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Use skin as DNA in damage assessment in stranded cetaceans

Avaliação dos danos em DNA utilizando pele de cetáceos encalhados

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ABSTRACT

Environmental contamination can affect natural populations causing tissue damage such as reduced genetic variability, limiting the ability of the species live with long-term changes in the environment. Cetaceans are considered biomarkers since they are more vulnerable to exposure to environmental contaminants. This study was to analyze the phases of cell cycle in the skin of cetaceans, DNA damage and relate the results with analysis of trace elements found in these animals. We used fragments of the skin of 30 cetaceans stranded and frozen for analysis of trace elements. We observed a large amount of iron and zinc, the latter being necessary for metalloprotease, proliferation of cutaneous cells, and deposition of collagen in wounds. Animals in an advanced state of decay showed a greater number of cells in all phases of the cell cycle, and this fact be related to a response of the organism in response to cell death.

Keywords: Cetaceans; Trace elements; Skin; Danos em DNA; Cell cycle;

RESUMO

A contaminação ambiental pode afetar as populações naturais causando danos aos tecidos, como redução da variabilidade genética, limitando a capacidade das espécies de conviver com mudanças de longo prazo no ambiente. Os cetáceos são considerados biomarcadores por serem mais vulneráveis à exposição a contaminantes ambientais. Este estudo teve como objetivo analisar as fases do ciclo celular na pele dos cetáceos, os danos ao DNA e relacionar os resultados com a análise de elementos traços encontrados nestes animais. Usamos fragmentos de pele de 30 cetáceos encalhados e congelados para análise de elementos traços. Observamos grande quantidade de ferro e zinco, sendo este último necessário para metaloprotease, proliferação de células cutâneas e deposição de colágeno em feridas. Animais em avançado estado de decomposição apresentaram maior número de células em todas as fases do ciclo celular, fato este relacionado a uma resposta do organismo à morte celular.

Palavras-chave: Cetáceos; Elementos traços; Pele; Ciclo celular;

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INTRODUCTION

Pollutants are extremely dangerous to aquatic ecosystems, since most of them are toxic for living organisms. It can be considered a serious threat to the biodiversity of this environment (Sarkar et al., 2006). The protection and conservation of marine animals enjoys worldwide public support. Heavy metals represent a particular problem for the marine environment. This is because they present, at the same time, toxicity, persistence, and bioaccumulation in the food chain (Marcovecchio, 2000; Marins et al., 2002).

Environmental contamination may affect the genetics of natural populations. In a molecular level, mutagens can interact with the DNA and form somatic lesions that may damage tissues and generate adverse effects to organic systems. These lesions can reduce reproductive success of the individuals, size of the population, and strangling of the population, leading to a reduction in the genetic viability of the populations (genetic effects on the population) (Bickham et al., 2000). The loss of genetic variability may limit the ability of the species to adapt to an environment subject to long-term changes, such as pollution and new diseases (Frankham, 2003).

Therefore, given the concern that pollutants may affect evolutionary processes (Dieter, 1993; Depledge, 1994), research in genetic ecotoxicology should be considered essential in the preservation of the genetic variability of these ecosystems (Stomp, 1994).

Marine mammals are increasingly considered bioindicators, because they point out to the prevalence and persistence of the pollutants in marine environments (Hoekstra et al., 2003). Given their biology and habitat, they are one of the best indicators of marine contamination (sentinel species) as they are more vulnerable to the exposure to environmental contaminants in the oceans, (Bossart, 2010).

The skin of the cetaceans shows special adaptations to the aquatic environment. The epidermis works as a primary protective barrier and represents the interface between the organism and its environment (Pfeiffer and Menon, 2002). According to Yang et al. (2002), the skin is one of the most important organs of the cetaceans. It lacks hair and glands (Tinker, 1988; Pfeiffer and Rowntree, 1996), making the excretion of some pollutants impossible (trace elements). Therefore, these compounds may accumulate in the skin, with some different characteristics (Yang et al., 2002). Cetaceans fulfill vital roles in marine ecosystems, and the diminishment of their numbers has had profound effects on some of those ecosystems.

In Brazil, studies on the level of contaminants in tissues (liver, fat, muscles, and kidneys) of aquatic mammals are being carried out in order to evaluate marine environments, correlate the levels of contamination with age, sex and changes in behavior of these animals (Becker et al., 1997; Kunito et al., 2002, 2004; Seixas et al., 2008, 2009; Moreira et al., 2009; Anzolin et al., 2012). However, there are few studies that analyze this data on the cell level.

Although skin biopsies were suggested as non-invasive tools to evaluate the ecotoxicological risks for marine mammals (Fossi and Massili, 1997), there is little information on trace elements in the skin of cetaceans.

Non-invasive studies, such as skin biopsies, are being carried out to evaluate ecotoxicological risks, like the determination of contaminants, the evaluation of the activity of the enzyme oxidase, and genotoxic damage to DNA in free-living cetaceans (Fossi and Massili, 1997; Fossi et al., 2000).

In order to better understand the consequences of genetic exposure, it is important to test hypotheses that focus on mechanisms and effects throughout the levels of biological organization, from the DNA of the populations to, ideally, the ecosystems (Anderson et al., 1994). Therefore, the objective of this study was to analyze the phases of cell cycle in the skin of cetaceans stranded in the northeast of Brazil, as well as the damage found in this DNA, and to correlate these results with the analysis of trace elements found in these animals.

MATERIAL AND METHOD

Animals

Fragments of the skin of 30 cetaceans stranded on the seaside of the states of Bahia, Sergipe and Alagoas (Figure 1) were used. They were part of the biological collection of the Fundação Mamíferos Aquáticos (FMA), with 18 *Sotalia guianensis*, 8 *Stenella clymene*, 3 *Megaptera novaeangliae,* and 1 *Ziphius cavirostris* (Table 1).

