ECOTOX - BRASIL

Ecotoxicol. Environ. Contam., v. 12, n. 1, 2017, 113-131
doi: 10.5132/eec.2017.01.14

EEC

Toxicity evaluation of PAHs in the sponge *Hymeniacidon heliophila:* field assessment and laboratory assays - a preliminary study

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(Received May 15, 2017; Accept October 30, 2017)

Abstract

Polycyclic aromatic hydrocarbons (PAHs) have been extensively studied in the aquatic environment, although the mechanism of action and biological effects of this xenobiotic in several species, including sponges, are not yet well known. In this context, the aims of the present study were to apply the sponge *Hymeniacidon heliophila* as a biomonitor for PAH contamination, and to determine PAH depuration and chrysene accumulation patterns through bioassays, evaluating biomarkers in order to verify sublethal responses to exposure. The monitoring of two Southeastern Brazilian areas, Quadrado da Urca and Itaipu beach, indicated bioconcentration of PAHs in sponges. A predominantly petrogenic character was observed in the evaluated areas. Uptake of chrysene and elimination of PAHs were evaluated in *H. heliophila*, through the biomarkers neutral red and reduced glutathione (GSH), as well as histological evaluations. In the depuration assay, sponges from Quadrado da Urca, a contaminated site, were transferred to aquaria and collected at 24, 48, 72, 96, 168, 240 and 336 h. No significant depuration occurred during the first 96 h, and no changes were verified in the evaluated biomarkers. However, the sponges were able to depurate PAHs from 96 h to 336 h, indicating that this species may reach a healthy status if the environment is uncontaminated. An accumulation bioassay was carried up to 96 h, with sponges from Itaipu beach, in order to evaluate their ability to accumulate chrysene and show immediate effects. Accumulation was intense during 72 h, followed by a decline at 96 h. Neutral red retention time declined with chrysene accumulation, and the decrease of accumulation at 96 h seems related to GSH production. Histological structures were proven to be useful biomarkers of sponge health for experimental conditions. Thus, *H. heliophila* is confirmed to be an adequate bioindicator PAHs exposure.

Keywords: bioassay, biomarkers, PAHs, *Hymeniacidon heliophila*, toxicity.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are derived from petrogenic (oil and derivatives) and pyrolytic (incomplete combustion of organic matter) sources. Due to their highly lipophilic character, these compounds may undergo bioaccumulation in marine organisms, if the species is unable to metabolize these contaminants efficiently. This occurs in invertebrates, in which the biotransformation process is, generally, slower than in vertebrates (Rey-Salgueiro *et al*., 2009; Batista *et al*., 2013).

High molecular weight PAHs (4-6 fused aromatic rings) present low acute toxicity, but display higher carcinogenic potential for aquatic organisms (Baird & Cann, 2012). Among PAHs, chrysene consists of four condensed benzene rings and is formed through the incomplete combustion of organic matter or pyrolysis of coal and crude oil, and is a very present contaminant in the environment, mainly due to the anthropic activities (ATSDR, 1990). The relatively low solubility of chrysene in seawater (2 μg L^{-1} at 25 ° C) promotes its adsorption to particulate material, followed by sedimentation (Neff, 2002). This compound possesses a bay region formed by the

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branches of the carbon chain in the sequence of benzene rings. The organization of the carbon atoms provides chrysene with a high degree of biochemical reactivity, giving it a potential carcinogenicity (Baird & Cann, 2012).

Sponges (Phylum Porifera) are widely distributed in marine systems, occurring mostly in shallow waters of the continental shelf in tropical areas (Muricy & Hajdu, 2006). Their high filtration rates (100 mL $h^{-1}g^{-1}$), as well as the ability to ingest particles ranging from 0.2 to 50 µm, allow for the efficient capture of contaminants in both dissolved and particulate phases (Vogel, 1977). Due to this property, sponges are highly exposed to mutagenic and carcinogenic substances naturally present in the environment or originated from anthropogenic sources, such as PAHs, and are considered pollution bioindicators.

Studies have shown that sponges can efficiently accumulate several contaminants, such as metals (Mestre *et al.,* 2012; Batista *et al*., 2014), PCBs (Pérez *et al*., 2003) and PAHs (Denton *et al*., 2006; Mahaut *et al*., 2013; Batista *et al*., 2013). Most of the studies that tested PAHs effects in laboratory used sponge species from the Northern temperate zone and from the Pacific Ocean (Zahn *et al*., 1983; Glyzina *et al*., 2002). Only a single study is available describing the *in situ* evaluation of marine sponges from the Southeastern Brazilian coast as bioindicators for PAH contamination (Batista *et al*., 2013).

