza*) during and after the algal bloom. Mullet were collected from Araruama Lagoon in June and July 2005 and August 2007, as well as from a reference lagoon with no algal blooms, Saquarema Lagoon. Several enzymatic activities in liver were analyzed, namely glutathione peroxidase (GPX), catalase (CAT), total glutathione S-transferase (GSTt), specific GSTπ (pi) and GSTμ (mi) classes and ethoxyresorufin-O-deethylase (EROD), as well as muscle acetylcholinesterase activity (AChE). In 2005, only EROD activity was significantly increased at Araruama Lagoon, indicating induction of the biotransformation enzyme system (CYP 1A1). GSTπ, on the other hand, was lower in the Araruama Lagoon, especially in 2005, when compared to mullet from Saquarema lagoon (reference area). These results highlight the importance of developing actions that go beyond the physical-chemical monitoring of these environments, since the study of ecological relationships and the physiology of organisms affected by algal blooms also include biochemical parameters to evaluate new approaches to icthyotoxicity.

Keywords Harmful Algae Bloom . Erod . Lagoa de Araruama . Mullet

Introduction

Araruama Lagoon, located in the state of Rio de Janeiro, southeastern Brazil is a hypersaline lagoon which,

Manildo M Oliveira (✉)
Laboratório de Ecotoxicologia e Microbiologia Ambiental – Instituto Federal de Educação, Ciência e Tecnologia Fluminense - IFF - Cabo Frio - Brazil
e-mail: mmoliveira@iff.edu.br

MF Ventura
Colégio Estadual Professora Sonia Regina Scudese/Colégio Estadual Olga Benário Prestes - Rio de Janeiro - Brazil

Aline S Fraga
Colégio Pedro II - Rio de Janeiro - Brazil

Maria Helena C Baeta Neves
Departamento de Oceanografia Biológica - Instituto de Estudos do Mar Almirante Paulo Moreira - Arraial do Cabo - Brazil

Rafael F Dantas
Laboratório de Bioquímica Experimental e Computacional de Fármacos Instituto Oswaldo Cruz - FIOCRUZ - Rio de Janeiro - Brazil

Rachel A Hauser-Davis
Laboratório de Avaliação e Promoção da Saúde Ambiental, Instituto Oswaldo Cruz, FIOCRUZ - Rio de Janeiro - Brazil

Renato M Lopes
Laboratório de Comunicação Celular, Instituto Oswaldo Cruz, FIOCRUZ - Rio de Janeiro - Brazil

João B Salles
Centro Universitário Estadual da Zona Oeste (UEZO) - Campo Grande - Brazil

Frederico F Bastos, Vera Lúcia F Cunha Bastos, Jayme Cunha Bastos
Departamento de Bioquímica – IBRAG - Universidade do Estado do Rio de Janeiro - Rio de Janeiro - Brazil

until the 80's, was considered oligotrophic, with its crystal-clear blue waters attracting many tourists to the region. However, significant disordered population growth occurred on the shores of the lagoon in subsequent decades, unaccompanied by basic sanitation programs, increasing local anthropogenic pressures (Bertucci et al. 2016). During the next 20 years, high sewage inputs significantly altered the area, shifting the lagoon from oligotrophic (low ammonia, nitrogen and phosphorus levels) to eutrophic (high ammonia, nitrogen and phosphorus levels) (Rosa et al. 2016).

Eutrophic conditions include increased primary phytoplankton production at the expense of background macroalgae, resulting in reduced light incidence at the bottom of lagoon, increasing local biochemical oxygen demands (BOD) and leading to lower dissolved oxygen levels in the water (Coutinho et al. 1999, Souza et al. 2003). Current nutrient concentrations at Araruama Lagoon are about ten-fold higher than those found previously (Mello 2007), and high nitrate and phosphate levels may have been the cause for a 2005 algae bloom at the lagoon, as observed in other aquatic environments (Souza et al. 2003).

Algal blooms are harmful to aquatic ecosystems due to high biomass production, leading to decreased oxygen levels and, consequently, alterations in trophic ecosystem balances (Glibert 2017), resulting in mortality events due to damage to invertebrate filtration/circulation systems and fish gills (Hallegraeff 2004; Viana et al. 2019) and ecological damage, affecting both fishing activities and tourist attractions (Sanseverino et al. 2016). In addition, certain algal species able to produce toxic metabolites may result in the bioaccumulation of these compounds in certain organisms, such as shellfish and fish (Busch et al. 2016; Magalhães et al. 2003; Moroño et al. 2003; Turki et al. 2014). When algae blooms present some or all of the aforementioned characteristics mentioned above, they are termed harmful algal blooms (HABs) (Zohdi and Abbaspour 2019).

In this regard, a wide variety of toxic metabolites are produced by distinct algal taxonomic groups, including okadaic acid and dinophysistoxin, which may lead to diarrheic shellfish poisoning (DSP); gonyautoxin and saxitoxin, which may cause paralytic shellfish poisoning (PSP); domoic acid, which can lead to amnesic shellfish poisoning (ASP); azaspiracid, a known cause of azaspiracid shellfish poisoning (AZP); ciguatoxins, which causes ciguatera fish poisoning (CFP); and cyanobacterial toxins, such as microcystins, nodularin, saxitoxin, anatoxin-a, anatoxin-a(s) and cylindrospermopsins (Backer et al. 2015; Berdalet et al. 2016; Valdiglesias et al. 2013; Zabaglo et al. 2016). The frequency of these harmful events has recently prompted several international strategies for the management, monitoring, ecological and genetic studies of these blooms, in order to protect both natural resources and human health (Eurohab 2002; Geohab 2001; Harrness 2005).

The use of biochemical biomarkers facilitates the ecotoxicological understanding of harmful algal bloom effects in aquatic organisms, (de Morais Calado et al. 2020; Dalzochio et al. 2016; Prego-Faraldo et al. 2016). For example, phase 1 (Cytochromes P-450 enzymes - CYPs) and phase 2 (Glutathione S-transferases - GSTs) biotransformation enzymes are important in assessments concerning xenobiotic toxicokinetic mechanism activation (Bonansea et al. 2017), while antioxidant enzymes (GPx and Catalase) act against the increased pro-oxidant state produced by xenobiotics (Nunes et al. 2015). In turn, acetylcholinesterase, responsible for promoting cholinergic synapses in brain and muscle, is significantly affected by the presence of several neurotoxins, leading to loss of homeostasis (Araújo et al. 2018).

In this context, the aim of the present study was to evaluate biochemical biomarkers, namely Glutathione peroxidase (GPX), catalase (CAT), Acethylcholinesterase (AChE), glutathione-S-transferase (GST, as Total, GSTμ and GSTπ) and ethoxyresorufin-O-deethylase (EROD), in mullet (*Mugil liza*) liver and muscle samples obtained during and after a Prasinophyceae (*Pseudoscourfieldia* sp) algal bloom in the hypersaline Araruama Lagoon, located in Southeastern Brazil.