Figure 1 –Distribution of the states and cities where the cetaceans used in this study were found stranded.

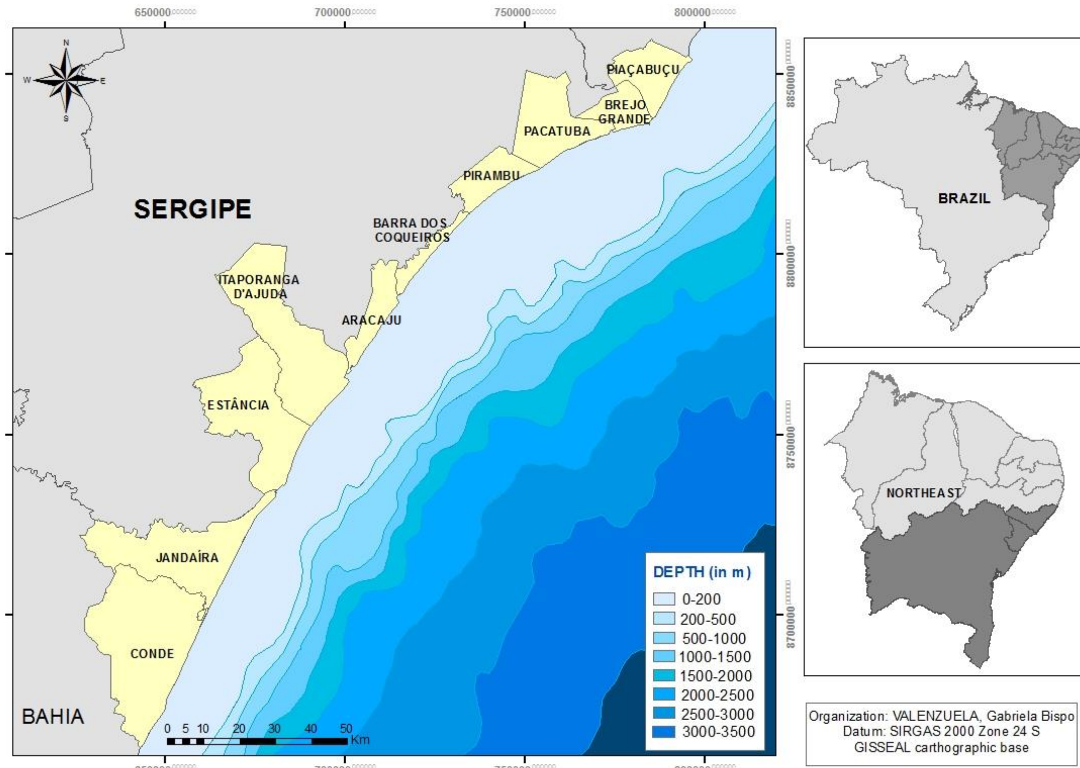


Table 1 – Distribution of the stranding sites of the specimens used in our study, in the north of states Bahia, Sergipe, and south of Alagoas, in Brazil.

Species	North of Bahia	Sergipe	South of Alagoas
Sotalia guianensis	04	13	01
Stenella clymene	02	06	-
Megaptera novaeangliae	01	02	-
Ziphius cavirostris	-	01	-

Initially, the stranded cetaceans were classified according to the status of carcass decomposition (SCD) criteria established by Geraci and Lounsbury (1993), as: CODE 1 (SCD 1) – live animal; CODE 2 (SCD 2) – carcass in good conditions (fresh); CODE 3 (SCD 3) – carcass in satisfactory status (decomposed, but with intact organs); CODE 4 (SCD 4) – decomposed (advanced decomposition); CODE 5 (SCD 5) – mummified carcass or rests of a skeleton. The animal was identified (species, sex and age group), if the status of the carcass allowed it. The body was measured according to the biometry

protocol of the REMANE (*Rede de Encalhes de Mamíferos Aquáticos do Nordeste* – IBAMA, 2005).

Trace elements analysis

For trace elements analysis, samples of tissue, fat, liver, muscle and kidneys were frozen at -20ºC. Initially, samples were thawed, weighted, dried to constant weight, completely digested in 2 mL of HNO_3 (Suprapur, Merck), and diluted in 10 mL of MiliQ water.

Concentrations of the elements Copper (Cu), Selenium (Se), Zinc (Zn), Mercury (Hg), Vanadium (V), Arsenic (As), Iron (Fe), Nickel (Ni), Chromium (Cr), Manganese (Mn) were determined by atomic absorption spectrophotometry (AAS-932 Plus; GBC; New Hampshire, IL, USA). The trace elements Cadmium (Cd) and Lead (Pb) were determined by means of electrothermal atomization (graphite furnace) to prevent the possible interference with the sodium found in the samples. The analyses were carried out in the *Universidade Federal de Sergipe,* and data were expressed as mg metal/kg of dry weight.

Digestion of the samples by collagenase

For cell digestion, samples of skin preserved in alcohol 70% were used. Initially, fragments of tissue were collected and frozen in liquid nitrogen. After one week, samples were placed in Petri dishes and washed in PBS for 24 hours.

After that, they were macerated, and 1 mL of collagenase type IV was placed in each plate. Samples were left in an incubator at 37ºC until digestion was complete. Then, they were filtered in 30-mesh membranes to obtain a cell suspension, and adjusted newbauer chamber at 10^5 cells/ml.