To more accurately assess exposure to PAHs in aquatic organisms, it is pertinent to detect these compounds in biological tissues or fluids. However, this biomonitoring parameter depends on the absorption, biotransformation and excretion mechanisms in these organisms. For example, the bioaccumulation process in sponges may vary depending on contaminant concentrations in the environment (Cebrian *et al*., 2003; Pérez *et al*., 2003).

The investigation of biomarkers allows for the evaluation of changes in the environment and exposure to chemicals. In this regard, the impact of PAHs exposure may be assessed by changes in structure and function of several cellular components, such as lysosomes, which accumulate a variety of compounds (Weyermann *et al*., 2005; Moore *et al*., 2006). Neutral Red Retention Time (NRRT) assay, an *in vitro* cytotoxicity assay, is used to evaluate the ability of cells to absorb and retain the cationic dye neutral red (3-amino-7-dimethylamino-2-methylphenazine hydrochloride), by quantifying the number of viable and intact cells after exposure to toxicants (Weyermann *et al*., 2005). Some studies have applied this technique to marine organisms exposed to xenobiotics, as a health status indicator (Moore *et al*., 2006; Pereira *et al*., 2014).

Reduced glutathione (GSH) plays an important role in the antioxidant defense system, operating in several cellular processes involved in maintenance of a large number of physiological functions in vertebrate and invertebrate cells. Decreases in glutathione expression may reduce the cellular capacity to destroy free radicals and reactive oxygen species, increasing the oxidative potential of the cell. GSH is a tripeptide (γ-glutamyl-L-cysteinyl-glycine) containing a sulfhydryl group (-SH) present in cysteine, conferring a high reducing capacity, and is used as a substrate for several enzymes of the glutathione group, including Glutathione S-transferase (GST) and Glutathione Peroxidase (GPX), for xenobiotic detoxification through conjugation reactions, that increase contaminant solubility in water. The intracellular induction of these enzymes can be triggered by a variety of xenobiotics, including pesticides, polychlorinated biphenyls and PAHs (Regoli & Giuliani, 2014). However, little is known about the concentration and distribution of GSH in sponges (Phylum Porifera). In one study, Bachinski *et al.* (1997), determined early markers for thermal stress in *Suberites domuncula*, and reported that trehalose, GSH, and GST can be considered adequate biomarkers in this situation, where GSH decreased significantly after 15 to 20 min heat exposure.

Histological techniques involve the preparation of tissues for study under microscopy, involving microtomy (cutting) and staining, which aims to contrast tissue structures. The action of most dyes is based on the interaction between the acid or basic radicals of the chemical elements of the dyes with the ionic radicals of the tissues, forming electrostatic bonds (Michalany, 1998). Specific dyes, such as picrosirius for collagen, are also available Hematoxylin stains acid structures in blue, such as cell nuclei, while eosin stains the cytoplasm and other basic structures in red (Michalany, 1998).

However, sponges do not have typical tissues, as found in other multicellular animals, and their cells still retain a high degree of totipotency (cell differentiation ability). These organisms use choanocytes, specialized cells with morphology and function similar to mammalian fibroblasts, to promote water circulation through a system of channels. Cholencytes secrete collagen, an extracellular matrix fibrillar protein, important for the support of the multicellular body (Muricy & Hajdu, 2006). The organic skeleton of the Porifera phylum is formed by spongin fibers, a modified type of collagen protein. Scarce studies are available relating pollutants and histological alterations in sponges, and no study has yet been conducted with the aim of characterizing histological alterations as a consequence of PAHs effects, although some studies have analyzed sponge tissues as ways of evaluating metal effects (Wagner *et al*., 1998; Efremova *et al*., 2002).

In this context, the goals of the present work were to apply the sponge *Hymeniacidon heliophila* as a PAHs biomonitor, to determine their depuration and accumulation patterns in through bioassays and evaluating biomarkers in order to verify sublethal responses to exposure.

MATERIAL AND METHODS

Chemicals

All the reagents used herein were obtained from Sigma-Aldrich®. Entellan was acquired from Merck®. Solvents

(pesticide grade) used in the chromatographic separations were purchased from Mallinckrodt®. The surrogate and internal standards were obtained from Accustandard. The chrysene standard was purchased from Dr. Ehrenstorfer GmbH. Filter membranes were acquired from Merck Millipore (GSWP14250).

