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Seahorse (*Hippocampus reidi*) as a bioindicator of crude oil exposure

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ABSTRACT

This study explored the suitability of the seahorse *Hippocampus reidi* (Ginsburg, 1933) for assessing biomarkers of genotoxic effects and its use as a sentinel organism to detect the effects of acute exposure to petroleum hydrocarbons. Fish were exposed to three concentrations of crude oil (10, 20 and 30 g/kg) for 96 h, and the activity of phase II biotransformation enzyme glutathione S-transferase (GST) was measured. In addition, we performed genotoxicity assays, such as comet assay, micronucleus (MN) test and nuclear abnormalities (NA) induction, on the erythrocytes of the fish species. Our results revealed that the inhibition of hepatic GST activity in *H. reidi* was dependent on increasing crude oil concentrations. In contrast, an increase in the damage index (DI) and MN frequency were observed with increased crude oil concentrations. These results indicate that the alkaline comet assay and micronucleus test were suitable and useful in the evaluation of the genotoxicity of crude oil, which could improve determinations of the impact of oil spills on fish populations. In addition, *H. reidi* is a promising "sentinel organism" to detect the genotoxic impact of petroleum hydrocarbons.

1. Introduction

Marine pollution monitoring programs have successfully included biological responses known as biomarkers as tools to assess potential toxic effects of pollutants (Cajaraville et al., 2000). Fish are aquatic vertebrates that have become suitable models to estimate possible risks in the aquatic environment because of their ability to efficiently metabolize and accumulate chemical pollutants (Çavas, 2013).

Seahorses (*Hippocampus spp.*) generally inhabit coastal habitats in temperate and tropical waters (Rosa et al., 2002; Teske et al., 2007). Seahorses have low population densities, and they are sparsely distributed. Seahorses are poor swimmers, and they anchor to vegetation or other substrates using their prehensile caudal tail. Seahorses rarely venture into the open sea to pursue prey or colonize new areas (Rosa et al., 2005; Vieira and Gasparini, 2007). This sedentary characteristic results in increased exposure to toxic substances when pollution events occur. Seahorses are subjected to acute contaminant exposure because of their low mobility and wide distribution. Other species with greater capacity for locomotion can escape the contaminated area more easily. In addition, seahorses have great potential as sentinel species and can be used to monitor marine ecosystems. Therefore, we chose this species as a bioindicator of contamination of marine and estuarine environments by petroleum and its derivatives. Polycyclic aromatic hydrocarbons (PAHs) are the most toxic components of petroleum products (Lima et al., 2007). PAHs that are released into the marine environment tend to be rapidly absorbed by suspended matter and sediment becoming available to fish and other marine organisms through the food chain (Perugini et al., 2007).

The most commonly used biochemical tests in fish studies are assays of liver enzymes that are involved in the detoxification of xenobiotics and their respective metabolites, like the glutathione S-transferase enzymes (GST) (Teles et al., 2005). Glutathione S-transferase (GST) is a phase II enzyme that plays an important role in the detoxification of endogenous (intracellular metabolites) and exogenous substances (drugs, pesticides, and other pollutants) through conjugation to glutathione (GSH), which converts a reactive lipophilic molecule into a water-soluble non-reactive conjugate to facilitate excretion (Hayes and Pulford, 1995; Richardson et al., 2008; Simonato et al., 2011). Glutathione S-transferase belongs to a multigene family that is present in all organisms, and the structural diversity within the GST family of isoenzymes allows the conjugation of a broad range of compounds (Robertson et al., 1986; Hedges and Kumar, 2002).

Two tests extensively used at the cellular level are the micronucleus (MN) test and the comet assay (Gustavino et al., 2005).

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The micronucleus (MN) test is a sensitive and rapid technique to detect structural and numerical chromosomal alterations that are induced by clastogenic and aneugenic agents in different cell types without prior knowledge of karyotype and cell turnover rate (Heddle et al., 1991; Jha, 2008; Santos et al., 2010). Recently, more attention has been directed to integrated analyses of micronuclei and other nuclear abnormalities (NA), and these nuclear abnormalities may be considered genotoxic analogues of micronuclei and complement MN scoring in routine genotoxicity surveys (Çavas and Ergene-Gozukara, 2005; Baršiene and Andreikenaite 2007; Rybakovas et al., 2009). The MN test is the most suitable technique for fish species (Al-sabti and Metcalfe, 1995; Çavas and Ergene-Gozukara, 2005; Udroiu, 2006).