Determination of the Phases of the Cell Cycle

Flow cytometry was used to determine parameters on the cell population, and to determine the percentages of cells in phases G0/G1 (quiescent cells, non-proliferative and mature), S (synthesis of genetic material); G2/M (cells with high capacity of proliferation and division), as well as those that underwent apoptosis or showed fragmented DNA.

Cells were treated with trypsin and centrifuged for 30 minutes at 2,000 rpm. The supernatant was discarded and the cells were resuspended in cytometry buffer (FACs Flow - BD).

The incidence of the light beam of the cytometer at a 90°-angle in each cell of the sample shows, according to the refraction of the beam, the morphological parameters of the cells, both in terms of size and internal complexity. The software received the data, which were plotted in the graph, with the distribution of the cells in different populations. The histograms (analyzed in MOD-FIT) of each sample determine, indirectly, the amount of molecules expressed per cell, that is, the intensity of fluorescence, as well as the percentage of fluorescence, which determines the percentage of cells that expresses each specific receptor.

Measurement of mitochondrial membrane potential

The mitochondrial membrane potential (MMP) was measured in a Rhodamine 123 efflux assay (Rho123) and monitored by flow cytometry. Rho 123 was added to 100 mg/L at 30 minutes before the end of the treatment, and later on, washed with PBS. Cells were analyzed using a FACScan flow cytometry system (Scalibur-Becton Dickinson, San Jose, CA). A total of 10,000 cells /sample were analyzed. Mean intensity of fluorescence and percentage of cells in each population (M1 e M2) were recorded.

Statistical analysis

Values were expressed as means ± standard deviation (SD). Statistical analysis was carried out using the Analysis of Variance (ANOVA), followed by a multiple comparison with Tukey-Kramer test, at a significance level of $p<0.05$.

RESULTS

Trace elements analysis

Samples from a total of 14 animals were analyzed for trace elements, with nine *Sotalia guianensis*, three *Stenella clymene*, and two *Megaptera novaeangliae*.

Table 2 shows the analysis of the results for the mean of each trace element in the respective samples, expressed in mg/kg of the mass of each organ analyzed.

Trace elements found in greater concentration in the organs were Fe (273.89±949.82) and Zn (32.44±20.43). In relation to the difference in the concentrations of trace elements in the organs analyzed, the samples of liver presented higher concentrations than the other organs.

Table 2 – Concentration of the trace elements (mg/kg) in samples of liver, fat, muscle and kidney in the specimens analyzed.

Trace elements	Liver	Fat	Muscle	Kidney	TOTAL
Cu	22.81±45.47	1.07±0.70	1.96±1.39	4.11±1.84	6.35±21.56
Se	4.84±2.44	2.28±1.94	2.05±1.19	2.41±1.40	2.77±2.04
Zn	54.10±12.66	28.34±23.04	25.01±17.13	27.17±10.93	32.44±20.43
Hg	0.87±0.27	0.51±0.35	0.64±0.46	1.01±1.39	0.72±0.71
V	0.62±0.30	0.24±0.21	0.24±0.13	0.28±0.14	0.33±0.25
Cd	5.28±3.29	1.94±1.44	2.09±1.39	2.88±1.30	2.85±2.27
As	4.06±5.12	0.91±1.34	1.25±1.63	1.78±1.59	1.82±2.84
Fe	403.55±313.42	32.52±39.70	569.63±1793.12	132.10±59.07	273.89±949.82
Pb	13.09±23.03	3.51±2.49	4.08±1.87	3.97±1.22	5.70±10.86
Ni	4.28±1.43	4.49±1.82	4.83±1.36	4.62±1.77	4.57±1.59
Cr	4.12±2.10	3.30±1.09	3.44±1.49	3.90±2.36	3.63±1.72
Mn	6.87±5.39	6.83±7.88	5.32±5.13	6.92±7.04	6.45±6.44

* Featured trace elements with the highest concentration

Cell cycle and fragmented DNA

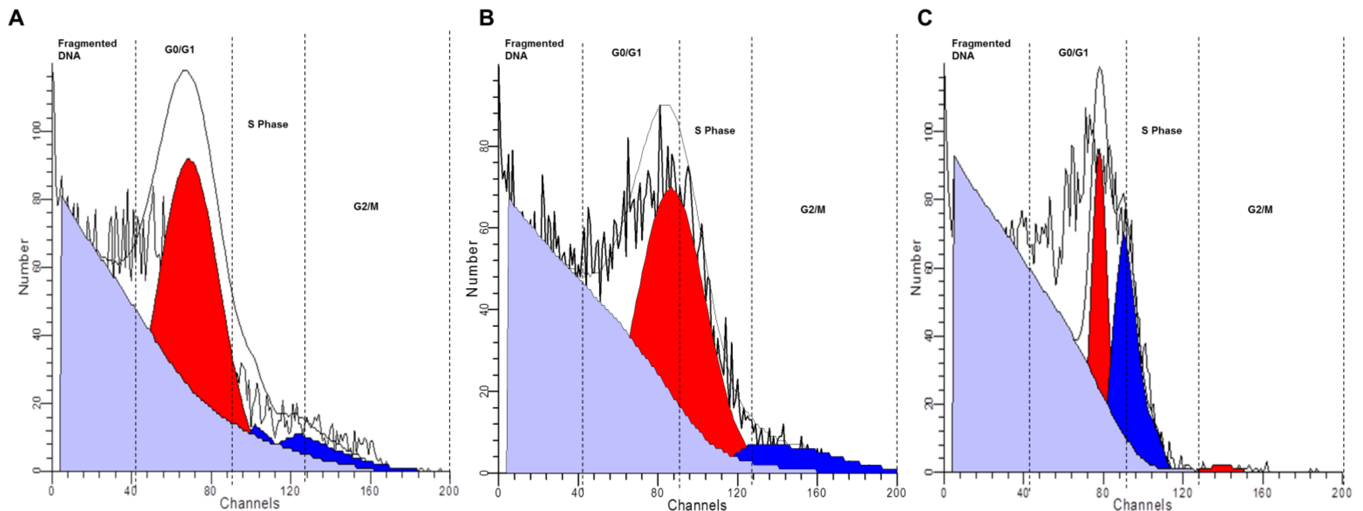
The analysis of the results was divided into two groups: species and code of the status carcass decomposition (SCD). These data are presented in Table 3.