Studied species

Hymeniacidon heliofila (Porifera: Halichondrida) is an orange sponge, with a form that varies from massive to entangling. The mesohyl in this species is dense and the skeleton, silicous. The coanosomal skeleton may be composed of ascending bundles of spikes grouped together by spongin and randomly dispersed spicules (Muricy & Hajdu, 2006). *H. heliophila* occurs from North Carolina (USA) to Santa Catarina (Brazil), in the intertidal region up to 15 m deep (Muricy & Hajdu, 2006), and is one of the first species to colonize hypertrophic areas in Guanabara Bay, a chronically polluted bay in Southeastern Brazil (Breves-Ramos *et al*., 2005).

This sponge seems to be a good candidate to integrate biomonitoring programs in coastal areas, since tissue PAHs levels reflect local contamination and its populations are quite stable in the environment during all seasons, which suggest a long-life cycle (Batista *et al*., 2014). Studies in degraded areas in the city of Rio de Janeiro, for example, have pointed *H. heliophila* as a pollution bioindicator, since it efficiently accumulates contaminants (Batista *et al*., 2013).

Sampling sites

Guanabara Bay is a complex estuarine system with a diversified water circulation pattern, multiple contaminant sources and a high population density occupation. It encompasses the second largest industrial park in the country, with 14,000 industries, which represent one of the main contamination factors of the bay (Marques-Junior *et al*., 2009) contributing to about 20% of the organic load. According to the Environmental Control Report of the Guanabara Bay (PDBG, 2000), approximately 10,000 tons of hazardous wastes are generated monthly in the Guanabara Bay watershed, comprising waste oils (31 %), heavy metals (11 %) and solvents (3 %). One of the main causes of the pollution in this ecosystem is domestic sewage (flow of 20 m^3s^{-1}), leading to increased nutrients and consequent eutrophication of the waters of the bay, leading to increased organic matter storage (Marque-Junior *et al*., 2009).

The coastal region of Itaipu is located outside of the Guanabara Bay, specifically in the west of its mouth. The coastal water mass is a mixture of waters from Guanabara Bay, the Itaipu and Piratininga lagoons and oceanic coastal waters, seasonally influenced by the resurgence of the central waters of the South Atlantic (Tubino *et al*., 2007). The Itaipu-Piratininga lagoon complex represents a specific habitat, suitable for the reproduction and/or growth of marine- or estuarine-dependent species. These lagoons have undergone extensive anthropic modifications with significant changes in their geomorphology, in turn altering local biota characteristics.

Studies have reported significantly higher PAHs levels at Guanabara Bay compared to Itaipu beach (Batista *et al*., 2013; Resende *et al*., 2017).

Sampling

Field study

Sponges were collected from Quadrado da Urca (n=3 individuals), an area located inside Guanabara Bay, and from Itaipu beach (n=2 individuals), an area located outside this bay (Fig. 1). PAHs levels in water samples and suspended solids (SS), in duplicate, were analyzed to evaluate the contamination status at both sites and to calculate bioconcentration factor (ratio between PAHs concentration in sponge and in water or suspended solids).

Laboratory study

Sponges were sampled from Quadrado da Urca and from Itaipu beach for depuration and accumulation bioassays, respectively. Individuals were immediately placed in a small aquarium containing local water for transport. Once in the laboratory, the sponges were cleaned in a container with seawater, removing impurities like sediment and epiphytic organisms.

Sponges Bioassays

The experiments were performed using aquaria containing 70 L of artificial seawater (marine salt Coralife - scientific grade). Oxygenation (aeration) was maintained with pumps (Sarlo Better) with 3000 L h^{-1} capacity. In each aquarium, specimens were held submerged by strings arrays and maintained at 21 °C ± 1 °C under a 12 h:12 h (light:dark) photoperiod. The Winkler method (Helm *et al*., 2012) was used to daily assess dissolved oxygen and ammonia was monitored routinely using an aquarium test for salt water (Nutrafin®). The organisms were neither acclimatized nor fed before and during bioassays. At each time interval, sampled individuals were removed from aquaria and entirely used, being that a small cut was separated for biomarkers and the remaining for PAHs analysis. Biomarkers were immediately evaluated, so that it could not interfere on their answers. For PAHs determination, the remaining sample was frozen at -80 °C and lyophilized (ModulyoD from Thermo). Sponges were weighted before and after the bioassays to verify possible biomass losses.