Materials and methods

Study area

Araruama Lagoon (Figure 1) is located in the state of Rio de Janeiro, between latitudes 22°50'S and 22°57'S and longitudes 42°00'W and 42°44'W. It covers 210 km^2 and extends to six cities, Saquarema, Araruama, São Pedro da Aldeia, Cabo Frio, Arraial do Cabo and Iguaba Grande. It is the largest permanent hypersaline lagoon in the world and is used for fishing, salt extraction and tourism. The Saquarema lagoonal system,

extending from 23°53'S, 42°39'W to 23°56'S, 42°28'W, forms five smaller lagoons: Fora (7.4 km^2), Jardim (2.0 km^2), Urussanga (12.6 km^2), Jaconé (4 km^2) and Boqueirão (0.6 km^2). Fish and algae samples were obtained from Boqueirão, within Araruama Lagoon, while the Fora lagoon, hereafter termed Saquarema, was used as a reference area, due to the absence of algal blooms. Both display similar salinity values.

Algae sampling and identification

Approximately 200 mL of water samples were collected in 2005 and 2007, using a 20 µm plankton net at Araruama Lagoon, transferred to 250 mL polyethylene bottles, and fixed with 4% formaldehyde buffered with sodium tetraborate.

Phytoplankton composition and abundance were assessed by the method described by Utermöhl (1958). Briefly, the samples were gently homogenized and placed in 50 mL cylinders, and organism quantification and identification were conducted using an inverted Olympus microscope with a 40 X objective lens after 48 hours of slow sedimentation. Algae genera were identified according to Tomas (1997).

Fish taxonomy, sampling and tissue processing

Sampled fish were classified according to Menezes (1983). The fish were caught by local fishers and immediately euthanized by spinal cord severing. No sexing was carried out. Livers and axial muscle portions were removed, immediately transferred to cryotubes and placed in liquid nitrogen. After thawing, the liver samples were dried on filter paper and individually weighed and homogenized in potassium phosphate 0.1 mol L^{-1} pH 7.0 containing 0.25 mol L^{-1} sucrose at 5°C (1 g liver + 4 mL buffer) using a Potter-Elvehjem apparatus (Potter 1955) in order to prepare soluble liver fractions. The homogenates were centrifuged (12,000xg/30 min/5ºC), and the supernatants subsequently re-centrifuged (105,000xg/90 min/5ºC). The final supernatants (soluble fraction, SF) and sediments (microsome, MS) were collected and stored at -20°C. Glutathione peroxidase (GPX), catalase (CAT), and glutathione-S-transferase (GST)

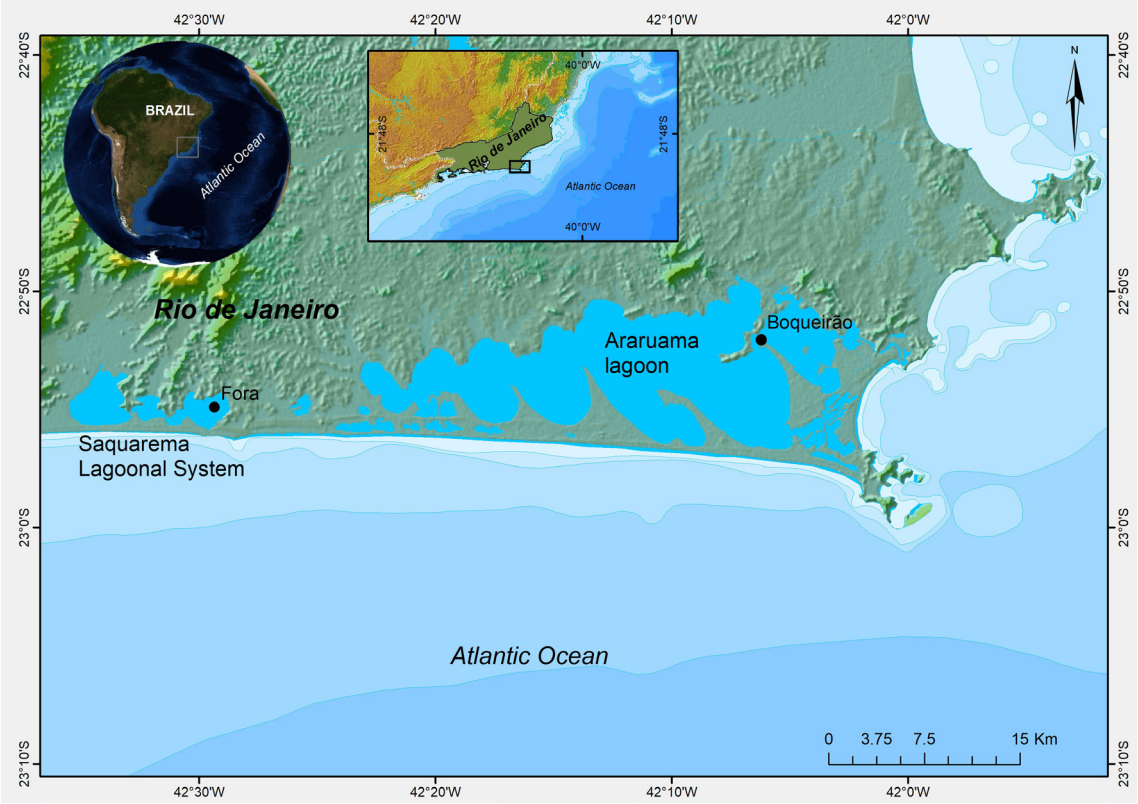

Fig. 1 Map of the study area, indicating the Boqueirão and Fora lagoons used as sampling sites in the present study

activities (Total, GSTμ and GSTπ) were assayed in the SF. The MS was used to assay ethoxyresorufin-O-deethylase (EROD), an enzyme related to the cytochrome P-450 1A family. Muscle samples were thawed, dried on filter paper, weighed individually, homogenized in Tris-HCl 10 mmol L^{-1} pH 7.0 containing 1% Triton X-100 and NaCl 1.0 mol L^{-1} (1 g muscle + 19 mL buffer), and stored at -20°C and submitted to the same process as livers and used to determine acetylcholinesterase (AChE) activities.

Total protein content

Total protein content was quantified by the method described by Peterson (1977) using bovine serum albumin as the external standard.

Enzymatic assays

GPX activity: The reaction medium contained potassium phosphate 0.1 mol L^{-1} pH 7.5, EDTA 1 mmol.L^{-1}, sodium azide 1 mmol.L^{-1}, dithiothreitol 1 mmol.L^{-1}, 8 mmol.L^{-1} GSH, 1 U.mL^{-1} glutathione reductase, 0.35 mmol $L^{-1}H_2O_2$, and up to 0.5 mL 0.48 mmol L^{-1} of NADPH (Regoli and Principato 1995). NADPH consumption (ε = 6,220 $M^{1}.cm^{-1}$ at 340 nm) was continuously recorded for 2 minutes at 25 °C.