The study of the phases of the cell cycle showed the comparison of the percentage of cells in the skin of the cetaceans (Figure 2 and Table 4). It was observed that the majority the population with the greatest number of cells was, in general, fragmented DNA (61.48±17.03), followed by the G0/G1 phase (81.85±10.37).

Table 3 – Species analyzed and status of carcass decomposition (SCD) of the animals analyzed.

Species	SCD 2		SCD 3		SCD 4		Total	
	N	%	N	%	N	%	N	%
Sotalia guianensis	6	33.3	10	55.6	2	11.1	18	60.0
Stenella clymene	6	75.0	2	25.0	-	-	8	26.7
Megaptera novaeangliae	1	33.3	-	-	2	66.7	3	10.0
Ziphius cavirostris	1	100	-	-	-	-	1	3.3
Total	**14**	**46.7**	**12**	**40.0**	**4**	**13.3**	**30**	**100**

Figure 2 – The histograms show the intervals in which cells were distributed in phases G0/G1, phase S, G2/M, and fragmented DNA. The inset represents the distribution of the cell population. **A.** SCD 2 – *Ziphius cavirostris*; **B.** SCD 3 – *Stenella clymene*; **C.** SCD 4 - *Sotalia guianensis*

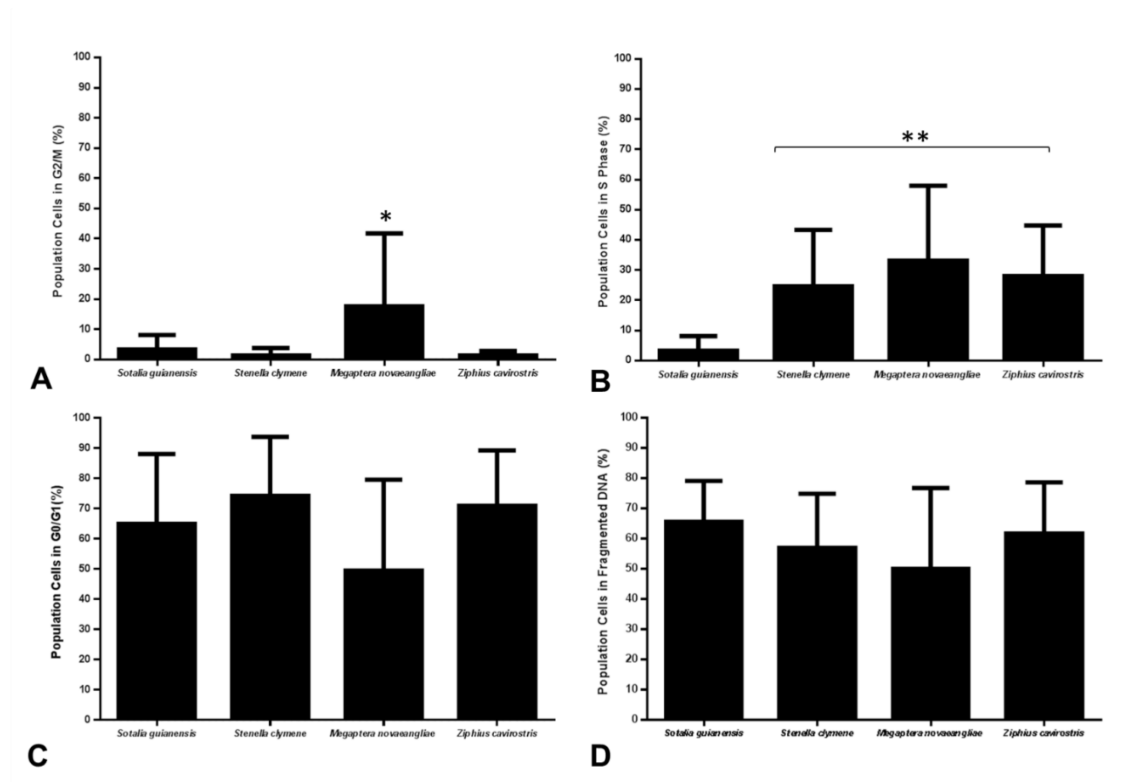


The species with the greatest percentage of cells in the phase G2/M and phase S was *Megaptera novaeangliae* (17.6±24.2 and 33.1±24.9). *Stenella clymene* presented a greater cell population in G0/G1 (74.2±19.6), and *Sotalia guianensis* presented greater population of fragmented DNA (65.4±13.8). ANOVA showed differences for phase S ($p<0.0001$) and G2/M ($p=0.0010$) (Figure 3).

Table 4 - Percentage of cells (mean ± SD) in the different phases of the cell cycle, per species.