Depuration assay

For the depuration assay, 24 individuals (approximately 20g each) were sampled from Quadrado da Urca and left to depurate (Fig. 1) in different aquaria containing 70 L of

artificial seawater. Three samples were taken at different time points (0, 24, 48, 72, 96, 168, 240 and 336 h) and were analyzed for biomarkers and PAHs.

Accumulation assay

For the accumulation assay, 10 individuals (approximately 20g each each) were sampled from Itaipu beach, generally a clean site comparing to Guanabara Bay. Sponges were exposed to chrysene dissolved in water (40 µg L^{-1} prepared in dimethylsulfoxide – DMSO). Two samples were taken at different time points (0, 24, 48, 72 and 96 h) and analyzed for biomarkers and chrysene concentrations (Fig. 1). Water samples from the aquaria were analyzed to check initial and final chrysene concentrations and verify sponges' bioaccumulation capacity (%).

PAH analysis

Sponge tissue samples (1.5 g dry weight) were Soxhlet-extracted in dichloromethane over 24 h, according to EPA Method 3540C (USEPA, 1996b). Clean-up steps were performed to reduce lipid content, according to Batista *et al.* (2013): 1) glass column (30 cm x 2.2 cm), packed with alumina, and; 2) gel-permeation chromatographic system composed of a GPC LC-10AD with fluorescence (254 nm) detector SPD-10AV (Shimadzu) and fitted with a CLNpak EV2000 Shodex column, using acetone:cyclohexane (3:7, v/v) for elution. Finally, the hydrocarbon fractions were separated in a glass column (30 cm x 1.3 cm) packed with alumina, silica and anhydrous Na_2SO_4, and the aromatic fraction was eluted with dichloromethane:hexane (1:1, v/v). Subsequently, the extracts were concentrated to 1 mL in a Turbo Vap II system (Caliper Life Sciences).

The PAH analysis in water samples (4 L of each sample) was based on EPA Method 3510C (USEPA, 1996a). Successive extractions (3x) with dichloromethane were performed by shaking (150 rpm) for 3 min with subsequent collection of the organic phase. For suspended solids evaluation, water samples (20 L of each sample) were filtered using a Millipore 142 mm filter holder until the filter membrane (142 mm, 0.22 µm pore, mix cellulose esters, Merck Millipore) reached saturation and the volume was measured. Each filter represents one sample. PAH Soxhlet extraction from membrane filters followed EPA Method 3540C (USEPA, 1996b). Hydrocarbon fractions in water and suspended solids samples were separated after PAH extraction, according to the same procedure adopted for sponge tissue samples.

Chrysene quantification followed EPA Method 8270D (USEPA, 2014) carried out on a system composed of a Trace-GC fitted with a J&W DB-5ms capillary column (30 m x 0.25 mm x 0.25 µm) and coupled to a Thermo Finnigan Polaris Q mass detector. Helium used as carrier gas was adjusted at 1.2 mL min^{-1} and the temperature program was set as follows: 50 °C during 5 min; 5 °C min^{-1} up to 80 °C; 6 °C min^{-1} from 80 °C to 280 °C and hold of 25 min at 280 °C. Injection volume was 1.4 µL. The surrogate standard *p*-terphenyl-d_{14} was added to the samples prior to PAH extraction to check