The single cell gel electrophoresis, or comet assay, is also a very sensitive genotoxicity test. The comet assay has been used for assessment of a broad spectrum of DNA damage in single cells (Singh et al., 1988; McKelvey-Martin et al., 1993; Tice et al., 2000), including DNA single- and double-strand breaks, alkali-labile sites and excision-repair events caused by simple and bulky DNA adducts at the individual cell level.

Therefore, the present study investigated the potential impact of crude oil contamination on the native marine fish species *Hippocampus reidi* using the response of phase II biotransformation enzyme, the alkaline comet assay and the micronucleus test in peripheral blood erythrocytes.

2. Materials and methods

2.1. Animals and acclimation

Hippocampus reidi specimens were obtained from a seahorse hatchery located at Serra municipality, Espirito Santo state, Brazil, and transferred to the Applied Ichthyology Laboratory (FISHLAB, Vila Velha University-UVV). Individuals were acclimated in the laboratory for 15 days in a 40 L glass aquaria with continuous aeration, in which 70% of the water was changed twice a week. Fish were fed frozen wild Mysidacea twice a day. Feeding was suspended 24 h before the animals were transferred to test aquaria.

2.2. Experimental design

A total of 24 adult specimens of *H. reidi*, approximately four months of age, were used in this study (8.77 ± 0.19 cm and 3.27 ± 0.20 g). *Hippocampus reidi* were transferred to individual test aquaria (4 L) with continuous aeration and a 12 h photoperiod after acclimation (15 days). Crude oil (230.7 mg of total PAH/kg, Table 1) was added to test aquaria after 24 h in three nominal concentrations: 10; 20 and 30 g/kg ($\approx$ 10, 20 and 30 mL crude oil per L of seawater) for 96 h. Crude oil was carefully added to water of each test aquarium, resulting in an oil layer on the water surface. This layer was slowly mixed into the water column due to the aeration and fish movements. These nominal concentrations derived from LC_{50} values for *Astronotus ocellatus* (Val et al., 2003) and from previous experiments in our Laboratory. The effects of crude oil were non lethal and could be experimentally detected using the current exposure period.

Six specimens were used in each crude oil concentration (one animal per aquarium). A control group (sea water without contaminant) was also set under similar exposure conditions. Experiments occurred in static conditions. The local animal and environmental ethic committees approved the experimental procedures.

Table 1
BTEX and PAH content of the crude oil used in this study and the water-soluble fraction of crude oil (WSF) at the initial and the end of the 96 h experimental period.

Compounds	Crude oil (mg/Kg)	WSF-0 h (μg/L)	WSF-96 h (μg/L)
BTEX			
Benzene	< 0.08	< 1.0	< 1.0
Ethyl benzene	115.34	< 1.0	< 1.0
Toluene	26.35	< 1.0	< 1.0
Xylene	118.78	< 1.0	< 1.0
PAHs			
Acenaphthene	< 0.02	< 0.01	< 0.01
Acenaphthylene	< 0.02	< 0.01	< 0.01
Anthracene	10.233	0. 159	0.134
Benz[a]anthracene	5.16	0.130	< 0.01
Benzo[b]fluoranthene	3.19	< 0.01	< 0.01
Benzo[ghi]perylene	1.11	0.034	< 0.01
Benzo[k]fluoranthene	< 0.02	0.056	< 0.01
Benzo[a]pyrene	3.90	0.074	< 0.01
Chrysene	17.22	0.337	< 0.01
Dibenz[ah]anthracene	1.11	< 0.01	< 0.01
Phenanthrene	126.14	3.619	1.76
Fluorene	42.75	1.456	0.20
Indeno(1, 2, 3-cd)pyrene	< 0.02	< 0.01	< 0.01
Naphthalene	235.56	< 1.0	< 1.0
Pyrene	19.91	0.384	0.19
Total PAHs	230.723	5076.149	2.309

2.3. Biological material sampling

Animals were sedated with a 0.1 g/L benzocaine solution at the end of the experiments. Blood was collected through a caudal vein puncture using heparinized syringes for the alkaline comet assay and MN test. Fish were sacrificed immediately after blood retrieval by cervical sectioning according to Winkaler et al. (2007), and hepatic tissue was excised and stored in liquid nitrogen for GST enzyme analysis.

