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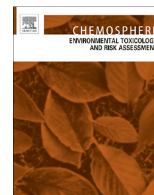

Biochemical responses in armored catfish (*Pterygoplichthys anisitsi*) after short-term exposure to diesel oil, pure biodiesel and biodiesel blends

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HIGHLIGHTS

- Diesel, pure biodiesel, B5 and B20 causes oxidative stress in armored catfish.
- Biodiesel increases GST activity as well as petroleum diesel.
- Diesel and biodiesel exposure did not change oxidative DNA damage levels.
- Biodiesel can represent a risk to aquatic biota.

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ABSTRACT

Biodiesel fuel is gradually replacing petroleum-based diesel oil use. Despite the biodiesel being considered friendlier to the environment, little is known about its effects in aquatic organisms. In this work we evaluated whether biodiesel exposure can affect oxidative stress parameters and biotransformation enzymes in armored catfish (*Pterygoplichthys anisitsi*, Loricariidae), a South American endemic species. Thus, fish were exposed for 2 and 7 d to 0.01 mL L^{-1} and 0.1 mL L^{-1} of pure diesel, pure biodiesel (B100) and blends of diesel with 5% (B5) and 20% (B20) biodiesel. Lipid peroxidation (malondialdehyde) levels and the activities of the enzymes glutathione S-transferase, superoxide dismutase, catalase and glutathione peroxidase were measured in liver and gills. Also, DNA damage (8-oxo-7, 8-dihydro-2′-deoxyguanosine) levels in gills and 7-ethoxyresorufin-*O*-deethylase activity in liver were assessed. Pure diesel, B5 and B20 blends changed most of the enzymes tested and in some cases, B5 and B20 induced a higher enzyme activity than pure diesel. Antioxidant system activation in *P. anisitsi* was effective to counteract reactive oxygen species effects, since DNA damage and lipid peroxidation levels were maintained at basal levels after all treatments. However, fish gills exposed to B20 and B100 presented increased lipid peroxidation. Despite biodiesel being more biodegradable fuel that emits less greenhouse gases, the increased lipid peroxidation showed that biofuel and its blends also represent hazards to aquatic biota.

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1. Introduction

Biofuels have been gradually used instead of fossil fuels because they are a renewable and less polluting energy source. In this context, petroleum-based diesel oil has been replaced by biodiesel which is a fatty acid methyl esters mixture derived from the transesterification of animal fats or vegetable oils (DeMello et al., 2007). Biodiesel can be used pure (B100) or blended at any level with petroleum-based diesel like B5 (5% biodiesel and 95% petroleum diesel), which is mandatory in Brazil since 2012, and B20 (20% biodiesel and 80% petroleum diesel), which is the most common biodiesel blend in the USA (Balat and Balat, 2010; Schröder et al., 2013). Although biodiesel emits less greenhouse gases (Leduc et al., 2009) and have been shown to be less harmful in toxicity tests in fish (*Oncorhynchus mykiss*) and microcrustaceans (*Daphnia magna*) (Khan et al., 2007), this fuel also presents some disadvantages like increased emissions of nitrogen oxides (Basha et al., 2009) and polycyclic aromatic hydrocarbons (PAHs) (Corrêa and Arbilla, 2006). Furthermore, the literature has demonstrated that biodiesel promotes mutagenic and genotoxic effects in *Salmonella* (Leme et al., 2012) and oxidative stress in male Sprague–Dawley rats (Poon et al., 2009).

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Oxidative stress occurs when reactive oxygen species (ROS) like superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^{\cdot}$) are produced in excess, leading to oxidization of cellular components. This state can cause different consequences in organisms such as loss of DNA integrity, leading to induction of mutations, chromosomal aberrations, birth defects and long-term effects such as cancer in vertebrates (Frenzilli et al., 2004). Hydroxyl radical interaction with DNA bases might lead to formation of modified bases such as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) (Valavanidis et al., 2009), culminating to GC→TA transversions (Kuchino et al., 1987). Another consequence of ROS is the lipid peroxidation, a process in which the cell membranes are oxidized leading to the formation of by-products such as malondialdehyde (MDA), a biomarker widely used to indicate injuries caused by oxidative stress (van der Oost et al., 2003; Almeida et al., 2005, 2007). In order to protect the cells against excessive ROS, the organisms have many antioxidant defenses, such as enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). These enzymes act removing ROS, thus promoting a protection against its harmful effects (Livingstone, 2001; van der Oost et al., 2003).

Armored catfish (Loricariidae) as those from the genus *Pterygoplichthys* are native from South America, but several members of this genus have been introduced in some parts of North and Central America and Asia (Nico and Martin, 2001; Nico et al., 2009). *Pterygoplichthys anisitsi* is an armored catfish occurring in South American freshwaters and it can be found throughout the Uruguay, Paraguay and Paraná River basins, inhabiting water bodies characterized by low oxygen concentrations (Cruz et al., 2009). This catfish is a benthic and non-obligatory air-breathing species that already shows to be responsive to exposure to diesel fuel, activating enzymes such as 7-ethoxyresorufin-*O*-deethylase (EROD) and SOD (Nogueira et al., 2011a), which makes them interesting in ecotoxicological studies.

We have already reported that biodiesel could be also deleterious to fish in a study done with Nile tilapias, in which biodiesel exposure elicited increased lipid peroxidation levels in their gills (Nogueira et al., 2011b). Considering that exposure to diesel, pure biodiesel and their blends B5 and B20 have been shown to promote oxidative stress and changes in biotransformation enzymes to Nile tilapia, in this work we were interested to evaluate whether these fuels affect oxidative stress parameters and biotransformation enzymes in *P. anisistsi*, a species not yet widely used in environmental toxicology studies, but that has demonstrated to be very resistant to pollutant exposure (Nogueira et al., 2011a; Rodrigues et al. submitted), thus being a potential sentinel organism in monitoring studies of highly impacted areas. In order to achieve our goals, we exposed armored catfish to 0.01 mL L^{-1} and 0.1 mL L^{-1} of pure diesel, pure biodiesel (B100) and the blends B5 and B20 for 2 and 7 d, and assessed the levels of 8-oxodGuo in gills, EROD activity in liver and lipid peroxidation (MDA) and activity of the enzymes GST, SOD, CAT and GPx in both gill and liver.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma. Pure biodiesel was obtained from Biodiesel Division of JBS S.A., Lins, Sao Paulo State, Brazil. Diesel oil was purchased from a gas station. The chemical characterization of pure biodiesel and pure diesel oil used in this study was the same as previously described (Nogueira et al., 2011b), since the same lot was used for both experiments.

2.2. Test organism and experimental conditions

Pterygoplichthys anisitsi was given by Aquaculture Center from ''Universidade Estadual Paulista'' (CAUNESP), campus of Jaboticabal, Sao Paulo State, Brazil. The weight and length of catfish specimens were respectively 158.01 ± 38.76 g and 19.86 ± 2.0 cm (mean ± standard deviation). Both males and females were used due to the absence of external sexual dimorphism among this specie. The animals were placed in individual tanks (20 L) with dechlorinated water at a controlled temperature (25 °C). Before the exposure began, the animals went through a period of 7 d of acclimatization. The fish were fed with commercial fish food once a day during the experimental period. This work had permission from the Ethics Committee for Animal Use in research of the ''Universidade Estadual Paulista'' (CEUA-IBILCE/UNESP).