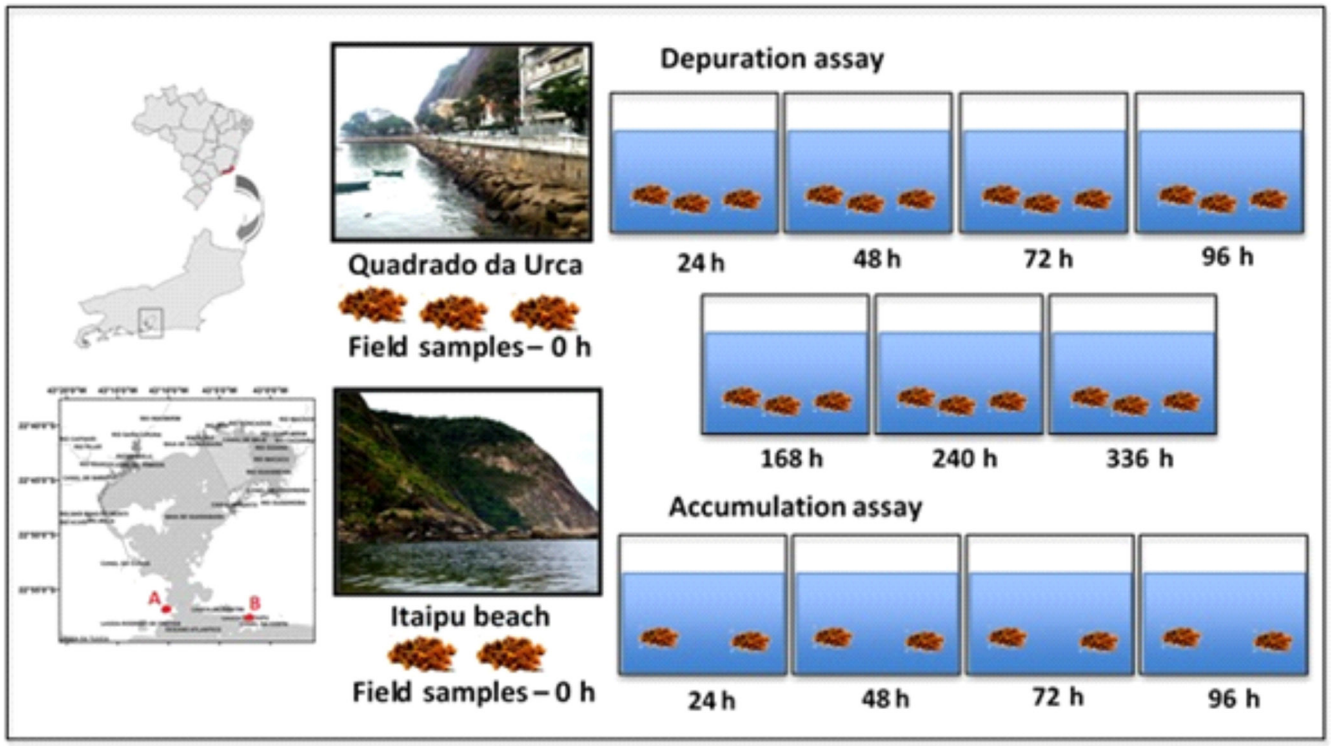

Figure 1. Diagram of the field study and laboratory assay applied in the present study. Map indicates sampling sites: A – Quadrado da Urca, B – Itaipu beach, with respective pictures and field samples – 0 h (n=3, n=2). Depuration assay – 3 individuals per aquarium (24, 48, 72, 96, 168, 240 and 336 h); Accumulation assay - 2 individuals per aquarium (24, 48, 72 and 96 h).

method recovery. A mixture containing naphthalene_d_8, acenaphthene_d_{10}, phenanthrene_d_{10}, perylene_d_{12} and chrysene-d_{12} was used as internal standard for quantification. Quality assurance procedures included successful analysis of a reference material (NIST 2974), analytical blank control, recovery control and daily verification of calibration curves.

Biomarkers

Neutral Red Retention Time (NRRT) assay

To perform cell extraction for the NRRT assay, sponge sections were transferred to Falcon tubes, containing CMFSW-EDTA (Calcium-Magnesium Free Seawater with EDTA), a saline medium containing 460 mmol L^{-1} NaCl, 7 mmol L^{-1} Na_2SO_4, 10 mmol L^{-1} KCl, 10 mmol L^{-1} Hepes buffer and 2.5 mmol L^{-1} EDTA in Milli-Q water. pH was adjusted to 8.0 with 1 mol L^{-1} NaOH. The tubes were slowly shaken for 30 min on a shaker (Thermolyne) to remove traces of residual calcium and to facilitate cell dissociation, and shaken again for 60 min, with a fresh solution. The cell suspension was obtained by filtration in nylon mesh (50-100 µm).

The neutral red assay was conducted using a modified protocol reported by Lowe et al. (1995) to check lysosomes cell viability. A physiological saline solution containing 20 mmol L^{-1} Hepes, 10 mmol L^{-1} $CaCl_2$, 53 mmol L^{-1} Na_2SO_4, 436 mmol L^{-1} NaCl and 10 mmol L^{-1} KCl, and pH adjusted to 7.36 with 1 mmol L^{-1} NaOH, was mixed with the sponge cell suspension (1:1, v/v). The mixture (30 µL) was transferred to microscope slides, which were maintained in a dark and humid chamber at 20 °C for 15 min, allowing for cell adhesion. A 30 µL aliquot of neutral red solution (neutral red 5% in physiological saline) was placed on the cell layer and the coverslip was set. After 15 min incubation, slides were systematically examined on an optical microscope under a x40 magnification at 30 min intervals to determine the time evidence of 50% of dye loss from the lysosomes into the cytosol. The Neutral Red Retention Time (NRRT) was calculated for each slide sequence.