	G2/M	Phase S	G0/G1	Fragmented DNA
Sotalia guianensis	3.3±4.8	31.9±20.7	63.9±23.1	65.4±13.8
Stenella clymene	1.2±2.6	24.6±18.7	74.2±19.6	57.2±18.2
Megaptera novaeangliae	17.6±24.2	33.1±24.9	49.3±30.3	49.8±27.1
Ziphius cavirostris	1.2±1.6	28±16.8	70.8±18.5	61.8±16.9

Figure 3 – Mean ± SD of the distribution of the cell population in the phases of the cell cycle in samples of skin of the four species of cetaceans analyzed. **A:** Cell populations in phase G2/M (p=0.0010); **B:** Cell populations in phase S (p<0.0001); **C:** Cell populations in phase G0/1; **D:** Cell populations with fragmented DNA.

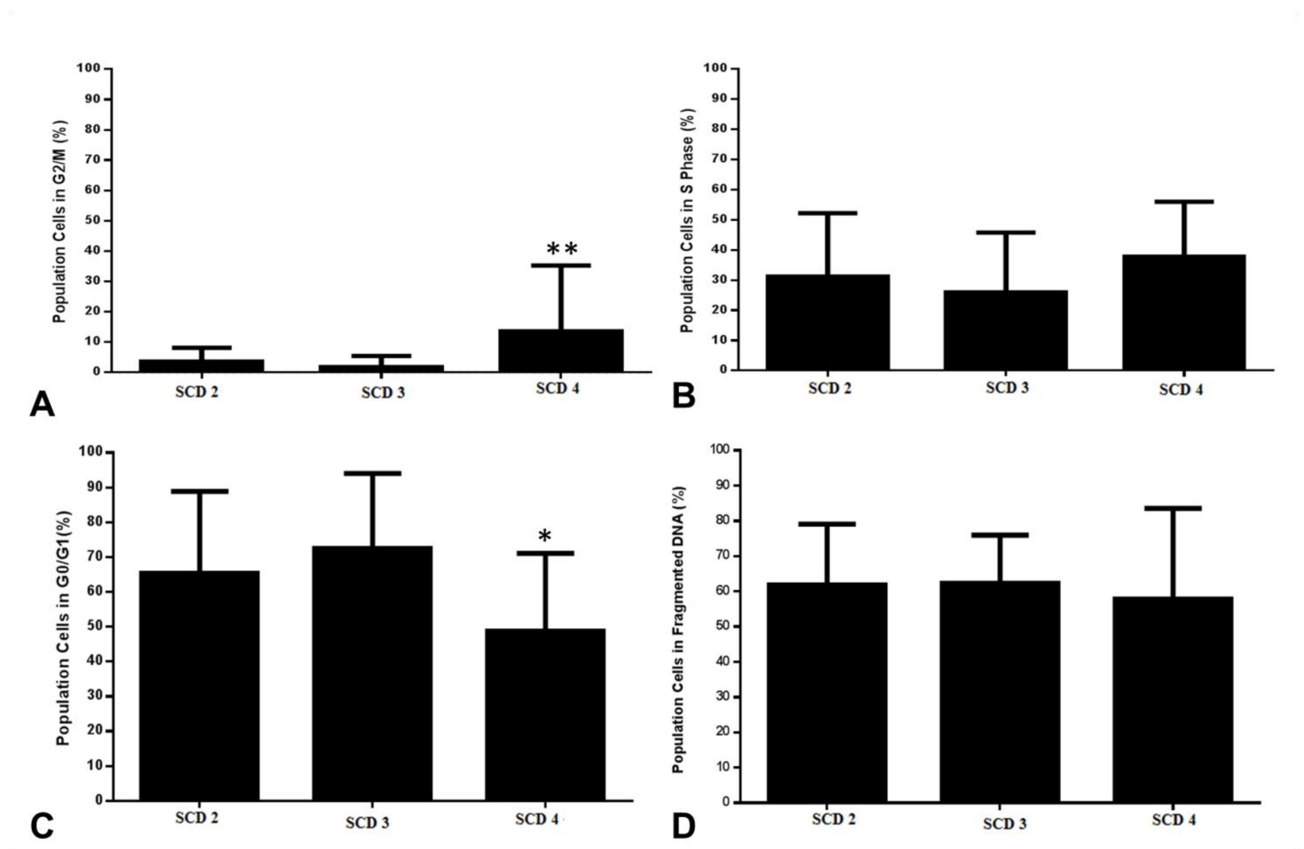


According to the status of carcass decomposition, decomposed carcasses (SCD 4) presented greater percentage of phase G2/M (13.4%±21.9) cells and phase S cells (37.8%±18.1). However, all carcasses in satisfactory status, with preserved organ architecture (SCD 3), presented greater percentage of cells in phase G0/G1 (71.3%±21.5) and fragmented DNA (62.3%±13.6). Carcasses in good status of preservation (SCD 2)

showed lower percentage of cells in all phases of the cell cycle than those in the other SCDs (Figure 4).

The comparison of the data on SCD by ANOVA showed significant differences in phase G0/G1 (p =0.0450) and G2/M (p=0.0058).

Figure 4 – Percentage of the distribution of cell populations in the phases of the cell cycle in skin samples, according to the status of carcass decomposition (SCD). **A:** Cell populations in phase G2/M (**p=0.0058); **B:** Cell populations in phase S; **C:** Cell populations in phase G0/G1 (*p=0.0450); **D:** Cell populations with fragmented DNA.