2.4. Experimental water and crude oil analysis

The saline water in acclimation and test aquaria was collected in Itapuã beach (20°21'13'' S, 40°17'02'' W), Vila Velha, ES, Brazil. Physical and chemical characteristics of the acclimation water, such as temperature, dissolved oxygen and salinity, were measured using a Multiparameter YSI, model 85 (Yellow Springs, USA).

The state oil company (Petrobras) donated the crude oil that was used in this study. Samples of water-soluble fraction of crude oil (1 L) were carefully withdrawn by siphoning at time 0 and 96 h after crude oil addition. An aliquot of crude oil or water soluble fraction of crude oil were transferred to the sample vial with a headspace of 20 mL for analyses of BTEX (Benzene, ethylbenzene, toluene and xylene) and 16 PAHs. Ten milliliters of ultrapure water and 20 mL of a solution of internal standard and surrogate Internal/ Surrogate Mix Standard VOA (5 mg/mL in methanol) were added. Samples were analyzed using gas chromatography with headspace sampling using an autosampler model TriPlus (Thermo Scientific) in a Trace GC Ultra (Thermo Scientific) coupled to a DSQ II mass spectrometer (Thermo Scientific) with the following settings: incubation sample of 24 min at 70 °C, 1 mL sample volume, split mode of injection (ratio 20), and chromatographic conditions and detection based on the EPA 8260 methods. The following reference standards were used: VOC liquid Mixture-Modified AccuStandard Cat M-502 A-R2-10X and Internal/Surrogate Standard Mix VOA Standard Accu CatM-8240/60-IS/ SS-10X. An aliquot of sample was added to a 10 mL volumetric flask, and the volume was completed with dichloromethane and transferred. This solution was withdrawn at a rate of 400 to 12 mL vials. The volume was increased to 1 mL with dichloromethane. Subsequently, 20 mL

of a solution of internal standard and surrogate Internal Standard Mix/ Method 8270-Standard Surrogate (25 mg/mL in dichloromethane) were added. Samples were analyzed using gas chromatography with liquid sample in a model TriPlus auto sampler (Thermo Scientific) in a trace GC Ultra (Thermo Scientific) coupled to a DSQ II mass spectrometer (Thermo Scientific) with the following settings: sample volume of 3 mL, the mode of injection was splitless, and the chromatographic conditions and detection were based on the EPA 8270 methods. The following benchmarks were used: Internal Standard Mix Standard Accu Cat Z-014J, Method 8270-Surrogate StandardAccu Standard CatM-8270-SS, Fluoranthene Absolute Cat Standard 70183, and SVOC EPA 525 506 540 Cal Mix Supelco Cat. The following PAHs were determined: acenaphthene, acenaphthylene, anthracene, benz[a] anthracene, benzo[b]fluoranthene, benzo [ghi] perylene, benzo[k] fluoranthene, benzo[a]pyrene, chrysene, dibenz[ah] anthracene, phenanthrene, fluorene, indeno (1, 2, 3-cd) pyrene, naphthalene and pyrene. The sum of the total quantity of all these compounds was considered the index of total PAH (TPAH).

2.5. Activities of Glutathione-S-transferase (GST) in the liver

Samples of liver tissue were homogenized in ten volumes (w/v) of ice-cold, 50 mM Tris–HCl, 0.15 M KCl, 0.1 mM PMSF buffer (pH 7.4) and centrifuged (9,000 × g) for 30 min at 4 °C to obtain supernatants for GST assays. Glutathione-S-transferase activity was determined as described previously by Ramos-Vasconcelos and Hermes-Lima (2003) using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. Changes in absorbance were recorded at 340 nm, and enzyme activity is expressed in μmol min^{-1} g^{-1} wet tissue.