Reduced Gluthatione (GSH) assay

GSH extraction was performed using the technique reported by Beutler *et al.* (1963), modified by Wilhelm-Filho *et al.* (2005). Sponge samples (200 mg) were homogenized in 0.1 mol L^{-1} Na_2HPO_4 buffer pH 7.0, containing 0.25 mol L^{-1} sucrose and centrifuged at 13500 rpm for 30 min at 4 ºC to precipitate high molecular weight proteins and cellular debris. After centrifugation, 150 µL of 5,5'-dithio-2-nitrobenzoic acid (DTNB) in 0.1 mol L^{-1} Na_2HPO_4 pH 8.0 were added to the supernatant diluted in ultrapure water (1:1, v/v). Following this procedure, samples were incubated in a microplate for 15 min, prior to reading on a spectrophotometer coupled to a microplate reader (SpectraMax 190 Hamilton) at $\lambda = 412$ nm. GSH concentrations were estimated using an analytical curve, prepared with 0.1 mol L^{-1} Na_2HPO_4 pH 7.0 using GSH as an external standard.

Histological analysis

Relative quantification of cavities (aquifer channels and choanocyte chambers) was performed by the analysis of sponge histological sections stained with hematoxylin/eosin (HE), while the analysis of collagen fibers was performed by the histochemical Picrosirius method, in order to verify possible alterations in tissue structure, such as decreases in cavities and collagen, due to PAH contamination in the field and chrysene exposure in the laboratory.

For both techniques, a sponge section was placed in Bouin's fixative solution for 6 h, in order to preserve tissue structures for subsequent treatments, and washed repeatedly with 70% ethanol for 30 min. Stages of sample preparation were then performed, namely embedment and cut and staining, according to the adopted methods for HE staining (Behmer *et al*., 1976) and the histochemical Picrosirius method (Dolber & Spach 1993). After staining, the slides were immersed in 0.01 mol L^{-1} HCl for one min followed by in 70% ethanol. To set the coverslip with resin Entellan, dehydration was necessary and was attained after placing the slides in an ethanol series (70, 100, 100 %) followed by xylene.

For histological sections stained with HE, an optical microscope Axioplan (Zeiss) with a 10x magnification, coupled to a MRM AxioCam camera and AxioVision software were used to serially photograph the sections, and a complete image was digitally assembled by photoshop (Adobe Photoshop CS 5.1). Collagen quantification, based on staining intensity was performed with an ApoTome (Zeiss) fluorescence microscope at a wavelength related to rhodamine (red color filter, Schott-Zeiss). Photograph processing and quantification were performed using the ImageJ software.

Data processing

PAHs concentrations were statistically analyzed for differences among individuals assayed at different times by a one-way ANOVA. Post-hoc comparisons were conducted (Tukey test) when differences occurred, considering a 95% confidence level (associated probability <0.05). Non-parametric Kruskall-Wallis and Mann-Whitney tests were applied as an alternative in case of assumption violations. The non-parametric Spearman's test was applied to search for correlations between the variables. All statistical methods were applied using the software STATISTIC 12.0. Diagnostic ratios were applied to the field samples to determine the source of PAH contamination. Bioconcentration factors (BCF) were estimated for field samples, as the ratio between each PAH concentration in sponges and water or suspended solids.

RESULTS AND DISCUSSION

Field samples

Mean concentrations of the sum of priority PAHs (∑16PAHs) and of the sum of 38 PAHs (∑TotalPAHs) in water and suspended solids samples were 72.8 ng L^{-1} and 103.7 ng L^{-1},

and 108.7 ng L^{-1} and 300.1 ng L^{-1}, respectively, in samples from Quadrado da Urca. Mean concentrations of the sum of priority PAHs (∑16PAHs) and of the sum of 38 PAHs (∑TotalPAHs) in water and suspended solids (SS) samples were 219.5 ng L^{-1} and 392.6 ng L^{-1} and 231.8 ng L^{-1} and 718.2 ng L^{-1}, respectively, in samples from Itaipu beach. The concentrations of the individual PAHs for both sampling sites are reported in Table 1.

