Citogenotoxic response of juvenile cobia *Rachycentron canadum* (Linnaeus, 1766) reared in two different systems

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Abstract

Aquaculture production is continuously growing worldwide, and marine fish farming in Brazil is still in its infancy. Intensive farming conditions may cause physiological stress to the cultured organism, which can be evaluated by citogenotoxic biomarkers. The aim of this study was to assess the genotoxic effect of the rearing conditions in red blood cells of juvenile cobia *Rachycentron canadum* by using comet assay and micronucleus and other nuclear abnormalities assay. Juvenile cobia were reared for 13 weeks in indoor tank with open water circulation and in near shore cage. The comet assay and the nuclear abnormalities assay detected higher DNA damage and higher nuclear abnormalities frequency in erythrocytes of fish reared in the indoor tank. Results showed that two methods are complementary. Additionally, cobia were injected with ß-naphthoflavone (BNF) at concentrations of $2mgkg^{-1}$ and $10mgkg^{-1}$ in laboratory controlled conditions, and maintained for 7 days in separate tanks to better understand the response mechanisms of this species to a toxic substance. The comet assay did not detect any significant differences between BNF injected and control fish, whereas nuclear abnormalities assay showed significant differences between BNF injected and the control groups. The damages identified by the comet assay are repairable breaks in the DNA strands, whereas nuclear abnormalities may be permanent. Possibly the period of maintenance after injection was enough to clean BNF from the organisms and to repair the breaks in the DNA strands. As cobia seems to respond very well to genotoxic elements, comet assay and nuclear abnormalities assay would be useful tools to monitor farming conditions.

Descriptors: Comet assay, *Rachycentron canadum*, Micronucleus, β-naphthoflavone, Aquaculture.

Resumo

A produção da aquicultura vem crescendo em todo o mundo e o cultivo de peixes marinhos no Brasil é ainda muito recente. Condições intensivas de aquicultura podem causar estresse fisiológico ao organismo do cultivo, o que pode ser avaliado por biomarcadores citogenotóxicos. O objetivo desse estudo foi avaliar o efeito genotóxico das condições de cultivo nos eritrócitos de juvenis de beijupirás *Rachycentron canadum* usando o ensaio cometa e o ensaio de micronúcleo e outras anormalidades nucleares. Juvenis de beijupirá foram cultivados por 13 semanas em um tanque *indoor* com circulação aberta de água e em um tanque-rede. O ensaio comera e o ensaio de anormalidades nucleares detectaram um maior dano ao DNA e uma maior frequência de anormalidades nucleares em eritrócitos de peixes cultivados no tanque *indoor*. Results showed that two methods are complementary. Além disso, beijupirás foram injetados com β-naftoflavona (BNF) nas concentrações de $2mgkg^{-1}$ e $10mgkg^{-1}$ em condições controladas de laboratório e mantidos por 7 dias em tanques separados para um melhor entendimento dos mecanismos de resposta dessa espécie a uma substância tóxica. O ensaio cometa não detectou nenhuma diferença significativa entre os peixes injetados com BNF e os da condição controle, enquanto que o ensaio das anormalidades nucleares apresentaram diferenças significativas entre peixes injetados com BNF e os do controle. Os danos identificados pelo ensaio cometa são quebras reparáveis na fita do DNA, enquanto que as anormalidades nucleares são permanentes. Possivelmente, o período de de manutenção dos peixes nos tanques após a injeção foi suficiente para limpar a BNF dos organismos e reparar as quebras na fita do DNA. Como os beijupirás parecem responder muito bem a compostos genotóxicos, o ensaio cometa e o ensaio de anormalidades nucleares podem ser ferramentas úteis para monitorar as condições de cultivo.

Descritores: Ensaio cometa, *Rachycentron Canadum*, Micronúcleo, β-naftoflavona, Aquicultura.