CAT activity: The reaction medium contained 1 mmol.L^{-1} EDTA, 20 mmol.L^{-1} H_2O_2, and up to 0.5 mL of potassium phosphate 0.1 mmol.L^{-1}, pH 7.5. The reaction was started with H_2O_2 (Lushchak et al. 2005). H_2O_2 consumption (ε = 43.6 $M^{-1}.cm^{-1}$ at 240 nm) was continuously recorded for 2 minutes.

Total GST activity: The reaction medium contained potassium phosphate 0.1 mol L^{-1} pH 7.5, 1-chloro-2,4-dinitrobenzene (CDNB) and 400 mmol.L^{-1}GSH. Conjugate formation (ε = 9.6 $M^{-1}.cm^{1}$ at 340 nm) was continuously recorded for 3 min (Habig et al. 1974).

GSTμ activity: The reaction medium contained potassium phosphate buffer 0.1 mol L^{-1}, pH 7.0, 1,2-dichloro-4-nitrobenzene (DCNB) 400 mmol L^{-1} and 50 mmol L^{-1} GSH. Conjugate formation (ε = 8.5 $M^{-1}.cm^{1}$ at 345 nm) was continuously recorded for 3 min (Habig et al. 1974).

GSTπ activity: The reaction medium contained potassium phosphate buffer 0.022 M pH 7.0, ethacrynic acid 6.25 mmol L^{-1} and 50 mmol L^{-1} GSH. Conjugate formation (ε = 5 $M^{-1}.cm^{1}$ at 270 nm) was continuously recorded for 3 min (Habig et al. 1974).

AChE activity: The reaction medium contained sodium phosphate 0.1 mol L^{-1} pH 7.5, dithionitrobenzoic acid 0.32 mmol L^{-1}, and 1.875 mmol L^{-1} acetylthiocholine. Thionitrobenzoic acid (TNB) formation (ε = 14,150 $M^{-1}.cm^{1}$ at 412 nm) was continuously recorded for 3 min (Ellman et al. 1961).

The activity of these enzymes was evaluated using a Shimadzu UV-160 spectrophotometer at 25-28 °C and expressed as International Units (U - μmol per minute) per wet tissue weight.

EROD activity: The reaction medium contained potassium phosphate 0.1 mol L^{-1} pH 7.8, magnesium chloride 5 mmol L^{-1}, 7ethoxyresorufin 2.5 mmol L^{-1}, and NADPH 0.25 mol L^{-1}. Resorufin formation was continuously recorded at 25-28 °C using a Hitachi F-3010 spectrofluorometer (excitation 550 nm; emission 582 nm; 5 nm bandpass) for 3 min. EROD activity was calculated using resorufin standards added to the reaction medium after the enzyme assay, and the results are expressed as Units (μmol per minute) per weight of MS proteins.

Statistical analyses

All assays were performed in duplicate and the coefficient of variation was always less than 10%. Student's T test ($P < 0.05$) was carried out using the GraphPad Prism® software.

Results and discussion

Araruama Lagoon presented a microalgae bloom from 2005 to 2007, with the prevalence of potentially harmful Prasinophyceae algae, majority among the phytoplankton species identified. Dinoflagellates (*Protoperidinium* sp. and *Prorocentrum* sp. as the prevailing genera) were the second most abundant group. In 2007, the Prasinophyceae bloom disappeared, and diatoms (*Pleurosigma* sp.) were the most prevalent and the dinoflagellates (*Protoperidinium* sp. and *Prorocentrum* sp.) were again verified as the the second

most abundant group (Table 1).

This bloom was well characterized in this lagoon in 2005, with chlorophyll-a values ranging between 109 and 159.23 µg.L^{-1} (Mello 2007), leading to concerns regarding the local aquatic environment health and that of the surrounding population. Fish and crustacean mortality events were also observed at Araruama Lagoon in 2005 during intense rainfall months, leading to increased freshwater supply and *in natura* sewage, and higher radiation levels associated with drops in dissolved oxygen levels (Oliveira et al. 2011). Other Prasinophyceae blooms occurring worldwide have also been reported as causing extreme mortality events. For example, Jones et al. (1994) followed a prasinophyte bloom caused by organisms belonging to the *Tetraselmis* genus in December 1993 in a small lake in Wellington Harbor in southern New Zealand, and reported the death of approximately half a ton of *Sardinops sagax* specimens. The dissolved oxygen levels measured at different points in this lake ranged between 1.2 and 7.4 ppm, and histological fish gill analyses indicated that phytoplankton adhering to the gills most likely reduced oxygen absorption on the gill surface, probably causing fish death.

In 2007, potentially toxic dinoflagellates were detected, including *Protoperidimium* sp and *Prorocentrum* sp, occurred in greater numbers in 2007 (*Protoperidimium* – 290.136 cells L^{-1} and *Prorocentrum* - 23.927 cells L^{-1}). In addition, *Protoperidinium* sp. (192 cells.mL-1) and *Prorocentrum* sp (0.8 cells.mL-1) were also present in 2005. It is known that some *Prorocentrum* species produce okadaic acid, a known carcinogen (Fujiki et al. 2018) and dinophysistoxin (the cause of diarrheic shellfish poisoning - DSP), a lipophilic polyether that causes diarrhea, nausea, vomiting, and stomach spasms in acute poisoning for mussel consumption (Huang et al. 2015).

Twenty-nine mullet were analyzed, 15 from Araruama Lagoon and 14 from Saquarema Lagoon. Biometric data for each year and lagoon are displayed in Table 2.

Enzymatic assays, both *in situ* and *in vivo*, are routinely applied in environmental and human health risk assessments as biomarkers of response to contaminant exposure, as these responses can reveal exposure, effect or susceptibility to chemicals and other stressors on the organism (Ghisi et al. 2017; Oost et al. 2003), and inform early biochemical changes that manifest before physiological damage occurs in affected aquatic organisms. Therefore, they can be used as an alternative to screen for the presence of phycotoxins and/or harmful effects.