The values determined for the sum of priority PAHs (∑16PAHs) in water are below the limits recommended by regulatory agencies (CONAMA, 2005; Buchman, 2008), of 300 ng L^{-1}. PAH levels at Itaipu beach were higher than at Quadrado da Urca, possibly due to the climatic conditions on the sampling day, since it rained during the week, increasing PAHs levels in water and suspended solids (SS). In addition, there may have been a contribution from Guanabara Bay waters, during tide ebb, since, on the sampling day, a South-Southwest current leaving the bay, entered the Itaipu region. In despite of PAHs levels at this site, it was believed that their signal could not interfere on accumulation assay, since the chrysene concentration applied in aquaria was at other magnitude.

The concentrations of the sum of priority PAHs (∑16PAHs) and of the sum of 38 PAHs (∑TotalPAHs) in the sponge samples from Quadrado da Urca ranged from 372.9 to 582.3 μg kg^{-1} and from 1286.4 to 2258.2 μg kg^{-1}, respectively. Concentrations of the sum of priority PAHs (∑16PAHs) and of the sum of 38 PAHs (∑TotalPAHs) ranged from 82.3 to 91.4 μg kg^{-1} and 781.0 to 1395.1 μg kg^{-1} in sponges collected from Itaipu Beach, respectively.

Pyrogenic sources are characterized, mostly, by the presence of parental and high molecular weight PAHs, while petrogenic sources are characterized by the presence of alkylated and low molecular weight PAHs, according to the criteria established by Boehm & Farrington (1984). In addition, it is possible to distinguish two types of oil for petrogenic sources: degraded, with a C0<C1<C2<C3 distribution, and recent, in a bell-shaped format.

The origin and profile of PAHs in water and suspended solids (SS) samples from Quadrado da Urca were assessed in this regard the exposure characteristics of the sponges in the environment (Fig. 2A). Water samples presented a PAHs typology that suggests pyrogenic influence due to the lower abundance of alkylated PAHs. In addition, the samples showed greater influence of heavy compounds, containing 4 to 6 benzenic rings, typically of pyrogenic origin, while the PM samples showed a C0<C1<C2<C3 distribution of fluorene, dibenzothiophene, phenanthrene and chrysene alkylates, indicating degraded oil. The PM samples presented 94% of the 16 priority PAHs as heavy compounds. A high proportion of alkylates was also present, mainly of light PAHs (2 to 3 benzenic rings), that reached up to 61% of alkylates. In relation to the total PAHs, the lower molecular mass alkylates reached up to 51%, which also characterizes the presence of material of petrogenic origin in the samples. Considering only alkylated PAHs, it was possible to verify the presence of petrogenic components in the PM samples.

The PAHs typology in the water and suspended solids (SS) samples (Fig. 2B) from Itaipu beach was analyzed with regard to the concentrations to which the sponges were exposed in the environment. The water samples presented pyrogenic influence, constituted mostly of parental PAHs and of compounds of 4 to 6 aromatic rings. Also at this site, the PM samples presented 68% of alkylated PAHs, and considering only the 16 priority PAHs, 92% of them were constituted by heavy compounds. There was also a high concentration of the alkyl compounds of light PAHs, which proportion was 59%, suggesting a petrogenic profile, with two types of oil: degraded, with distribution C0<C1<C2<C3, and recent, in bell format, as is the case of phenanthrene series (Fig. 2B). These two types of oil indicate natural sources for the alkylated series. This indicates a mixture of sources at Itaipu, with a greater contribution of petrogenic hydrocarbons.

The typology of PAHs in sponge samples indicates contamination by petrogenetic sources, since alkyl compounds represented the majority of PAHs, 71% in samples from Quadrado da Urca and 93% in samples from Itaipu. In addition, there is an indication of petrogenetic contamination by two types of oil: degraded, with distribution C0 <C1 <C2 <C3, and recent, bell-shaped, as is the case of the phenanthrene and dibenzothiophene series (Fig. 3).