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INTRODUCTION

The world per capita fish consumption is growing since 1960 (FAO, 2016), but fishery production by capture has been almost static since 1980 whereas aquaculture production has been increasing in the last decades to supply that demand. There is an increasing interest in cobia (*Rachycentron canadum*) as a marine fish species for aquaculture (Estrada et al., 2016). Cobia are large coastal pelagic fish of the family Rachycentridae and are distributed worldwide in tropical and subtropical seas, excepting in the eastern Pacific (Shaffer and Nakamura, 1989). They feed on invertebrates and fishes, but they prefer crustaceans (Shaffer and Nakamura, 1989). Sexual maturity is attained by males in the second year of life and by females on the third one (Shaffer and Nakamura, 1989). Cobia farming is expected to develop worldwide due to the high growth rate (Liao and Leano, 2007), easiness to spawn in captivity (Arnold et al., 2002; Franks et al., 2001; Souza Filho and Tosta, 2008), adaptability to captivity, positive response to vaccination (Lin et al., 2006) and high meat quality (Craig et al., 2006; Liao et al., 2004; Liao and Leaño, 2007). Farming techniques are still being developed, but cobia are normally cultured in outdoor earthen pounds or inshore cages until they reach *ca.* 1000g, when they could be transferred to offshore cages (Liao et al., 2004). The main constraint of producing early juveniles in outdoor ponds is that it may be limited by environmental conditions, so other alternatives, such as indoor tanks, are being studied (Webb Jr et al., 2007).

Biomarkers are widely used in environmental monitoring (Van der Oost et al., 2003), as they may detect many hazardous effects of exogenous and endogenous stressors on the health of the organisms, and they can also be useful for monitoring aquaculture procedures. During the development of rearing protocols, biomarkers can help to detect and to understand the effects of certain setbacks that may happen during the process, such as hypoxia, nitrogen compounds, contaminants and environmental variations (Kalantzi et al., 2016; Mahfouz et al., 2015; Prunet et al., 2012; Rodrigues et al., 2011). Genotoxicity biomarkers are largely used and DNA damage has already been associated with decreased growth rates, developmental abnormalities and reduced survival of larval, juvenile and adult forms of a wide variety of aquatic organisms (Lee and Steinert, 2003). Simple methodologies are constantly being improved to detect genotoxicity. Among them, the comet assay is currently used to detect DNA strand breaks by an electrophoresis that separates the fragmented DNA from the intact one. Another reliable method, the micronucleus and other nuclear abnormalities assay identifies and quantifies permanent genetic abnormalities in the nucleus (Carrasco et al., 1990).

To endorse the comprehension of data obtained by those techniques under the influence of fluctuating environmental conditions, the standardization of physiological responses in laboratory is recommended. The interpretation of biomarkers in environmental monitoring is more efficient when laboratory complementary tests are performed with reference substances to evaluate the organism response under controlled conditions (Van der Oost et al., 2003).

The objective of this study was to assess the effectiveness of the comet assay and the micronucleus and other nuclear abnormalities to evaluate the responses of the DNA as biomarkers of juvenile cobia cultured in two systems, i.e., an open water circulation indoor tank and in a nearshore cage. A preliminary study to establish a laboratory control of these biomarkers was undertaken with cobia injected with the polycyclic hydrocarbon compound (PAH) β-naphthoflavone (BNF), used as a reference contaminant with known genotoxic properties (Pacheco and Santos, 2002).

MATERIAL AND METHODS

Experimental animals and rearing systems

Cobia fingerlings (*ca.* 1 g) were purchased from a commercial hatchery (Redemar Alevinos, Ilhabela, SP - Brazil), in December 2014, transported to "Clarimundo de Jesus" Research Station, Oceanographic Institute, University of São Paulo (Ubatuba - SP - Brazil). When fish reached about 400g, a total of 99 individuals were distributed into two rearing systems, i.e., an indoor tank with open water circulation system (18L.min^{-1} exchange rate) and a nearshore cage, at a density of 1.5kg.m^{-3} and reared for 13 weeks. At this size, all the organisms were juveniles but developed enough to diminish mortality due to handling. The experiment finished before they reached maturity. During the experiment, cobia were fed a commercial feed every morning. To calculate total feed intake the ration was weighed before and after feeding. Water temperature, salinity, total dissolved oxygen, ammonia (N-NH_4^+) and nitrite (NO_2^-) concentrations were daily measured and monitored in both systems. At the end of the experiment, all the fish were individually weighed to estimate somatic growth rate (SGR - 1) for both treatments. Then, 300μL blood from the Cuvier Duct