Catalase (CAT) and glutathione peroxidase (GPX)

No significant differences were observed for either CAT or GPx between years and lagoons, although

Table 1 Prevalence (%) of the main phytoplankton groups determined at Araruama Lagoon in 2005 and 2007

Divisons	Class	Order	Family	Genus mains	Sampling years (%)	
					2005	2007
Chlorophyta	Prasinophyceae	Chlorodendrales	Chlorodendraceae	*Pseudoscourfieldia* sp.	94.8	0
Chysophyta (Diatoms)	Bacillariophyceae	Naviculales	Pleurosigmataceae	*Pleurosigma* sp.	0.73	83
Pyrrophyta (Dinoflagellates)	Dinophyceae	Peridiniales	Protoperidiniaceae	*Protoperidinium* sp.	4.43	17
		Prorocentrales	Prorocentraceae	*Prorocentrum* sp.		
Cyanobacteria	-	-	-	-	0.026	0
Total counts (cell.mL^{-1})					26.45	1.884

Table 2 Biometric data from Mugil liza specimens sampled from Araruama and Saquarema Lagoons

Year	Lagoon	Sampling dates (dd/mm/yy)	N	Total length (cm)	Total weight (g)
2005	Saquarema	29/07/05	5	31±11	288±30
	Araruama	21/06/05- 28/07/05	7	44±24	1.008±49
2007	Saquarema	24/07/07	9	27 ± 2	316 ± 38
	Araruama	20/08/07	8	Nd*	372±78

*nd: not determined

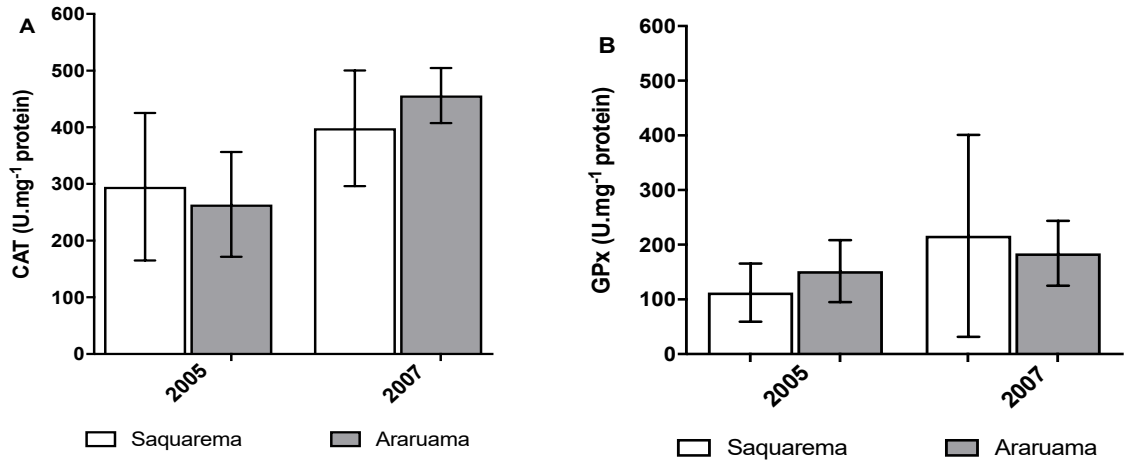

Fig. 2 Catalase (A) and glutathione peroxidase (B) activities in mullet liver sampled from Saquarema Lagoon and Araruama Lagoon. Each column and bar represents the means and standard deviations, respectively

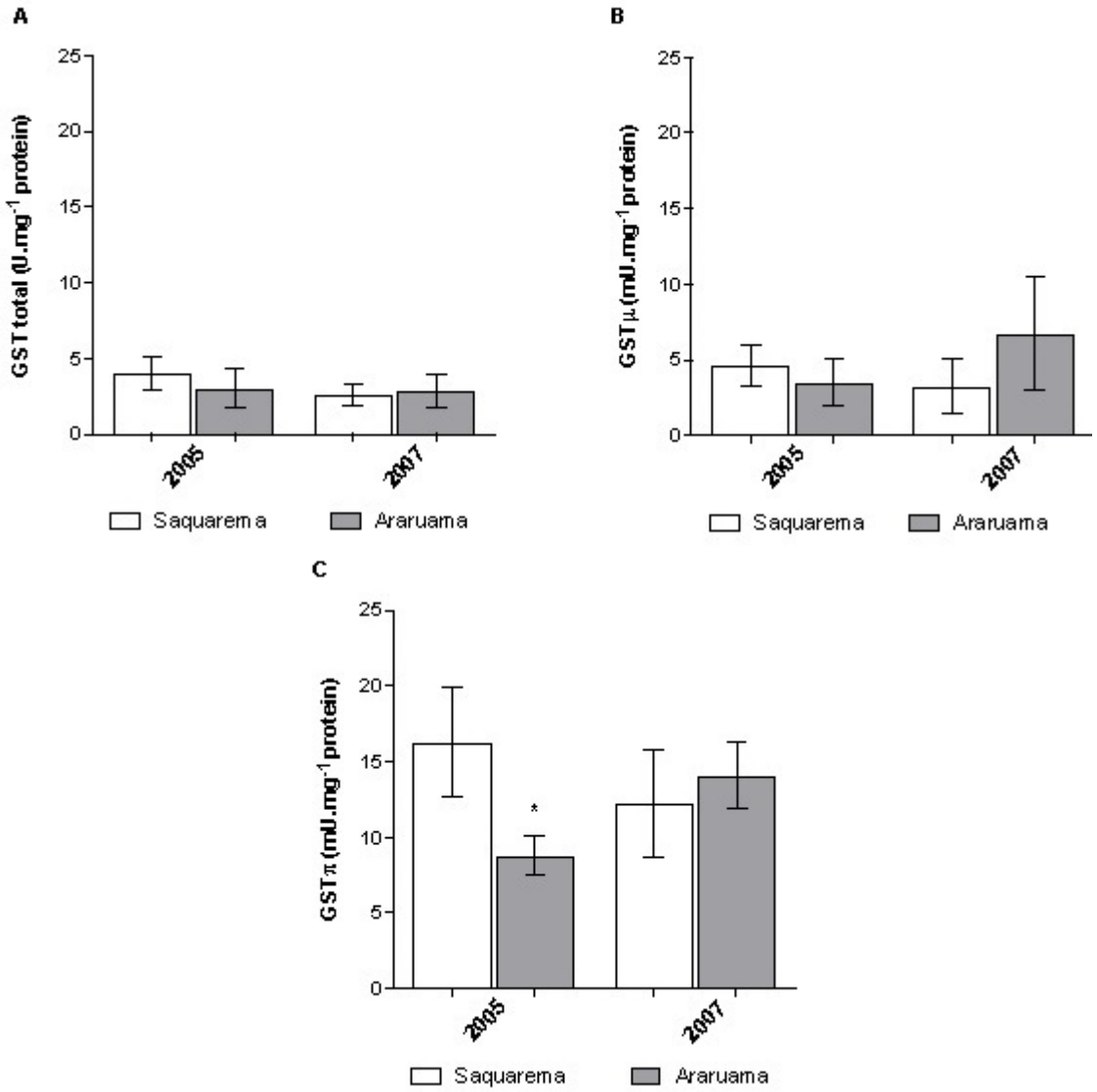

Fig. 3 Total glutathione S-transferase (GST)(A), mi (GSTμ)(B) and pi (GSTπ)(C) classes in mullet liver sampled from Saquarema Lagoon and Araruama Lagoon. Each column and bar represents the means and standard deviation, respectively.
*Significant difference between the lagoons during the same year (Student's T test, $P < 0.05$)