2.3. Experimental procedure

Five fish were used per treatment, totaling forty-five animals for each exposure period (2 and 7 d). Each fish was individually exposed to treatment in aquariums of 17 L (real replica). Thus, one group remained in aquaria without contaminant (controls) and the other groups were exposed to diesel, B5, B20 and B100 at concentrations of 0.01 mL L^{-1} and 0.1 mL L^{-1}, with no water change (static system) After these exposure periods, fish were anesthetized with benzocaine (90 mg L^{-1} directly dissolved in water) and had their liver and gills removed and immediately stored at −80 °C. Levels of NH_3 and the water pH were monitored after 2 and 7 d of exposure to the contaminants.

2.4. Biochemical analyses

2.4.1. Enzyme assays and protein quantification

Liver and gills were homogenized (1:4, w/v) in Tris buffer 0.05 M (pH 7.4) containing sucrose 0.005 M, KCl 0.015 M and protease inhibitor (PMSF) 0.001 M. The homogenized samples were centrifuged at 10 000*g* for 20 min at 2 °C. The supernatant was collected and centrifuged at 50 000*g* for one hour at 2 °C. The resulting supernatant fraction was used for GST, SOD, CAT and GPx assays. For quantification of EROD activity, the pellet of liver samples was suspended in 100 μL of Tris buffer 0.1 M (pH 7.5), containing EDTA 0.001 M, dithiothreitol 0.001 M, KCl 0.1 M, and 20% glycerol.

The GST (Keen et al., 1976), SOD (McCord and Fridovich, 1969), CAT (Beutler, 1975) and GPx (Sies et al., 1979) activities were measured spectrophotometrically (Thermo Scientific Evolution 300).

EROD activity was measured in liver using the Burke and Mayer method (1974), with some modifications. The assay mixture contained 1950 μL of potassium phosphate buffer 0.08 M (pH 7.4), 20 μL of 7-ethoxyresorufin 3.35×10^{-4} M, 20 μL of NADPH 0.02 M and 10 μL of microsomal liver extract. The reaction was observed for 3 min at 30 °C. The analyses were done in a Thermo spectrofluorimeter.

Protein levels were measured by Bradford (1976) method using bovine serum albumin as standard.

2.4.2. Lipid peroxidation

In order to assess lipid peroxidation levels, the product formed from malondialdehyde (MDA) and thiobarbituric acid (TBA) combination was detected by high performance liquid chromatography coupled to UV/Vis detector (Almeida et al., 2003, 2004). For this analysis 100 mg of liver or gills samples were homogenized in 0.3 mL of Tris buffer 0.1 M (pH 8.0). Then 0.3 mL of a TBA solution (40 mg in 10 mL of HCl 0.2 M) was added to the homogenized sample and the resulted mixture was heated at 90 °C for 40 min. Next,

1 mL of n-butanol was added and samples were centrifuged at 3500 rpm for 3 min to extract the MDA–TBA derivatives. The supernatant was collected and the MDA–TBA derivatives were quantified by HPLC at 532 nm, in terms of a malondialdehyde (MDA) standard calibration curve that had been previously prepared using the same procedure used for the samples.

The HPLC system consisted of ESA584 pump and an ESA526 UV/Vis detector. The column ACE 5 C18 (250×4.6 mm, 5 μm) was used for this analysis. Chromatogram monitoring and peak identification and quantification were performed using the EZ Chrom Elite software (Agilent Technologies). The mobile phase (potassium phosphate 0.05 M, pH 7.0, with 40% methanol) was pumped at an isocratic flow of 1 mL min^{-1}.

2.4.3. Analysis of 8-oxodGuo

The DNA was extracted from the gills and hydrolyzed following the method of Ravanat et al. (2002). The 8-oxodGuo levels were measured by high performance liquid chromatography coupled to electrochemical detection (HPLC–EDC) (Almeida et al., 2003). An electrochemical coulometric detector (ESA Coulochem III, Massachussetts, USA) with potentials set at 120 and 280 mV in electrodes 1 and 2, respectively, was used to evaluate 8-oxogdG levels. The HPLC system and the column were the same used in the MDA assay. The ESA526 UV/Vis detector was set at 254 nm to measure the amount of dGuo. The mobile phase consisted of potassium phosphate buffer 0.05 M (pH 5.5) containing 5% methanol and was pumped at an isocratic flow rate of 1 mL min^{-1}. A standard calibration curve of 8-oxodGuo and dGuo was constructed for quantification of these compounds in hydrolyzed DNA samples.

2.4.4. Statistical analyses

Tests of normality (Shapiro–Wilk) and homogeneity (Levene) were performed. One-way ANOVA followed by Fisher LSD test was used to compare treatments of the same exposure period. Comparisons were made between both exposure period for the same treatment (same concentration and contaminant) using Student-*t* test. Kruskall–Wallis test was performed for non-parametric data for the first case and Mann–Whitney in the second case. Differences were considered significant only for $p < 0.05$. Analyses were performed with software Statistica v. 7.1.

2.4.5. Integrated biomarker response (IBR) calculation

The integrated biomarker response (IBR) was calculated following the method described by Beliaeff and Burgeot (2002). The biomarker score was represented in a star plot in which the radial coordinate corresponds to the score and all biomarkers were considered for IBR calculation. This analysis was done in order to provide a data overview. The IBR analysis was performed with R v. 2.15.2.

3. Results

No mortality was observed during the exposure experiments. The water pH and NH_3 levels after 2 d of exposure were 7.98 ± 0.38 and 0.55 ± 0.13 mg L^{-1}, respectively. After 7 d of exposure, pH and NH_3 levels were 8.06 ± 0.28 and 0.76 ± 0.19 mg L^{-1}, respectively. Despite the inexistence of studies regarding toxic effects of unionized ammonia to *P. anisitsi*, the reported LC50 values for NH_3 levels at pH 8.0 for most catfish species such as the channel catfish *Ictalurus punctatus* (24 h) and the silver catfish *Rhamdia quelen* (96 h) were above 1.80 mg L^{-1} (Tomasso et al., 1980; Sheehan and Lewis, 1986; Miron et al., 2008). So it can be supposed that NH_3 levels measured in this work did not interfere with biomarker responses. Anyway, there were no differences in NH_3 levels between treatments and control.

3.1. EROD activity

EROD activity was increased in the liver of fish exposed for 2 and 7 d to pure diesel oil, B5 and B20 when compared to control groups. In the case of animals treated with B100, there was increased EROD activity only among those exposed to the higher concentration for 7 d (Table 1). For 2 d, the lowest concentration of diesel fuel, B5 and B20 induced higher enzyme activity than the same treatment at higher concentrations. The same occurred for catfish exposed to B5 for 7 d.

The EROD activity in catfish after 2 d of treatment was lower in the groups exposed to pure diesel at 0.1 mL L^{-1} and B5 at 0.01 mL L^{-1}, but higher for the groups exposed to B20 0.01 mL L^{-1} in relation to the same groups after 7 d of exposure. In the second day of exposure, we observed no differences in EROD activity between the groups exposed to diesel, B5 and B20 at 0.01 mL L^{-1} and between the same groups for the concentration of 0.1 mL L^{-1}. On the seventh day, this similar EROD induction occurred only in the group exposed to the highest concentration of diesel and B5: EROD induction in fish exposed to pure diesel at 0.01 mL L^{-1} was lower than that observed for the tilapias exposed to B5 at the same concentration, but higher when compared to the group exposed to B20 at the same concentration.