from each of these cobia were collected for the comet assay and for the micronucleus and other nuclear abnormalities assay. The growth rate ($g.day^{-1}$) was calculated by the subtraction of final and initial average weight divided by the number of days of the experiment. Fulton's condition factor (K - *2*), that is a very common morphometric index used for small samples (Sutton et al., 2000), and hepatosomatic index (HSI - *3*) were calculated for each treatment (n=10). Results are presented as mean ± standard error. Data were tested for normality by D'Agostino-Pearson test and submitted to *t*-test comparison. Differences were considered significant at $p<0.10$.

$$1: SGR(\%) = (e^{g} - 1) \times 100,$$

$g = (\ln(W_2) - \ln(W_1)) \div (t_2 - t_1)$, W_2 and W_1 are average weight at time t_2 and t_1

$$2: K = 100 \times (W \div L^3),$$ W is the whole body wet weight and L is the standard length

$$3: HSI = \frac{liver\ weight}{body\ weight} \times 100$$

Comet assay

The comet assay was performed as described by Singh et al. (1988) with modifications proposed by Gontijo and Tice (2003) and Tice et al. (2000). Briefly, one drop of blood sample was mixed with 1mL of PBS (phosphate saline buffer, pH 7.4, 0.01M) and then this suspension was diluted at 10% in PBS, because of the large number of erythrocytes. An aliquot of 20µL of the diluted sample was mixed in 120µL of 1.0% low-melting point agarose (LMP, 37°C) dissolved in PBS and spread onto the surface of a glass slide previously covered with 1.5% normal melting point agarose (NMP, 60°C) dissolved in distilled water. Slides were covered with cover slips and kept at 4°C for 30min to solidify the LMP gel. Cover slips were removed and slides were immersed into a lysing solution (2.5M NaCl, 100mM Na_2EDTA and 10mM Tris, pH 10, 1% Triton X-100 and 10% DMSO) for 2h at 4°C. Slides were then rinsed with cold distilled water and submitted to unwinding under alkaline conditions (300 mM NaOH, 1 mM EDTA, pH >13) in electrophoresis chamber for 5min at 4°C. After the unwinding, electrophoresis was performed for 20min at 20V (0.74V/cm) and 300mA. After electrophoresis, slides were neutralized by rinsing with a buffer solution (0.4M Tris, pH 7.5). The silver staining method was used, as described by García et al. (2004).

A positive control of the comet assay was carried out to check the reliability of the technique. Before lysing, slides with cells were exposed *in vitro* to hydrogen peroxide (H_2O_2) at increasing concentrations (5µM, 10µM, 20µM and 40µM) for 30min as described by Wojewódzka et al. (2002). The slides were then rinsed with PBS and treated in the same way as the other slides.

Comets were photographed under an optical microscope and the images were classified by visual analysis and by the CometScoreTM (TriTek Corporation®) software. One hundred cells were analyzed on each one of the two slides prepared per fish. By the visual analysis, cells were classified in five types and the Damage Index (DI) was calculated by the following expression:

$$DI = (0 \times N_0) + (1 \times N_1) + (2 \times N_2) + (3 \times N_3) + (4 \times N_4)$$

where N_0 number of comets classified as 0; N_1 number of comets classified as 1; N_2 number of comets classified as 2; N_3 number of comets classified as 3; N_4 number of comets classified as 4. In the image analysis software, the tail moment was the chosen parameter, because it includes both the DNA percentage in the tail and the tail's length.

Micronucleus and others nuclear abnormalities assay

Immediately after sampling, blood smears were prepared for the micronucleus and other nuclear abnormalities assay. After the smear dried, slides were fixed in methanol 100% for 10min, stained with Giemsa 10% for 30min, rinsed with distilled water and left to air-dry. Slides were analyzed under an optical microscope and 1,000 cells were counted. The frequencies of reniform, lobed, segmented and micronucleus cells were calculated. The total erythrocyte nuclear abnormalities (ENA) was also calculated as the sum of all abnormalities.