2.6. Alkaline comet assay

Blood samples were diluted 1:120 (v/v) in RPMI 1640 medium and used immediately (RPMI-Roswell Park Memorial Institute). The alkaline comet assay was performed as described previously by Tice et al. (2000) and Andrade et al. (2004), with some modifications. Briefly, 5 μL of each diluted blood sample was added to 95 μL of 0.75% (w/v) molten low melting point agarose, and a portion of the mixture was spread on a microscope slide that was pre-coated with 1.5% (w/v) normal melting point agarose and topped with a coverslip. Coverslips were removed after the agarose solidified, and the slides were immersed in a lysis solution (2.5 M NaCl, 100 mM EDTA and 10 mM Tris, pH 10.0-10.5) containing 1% Triton X-100 and 20% DMSO. Slides were maintained in this lysis solution (4 °C) and protected from light for at least 2 h and at most 3 h. Subsequently, slides were incubated in freshly prepared alkaline buffer (300 mM NaOH and 1 mM EDTA, pH ≥ 13, which was experimentally determined) for 20 min for DNA unwinding. Electrophoresis (15 min at 300 mA and 25 V) was performed in the same buffer. Every step was performed under indirect yellow light. Slides were neutralized in Tris 400 mM (pH 7.5) after electrophoresis, rinsed three times in distilled water, and dried overnight at room temperature. Slides were fixed for 10 min in trichloroacetic acid (15% w/v), zinc sulfate (5% w/v), glycerol (5% v/v), rinsed three times in distilled water, and dried for 2 h at 37 °C. Dry slides were re-hydrated for 5 min in distilled water and stained (sodium carbonate 5% w/v, ammonium nitrate 0.1% w/v, silver nitrate 0.1% w/v, tungstosilicic acid 0.25%, formaldehyde 0.15% w/v, freshly prepared in the dark) under constant shaking for 35 min. Stained slides were rinsed twice with distilled water, submerged in the stop solution (acetic acid 1%), rinsed again, and immediately coded for analysis. A total of 100 cells from each replicate were randomly chosen (50 from each duplicate slide) and analyzed under an optical microscope (100 × magnification) to calculate image length (IL). Analysis of the slides involved 100 cells/animal using a visual classification based on the migration of DNA fragments from the nucleus. The cells were classified into class 0 (no damage), class 1 (little damage-the tail is smaller than the nucleus), class 2 (medium damage-tail length is between 1 and 2 times the nucleus diameter), class 3 (extensive damage-tail length is over 2 times the nucleus diameter), and class 4 (apoptosis) (Kobayashi et al., 1995; Speit and Hartmann, 1999). The damage index (DI) for each fish was calculated as the sum of the number of nucleoids that were observed for each damage class multiplied by the value of its respective damage class (0, 1, 2, 3 or 4). Results are expressed as the mean Damage Index (DI) for each treatment group, where 0 represents the absence of damage and 400 indicates the highest damage score.

2.7. Measurement of micronucleus (MN) and nuclear abnormality (NA)

Peripheral blood samples were obtained from the caudal vein and smeared on clean slides. Slides were air-dried after fixation in pure ethanol for 20 min, and the smears were stained with a 10% Giemsa solution for 25 min. Small, non-refractive, circular or ovoid chromatin bodies that showed the same staining pattern as the main nucleus were considered MN (Al-Sabti and Metcalfe, 1995). NAs were classified according to Carrasco et al. (1990) and Smith (1990), as adapted by Pacheco and Santos (1996). These authors scored nuclear lesions into one of the following categories: lobed nuclei (L), dumbbell-shaped or segmental nuclei (S), and kidney-shaped nuclei (K). The final result in each group is expressed as the mean value (‰) of the sums (MN+L+S+K) of all the observed individual lesions.