3.2. GST activity

The GST activity was increased in catfish liver exposed to pure diesel oil 0.01 mL L^{-1}, B5 0.1 mL L^{-1} and both concentrations of B20 and B100 after 2 d of treatment (Table 1). GST activity decreased from the second to the seventh day of exposure to the two concentrations of B20 and B100. Furthermore, the hepatic activity of GST was decreased after 7 d on animals exposed to the lowest concentration of B20 when compared to control group.

In gills, all treatments promoted significant increases in GST activity when compared to control group after 2 d (Table 2). After 7 d, there was an increase in GST activity in the catfish gills contaminated with 0.01 and 0.1 mL L^{-1} of B20 and B100 and 0.1 mL L^{-1} of pure diesel oil. The GST activity decreased from 2 to 7 d in the groups exposed to 0.01 mL L L^{-1} of diesel oil and B5, while GST activity was increased in animals exposed to B100.

In addition, the B20 0.01 mL L^{-1} was able to induce higher GST activity than the diesel and biodiesel B5 in the catfish gills after 7 d. The same was observed for B20 0.1 mL L^{-1}, in which the group exposed to this treatment had higher enzyme activity compared to the same concentration of diesel and B5. Another fact is that both concentrations of B100 also induced higher GST activity in gills compared to all other contaminants, except for B20 0.01 mL L^{-1}.

3.3. SOD activity

In fish livers, SOD activity increased on the seventh day of exposure to all treatments, except for animals exposed to the lower concentration of B100 (Table 1). During this period of exposure, SOD activity was higher in the group exposed to B5 0.01 mL L^{-1} compared to all other treatments. Also, SOD activity increased from the second to the seventh day in animals exposed to B5 and to B100 at 0.1 mL L^{-1}.

The SOD activity was also changed in the *P. anisitsi* gills as a result of exposure to the contaminants (Table 2). SOD activity increased in fish exposed to the B20 0.1 mL L^{-1} and to both concentrations of B100 within 2 d when compared to the control group. The same results occurred on the concentration of 0.01 mL L^{-1} of diesel fuel, B20 and B100 within 7 d of the experiment. In addition, SOD activity was higher in 2 d for B20 and B100 0.1 mL L^{-1} than in 7 d of exposure to the same treatment.

Table 1
Activities of EROD, GST, SOD, CAT and GPx, and lipid peroxidation levels in liver of *P. anisitsi* exposed to 0.01 and 0.1 mL L^{-1} of pure diesel, B5 (5% biodiesel + 95% diesel), B20 (20% biodiesel + 80% diesel), and pure biodiesel for 2 and 7 d.

Period	Treatment	Concentration (mL L^{-1})	Biomarkers					
			EROD*	GST**	SOD**	CAT**	GPx**	MDA***
2 d	Control	–	0.93 ± 0.55	0.23 ± 0.04	8.15 ± 0.70	81.65 ± 27.35	0.014 ± 0.004	0.76 ± 0.13
	Diesel	0.01	4.17 ± 0.95[a,b]	0.33 ± 0.10[a]	10.16 ± 4.83	128.71 ± 47.65[a]	0.025 ± 0.006[a]	1.11 ± 0.39
	Diesel	0.1	2.09 ± 0.54[a,c]	0.29 ± 0.06	3.32 ± 2.00	131.40 ± 36.54[a]	0.019 ± 0.005	0.91 ± 0.25
	B5	0.01	5.07 ± 1.01[a,b,c]	0.26 ± 0.07	3.72 ± 0.54	104.63 ± 12.41[c]	0.021 ± 0.009	0.62 ± 0.25
	B5	0.1	2.53 ± 0.63[a]	0.33 ± 0.04[a]	2.97 ± 0.55[c]	115.74 ± 31.73[a]	0.020 ± 0.005[c]	0.80 ± 0.33
	B20	0.01	4.57 ± 0.87[a,b,c]	0.48 ± 0.17[a,c]	3.38 ± 0.50	118.72 ± 20.19[a]	0.028 ± 0.003[a,c]	1.21 ± 0.31
	B20	0.1	2.82 ± 1.30[a]	0.40 ± 0.07[a,c]	3.96 ± 0.63	139.33 ± 29.39[a]	0.022 ± 0.007[a]	0.80 ± 0.33
	B100	0.01	0.47 ± 0.20	0.73 ± 0.16[a,c]	16.21 ± 8.65	151.48 ± 13.02[a,c]	0.019 ± 0.003[b]	0.66 ± 0.36
	B100	0.1	0.86 ± 0.30	0.55 ± 0.17[a,c]	17.01 ± 3.50[c]	130.92 ± 30.80[a]	0.029 ± 0.009[a]	0.98 ± 0.35
7 d	Control	–	0.48 ± 0.20	0.21 ± 0.06	7.13 ± 2.77	92.66 ± 6.84	0.015 ± 0.002	0.88 ± 0.74
	Diesel	0.01	3.08 ± 1.64[a]	0.24 ± 0.02	13.63 ± 5.70[a]	102.32 ± 25.14	0.018 ± 0.005	0.31 ± 0.33
	Diesel	0.1	4.05 ± 0.53[a]	0.21 ± 0.07	12.77 ± 1.40[a]	116.26 ± 22.32	0.021 ± 0.003	0.35 ± 0.21
	B5	0.01	6.64 ± 1.07[a,b]	0.30 ± 0.07	18.51 ± 4.07[a,b]	151.61 ± 28.10[a,b]	0.025 ± 0.005[a]	0.67 ± 0.43
	B5	0.1	3.80 ± 1.10[a]	0.21 ± 0.10	13.54 ± 2.27[a]	126.17 ± 12.84[a]	0.029 ± 0.007[a]	1.24 ± 0.47
	B20	0.01	1.79 ± 0.62[a,b]	0.11 ± 0.08[a]	13.20 ± 5.34[a]	117.32 ± 15.04[a]	0.016 ± 0.002	0.70 ± 0.40
	B20	0.1	2.69 ± 0.24[a]	0.16 ± 0.05	13.62 ± 3.20[a]	121.12 ± 18.43[a]	0.021 ± 0.004	0.76 ± 0.59
	B100	0.01	0.69 ± 0.36	0.18 ± 0.07	11.63 ± 3.92	100.03 ± 13.30	0.017 ± 0.002	0.45 ± 0.31
	B100	0.1	0.74 ± 0.23[a]	0.23 ± 0.11	12.21 ± 2.42[a]	115.11 ± 17.49	0.016 ± 0.003	0.76 ± 0.42

Note: Data expressed as mean ± standard deviation.
* Activity expressed as pmol min^{-1} mg^{-1} protein.
** Activities expressed as U mg^{-1} protein.
*** Concentration expressed as nmol g^{-1} tissue.
[a] Significant difference compared to the control of the same period.
[b] Significant difference comparing different concentrations of the same treatment.
[c] Significant difference between the exposure periods of the same group.

Table 2
Activities of GST, SOD, CAT and GPx, lipid peroxidation levels and oxidative DNA damage content in gills of *P. anisitsi* exposed to 0.01 and 0.1 mL L^{-1} of pure diesel, B5 (5% biodiesel + 95% diesel), B20 (20% biodiesel + 80% diesel), and pure biodiesel for 2 and 7 d.