Araruama Lagoon CAT activities were slightly higher when compared to Saquarema Lagoon after the bloom (Figure 2). GPx and CAT are responsible for the control of hydrogen peroxide production in cells due to increased production of reactive oxygen species (ROS) (Ighodaro and Akinloye 2018). ROS modify important cellular macromolecules, primarily proteins, lipids and DNA, by carbonylation, lipid peroxidation and mutations ("adduct" formation), respectively (Lushchak 2016). These enzymes have been routinely applied in environmental studies in polluted environments as oxidative stress biomarkers (Lushchak 2016; Mazmanci and Çavas 2010; Ribalta et al. 2015). For example, Mazmanci and Cavas (2010) injected Nile tilapia (*Oreochromis niloticus*) with domoic acid (1, 5 and 10 µg.g^{-1} body weight), a phycotoxin produced by some *Pseudonitzschia* species, and observed increased superoxide dismutase (SOD), CAT, GPx and glutathione reductase (GRD) activities in liver and gills, in addition to increased lipid peroxidation. However, no significant differences in GPx activity were noted when comparing specimens from both lagoons in 2007. On the other hand, CAT activities in liver seem to indicate of pro-oxidant status in *Mugil liza* sampled from Araruama Lagoon during the same year. Although Saquarema lagoon in this

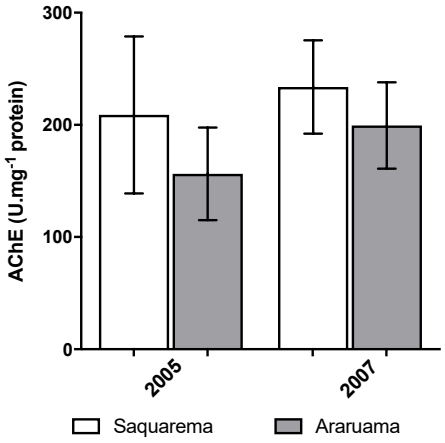

Fig. 4 Acetylcholinesterase (AChE) in mullet muscle of mullet sampled from Saquarema Lagoon and Araruama Lagoon.
*Significant difference between lagoons during the same year (Student's T test, P <0.05).

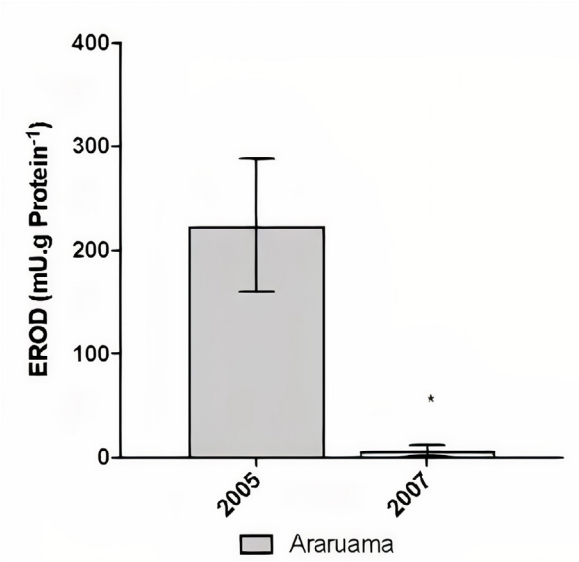

Fig. 5 Ethoxyresorufin O-deethylase (EROD) levels in mullet liver microsome collected at Araruama Lagoon. Each column and bar represents the means and standard deviation, respectively.
*Significant difference between the lagoons during the same year (Student's T test, P <0.05)

study is considered a reference area due to the non-occurrence of phytoplankton blooms, it is not free of other impacts. For example, biochemical oxygen demand (BOD) and ammonia levels remained high in the lagoon even after opening the bar that connects the lagoon with the sea (Carloni et al. 2010), BOD and ammonia levels remained high in the lagoon, due to the concomitant increase of the sewage supply from the Bacaxá River (main tributary) and the diffuse sewage input from residences located in the surroundings. These variations in dissolved oxygen levels and consequent redox potential alterations in the environment can cause increased oxidative stress (Lushchak 2011).

Glutathione transferase (GST)

This biomarker can indicate alterations in xenobiotic metabolism. In 2005, during the Prasinophytes bloom, total GST and GSTμ liver activities were statistically similar compared to specimens sampled from Saquarema Lagoon ($P > 0.05$). Araruama Lagoon displayed an increase in GST π activity over the study years, with a significant difference ($P < 0.05$) in 2005 when compared to Saquarema Lagoon (Figure 3).
Glutathione S-transferases (GST) are involved in xenobiotic biotransformation, phase II detoxification (Glisic et al. 2015), and are recognized as an important tool for ecotoxicological research in several classes of organisms, such as mussels, fish and mammals (Liu et al. 2015; Nóvoas-Valiñas et al. 2002; Satoh 2018). Ventura et al. (2002) found in the soluble fraction of liver *Orthopristis ruber*, low levels of total GST activity in fish of the bays of Guanabara and Sepetiba compared to activities of fishes in the Ilha Grande. These authors assume that this decline may be related to levels of contaminants that cause oxidative stress which, when reduced, increasing the consumption of GSH, reducing the ability of the combination of GST isoenzymes by reduction of its substrate.

Acetylcholinesterase (AChE)

Somewhat lower AChE activities were found for both lagoons in 2005 compared to 2007, but without significant differences (Figure 4).

Acetylcholinesterase (AChE) is a known biomarker of exposure and effect of organophosphates and carbamates (Albendín et al. 2017; Lopes et al. 2018), and a biomarker for anatoxins, for example in *in vitro* studies as a biosensor to the toxic compounds produced by cyanobacteria from the Anabaena genus (Devic et al. 2002; Dos Santos et al. 2019; Yunes 2019). Despite the lower trend in AChE activity in 2005, AChE activities in this study were similar in both lagoons and in the same order of magnitude as observed other studies (Napierska and Podolska 2005), indicating that this enzyme was not affected by the phytoplankton changes at Araruama Lagoon during the study period.