2.8. Statistical analysis

All data were analyzed for homogeneity of variances using Levene's test, and the normality of the residuals was evaluated using the Shapiro–Wilk test before parametric tests were performed. Simple linear regression was calculated to express the relationships between different measured biomarkers (GST activity, damage index and micronucleus frequency) and crude oil concentrations. Nuclear abnormality (NA) data did not comply with the parametric assumption of normality and homogeneity of variance after various transformations. Therefore, logistic regression was used to analyze the influence of crude oil concentrations for genotoxicity endpoint. A p value ≤ 0.05 was considered statistically significant for all of the above-mentioned tests.

3. Results

3.1. Analysis of oil and water

The presence of xylene and ethylbenzene dominated the composition patterns of BTEX compounds in crude oil, followed by toluene and benzene (Table 1). The water-soluble fraction (WSF) contained very low concentrations of these compounds, which remained stable during the 96 h of the experiment. Naphthalene levels represented the highest concentration of the 16 analyzed PAHs in the crude oil, followed by phenanthrene (Table 1). A small decrease in total PAHs was seen in WSF after 96 h, and reductions in the concentrations of the different classes of compounds of interest were also detected as follows: pyrene concentration was reduced by 48% after 96 h, phenanthrene was reduced by 47%, and fluorene was reduced by 14%. Physical and chemical characteristics of the experimental water in test and control aquaria remained stable during the experimental period. The results are reported as the mean ± standard deviation: temperature = 22 ± 1 °C, oxygen =

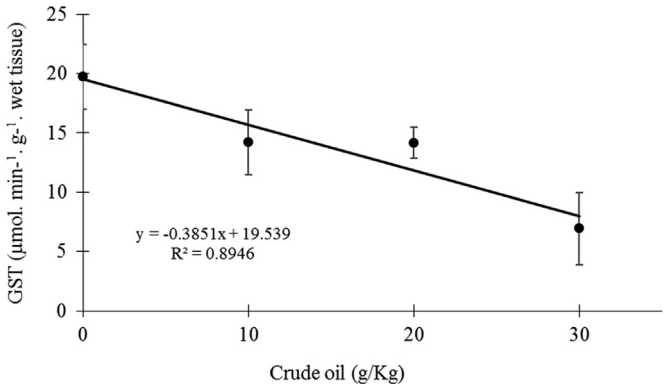

Fig. 1. Relationship between liver glutathione-S-transferase (GST) activity and crude oil concentrations in *Hippocampus reidi* after 96 h exposure ($p \leq 0.01$).

7.0 ± 0.5 mgO_2 / L and salinity $= 28 \pm 1‰$.

3.2. Tissue enzyme analysis (GST)

Glutathione-S-transferase activity (GST) was detected in *H. reidi* of the control group and groups exposed to the 10, 20 and 30 g/kg of crude oil for 96 h. A significant ($p \leq 0.01$; $r^2 = 0.895$) relationship between GST activity inhibition and increased crude oil concentration was observed (Fig. 1).

3.3. Comet assay

All crude oil concentrations produced DNA strand breaks in fish, and a significant ($p \leq 0.01$; $r^2 = 0.938$) relationship between the DNA damage index and crude oil concentration was observed (Fig. 2), suggesting that exposure to high concentrations of petroleum increased the DNA damage index (DI) in *H. reidi*.

3.4. Micronucleus (MN) test and nuclear abnormality (NA)

Micronucleus frequency was observed in fish that were exposed to all crude oil concentrations. A significant ($p \leq 0.01$; $r^2 = 0.774$) relationship between genotoxicity endpoint and crude oil concentration was found, with increases in MN frequency over tested crude oil concentrations (Fig. 3). Similar results were observed for comet assay. However, logistic regression to other NA data was not significantly related with crude oil concentrations: lobed nuclei ($p \leq 0.07$; $g = 3.228$); dumbbell-shaped or segmental nuclei ($p \leq 0.64$; $g = 0.223$) and kidney-shaped nuclei ($p \leq 0.482$; $g = 0.495$). These results suggest that crude oil concentrations did not affect these parameters under the present experimental conditions.