Period	Treatment	Concentration (mL L^{-1})	Biomarkers					
			GST*	SOD*	CAT*	GPx*	MDA**	8-Oxo-dGuo***
2 d	Control	–	0.08 ± 0.01	7.45 ± 1.03	2.56 ± 0.71	0.030 ± 0.007	0.60 ± 0.24[c]	20.58 ± 10.71
	Diesel	0.01	0.13 ± 0.03[a,c]	7.93 ± 1.18	3.13 ± 0.68	0.031 ± 0.006	0.75 ± 0.27	59.94 ± 43.70
	Diesel	0.1	0.12 ± 0.01[a]	7.11 ± 0.44	2.82 ± 1.06	0.033 ± 0.005	0.74 ± 0.28	40.37 ± 30.01
	B5	0.01	0.12 ± 0.01[a,c]	6.87 ± 0.97[b]	2.89 ± 0.60	0.031 ± 0.004	0.68 ± 0.14	51.92 ± 29.64
	B5	0.1	0.11 ± 0.02[a]	8.42 ± 0.97	2.41 ± 0.50	0.030 ± 0.007	0.83 ± 0.18	72.94 ± 50.47
	B20	0.01	0.12 ± 0.02[a]	8.86 ± 0.83	3.34 ± 0.82	0.028 ± 0.008	0.86 ± 0.17	32.93 ± 14.70
	B20	0.1	0.13 ± 0.03[a]	9.94 ± 1.48[a,c]	2.57 ± 0.33	0.027 ± 0.004	0.89 ± 0.20[a]	50.55 ± 29.00
	B100	0.01	0.11 ± 0.01[a]	9.91 ± 1.31[a]	2.87 ± 0.37	0.025 ± 0.005	0.84 ± 0.16	34.67 ± 12.78
	B100	0.1	0.10 ± 0.01[a,c]	10.41 ± 1.10 [a,c]	3.26 ± 1.03	0.026 ± 0.004	1.12 ± 0.14[a]	33.14 ± 8.12
7 d	Control	–	0.08 ± 0.02	6.34 ± 0.78	2.86 ± 0.31	0.029 ± 0.007	1.25 ± 0.18	17.98 ± 9.35
	Diesel	0.01	0.09 ± 0.02	8.46 ± 1.13[a]	3.43 ± 0.65	0.030 ± 0.010	1.35 ± 0.20	48.94 ± 32.76
	Diesel	0.1	0.11 ± 0.01[a]	7.34 ± 1.94	2.95 ± 0.92	0.032 ± 0.003	1.42 ± 0.73	55.65 ± 39.18
	B5	0.01	0.08 ± 0.02	5.47 ± 1.60	2.98 ± 0.78	0.029 ± 0.005	1.04 ± 0.24	91.41 ± 21.13
	B5	0.1	0.10 ± 0.02	5.98 ± 1.60	2.17 ± 0.68	0.026 ± 0.007	0.98 ± 0.14	42.93 ± 17.25
	B20	0.01	0.12 ± 0.02[a]	8.17 ± 1.08[a]	3.13 ± 0.90	0.027 ± 0.008	1.37 ± 0.25	33.76 ± 17.83
	B20	0.1	0.14 ± 0.02[a]	6.58 ± 1.08	2.30 ± 0.37	0.029 ± 0.009	1.33 ± 0.11	26.42 ± 7.61
	B100	0.01	0.12 ± 0.03[a]	8.20 ± 0.93[a]	2.99 ± 1.26	0.031 ± 0.014	1.33 ± 0.35	29.39 ± 10.20
	B100	0.1	0.13 ± 0.02[a]	7.09 ± 0.93	2.83 ± 0.82	0.026 ± 0.006	1.53 ± 0.37	17.44 ± 6.45

Note: Data expressed as mean ± standard deviation.
* Activities expressed as U mg^{-1} protein.
** Concentration expressed as nmol g^{-1} tissue.
*** Concentration expressed as 8-oxodGuo residues/10^6 dGuo.
[a] Significant difference compared to the control of the same period.
[b] Significant difference comparing different concentrations of the same treatment.
[c] Significant difference between the exposure periods of the same group.

3.4. CAT activity

The hepatic catalase activity of most groups exposed for 2 d was increased compared to control, except for animals exposed to B5 0.01 mL L^{-1}. After 7 d, animals exposed to 0.01 and 0.01 mL L^{-1} of B5 and B20 also presented increased CAT activity compared to the control group, while no difference was observed for animals exposed to pure diesel and B100. Comparing only the animals exposed to the two concentrations of B5 for 7 d, it was observed that the lower concentration induced a significantly higher CAT activity. Indeed, when comparing same treatments along time, it was observed that CAT activity increased from the second to the seventh day in animals exposed to 0.01 mL L^{-1} of B5, but decreased in animals exposed to 0.01 mL L^{-1} of B100. In gills, catalase activity was not altered by any treatment (Table 2).

3.5. GPx activity

The hepatic GPx activity was significantly higher in tilapias exposed for 2 d to pure diesel at 0.01 mL L^{-1}, B20 at 0.01 and 0.1 mL L^{-1}, and B100 0.1 mL L^{-1}, compared to control values. After 7 d, only B5 at both concentrations caused an increased in GPx activity. Along time, GPx decreased from the second to the seventh day for B5 0.1 mL L^{-1} and B20 0.01 mL L^{-1} treatments. In gills, GPx activity was unchanged after all treatments (Table 2).

3.6. MDA levels

We found no differences in the MDA levels in catfish liver among the treatments of 2 d and 7 d (Table 1). However, there was an increase in lipid peroxidation levels in catfish gills exposed to 0.1 mL L^{-1} of B20 and B100 after 2 d when compared these treatments with the control group (Table 2).

3.7. 8-oxodGuo

The 8-oxodGuo levels were unchanged in gills (Table 2) in both exposure periods by any contaminant.

3.8. Integrated biomarkers response

In liver, the IBR for concentration 0.01 mL L^{-1} was higher in 2 d than in 7 d of treatment. In this tissue for both concentrations we could notice an increase in the standardized biomarker responses value for B5 from 2 to 7 d of exposure and a decrease for the other treatments. In gills we could observe a temporal increase in the standardized biomarker response for B100 0.01 mL L^{-1} and diesel 0.1 mL L^{-1} (Fig. 1).