β-naphthoflavone (BNF) injection experiment

As a preliminary control trial, juvenile cobia (ca. 150g) were injected with β-naphthoflavone (BNF) to observe their response to a recognized genotoxic compound. The BNF was diluted in corn oil and four groups of three fish each were tested. The first and the second one were control groups: one fish group was just transferred to the tanks and the other group was injected with corn oil. The other two groups were injected with a low ($2mg.kg^{-1}$) and

a high (10mg.kg^{-1}) concentration of BNF. Groups were kept in 500L tanks separately for 7 days. This procedure was performed twice. Tanks were in a recirculation water system with water filtration and water exchange rate of 200L.h^{-1}. Water temperature, salinity, total dissolved oxygen and percentage of oxygen saturation were measured daily. At the end of the 7-day period, blood was sampled from all individuals for the comet assay and micronucleus and others nuclear abnormalities assay. Individual HSI and K factor were also calculated. Results are presented as mean ± standard error. Data was tested for normality by Kolmogorov-Smirnov test and submitted to analysis of variance (ANOVA) followed by Newman-Keuls comparison test. Differences were considered significant at $p<0.10$.

RESULTS

Water temperature, salinity and dissolved oxygen concentration presented similar values in both rearing systems (Table 1). N-NH_4^+ and NO_2^- concentrations were undetectable in the nearshore cage but high in the indoor tank. The SGR and the daily growth rate of cobia were similar in both systems, in spite of an indication of being higher in nearshore cage (Table 2). The initial and final average weight of fish from the indoor tank were 470.71±15.92g and 884.28±35.80g and for the nearshore cage were 466.98±26.69g and 929.48±66.49g. K factor (Table 2) did not differ between rearing systems (1.34±0.05 in cobia in the nearshore cage and 1.32±0.04 in cobia in the indoor tank). The HSI (Table 2) was significantly higher (p=0.0052) in cobia from the indoor tank (3.17±0.16) than that from the nearshore cage (2.37±0.18).

Positive control demonstrated an increasing DNA damage with H_2O_2 concentration (Figure 1). Statistical difference was not detected only between the concentrations of 5 and 10µM.

In both visual and software analysis, the DNA damage detected by comet assay was significantly higher in cobia reared indoor than in the outdoor cage, (Figures 2A and B). Frequency of abnormalities in cobia reared in the indoor tank also tended to be higher than in the outdoor cage (Figure 2C). The frequencies of micronuclei, lobed nuclei, segmented nuclei and total ENA of fish from the indoor tank were significantly higher.

K factor and HSI of fish injected with different concentrations of BNF did not present significant differences (Table 3). Seven days after the BNF injection in cobia, no significant differences in DNA damage could be detected by the comet assay. In all groups, DNA damage may be considered low, when compared to the results of the positive control and also to the rearing experiment (Figure 3 A and B). Nevertheless, the micronuclei and other abnormalities assay detected reniform and lobed nuclear abnormalities and ENA frequencies significantly higher in fish from the injected groups than in fish from the control ones (Figure 3 C). DNA damage detected by the comet assay may be repaired with time, but the micronuclei and other nuclear abnormalities are permanent, as further discussed.

DISCUSSION

Although cobia aquaculture is already being carried out in Taiwan for more than a decade (Liao et al., 2004), there are still many aspects that must be improved for the expansion of this activity around the world. In Taiwan, juveniles are reared in ponds and nearshore cages until they had grown enough to be transferred to offshore cages (Liao et al., 2004). However, growth of juveniles in earthen ponds may limit the production depending on

Table 1. Physical chemical parameters of the two reared systems where juveniles of cobia were kept during the experiment (13 weeks). Results are presented by mean ± standard error.

	Temperature (°C)	Salinity	Dissolved oxygen (mg/L)	N-NH_4^+ (mg/L)	NO_2^- (mg/L)
Indoor tank	23.74±0.15	34.12±0.08	6.27±0.06	0.11±0.03	0.56±0.05
Nearshore cage	24.38±0.17	33.89±0.19	5.80±0.06	-	-

(-): Not detectable.