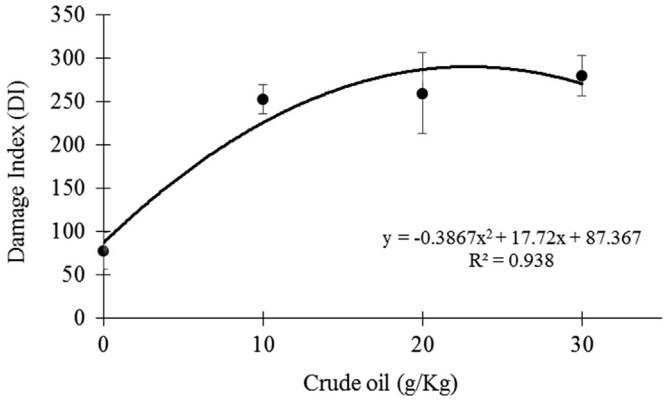

Fig. 2. Relationship between the DNA damage index (DI) and crude oil concentrations in peripheral erythrocytes of *Hippocampus reidi* exposed to crude oil for 96 h ($p \leq 0.01$).

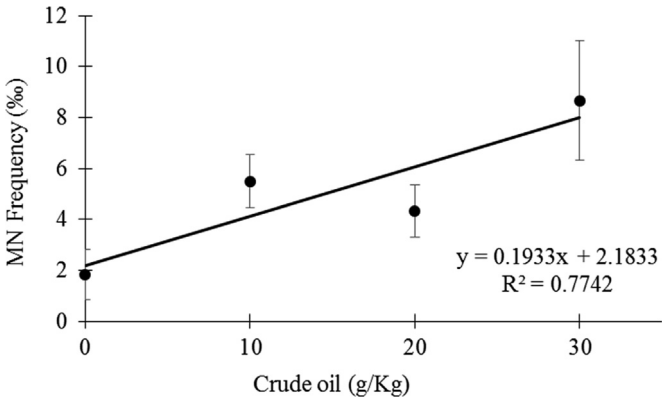

Fig. 3. Relationship between micronucleus frequencies (MN) and crude oil concentrations in peripheral erythrocytes of *Hippocampus reidi* exposed to crude oil for 96 h ($p \leq 0.482$).

4. Discussion

4.1. Tissue enzyme analysis (GST)

Liver GST activity after fish exposure for 96 h to different crude oil concentrations was measured to assess whether crude oil activated or not the detoxification systems in *H. reidi*. Glutathione-S-transferase levels decreased in a dose-dependent manner. Inhibition of GST activity indicates the incapacity to efficiently conjugate PAHs that are present in crude oil using the glutathione pathway or an alternative pathway, such as uridine diphosphate glucuronyltransferase (UDP-GT) or sulfotransferase conjugation (Silva et al., 2013). Another possible explanation for our findings is that PAHs compounds inhibited the substrate (reduced glutathione, GSH) as this enzyme serves as a substrate for conjugation with electrophilic intermediates under the catalytic action of GST (Habig et al., 1974). Variations of GSH levels result in variations of GST levels. Severe oxidative stress may suppress GSH levels (due to a loss of adaptive mechanisms) and cause oxidation of GSH to GSSG with a consequent decrease in GST level (Oliva et al., 2010).

An increase in hepatic GST activity after fish exposure to PAHs has been reported in many studies (Bello et al., 2001; Ahmad et al., 2004; Jee and Kang, 2005; Jifa et al., 2006; Vieira et al., 2008; Oliva et al., 2010; Han et al., 2014), but several studies have shown a decrease in GST activity (Oikari and Jimenez, 1992; van der Oost et al., 1994; Lemaire et al., 1996; Tuvikene et al., 1999; van Schanke et al., 2001; Wang et al., 2006; Vieira et al., 2008; Sun et al., 2008; Luís and Guilhermino, 2012; Palanikumar et al., 2012; Silva et al., 2013). Species specificity regarding biotransformation mechanisms, the preferential metabolic pathways of compounds, and different exposure conditions, including different exposure time and intensity, were suggested as responsible factors for the observed differential responses (Almeida et al., 2012).