4. Discussion

According to Demirbas (2009), biodiesel has become more attractive recently because of its environmental benefits. However, in accordance to our previous work performed with *O. niloticus* (Nogueira et al., 2011b) biodiesel and its blends with diesel oil were also able to produce changes in the biomarker responses of *P. anisitsi.*

The cytochrome P4501A is highly induced in fish species such as *O. niloticus* (Trídico et al., 2010; Costa et al., 2011), *Sparus aurata* (Kopecka-Pilarczyk and Correia, 2009) when exposed to PAHs. In this study, we observed that the EROD activity was also induced in *P. anisitsi* exposed to all concentrations of diesel fuel, B5 and B20 in the same way as it was in another experiment with this specie exposed to 0.1 mL L^{-1} and 0.5 mL L^{-1} of diesel oil (Nogueira et al., 2011a). These results differ from other studies in which EROD activity was not detected in *Pterygoplichthys* sp. (Parente et al., 2011) and in two other species of armored catfish (*Hypostomus affinis* and *Hypostomus auroguttatus*) (Parente et al., 2009) injected with beta-naphthoflavone. However, *Pterygoplichthys* sp. has demonstrated the gene CYP1A, CYP1A mRNA and CYP1A protein. According to Parente et al. (2011), *Pterygoplichthys* sp. has six exclusive amino acid substitutions and for this reason there is a reduced frequency of correctly oriented substrate ethoxyresorufin in the active site preventing the detection of EROD activity. In fact, when comparing *P. anisitsi* from our current work with *O. niloticus* (Nogueira et al., 2011a) the EROD activity was around 10 times lower for catfish. However in our studies the EROD activity was detectable even in the control group. The reasons for these discrepancies should be further investigated, but it could be possible that our *Pterygoplichtys anisitsi* is not the same specie used by Parente's group, the so-called *Pterygoplichthys* sp.

We could notice that the EROD activity was higher in the group exposed for 2 d to the lower concentration of diesel oil, biodiesel B5, B20 and the same was observed for fish exposed for 7 d to B5. It was demonstrated that higher concentrations of diesel oil could inhibit the EROD activity in *O. niloticus* (Nogueira et al., 2011b) and the same could occur for the catalytic activity of CYP1A in this catfish specie. This response could be due to the presence of other components together with PAH in the diesel composition, which promotes inhibitory effect on CYP1A, as we previously proposed. Moreover, it was recently reported that PAH with two or three aromatic rings such as naphthalene, phenanthrene and fluoranthene are able to inhibit EROD activity (Pathiratne and Hemachandra, 2010). According to our previous analysis of PAH in the same diesel oil used in the present study (Nogueira et al., 2011b), such molecules were present in this fuel composition at high concentrations, which could be the main cause for the less EROD activity induction.

When comparing EROD activity of animals exposed to higher concentrations of diesel oil with B5 and B20 after 2 d, there were no differences. The same results occurred between animals exposed to lower concentrations after 2 d and fish exposed to higher concentrations of diesel oil and B5 after 7 d of treatment. A hypothesis for the lack of differences when comparing treatments with the same concentration would be the biodiesel concentration in B5 and B20. Despite B5 contains more PAHs compared to B20, the higher concentration of biodiesel in B20 probably increases the lipophilicity of these contaminants and promote an increase of PAH absorption and consequently an increase the activity of EROD in the same way the pure diesel oil did. Another hypothesis would be that the different proportions of diesel/biodiesel from contaminants are not sufficient to generate the differential effects between the treatments. However, after 7 d the enzyme activity was significantly lower in fish exposed to B20 than in groups exposed to B5 and diesel (when comparing the same concentrations). Initially, the biodiesel B20 would promote a high induction of EROD due to biodiesel in its composition that enhance the absorption rate of PAH present in diesel oil fraction of this mixture. However, since the diesel oil concentration in B20 is lower compared to pure diesel oil and B5, the substances present in B20 would be detoxified faster than other fuels and EROD activity back to baseline levels earlier. These finds are similar to our previous work in which Nile tilapias were exposed to the same experimental conditions as the armored catfish were (Nogueira et al., 2011b).

Further, even with a slight increase in EROD activity after 7 d in those catfish exposed to the highest concentration of B100, this biomarker can be used to distinguish pure biodiesel contamination from pure diesel and biodiesel blends since diesel promotes a more significant increase in EROD activity when compared with control and B100 groups.

GST induction is part of an adaptive response mechanism to chemical stress (Gadagbui and James, 2000), involving the detoxification of reactive intermediates and oxygen radicals (van der Oost et al., 2003). In some studies, the activity of GST was increased in fish species like *Carassius auratus* (Zhang et al., 2004), *Oreochromis niloticus* (Trídico et al., 2010) and *Prochilodus lineatus* (Simonato et al., 2011) after petroleum derivate exposure. In the catfish liver, this enzyme responded quickly to the presence of contaminants in the water, right at the second day of exposure, but at the seventh day, the enzyme activity had already been reduced. In their gills, the GST was increased quickly in all treatments in relation to the control group and this increase was maintained for 7 d, for almost treatments. Interestingly, B20 and B100 were responsible for causing the highest increase in gill and liver GST activity. As commented before, it is possible that higher amount of fats present in B20 composition increases absorption of toxic compounds present in diesel oil fraction, which can explain why the GST increase

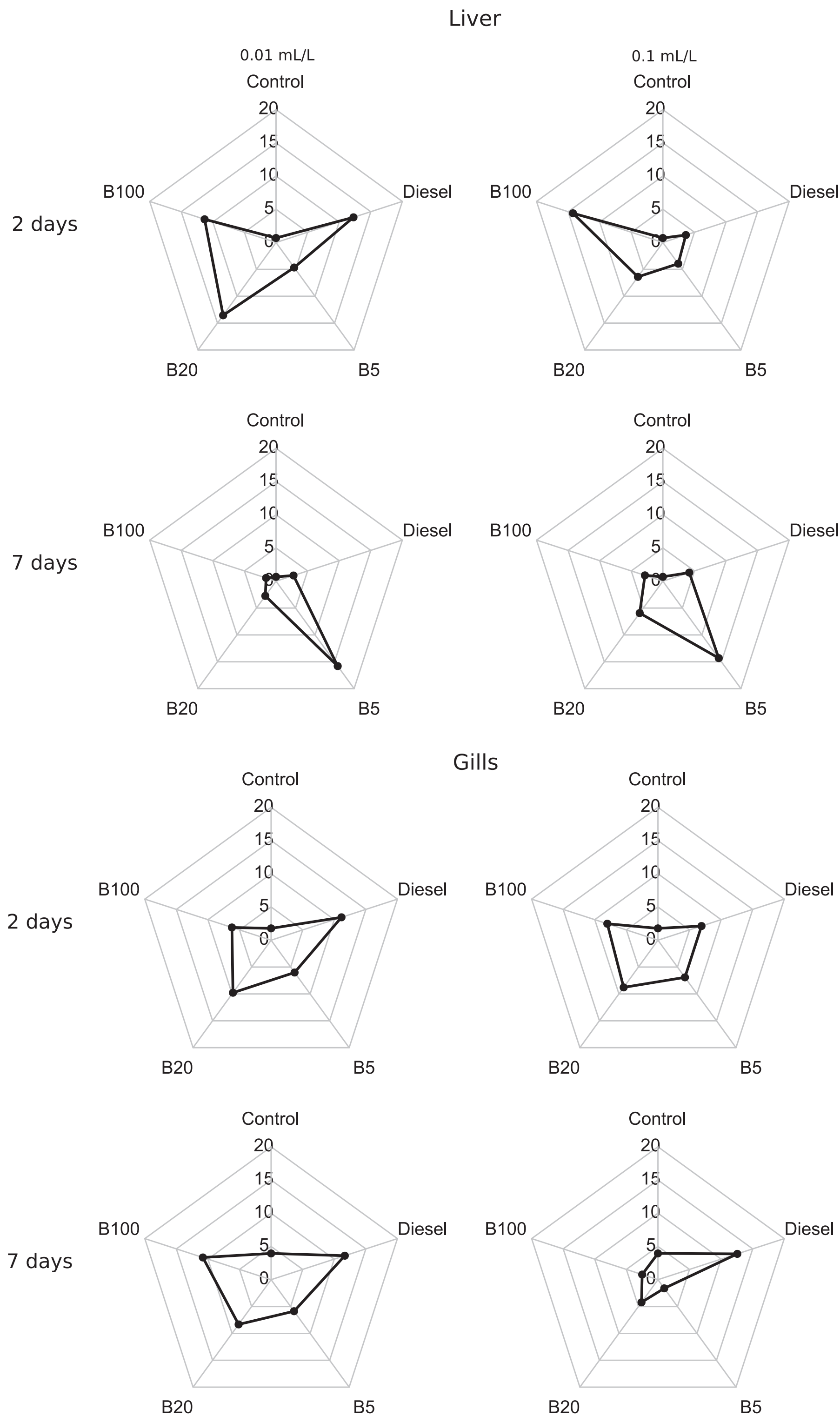

Fig. 1. Integrated biomarker response (IBR) star plots for liver and gills of *Pterygoplichthys anisitsi*.