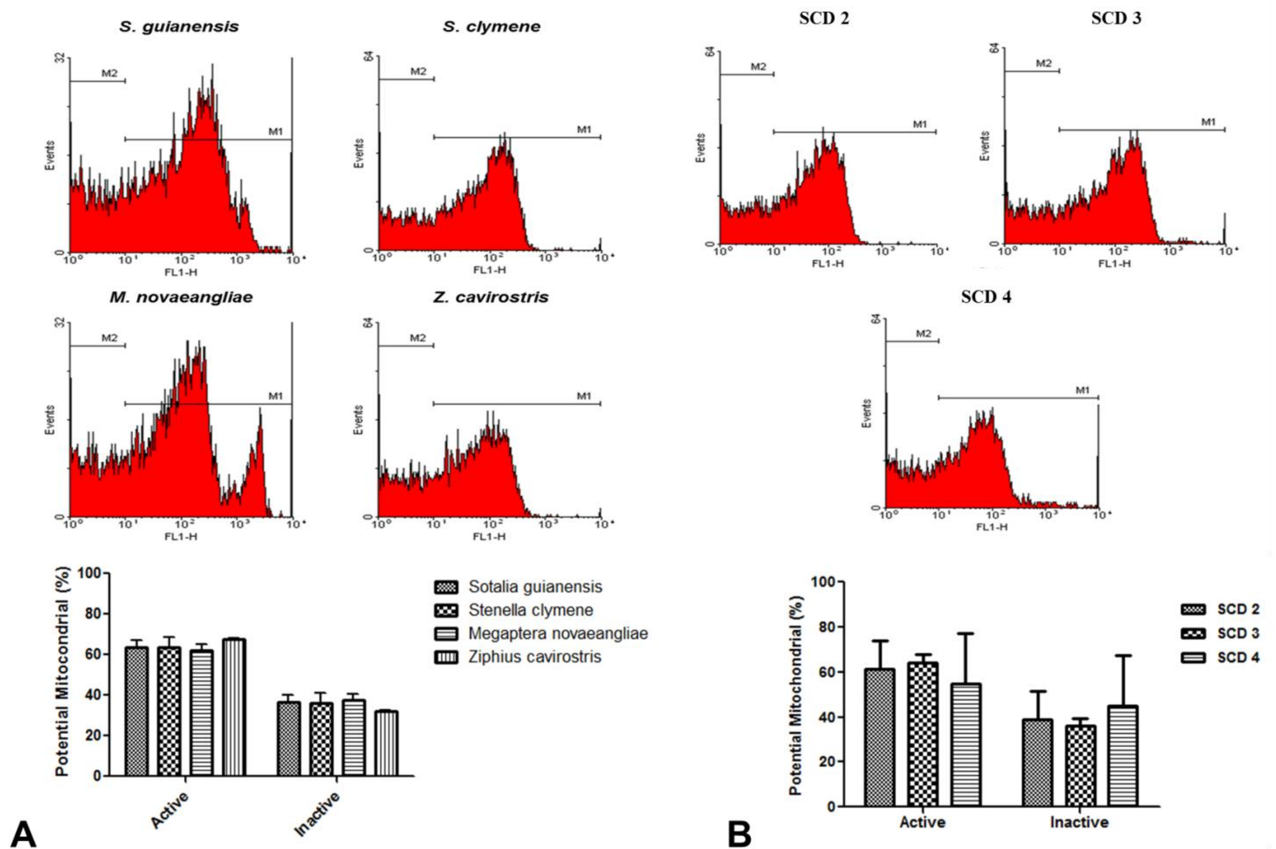


Evaluation of mitochondrial membrane potential

The evaluation of the mitochondria membrane potential has the main objective of evaluating the viability and metabolic activity of the mitochondria.

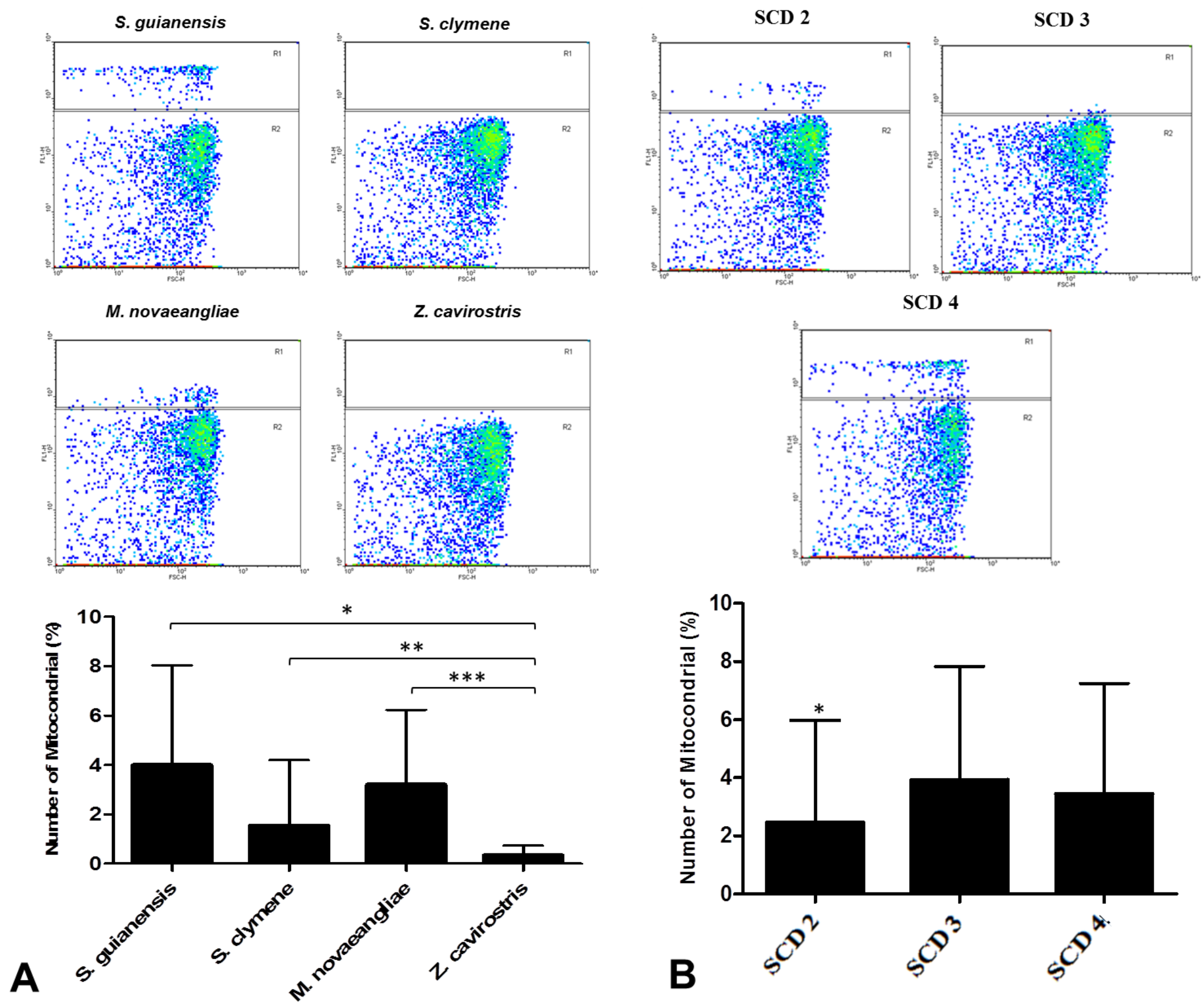
In our analyses, we observed that both active (M1) and inactive potential (M2) showed significant statistical differences among the species and status of carcass decomposition, ***p<0.0001 (Figure 5).