Ethoxyresorufin-O-deethylase, EROD

Cytochrome p-450 1A (CYP 1A) activity was significantly higher in 2005 when compared to Saquarema Lagoon, with a significant decrease in 2007 (Figures 5). Enzymes belonging to the cytochrome P-450 1A (CYP 1A) family of microsomal monooxygenases are involved in the biotransformation of nonpolar xenobiotics (phase I detoxification), which include polycyclic aromatic hydrocarbons (PAHs), dioxins and polychlorinated biphenyls (PCBs) (Gagnon and Rawson 2017; Hanno et al. 2010). EROD activity in microsomal *Mugil liza* fractions was, unfortunately, analyzed only at both lagoons in 2005, due to logistic reasons, and was higher at Araruama Lagoon. EROD was significantly higher in 2005 than in 2007, with similar values to other environments polluted by xenobiotics, and probably due to algal bloom effects (Gagnon and Rawson 2017; Parente et al. 2008). Prasinophyte species, such as *Tetraselmis suecica*, produce high concentrations of polyunsaturated fatty acids (PUFAs), with arachidonic acid being one of the most abundant (Sathasivam et al. 2017; Servel et al. 1994). This compound is a proven CYP1A inducer in some fish species (Søfteland et al. 2016; Schlezinger et al. 1998), and may be the cause for the high EROD activities in *Mugil liza* specimens from Araruama Lagoon in 2005. This has been observed in other cases, for example in certain fish species found in Brazilian reservoirs, such as the Pearl Cichlid (*Geophagus brasiliensis*), which presented increased EROD (CYP1A1) activity in an environment with the presence of a saxitoxin-producing cyanobacterium, *Cylindrospermopsis raciborskii* (Clemente et al. 2010). However,

other studies found no induction of hepatic EROD in fish species (*Diplodus sargus*) exposed to saxitoxins for 2 to 6 days (Costa et al. 2012). EROD activity at Araruama lagoon in 2007 was significantly lower than in 2005, following the decrease in the number of *Pseudoscourfieldia* sp. In 2007, prasinophytes no longer appeared in the water samples, and the prevalence of diatoms, followed by dinoflagellate was observed, which is probably the cause of the significantly lower EROD values found during this sampling, close to those found in locations used as controls in other studies (Parente et al. 2008; Morado et al. 2018).

Two biomarkers, enzymes belonging to the detoxification metabolism of xenobiotic phase I (CYP 1A1) and phase II (GSTπ), exhibited changes that can be correlated to the occurrence of an algal bloom in 2005 at Araruama lagoon. As this historic event is noted in similar situations worldwide, we offer this record so that it can be the subject of discussion concerning future events in this environment.

Conclusions

The current study was an attempt to monitor HABs by applying an ecotoxicological approach to a native fish species (mullet) from an affected environment (Araruama Lagoon). EROD activity in mullet from Araruama Lagoon was high, indicating CYP1A induction during the *Pseudoscourfieldia sp* bloom in 2005, while two years after the bloom period decreased EROD activity was observed. Still in 2005, GST shows low liver activity in mullets collected in the Araruama lagoon compared to collect in Saquarema lagoon. This activity was higher in 2007, just like CAT. GPx, GST total, GSTμ and AChE, however, was not affected by the phytoplankton changes at Araruama Lagoon during the study period. The use of biochemical biomarkers seems to be adequate for monitoring organism health during and after algal blooms, and, alongside traditional phycotoxin detection test may indicate specific bloom effects. In addition, the monitoring of the ecological succession of the phytoplankton community at Araruama Lagoon in bloom-free conditions should be implemented, in order to determine which species are normally predominant in this environment and better understand these increasing events in coastal environments.

List of abbreviations AChE: acetylcholinesterase; ASP: amnesic shellfish poisoning; AZP: azaspiracid shellfish poisoning; BOD: biochemical oxygen demands; CAT: catalase; CFP: ciguatera fish poisoning; CDNB: 1-chloro-2,4-dinitrobenzene; CYP 1A1: Cytochrome p-450 1A1; DCNB: 1,2-dichloro-4-nitrobenzene; DSP: diarrheic shellfish poisoning; EDTA: Ethylenediamine tetraacetic acid; EROD: ethoxyresorufin-O-deethylase; GPX- glutathione peroxidase; GSH: Glutathione (γ-L-Glutamyl-L-cysteinylglycine); GRD: glutathione reductase; GSTt: total glutathione S-transferase; GSTπ: Pi class glutathione S-transferase; GSTμ: Mi class glutathione S-transferase; HABs - harmful algal blooms; H_2O_2: Hydrogen peroxide; NADPH: Nicotinamide adenine dinucleotide phosphate; PAHs: polycyclic aromatic hydrocarbons; PCBs: polychlorinated biphenyls; PSP: paralytic shellfish poisoning; PUFAs: polyunsaturated fatty acids; ROS: reactive oxygen species; SOD: superoxide dismutase; TNB: Thionitrobenzoic acid.

Acknowledgments The authors would like to acknowledge the logistical support of the Lagos São João Consortium, the Iguaba Fishermen Colony, the São Pedro d'Aldeia Fisher's Association, the Saquarema fishing colony (Z-24), Environmental Management Specialist Debora Gomes Figueiredo, Dr. Eduardo B. Fagundes Netto for the taxonomic classification of the mullets and Dr Moacelio Verânio Silva Filho (*in memoriam*) for unconditional support in conducting work. The authors would also like to thank CNPq for financial support.

The authors dedicate this work to Dr. Moacelio veranio Silva Filho.

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

Authors' contributions MMO, AMFV, RFD, ASF, JBS, FFB - Carried out the experimental assays. MHCBN - performed taxonomic studies. RML, RAHD - Data analysis and curation, validation, statistics, final text revision. VLFCB, JCB - conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

References

Albendín G, Arellano JM, Mánuel-Vez MP, Sarasquete C, Arufe MI (2017) Characterization and in vitro sensitivity of cholinesterases of gilthead seabream (*Sparus aurata*) to organophosphate pesticides. Fish Physiol Biochem 43(2):455-464

Araújo MC, Assis CRD, Silva KCC, Souza KS, Azevedo RS, Alves MHME, Silva LC, Silva VL, Adam ML, Carvalho Junior LB, Bezerra RS, Oliveira MBM (2018) Characterization of brain acetylcholinesterase of bentonic fish *Hoplosternum littorale*:

Perspectives of application in pesticides and metal ions biomonitoring. Aquat Toxicol 205:213-226
Backer L, Manassaram-Baptiste D, LePrell R, Bolton B (2015) Cyanobacteria and algae blooms: review of health and environmental data from the harmful algal bloom-related illness surveillance system (HABISS) 2007–2011. Toxins 7(4):1048-1064
Berdalet E, Fleming LE, Gowen R, Davidson K, Hess P, Backer LC, Enevoldsen H (2016) Marine harmful algal blooms, human health and wellbeing: challenges and opportunities in the 21st century. J Mar Biol Assoc UK 96(1):61-91
Bertucci TCP, Silva EP, Marques JR. AN, Monteiro NC (2016) Turismo e urbanização: os problemas ambientais da lagoa de araruama - Rio de Janeiro. Ambient Soc 19(4):59-80
Bonansea RI, Marino DJ, Bertrand L, Wunderlin DA, Amé MV (2017) Tissue specific bioconcentration and biotransformation of cypermethrin and chlorpyrifos in a native fish (*Jenynsia multidentata*) exposed to these insecticides singly and in mixtures. Environ Toxicol Chem 36(7):1764-1774
Busch JA, Andree KB, Diogène J, Fernández-Tejedor M, Toebe K, John U, Krock B, Tillmann U, Cembella AD, (2016) Toxigenic algae and associated phycotoxins in two coastal embayments in the Ebro Delta (NW Mediterranean), Harmful Algae 55:191-201
Carloni FBBA, Wasserman JC, Pereira LFM, Moreira MF (2010) Mudanças na qualidade da água de uma laguna do leste fluminense, geradas pela abertura permanente de conexão com o mar. Geochim Bras 24:29-40
Clemente Z, Busato RH, Oliveira Ribeiro CA, Cestari MM, Ramsdorf WA, Magalhães VF, Wosiack AC, Silva De Assis HC (2010) Analyses of paralytic shellfish toxins and biomarkers in a southern Brazilian reservoir. Toxicon 55:396–406
Costa PR, Pereira P, Guilherme S, Barata M, Nicolau L, Santos MA, Pacheco M, Pousão-Ferreira P (2012) Biotransformation modulation and genotoxicity in white seabream upon exposure to paralytic shellfish toxins produced by *Gymnodinium catenatum*. Aquat Toxicol 106-107: 42– 47
Coutinho R, Ribeiro P, Kjerfve B, Knoppers B, Muehe D, Valentin JL (1999) Araruama uma lagoa ameaçada. Ciência Hoje 25:24-31
Dalzochio T, Rodrigues GZP, Petry IE, Gehlen G, da Silva LB (2016) The use of biomarkers to assess the health of aquatic ecosystems in Brazil: a review. Int Aquat Res 8(4):283-298
De Morais Calado SL, Santos GS, Vicentini M, Bozza DC, Prodocimo V, de Magalhães VF, Assis HCS (2020) Multiple biomarkers response in a Neotropical fish exposed to paralytic shellfish toxins (PSTs). Chemosphere 238:124616
Devic E, Li D, Dauta A, Henriksen P, Codd GA, Marty JL, Fournier D (2002) Detection of anatoxin-a (s) in environmental samples of cyanobacteria by using a biosensor with engineered acetylcholinesterases. Appl Environ Microbiol 68(8):4102-4106
Dos Santos DS, Rosa ME, Zanatta AP, Oliveira RS, de Almeida CGM, Leal AP, Pinto E (2019) Neurotoxic effects of sublethal concentrations of cyanobacterial extract containing anatoxin-a (s) on *Nauphoeta cinerea* cockroaches. Ecotox Environ Safe 171:138-145
Ellman GL, Courtne YKD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88-95
Eurohab (2002) Harmful algae news. An IOC Newsletter on toxic algae and algal blooms (UNESCO). Special Issue, PP. 1-6
Fujiki H, Sueoka E, Watanabe T, Suganuma M (2018) The concept of the okadaic acid class of tumor promoters is revived in endogenous protein inhibitors of protein phosphatase 2A, SET and CIP2A, in human cancers. J Cancer Res Clin Oncol 144(12):2339-2349
Gagnon MM, Rawson CA (2017) Bioindicator species for EROD activity measurements: A review with Australian fish as a case study. Ecol Indic 73:166-180
Geohab (2001) Global ecology and oceanography of harmful algal blooms, science plan. SCOR and IOC, Baltimore and Paris.
Ghisi NC, Oliveira EC, Guiloski IC, Lima SB, Silva de Assis HC, Longhi SJ, Prioli AJ (2017) Multivariate and integrative approach to analyze multiple biomarkers in ecotoxicology: A field study in Neotropical region, Sci Total Environ 609:1208-1218
Glibert PM (2017) Eutrophication, harmful algae and biodiversity—Challenging paradigms in a world of complex nutrient changes. Mar Pollut Bull 124(2):591-606
Glisic B, Mihaljevic I, Popovic M, Zaja R, Loncar J, Fent K, Kovacevic R, Smital T (2015) Characterization of glutathione-S-transferases in zebrafish (Danio rerio), Aquat Toxicol: 158:50-62
Habig WH, Pabst MJ, Jacoby WB (1974) Glutathione S-transferases: The first step in mercapturic acid formation. J Biol Chem 249:7130-7139
Hallegraeff GM (2004) Harmful algal blooms: A global overview, in: Hallegraeff GM, Anderson DM, Cembella DM (Eds.), 2nd revised edition. Manual on harmful marine microalgae. Intergovernmental Oceanographic commission/UNESCO, Paris, pp. 25-49
Hanno K, Oda S, Mitani H (2010) Effects of dioxin isomers on induction of AhRs and CYP1A1 in early developmental stage embryos of medaka (*Oryzias latipes*), Chemosphere 78(7):830-839
Harrness (2005) Harmful algal research and response. A National Environmental Science Strategy 2005-2015, in: Ramsdell JS, Anderson DM, Glibert PM (Eds.). Ecological Society of America, Washington DC, p. 96
Huang L, Zou Y, Weng HW, Li HY, Liu JS, Yang WD (2015) Proteomic profile in Perna viridis after exposed to *Prorocentrum lima*, a dinoflagellate producing DSP toxins. Environ Poll 196:350-357
Ighodaro OM, Akinloye OA (2018) First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J Med 54(4):287-293
Jones JB, Rhodes LL (1994) Suffocation of pilchards (*Sardinops sagax*) by a green microalgal bloom im Wellington Harbour, New Zealand. New Zeal J Mar Fresh 28, 379-383.
Liu H, He J, Zhao R, Chi C, Bao Y (2015) A novel biomarker for marine environmental pollution of pi-class glutathione S-transferase from *Mytilus coruscus*, Ecotox Environ Safe 118, 47-54.
Lopes RM, Bezerra ALA, Hauser-Davis RA, Oliveira MM, Salles JB, Cunha Bastos VLF, Cunha Bastos J (2018) The importance of an efficient extraction protocol for the use of fish muscle cholinesterases as biomarkers, Talanta, 179, 769-774.
Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. Aquat Toxicol 101, 13–30.
Lushchak VI (2016) Contaminant-induced oxidative stress in fish: a mechanistic approach. Fish Physiol Biochem, 42(2), 711-747.
Lushchak VI, Bagnyukova TV, Lushchak OV, Storey JM, Storey KB (2005) Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (*Cyprinus carpio*) tissues. Int J Biochem Cell Biol 37, 1319–1330.
Magalhães VF, Marinho MM, Domingos P, Oliveira AC, Costa SM, Azevedo LO, Azevedo SMFO (2003) Microcystins (cyanobacteria hepatotoxins) bioaccumulation in fish and crustaceans from Sepetiba Bay (Brasil, RJ). Toxicon 42:289–295
Mazmanci B, Çavas T (2010) Antioxidant enzyme activity and lipid peroxidation in liver and gill tissues of Nile tilapia (*Oreochromis*