was higher in B20 compared to B5 and pure diesel. However, B100 did not contain significant amounts of PAH (Nogueira et al., 2011b), so GST increase in these fish is not expected as a response due to PAH exposure. It is possible that some other substances present in these fuel or by-products of its biotransformation metabolism could lead to GST activation.

Furthermore, GST activity was kept high for longer in gills than in livers probably due to close contact with the contaminated water and the by-products, which are being formed and eliminated in the water along the time. Therefore, since the reactive intermediates would be present in higher amounts in the gills, the GST was kept active for longer in this tissue. Moreover, it was noticed that MDA levels increased in the fish gills exposed to higher concentrations of B20 and B100, thus the GST induction could be also a response to increased lipid peroxidation levels in fish. It has been reported that some GST isoforms have peroxidase activity (Fiander

and Schneider, 1999; Collinson and Grant, 2003), which could be acted against lipid hydroperoxides generated due ROS production during fuel exposure.

The ROS production can also explain the activation of antioxidant system in *P. anisitsi*. The hepatic SOD, CAT and GPx responded significantly to treatments, showing that the antioxidant system was activated. In their gills, SOD increased in response to lower concentrations of diesel oil and the two concentrations of B20 and B100. The catalase and GPx in the same tissue did not change, which could be compensated by the activation of GST, as previously commented. The increased activity of antioxidant enzymes in fish exposed to diesel oil, B5, B20 and B100 demonstrates that there was an increase in ROS in the liver and gills, but the armored catfish showed no signs of lipid peroxidation in the liver, indicating that antioxidant defenses were efficient to counteract ROS. On the other hand, the antioxidant system could not prevent lipid peroxidation in their gills when the animals were exposed for 2 d to the higher concentration of B20 and B100. Nevertheless, after 7 d of exposure, the lipid peroxidation in gills decreased returning to levels similar to the control group, demonstrating an effective support of the antioxidant enzymes to avoid oxidative damage.

Fish *Prochilodus lineatus* exposed to gasoline (Simonato et al., 2011) and mussels *Mytilus galloprovincialis* exposed to benzo[a]-pyrene (Maria and Bebianno, 2011) showed high levels of lipid peroxidation in the gills, showing that PAH are able to produce oxidative damage. Considering that B100 does not contains PAH, it can hypothesized that other components are being responsible to trigger ROS generation in exposed fish, like acid compounds and peroxides generated due to biodiesel auto-oxidation (National Renewable Energy Laboratory, 2009). Leite et al. (2011) found an increase in the methanol concentration of water-soluble fraction of biodiesel obtained of castor oil from day 1 up to day 120 of incubation due to the reversion of the transesterification process in the presence of water. According to the data provided by industry that produced the biodiesel used in our work, there is no methanol in its composition. But, in the same way as biodiesel from castor oil, the methanol concentration could have increased in biodiesel from animal fat. So, since methanol is a toxic substance, this would also explain the effects promoted by B100 in catfish. However, to validate this hypothesis more tests are needed.

Lipid peroxidation is a process that occurs spontaneously in biodiesel produced from animal fat, since it can be detected by low levels of malondialdehyde in their composition (Nogueira et al., 2011b). As biodiesel probably should be easily absorbed due to the presence of lipophilic compounds such as monoglycerides and diglycerides in their formulation and lipid peroxidation is a chain reaction, the oxidative processes that occur in pure biodiesel would be the trigger to induce lipid peroxidation in fish gills. This would be one of the factors that explain the increased presence of MDA in the catfish gills exposed to B100.

The temporal decrease in the standardized biomarker response in liver for diesel, B20 and B100 after 2 d of exposure was probably due to a high input of toxic compounds in the organism. Over time the toxic compounds were metabolized and excreted and the IBR decreased. In the opposite way, B5 presented an increase in standardized biomarker response from 2 d to 7 d of exposure for both concentrations, showing that this blend takes more time to be metabolized and keeping the biomarker responses high over time.

In gills, we could notice that the IBR decreased over time for concentration 0.1 mL L^{-1} because the biomarkers in general decrease the responses levels to B5, B20 and B100. Just diesel oil kept the biomarker responses in a high level, showing that in the gills, unlike what happened in the liver, this contaminant takes more time to be metabolized and excreted. It is interesting to observe that B100 0.01 mL L^{-1} in gills also presented an increase in the standardized biomarker response. As already mentioned, MDA could be generated spontaneously in biodiesel B100 from animal fat. The lipid peroxidation that happens in B100 could act as a trigger for lipid peroxidation in gills, since gills are in direct contact with water contaminated by B100. As lipid peroxidation was observed in gills exposed to B100, probably the organism should be counteract ROS to prevent oxidative damage, which could explain the increased value over time in B100 standardized biomarker response.

Reactive oxygen species can cause specific oxidative DNA damage and play a leading role in initiation and promotion of carcinogenesis (Valavanidis et al., 2009). However, probably due to the antioxidant system has been activated efficiently in the catfish, the 8-oxo-dGuo levels in gills exposed to all treatments were similar to the basal levels in control groups, similar to that occurred with gill of mussels *Mytilus galloprovincialis* exposed to B[a]P-contaminated feed (Akcha et al., 2000). In addition, DNA repair mechanisms also could be activated, resulting in a smaller number of oxidative damage in guanines. However, this hypothesis needs to be further tested.

Compared to Nile tilapias (Nogueira et al., 2011b), the increase in the catfish antioxidant system in response to contaminants exposure probably turns this fish specie more resistant to contamination. Even when the catfish were exposed to high diesel oil concentration (0.5 mL L^{-1}), the SOD activity was kept increased and the fish did not die after 7 d of exposure, unlikely what happened with Nile tilapia that died after 7 d of exposure under the same conditions, demonstrating a higher resistance of *P. anisitsi* to this type of contaminant. Despite being a benthic animal, *P. anisitsi* is an air breathing catfish specie (Cruz et al., 2009). When they rise to the surface to breathe, they make contact with the insoluble portion of diesel and biodiesel, which means that these animals could be more affected by this type of compound. It is important to emphasize that the presence of sediment was not considered in our experiments. As the sediment in the environment can retain many hydrophobic compounds (Jantunen et al., 2008), it is possible that this compartment could contribute to the access of benthic animals to more biodiesel and diesel oil fractions, but this needs to be further investigated.