Figure 5 – Histogram presenting the mean ± sd of the active (M1) and inactive (M2) mitochondria membrane potential, according to the species (A) and status of carcass decomposition (B).



The number of hyperactive mitochondria showed significant differences between the species *Sotalia guianensis* and *Ziphius cavirostris* (*p=0.0288); *Stenella clymene* and *Ziphius cavirostris* (p=0.0442); and *Megaptera novaeangliae* and *Ziphius cavirostris* (*p=0.0159). As for the status of carcass decomposition, there was a significant difference only in relation to SCD 2 (*p=0.0463).

Figure 6 – Graphics dot plot percentage of the number of hyperactive mitochondria, according to the species (A) and status of carcass decomposition (B).



DISCUSSION

The Brazilian coast is 8,000 km long and has more than 50 species of cetaceans recorded (Ab'Saber, 2001; Pinedo et al., 2002; Monteiro-Filho et al. 2002; IBAMA, 2001). In our study, four of these species were used: *Sotalia guianensis*, *Stenella clymene*, *Megaptera novaeangliae,* and *Ziphius cavirostris*. These animals are endangered due to the impact caused by human occupation and by the, most of the times, inappropriate use of marine resources.

Trace elements are found all over nature. They may come from rocks or other sources besides the soil, such as atmospheric precipitation, ashes, limestone, chemical fertilizers, and organic fertilizers. Most of them are observed in trace amounts. From the

biological viewpoint, trace elements may be classified in two different forms: essential and non-essential (EISLER, 2000). When found in excess, essential elements may cause effects that are deleterious to aquatic and terrestrial organisms, making them contaminant agents or pollutants of the soil and water (EISLER, 2000).

Studies on the geochemical characterization of the Brazilian continental platform showred that trace elements found in the greatest concentrations are mainly Zn, Cu, and Cd (Maia, 2004; Lacerda and Molissari, 2006; Aguiar, Marins and Almeida, 2007; Abílio et al., 2004).

Aquatic mammals are described as sentinel animals because they have a long life expectancy, are predators on the top of the food chain, and store fat, which works as a deposit of chemical anthropogenic products and toxins (Aguirre; Tabor, 2004; Bonde; Aguirre, 2004; Bossart, 2006; 2007; Jessup et al., 2004; 2007; Moore, 2008; Reddy; Dierauf; Gulland, 2001). In Brazil, studies of the contaminants on tissue level (liver, fat, muscles, and kidneys) of aquatic mammals are being carried out in order to evaluate marine environments. These studies correlate the levels of contamination with age, sex and changes in behavior of these animals (Becker et al.,1997; Kunito et al., 2002, 2004; Seixas et al., 2008, 2009; Moreira et al., 2009; Anzolin et al., 2012). However, there are few studies that analyze this data on the cell level.

Although skin biopsies were suggested as non-invasive tools to evaluate the ecotoxicological risks for marine mammals (Fossi and Massili, 1997), there is little information on trace elements on the cetacean sten. These data described *Phocoenoides dalli* in Japan (Yang et al., 2002); *Stenella coeruleoalba,* in the Mediterranean (Augier et al., 1993); and *Phocoeana phocoeana* in Greenland (Paludan-Muller et al., 1993). In all studies cited, trace elements found in the greatest concentration in the skin were Zn and Se, similar to our study, in which Fe and Zn elements trace were found in the greatest concentrations, respectively, 70.88% and 13.6% of the total analyzed; Se metal accounted only for 1.9%.

According to Yang et al. (2002), the high concentration of these two elements in the skin are related to three functions: protection against UV radiation, promotion of wound recovery, and reduction of the mortality caused by trauma. A large amount of Zn is necessary for the metalloproteinases, proliferation of cutaneous cells, and deposition of collagen in wounds (Gemeul et al., 1998; Nezu et al., 1999).