Table 2. Biometric parameters of cobia juveniles reared for 13 weeks in two different systems. Results are presented by mean ± standard error. Different letters represent statistic difference.

	Growth rate (g/day)	SGR	K	HSI
Indoor tank	4.6	0.66	1.32±0.04 [a]	3.17±0.16 [a]
Nearshore cage	5.12	0.73	1.34±0.05 [a]	2.37±0.18 [b]

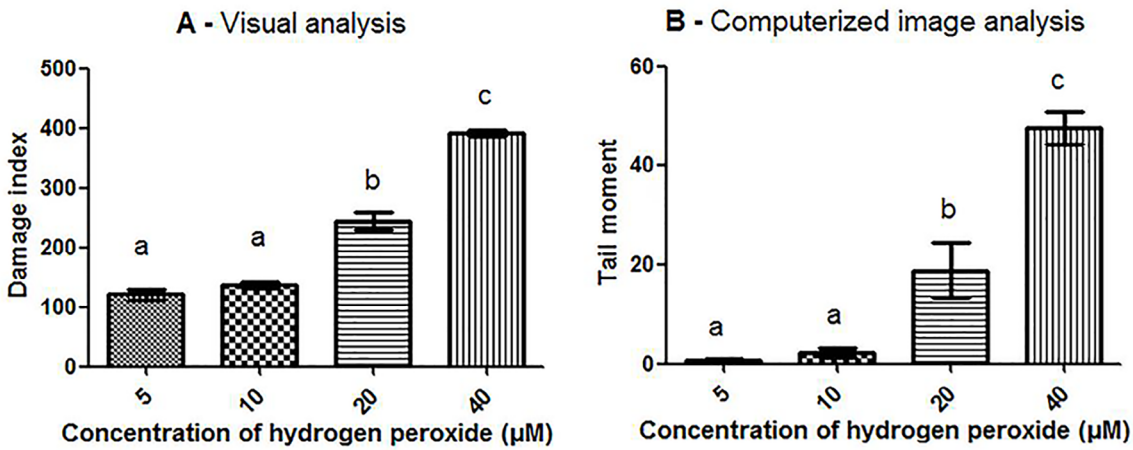

Figure 1. Results of positive control of the comet assay in juvenile cobia. Variation of damage index (A) and of tail moment (B) as function of concentration of H_2O_2. Different letters represent groups significantly different and the error bars represent the standard error.

the environmental conditions, which can reduce profits (Webb Jr. et al., 2007). In the present study, we reared cobia in indoor tank and in nearshore cage and compared the state of the organisms using citogenotoxic techniques and morphometric indices.

Cobia SGR were slightly lower in this study than previously reported (0.82) for individuals of the same weight range (Sampaio et al., 2011). This difference could be related to the diet, as in the present study fish were fed a commercial diet, whereas Sampaio et al. (2011) fed fish with frozen sardine. Statistical significance of the differences of SGR and the growth rate between both rearing systems was not evaluated as they were calculated as single values by the average of fish weight from each tank. Nevertheless, the values of both rates indicate higher growth of fish from the nearshore cage. In the same direction, results from the comet assay, from the micronucleus and other abnormalities assay and the HSI show that cobia reared in nearshore cage were in a better physiological state than fish in the indoor tank. Possibly, fish exposed to less stressors, such as high concentration of $N\text{-}NH_4^+$ and NO_2^-, may spend less energy on metabolism and channel it to growth.

The K factor assumes that individuals present isometric growth, so that comparing fish of the same length, the heavier will be the one with the best health conditions. It is established that healthy fish have K >1 (Kerambrun et al., 2013; Saraiva et al., 2015). In this study, cobia from both systems presented K >1, indicating that they were in good general conditions. Further to K factor, HSI is also a simple initial screening index largely used (Van der Oost et al., 2003). Fish exposed to contaminants usually present a higher HSI in comparison to those from pristine areas, because there is an increased detoxification activity of the liver in response to the presence of the toxic compounds (Dragun et al., 2013; Machado et al., 2014; Pereira et al., 1993; Van Dyk et al., 2012). Higher HSI could also be related to reproduction, but all the fish used in this work were not mature. All of them were less than one year old, and it is already known that male cobia become mature on the second year and females on the third year of life (Shaffer and Nakamura, 1989). In this way, the higher HSI found in cobia in the indoor tank could be related to a response to some toxic material, such as to the higher concentration of $N\text{-}NH_4^+$ and/or NO_2^-. Cobia are considered to be highly tolerant to elevated concentrations of these compounds (Atwood et al., 2004; Rodrigues et al., 2007), but, to compensate the stress, they could have set in motion some physiological mechanisms that affected HSI.