In general, all contaminants affected the fish species used in some way. The blends B5 and B20 changed most of the enzymes tested and in some cases induced more enzymatic activity than diesel oil. As already discussed above, biodiesel is likely to increase the lipophilicity of the mixture, causing an increase in the absorption of toxic compounds present in diesel oil. This could explain that although biodiesel blends have lower concentrations of diesel oil in its composition, they would promote similar or worse damage than diesel.

5. Conclusions

P. anisitsi is a fish specie that has not been used in ecotoxicological studies. This work shows that this specie is resistant to oxidative damage because their antioxidant system responds quickly to the presence of contaminants. Taking into account the concentrations and exposure periods used, catalase and GPx in their gills were the only enzymes that have not responded to treatment. Thus, this specie of fish can be used as sentinel organism for detecting diesel oil and biodiesel contamination in freshwaters environments. Regarding contaminants, diesel oil, B5 and B20 blends and pure biodiesel promoted alteration of several biochemical parameters tested in *P. anisitsi*. Biodiesel is likely to increase the lipophilicity of the mixture, causing an increase in the absorption of toxic compounds present in diesel oil, a fact that should be contributing for the changes observed in biochemical parameters of fish exposed to B5 and B20. In the case of pure biodiesel, further

research is needed to better address which compounds are present in its composition that are able to promote changes in aquatic organisms, since it changed the activities of antioxidant defenses and GST of catfish, and also induced lipid peroxidation. Finally, according to our results, biodiesel from animal fat and their mixtures at the concentrations tested in this study can cause oxidative stress and enzymatic changes in *P. anisitsi* as much as pure diesel. Therefore, even if a fuel considered more environmentally friendly, it has been shown that biodiesel presents a risk to aquatic biota, corroborating our previous study with Nile tilapia. Therefore, procedures must be adopted to reduce the risks of accidents and inappropriate discharges into the environment during production, transport and use of this fuel. Moreover, our findings highlight the importance of new studies related to exposure of aquatic organisms to biodiesel and its blends to better understand the mechanisms of toxicity that these compounds may generate.

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References

Akcha, F., Burgeot, T., Budzinski, H., Pfohl-Leszkowicz, A., Narbonne, J.F., 2000. Induction and elimination of bulky benzo[a]pyrene-related DNA adducts and 8-oxodGuo in mussels *Mytilus galloprovincialis* exposed *in vivo* to B[a]P-contaminated feed. Mar. Ecol. Prog. Ser. 205, 195–206.

Almeida, E.A., Bainy, A.C.D., Dafre, A.L., Gomes, O.F., Medeiros, M.H.G., Di Mascio, P., 2005. Oxidative stress in digestive gland and gill of the brown mussel (*Perna perna*) exposed to air and re-submersed. J. Exp. Mar. Biol. Ecol. 318, 21–30.

Almeida, E.A., Bainy, A.C.D., Loureiro, A.P.M., Martinez, G.R., Miyamoto, S., Onuki, J., Barbosa, L.F., Garcia, C.C.M., Prado, F.M., Ronsein, G.E., Sigolo, C.A., Brochini, C.B., Martins, A.M.G., Medeiros, M.H.G., Di Mascio, P., 2007. Oxidative stress in *Perna perna* and other bivalves as indicators of environmental stress in the Brazilian marine environment: antioxidants, lipid peroxidation and DNA damage. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. 146, 588–600.

Almeida, E.A., Miyamoto, S., Bainy, A.C., Medeiros, M.H., Di Mascio, P., 2004. Protective effect of phospholipid hydroperoxide glutathione peroxidase (PHGPx) against lipid peroxidation in mussels *Perna perna* exposed to different metals. Mar. Pollut. Bull. 49, 386–392.

Almeida, E.A., Marques, S.A., Klitzke, C.F., Bainy, A.C.D., Medeiros, M.H.G., Di Mascio, P., Loureiro, A.P.M., 2003. DNA damage in digestive gland and mantle tissue of the mussel *Perna perna*. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 135, 295–303.

Balat, M., Balat, H., 2010. Progress in biodiesel processing. Appl. Energy 87, 1815–1835.

Basha, S.A., Gopal, K.R., Jebaraj, S., 2009. A review on biodiesel production, combustion, emissions and performance. Renew. Sust. Energy Rev. 13, 1628–1634.

Beliaeff, B., Burgeot, T., 2002. Integrated biomarker response: a useful tool for ecological risk assessment. Environ. Toxicol. Chem. 21, 1316–1322.

Beutler, E., 1975. Red Cell Metabolism: A Manual of Biochemical Methods. Grune & Stratton, New York.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Burke, M.D., Mayer, R.T., 1974. Ethoxyresorufin: direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug. Metab. Dispos. 2, 583–588.

Collinson, E.J., Grant, C.M., 2003. Role of yeast gluta-redoxins as glutathione S-transferases. J. Biol. Chem. 278, 22492–22497.

Corrêa, S.M., Arbilla, G., 2006. Aromatic hydrocarbons emissions in diesel and biodiesel exhausts. Atmos. Environ. 40, 6821–6826.

Costa, J., Ferreira, M., Rey-Salgueiro, L., Reis-Henrique, M.A., 2011. Comparision of the waterborne and dietary routes of exposure on the effects of Benzo(a)pyrene on biotransformation pathways in Nile tilapia (*Oreochromis niloticus*). Chemosphere 84, 1452–1460.

Cruz, A.L., Pedretti, A.C.E., Fernandes, M.N., 2009. Stereological estimation of the surface area and oxygen diffusing capacity of the respiratory stomach of the air-breathing armored catfish *Pterygoplichthys anisitsi* (teleostei: Loricariidae). J. Morphol. 270, 601–614.

DeMello, A.J., Carmichael, C.A., Peacock, E.E., Nelson, R.K., Arey, J.S., Reddy, C.M., 2007. Biodegradation and environmental behavior of biodiesel mixtures in the sea: an initial study. Mar. Pollut. Bull. 54, 894–904.

Demirbas, A., 2009. Progress and recent trends in biodiesel fuels. Energy Convers. Manage. 50, 14–34.

Fiander, H., Schneider, H., 1999. Compounds that induce isoforms of glutathione S-transferase with properties of a critical enzyme defense against oxidative stress. Biochem. Biophys. Res. Commun. 262, 591–595.

Frenzilli, G., Scarcelli, V., Del Barga, I., Nigro, M., Förlin, L., Bolognesi, C., Sturve, J., 2004. DNA damage in eelpout (*Zoarces viviparus*) from Göteborg harbour. Mutat. Res. 552, 187–195.

Gadagbui, B., James, M., 2000. Activities of affinity-isolated glutathione S-transferase (GST) from channel catfish whole intestine. Aquatic Toxicol. 49, 27–37.

Jantunen, A., Tuikka, A., Akkanen, J., Kukkonen, J.V.K., 2008. Bioaccumulation of atrazine and chlorpyrifos to *Lumbriculus variegatus* from lake sediments. Ecotoxicol. Environ. Saf. 71, 860–868.

Keen, J.H., Habig, W.H., Jakoby, W.B., 1976. Mechanism for the several activities of the glutathione S-transferases. J. Biol. Chem. 251, 6183–6188.

Khan, N., Warith, M.A., Luk, G., 2007. A comparison of acute toxicity of biodiesel, biodiesel blends, and diesel on aquatic organisms. J. Air Waste Manage. 57, 286–296.