According to Hicks et al. (1985) the period and rate of sloughing of the dermis in *Tursiops truncatus* was estimated as longer and quicker, respectively, than that of

epidermal cells in humans. The cell growth kinetics in the dermis was also confirmed in the present study by the increase in the capacity of synthesis (Phase S) and presence of new populations of cells in proliferation (G2/M).

During their evolution, cetaceans developed a mechanism to prevent bioincrustation, with a epidermal structure differentiated to produce a zymogel that is a complex of extracellular enzyme aggregates composed of glycoproteins that make it difficult for organisms to adhere to their skin (Baum et al., 2001). The presence of organic and inorganic molecules contaminating the structure of the zymogel changes several biological systems, such as sensory perception, damage to metabolism, adhesion, and locomotion.

Another characteristic of these animals is the germinative layer of the dermis, which has a greater number of proliferative cells (Brown et al., 1983). However, this finding was not observed in our study, once greater concentration of populations in phase G0/G1 were observed, both when samples analyzed in relation to the status of carcass decomposition than when analyzed by species.

In relation to the organs, the liver showed the higher concentration of trace elements, similar to other studies. This fact may be related to cytochrome P450, which takes part in the metabolization of drugs, steroids and carcinogens, making the excretion of these compounds easier by the insertion of one atom of molecular oxygen in them. However, it may undergo the consequences of this conversion of chemical products in highly reactive molecules, for adducts in supramolecular structures may be formed and/or undesirable cell damage may occur (Devlin, 2002; Galli and Feijoo, 2002).

Cytochromes P450 are a superfamily of heme-containing monoxygenases that metabolize a large number of compounds. While a limited number of P450s are involved in the biosynthetic pathways of steroid and bile acid production, most P450s metabolize foreign compounds or xenobiotics, including drugs, toxicants and chemical carcinogens. Xenobiotic-metabolizing cytochromes P450 are found at highest levels in the liver, the mammalian metabolic clearing-house for both exogenous and endogenous chemicals. Many metabolites generated by P450s are subjected to conjugation with either glucuronic acid, sulfate, glutathione, or acetyl groups by phase 2 enzymes (glutathione S-transferases, UDP-glucuronosyltransferases, N-acetyltransferases and sulfotransferases). This can result in transformation of a hydrophobic molecule to a more water soluble derivative that is easier to eliminate by renal and biliary excretion. In some cases, oxidation can lead to highly reactive electrophilic metabolites than can covalently react

with protein, RNA and DNA, resulting in cell toxicity and, in some cases, cell transformation and DNA damage.

Oxidative stress is the result of an increase in intracellular pro-oxidant species, such as H_2O_2, hydroxy radicals and superoxide anion radical. High intracellular levels of reactive oxygen species, can lead to damaged mitochondria, DNA modification, lipid peroxidation, elevated cytokine production and even cell death. Lipid hydroperoxides damage the mitochondria, resulting in membrane permeability transition pore damage, loss of ATP, and release of apoptotic-inducing factors.

P450 has been detected in large amounts in dolphins that accumulated greater concentrations of polychlorinated biphenyls in contaminated environments of Florida (Wilson et al., 2007). An ecotoxicological study of skin and genic expression in striped dolphins (*Stenella coeruleoalba*) reported the role of gene E2F-1 in the regulation of the cell cycle and apoptosis. These researchers proposed that this gene is a possible biomarker of exposure to general stress. The ability of HSP70 to respond to multiple stressors does not provide a specific and clear cause-effect response (Panti et al., 2011).

Apoptosis is essential for the maintenance of the development process of living beings, it is a programmed death, it is important to eliminate defective cells, and occurs under normal physiological conditions (Saraste and Pulkki, 2000). There are many molecules involved on the control of apoptosis activation pathways. Antiapoptotic proteins and pro-apoptotic caspases addition, involvement of pro-protein and anti-apoptotic family, such as Bcl-2 are involved on this control (Jacobson et al, 1993).

The mitochondrion is a critical organelle that regulates the pathway of activation of the apoptosis cascade. These organelles are necessary to the efficient metabolism of energy, the production of membrane lipids and the growth of cells but also are mostly determinants of life and cell death (Arends and Wyllie, 1991).

The involvement of mitochondria in apoptosis may are related to a number of different mechanisms (Zhuang et al, 1998a; Dinsdale et al, 1999; Finucane et al, 1999). Other mechanisms, include the loss of mitochondrial membrane potential; variations in cellular oxidation-reduction (redox) potential (Jacobson et al, 1993) although apoptosis is not dependent on oxidative phosphorylation, or the presence of mitochondrial DNA, mitochondria were shown as coordinators of apoptosis in several cell (Frade and Michaelidis, 1997; Kroemer, 1997, Zhuang et al, 1998b; Green and Reed, 1998).

Mitochondria are dynamic organelles that have been shown to have a central role in the apoptosis process. The reduction in transmembrane electric potential of

mitochondria is known as a trigger for cell death by apoptosis, and this mechanism may be associated with the intrinsic apoptosis signaling pathway (Galluzzi et al., 2011). One of the hypotheses of this study is that in animals in SCD 3 and SCD 4 there is greater possibility that trace elements may change the functionality of the mitochondria. Therefore, the possibility of cell death or toxicity is increased. In our study, SCD 4 animals showed greater number of cells in all phases of the cell cycle, and this fact may be related to a response of the organism in a cell death reaction.

Molecular and biochemical changes are the first biological responses, and are related to the essence of the mechanism of toxic action of the contaminants, because they represent the molecular basis of toxicity (Walker et al., 1996). Therefore, it is suggested that new studies are carried out at the cell level and with specific biomarkers, such as P450, for example, to improve the correlation of trace element concentrations in the skin and organs of animals stranded on the Brazilian seaside.

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