Culture conditions, mainly the intensive one, can cause DNA damage by the production of reactive species of oxygen (ROS) due to stress (Sahin et al., 2014). DNA damage in aquatic organisms can negatively affect growth rates, cause abnormal development and lead to higher mortality rates (Lee and Steinert, 2003), therefore, the evaluation of genotoxic damage in farmed organisms can be an important tool to monitor the quality of the rearing system. Nuclear abnormalities result from permanent damage to the chromosomes during the cell division. On the other hand, DNA strand breaks occur in the DNA polymer itself and can be repairable. Therefore, the use of these two techniques together allows a broader view of the

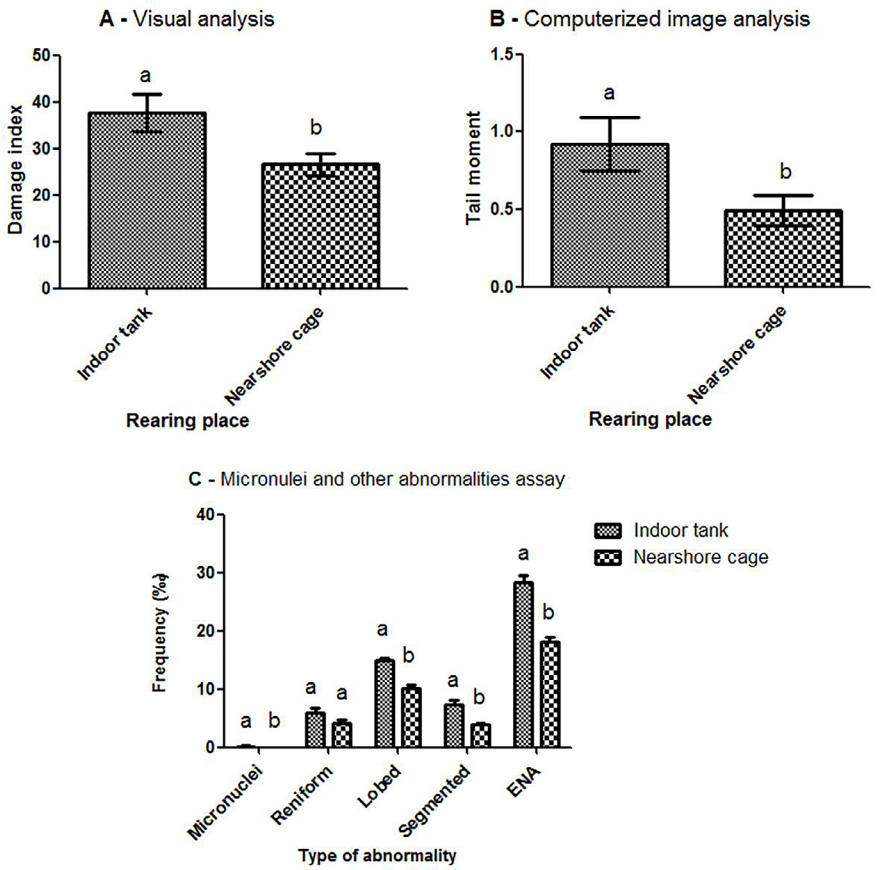

Figure 2. Damage index (A) and tail moment (B) obtained by the comet assay and the nuclear abnormalities frequency (C) of juvenile cobia reared in two different systems. Different letters represent groups significantly different and the error bars represent the standard error.

performance of genotoxic stressors (Martins and Costa, 2015). It is also important to highlight that the use of the sum of all abnormalities, or ENA, is recommended as it is noticeable, after a long time of empirical experience, that they are more efficient than micronuclei alone (Ayllon and Garcia-Vazquez, 2001; Van der Oost et al., 2003; Strunjak-Perovic et al., 2009; Santos et al., 2017).