Kopecka-Pilarczyk, J., Correia, A.D., 2009. Biochemical response in gilthead seabream (Sparus aurata) to in vivo exposure to a mix of selected PAHs. Ecotoxicol. Environ. Saf. 72, 1296–1302.

Kuchino, Y., Mori, F., Kasai, H., Inoue, H., Iwai, S., Miura, K., Ohtsuka, E., Nishimura, S., 1987. Misreading of DNA templates containing 8-hydroxydeoxyguanosine at the modified base and at adjacent residues. Nature 327, 77–79.

Leduc, S., Natarajan, K., Dotzauer, E., McCallum, I., Obersteiner, M., 2009. Optimizing biodiesel production in India. Appl. Energy 86, 125–131.

Leite, M.B.N.L., Araújo, M.M.S., Nascimento, I.A., Cruz, A.C.S., Pereira, S.A., Nascimento, N.C., 2011. Toxicity of water soluble fractions of biodiesel fuels derived from castor oil, palm oil, and waste cooking oil. Environ. Toxicol. Chem. 30, 893–897.

Leme, D.M., Grummt, T., de Oliveira, D.P., Sehr, A., Renz, S., Reinel, S., Ferraz, E.R.A., de Marchi, M.R.R., Machado, M.C., Zocolo, G.J., Marin-Morales, M.A., 2012. Genotoxicity assessment of water soluble fractions of biodiesel and its diesel blends using the *Salmonella* assay and the in vitro MicroFlow® kit (Litron) assay. Chemosphere 86, 512–520.

Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Pollut. Bull. 42, 656–666.

McCord, J.M., Fridovich, I., 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244, 6049–6055.

Maria, V.L., Bebianno, M.J., 2011. Antioxidant and lipid peroxidation responses in *Mytilus galloprovincialis* exposed to mixtures of benzo(a)pyrene and copper. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 154, 56–63.

Miron, D.S., Moraes, B., Becker, A.G., Crestania, M., Spanevello, R., Loro, V.L., Baldisserotto, B., 2008. Ammonia and pH effects on some metabolic parameters and gill histology of silver catfish, *Rhamdia quelen* (Heptapteridae). Aquaculture 277 (3–4), 192–196.

National Renewable Energy Laboratory, 2009. Biodiesel Use and Handling Guide, fourth ed.

Nico, L.G., Loftus, W.F., Reid, J.P., 2009. Interactions between non-native armored suckermouth catfish (Loricariidae: *Pterygoplichthys*) and native Florida manatee (*Trichechus manatus latirostris*) in artesian springs. Aquat. Invas. 4, 511–519.

Nico, L.G., Martin, T.R., 2001. The South American suckermouth armored catfish, *Pterygoplichthys anisitsi* (Pisces: Loricariidae), in Texas, with comments on foreign fish introductions in the American Southwest. Southwest. Nat. 46, 98–104.

Nogueira, L., Rodrigues, A.C.F., Trídico, C.P., Fossa, C.E., Almeida, E.A., 2011a. Oxidative stress in Nile tilapia (*Oreochromis niloticus*) and armored catfish (*Pterygoplichthys anisitsi*) exposed to diesel oil. Environ. Monit. Assess. 180, 243–255.

Nogueira, L., Sanches, A.L.M., Silva, D.H.G., Ferrizi, V.C., Moreira, A.B., Almeida, E.A., 2011b. Biochemical biomarkers in Nile tilapia (*Oreochromis niloticus*) after short-term exposure to diesel oil, pure biodiesel and biodiesel blends. Chemosphere 85, 97–105.

Parente, T.E.M., De-Oliveira, A.C.A.X., Beghini, D.G., Chapeaurouge, D.A., Perales, J., Paumgartten, F.J.R., 2009. Lack of constitutive and inducible ethoxyresorufin-O-deethylase activity in the liver of suckermouth armored catfish (*Hypostomus affinis* and *Hypostomus auroguttatus*, Loricariidae). Comp. Biochem. Physiol. C Toxicol. Pharmacol. 150, 252–260.

Parente, T.E.M., Rebelo, M.F., Da-Silva, M.L., Woodin, B.R., Goldstone, J.V., Bisch, P.M., Paumgartten, F.J.R., Stegeman, J.J., 2011. Structural features of cytochrome P450 1A associated with the absence of EROD activity in liver of the loricariid catfish *Pterygoplichthys sp.* Genetics 489, 111–118.

Pathiratne, A., Hemachandra, C.K., 2010. Modulation of ethoxyresorufin O-deethylase and glutathione S-transferase activities in Nile tilapia (Oreochromis niloticus) by polycyclic aromatic hydrocarbons containing two to four rings: implications in biomonitoring aquatic pollution. Ecotoxicology 19, 1012–1018.

Poon, R., Valli, V.E., Rigden, M., Rideout, G., Pelletier, G., 2009. Short-term oral toxicity of three biodiesels and an ultra-low sulfur diesel in male rats. Food Chem. Toxicol. 47, 1416–1424.

Ravanat, J.L., Douki, T., Duez, P., Gremaud, E., Herbert, K., Hofer, T., Lasserre, L., Saint-Pierre, C., Favier, A., Cadet, J., 2002. Cellular background level of 8-oxo-7,8-dihydro-2′-deoxyguanosine: an isotope based method to evaluate artefactual oxidation of DNA during its extraction and subsequent work-up. Carcinogenesis 23, 1911–1918.

Schröder, S., Bünger, J., Munack, A., Knothe, G., Krahl, J., 2013. Exhaust emissions and mutagenic effects of diesel fuel, biodiesel and biodiesel blends. Fuel 103, 414–420.

Sheehan, R.J., Lewis, W.M., 1986. Influence of pH and ammonia salts on ammonia toxicity and water balance in young channel catfish. Trans. Am. Fish. Soc. 115, 891–899.

Simonato, J.D., Fernandes, M.N., Martinez, C.B.R., 2011. Gasoline effects on biotransformation and antioxidant defenses of the freshwater fish *Prochilodus lineatus*. Ecotoxicology 20, 1400–1410.

Sies, H., Koch, O.R., Martino, E., Boveris, A., 1979. Increased biliary glutathione disulfide release in chronically ethanol-treated rats. FEBS Lett. 103, 287–290.

Tomasso, J.R., Goudie, C.A., Simco, B.A., Davis, K.B., 1980. Effects of environmental pH and calcium on ammonia toxicity in channel catfish. Trans. Am. Fish. Soc. 109, 229–234.

Trídico, C.P., Rodrigues, A.C.F., Nogueira, L., Silva, D.C., Moreira, A.B., Almeida, E.A., 2010. Biochemical biomarkers in *Oreochromis niloticus* exposed to mixtures of benzo[a]pyrene and diazinon. Ecotoxicol. Environ. Saf. 73, 858–863.

Valavanidis, A., Vlachogianni, T., Fiotakis, C., 2009. 8-hydroxy-2′-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. J. Environ. Sci. Heal. C 27, 120–139.

van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.

Zhang, J.F., Wang, X.R., Guo, H.Y., Wu, J.C., Xue, Y.Q., 2004. Effects of water-soluble fractions of diesel oil on the antioxidant defenses of the goldfish, *Carassius auratus*. Ecotoxicol. Environ. Saf. 58, 110–116.