The glutathione-S-transferase activity results reveal that the biochemical responses to pollutant mixtures are complex because the absence of a specific effect does not always reflect the absence of contamination. Furthermore, the antagonistic effects on liver biotransformation that are promoted by different xenobiotics in complex mixtures are well known. Finally, the role of GST in PAH detoxification in fish deserves further research (van der Oost et al., 2003; Oliva et al., 2010).

4.2. Comet assay

Comet assays were used on blood samples to detect the genotoxic effects of crude oil, and the results were validated for use in *H. reidi*. This method successfully detected an increase in DNA strand breaks in whole nucleated blood samples of sea horse specimens exposed to all three (10, 20 and 30 g/kg) crude oil concentrations tested in this study. The results indicated that the amount of DNA damage was linked to increased concentrations of crude oil. Zhu et al. (2005) demonstrated that the degree of DNA integrity is a sensitive indicator of genotoxicity and an effective biomarker for environmental monitoring.

The results of this investigation are similar to previous studies, i.e., comet assay endpoints responded in a dose-dependent manner to increasing concentrations of contaminants (Gontijo et al., 2003; Andrade et al., 2004; Santos et al., 2010; Otter et al., 2012; Delunardo et al., 2013). Therefore, comet assays on *H. reidi* erythrocytes is an excellent assay for a rapid in vitro evaluation of PAH contamination.

4.3. Micronucleus (MN) assay and nuclear abnormality (NA)

Evaluation of MN induction in erythrocytes of *H. reidi* exposed to crude oil revealed that this test is efficient for the detection of the genotoxic potential of in crude oil compounds. Variation in MN frequency was dependent on increasing crude oil concentrations, which indicates that a higher induction of cytogenetic damage occurs with the availability of environmental contaminants.

The literature supports these results. Siu et al. (2004) used the comet assay and MN test in green-lipped mussels (*Perna viridis*) that were exposed to waterborne benzo [a] pyrene and found a dose-dependent relationship between the levels of DNA damage and environmentally relevant PAH concentrations. Dose-dependent responses in MN formation in liver cells of European sea bass (*Dicentrarchus labrax*) and eel (*Anguilla anguilla*) were also observed after exposure to naphthalene and to water-soluble fraction of oil (Pacheco and Santos, 2001; Gravato and Santos, 2003; Teles et al., 2003).

Some studies have described the presence of other erythrocytic abnormalities in fish cells that were exposed to contaminants (Çavas and Ergene-Gozukara, 2005; Ergene et al., 2007; Kochhann et al., 2013) and xenobiotic contaminants (Pacheco and Santos, 1997; Serrano-Garcia and Montero-Montoya, 2001). However, NA frequency data in the present study were not significantly related to crude oil concentrations, which showed the lack of sensitivity of these biological markers for these experimental conditions. Some studies have also shown that there is not always an association between exposure to genotoxic agents and changes in MN and NA frequencies (Bombail et al., 2005; Cavalcante et al., 2008). de Flora et al. (1993), Monteiro et al. (2011) and Baršiene et al. (2012) proposed the use of both tests (MN and NA) in fish erythrocytes as an appropriate biomarker for exposure to genotoxic agents, especially contaminants with clastogenic properties.

5. Conclusion

Our results indicate that the inhibition of hepatic GST activity in *H. reidi* was dependent on increasing crude oil concentrations, which indicates a dose-dependent relationship. These results indicated that care should be taken when using this enzyme as a biomarker in ecosystems that are contaminated with PAHs because PAHs might exert opposite effects on GST activity. The comet assay and MN test were adequate and useful for the evaluation of genotoxicity of crude oil, but the frequency of other nuclear abnormalities (NA) was the least efficacious indicator of genotoxicity. Our results will hopefully stimulate future experiments on this subject, which will provide a clearer understanding of the genetic effects of oil spills on neotropical fish. Our results also demonstrated that *H. reidi* may be an excellent bioindicator. This neotropical fish has low mobility and does not migrate from contaminated areas. Therefore, this fish is likely to be exposed to oil spills. Seahorses in general may have great potential for use as sentinel species to monitor marine environments.

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