As it was the first time that the comet assay was applied to cobia, it was necessary to perform a positive control *in vitro*, which also aimed to verify the effectiveness of the method. Increasing concentrations of H_2O_2 (5, 10, 20 and 40µM) were tested and DNA damage also increased according to the concentrations, in both visual and image analysis software, indicating that the technique is reliable and successfully applied to this species. It is also important to emphasize that cobia presented a well-defined and precise response with low variation in the results.

Although cobia reared in indoor tank presented a significantly higher genotoxic damage compared to cobia reared in nearshore cage, the DNA damage was small when compared to the damage caused by the lowest H_2O_2 concentration. This is a highly desirable result as the comet assay in cobia was sensitive enough to detected initial stages of DNA damage and it could be used to avoid the consequences of prolonged exposure to genotoxic factors. The results of micronuclei and others abnormalities assay are complementary to comet assay and fish from the indoor tank also had a significantly higher frequency of nuclear abnormalities. These values and the higher HSI in fish from the indoor tank can be a consequence of higher concentration of $N\text{-}NH_4^+$ and/or NO_2^- in the water, among others undetected factors, although the nitrogen compounds were under or near the limits stipulated by the Brazilian legislation for saline waters used for fishery and rear fish (CONAMA 357, 2005). It's very important to highlight that these data does not discard the feasibility of rearing juvenile cobia in indoor tanks as the high nitrogenous compounds concentrations can be avoided with time, but our results indicate the efficiency of both assays

Table 3. Biometric parameters of cobia juveniles reared exposed with β-naphtoflavone for seven days. Results are presented by mean ± standard error.

	K	HSI
Water control	1.04±0.03	5.58±0.36
Oil control	1.09±0.03	5.69±0.49
BNF 2mg.kg^{-1}	1.05±0.02	5.30±0.44
BNF 10mg.kg^{-1}	1.07±0.05	5.47±0.26

to detect the effect of frequent stressors and monitor the health of the farmed fish.

Experiments with exposures to known toxic compounds under controlled laboratory conditions are important to understand the reaction of each species to stress situations. The BNF is a synthetic polycyclic aromatic hydrocarbon (PAH) normally used in studies for the evaluation of the consequences of fish exposure to PAHs (Pacheco and Santos, 2002). There are several studies on marine fish exposed to or injected with BNF and comet assay and frequency of nuclear abnormalities (Ahmad et al., 2005; Gravato and Santos, 2002a; Gravato and Santos, 2002b; Nigro et al., 2002; Pacheco and Santos, 1998; Pacheco and Santos, 2002), but no study had been reported for cobia.

Both K factor and the HSI did not show significant differences between fish injected with BNF and fish of the control groups, which was not expected. K values in cobia from the control groups and those injected with BNF presented, on average, values higher than 1, but when analyzed individually, in all groups there were individuals with values lower than 1. In relation to HSI, the average values found for all the four groups were higher in comparison to the HSI previously reported for cobia (Trushenski et al., 2012; Wang et al., 2016; Zhou et al., 2011; Zhou et al., 2012). Possibly, HSI measured in our study was already close to extreme values, preventing a further modulation by the BNF. It is known that these biomarkers are less sensitivity to a specific stressor and that they can be affected by other factors than toxic compounds, such as nutritional status, diseases and seasonal variability (Van der Oost et al., 2003).