niloticus) following *in vivo* exposure to domoic acid. Toxicon 55:734–738
Mello TBM (2007) Caracterização biogeoquímica da Lagoa de Araruama, RJ. Master's dissertation in Geochemistry. Federal University Fluminense, 82 p.
Menezes NA (1983) Guia prático para conhecimento e identificação de tainhas e paratis (Pisces, Mugilidae) do litoral brasileiro. Rev Bras Zool 2:1-12
Morado CN, Parente TEM, Araújo FG, Paumgartten FR, Gomes ID (2018) Induced CYP1A activity and DNA damage in fish from the middle Paraíba do Sul River basin, southeastern Brazil. Acta Sci Biol Sci 40:1-7
Moroño A, Arévalo F, Fernández ML, Maneiro J, Pazos Y, Salgado C, Blanco J (2003) Accumulation and transformation of DSP toxins in mussels *Mytilus galloprovincialis* during a toxic episode caused by *Dinophysis acuminata*. Aquat Toxicol 62:269-280
Napierska D, Podolska M (2005) Biomarkers of contaminant exposure: results of a field study with flounder (*Platichthys flesus*) from the southern Baltic Sea. Mar Pollut Bull 50:758-767
Nóvoas-Valiñas MC, Pérez-López M, Melgar MJ (2002) Comparative study of the cytosolic Glutathione S-transferases from two salminid species: atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). Comp Biochem Physiol Part C 131:207-213
Nunes B, Campos JC, Gomes R, Braga MR, Ramos AS, Antunes SC, Correia AT (2015) Ecotoxicological effects of salicylic acid in the freshwater fish *Salmo trutta* fario: antioxidant mechanisms and histological alterations. Environ Sci Pollut 22(1):667-678
Oliveira MM, Baeta Neves, MHC, Albano, RM, Bastos JC, Silva Filho MV (2011) Presença de microcistina durante eventos de florações de microalgas na Lagoa de Araruama. Bol Observ Amb Alb Rib Lam 5:35-45
Oost RV, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:57-149
Parente TEM, Oliveira ACAX, Paumgartten FJR (2008) Induced cytochrome P450 1A activity in cichlid fishes from Guandu River and Jacarepaguá Lake, Rio de Janeiro, Brazil. Environ Pollut 152:233-238
Peterson GL (1977) A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 83:346-356
Potter VR (1955) Tissue Homogenates, in: Colowick SP, Kaplan NO (Eds.), Methods in Enzymology, pp. 10-15
Prego-Faraldo MV, Méndez J, Laffon B, Valdiglesias V (2016) Cellular and molecular biomarkers for assessing the harmful effects of marine toxins in bivalve mollusks. Environ Res J 10(3):337-356
Regoli F, Principato G (1995) Glutathione, glutathione-dependent and antioxidant enzymes in mussel, *Mytilus galloprovincialis*, exposed to metals under field and laboratory conditions: implications for the use of biochemical biomarkers. Aquat Toxicol 31:143-164
Ribalta C, Sanchez-Hernandez JC, Sole M (2015) Hepatic biotransformation and antioxidant enzyme activities in Mediterranean fish from different habitat depths. Sci Total Environ 532:176-183
Rosa JCL, Alberto MD, Ribas WMM, Baeta Nevas MHC, Fernandes LDA (2016) Spatial variability in the icthyoplankton structure of a subtropical hypersaline lagoon. Braz J Ocean 64(2):149-156
Sanseverino I, Conduto D, Pozzoli L, Dobricic S, Lettieri T (2016) Algal bloom and its economic impact. European Commission, Joint Research Centre Institute for Environment and Sustainability.
Sathasivam R, Radhakrishnan R, Hashem A, Abd_Allah EF (2017) Microalgae metabolites: A rich source for food and medicine. Saudi J Biol Sci 26:709-722
Satoh K (2018) Strong carcinogenic stress response induction of preneoplastic cells positive for GST-P in the rat liver: Physiological mechanism for initiation. Life Sci 200:42-48
Schlezinger JJ, Parker C, Zeldin DC, Stegeman JJ (1998) Arachidonic acid metabolism in the marine fish *Stenotomus chrysops* (Scup) and the effects of cytochrome P450 1A inducers. Arch Biochem Biophys 353:265-275
Servel MO, Claire C, Derrien A, Coiffard L, Deroeckholtzhauer Y (1994) Fatty-acid composition of some marine microalgae. Phytochemistry 36:691-693
Søfteland L, Berntssen MH, Kirwan JA, Størseth TR, Viant MR, Torstensen BE, Olsvik PA (2016) Omega-3 and alpha-tocopherol provide more protection against contaminants in novel feeds for Atlantic salmon (*Salmo salar* L.) than omega-6 and gamma tocopherol. Toxicol Rep 3:211-224
Souza MFL, Bjorn K, Knoppers B, Souza WFL, Damasceno RN (2003) Nutrient budgets and trophic state in a hypersaline coastal lagoon: Lagoa de Araruama, Brazil. Estuar Coast Shelf Sci 57:843-858
Tomas CR (1997) Identifying marine phytoplankton. Academic Press, San Diego
Turki S, Dhib A, Fertouna-Bellakhal M, Frossard V, Balti N, Kharrat R, Aleya L (2014) Harmful algal blooms (HABs) associated with phycotoxins in shellfish: What can be learned from five years of monitoring in Bizerte Lagoon (Southern Mediterranean Sea)? Ecol Eng 67:39-47
Utermohl H (1958) Zur vervollkommnung der quantitativen phytoplankton-methodic. Verh Internat Verein Theor Angew Limnol 9:1-39
Valdiglesias V, Prego-Faraldo M, Pásaro E, Méndez J, Laffon B (2013) Okadaic acid: more than a diarrheic toxin. Mar Drugs 11(11):4328-4349
Ventura EC, Gaelzer LR, Zanette J, Marques MRF, Bainy ACD (2002) Biochemical indicators of contaminant exposure in spotted pigfish (*Orthopristis rubber*) caught at three bays of Rio de Janeiro coast. Mar Environ Res 5:775-779
Viana TV, Fistarol GO, Amario M, Menezes RB, Carneiro BLR, Chaves DM, Hargreaves PI, Silva-Lima AW, Valentin JL, Tenenbaum DR, Arruda EF, Paranhos , Salomon PS (2019) Massive Blooms of *Chattonella subsalsa* Biecheler (Raphidophyceae) in a Hypereutrophic, Tropical Estuary—Guanabara Bay. Brazil Front Mar Sci 6:85
Yunes JS (2019) Cyanobacterial Toxins. In Cyanobacteria. Academic Press. pp. 443-458
Zabaglo K, Chrapusta E, Bober B, Kaminski A, Adamski M, Bialczyk J (2016) Environmental roles and biological activity of domoic acid: A review. Algal Res 13:94-101
Zohdi E, Abbaspour M (2019) Harmful algal blooms (red tide): a review of causes, impacts and approaches to monitoring and prediction. Int J Environ Sci Te 16(3):1789-1806

Publisher's Note