Comparing the results of comet assay to the micronucleus and other nuclear abnormalities assay in cobia, we can suppose that the BNF could also have caused DNA strand breaks, but the period of seven days after the injection was probably enough to repair the damages (Shugart et al., 1992). *Anguilla anguilla* presented higher DNA damage after injection with BNF at the same conditions as in our study (Nigro et al., 2002) indicating that repairing mechanisms could be faster in cobia. As also occurred in our study, *Anguilla anguilla* and *Dicentrarchus labrax* exposed to BNF showed a higher frequency of nuclear abnormalities when compared to the control groups (Gravato and Santos, 2002b; Pacheco and Santos, 2002). Nuclear abnormalities are not reparable and altered nuclei could be detected meanwhile the damaged cells were still present in the circulatory system of the fish (Van der Oost et al., 2003). It's important to highlight that the life span of fish erythrocytes vary between 13 and 500 days (Witeska, 2013), so the time of the experiment was probably not enough to replace all the cells. DNA damages and nuclear abnormalities in cobia injected with BNF were lower and similar to those obtained for fish from the nearshore cage. This fact would further strengthen the assumption that the DNA strand breaks caused by BNF was repaired over the 7 days of maintenance after the injection of the contaminant. Further investigation is warranted to test this hypothesis. Nevertheless, data once more indicates that cobia responses were fast and sensitive to genotoxic compounds.

Comet assay and the micronucleus and other nuclear abnormalities assay demonstrated to be effective methods to detect the genotoxic effects of the environment in cobia. These techniques proved to be sensitive and quick to perform, so they could be used to monitoring cobia farming, with no need to sacrifice the individuals for sampling.

CONCLUSION

Cobia reared on nearshore cage presented a lower DNA damage and HSI, indicating a better physiological state than those reared in an indoor tank. Data indicates that comet assay and micronuclei and other nuclear abnormalities assay are sensitive and useful methods to monitor cobia during rearing.

The BNF exposure trial was preliminary, or a first approach to a positive laboratory control for this species. Cobia has shown to respond to BNF exposure by the higher frequency of nuclear abnormalities compared to the control groups. Further studies with different concentrations of BNF with a broad variation of time, to study DNA strand damage and repair are warranted.

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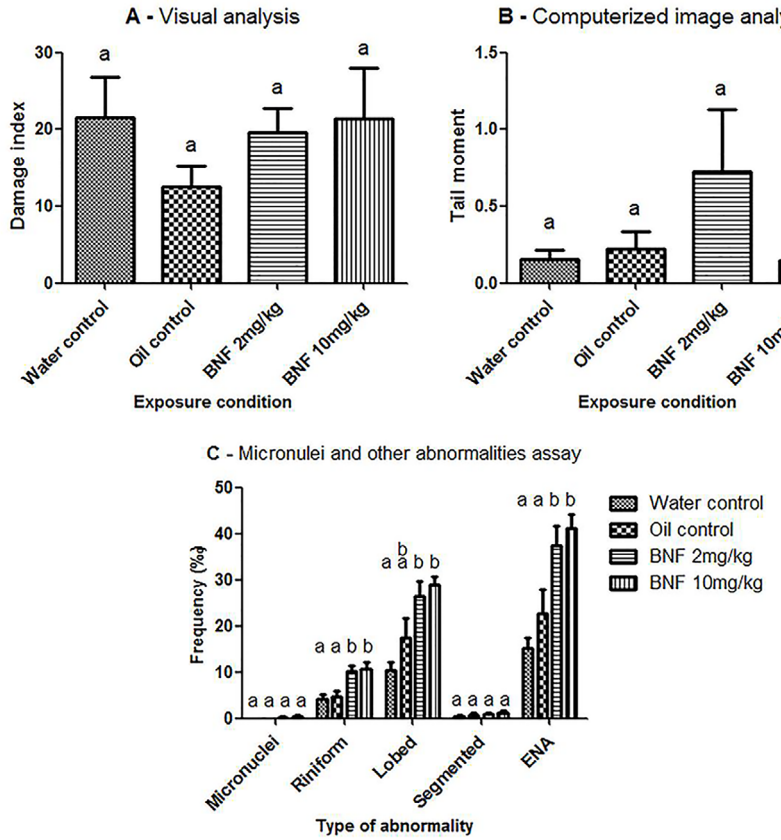

Figure 3. Damage index (A) and tail moment (B) obtained by the comet assay and the nuclear abnormalities frequency (C) of juvenile cobia injected with β-naphthoflavone (BNF). Different letters represent groups significantly different and the error bars represent the standard error.

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