The evaluation of contamination sources in water, suspended solids and sponge samples was performed using diagnostic ratios (Fig. 4). A large number of diagnostic ratios based on individual PAHs are applied, mainly with bi-plot diagrams such as Fl/(Fl + Py) and BF1/(BF1 + BePy), to evaluate the relative contribution of petrogenic and/or pyrogenic PAHs in the environment, thus determining the origin of these compounds. In addition, the use of diagnostic ratios makes it possible to evaluate the type of material burned, such as oil, diesel, coal and biomass, among others (Wang *et al*., 1999; Yunker *et al*., 2002). However, uncertainties inherent to the application of these indices in environmental samples exist, generated by several factors, such as combustion temperature, differentiated PAH degradation and the presence of materials and oils from different sources, which may influence the original signature of specific PAHs sources (Wang *et al*., 1999). The sponges sampled at Itaipu showed a petrogenetic source contamination trend, in contrast to the water samples. This can be explained by the fixation site of sponges, which is localized in the intertidal zone, facilitating the filtration of superficial water, where the oil film is. One sponge sample from Quadrado da Urca displayed this profile, while the others were close to this threshold.

Cross-plots were used to evaluate the type of material and determine the possible source of the samples as pyrogenic, petrogenetic or source mixture (Fig. 4). Most samples presented a characteristic distribution for vegetation, wood and coal combustion sources. However, the distribution profile of the PAHs indicates a mixture of sources at Itaipu and petrogenetic contamination at Quadrado da Urca. A problem related to the use of diagnostic ratios for the determination

Table 1. Mean PAHs concentrations in field samples (water, suspended solids and sponges) from Quadrado da Urca and Itaipu beach and bioconcentration factor in sponges by water and suspended solids at each site.

	Quadrado da Urca						Itaipu beach					
	Water	Sponge	BCF	SS	Sponge	BCF	Water	Sponge	BCF	SS	Sponge	BCF
N[a]	5.6	2.8	0.5	ND	2.8	ND	2.1	4.6	2.2	ND	4.6	ND
C1N	ND	5	ND	ND	5	ND	ND	4.4	ND	ND	4.4	ND
C2N	ND	20.7	ND	ND	20.7	ND	2.3	52.9	22.9	ND	52.9	ND
C3N	2.1	10.9	5.2	2.8	10.9	3.8	2.7	10	3.7	2.2	10	4.5
C4N	2	24.8	12.2	4.1	24.8	6.1	3.5	12.5	3.6	4	12.5	3.1
ACF[a]	ND	4.3	ND	ND	4.3	ND	2.7	ND	ND	ND	ND	ND
ACE[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
F[a]	ND	3	ND	ND	3	ND	ND	ND	ND	ND	ND	ND
C1F	ND	4.2	ND	2.4	4.2	1.8	ND	4.7	ND	7.6	4.7	0.6
C2F	ND	22.6	ND	6.2	22.6	3.6	3.4	21.6	6.3	29	21.6	0.7
C3F	ND	76.3	ND	26.6	76.3	2.9	25.8	67.9	2.6	77.5	67.9	0.9
DBT	ND	2.7	ND	ND	2.7	ND	ND	ND	ND	0.9	ND	ND
C1DBT	ND	15.2	ND	2.6	15.2	5.8	ND	5.2	ND	3	5.2	1.8
C2DBT	ND	68.4	ND	8.7	68.4	7.9	3.3	22.6	6.9	12.2	22.6	1.8
C3DBT	2.3	119.3	51.7	15	119.3	8	4.9	27.5	5.6	15.4	27.5	1.8
Ph[a]	5.1	20.2	4	5.9	20.2	3.4	3.6	8.5	2.4	14.9	8.5	0.6
C1Ph	4	43.8	10.9	8.3	43.8	5.3	8.1	18.4	2.3	24.6	18.4	0.7
C2Ph	4.3	148.1	34.8	19.2	148.1	7.7	10.4	68.8	6.6	38.3	68.8	1.8
C3Ph	3.9	202.6	51.5	23.3	202.6	8.7	8.4	814.2	96.5	149	814.2	5.5
C4Ph	3.3	178.6	54.6	31.4	178.6	5.7	5.6	111.1	19.8	42.2	111.1	2.6
A[a]	ND	8	ND	ND	8	ND	ND	ND	ND	3.2	ND	ND
Fl[a]	12.6	50.9	4	15.1	50.9	3.4	32.8	7.1	0.2	34.2	7.1	0.2
Py[a]	10.9	53.8	4.9	15.2	53.8	3.5	39.8	9.9	0.2	44.7	9.9	0.2
C1Py	5.5	53.2	9.7	12.1	53.2	4.4	25.8	22	0.9	37.2	22	0.6
C2Py	3.8	70.3	18.4	11.4	70.3	6.2	11.9	21.8	1.8	21.7	21.8	1
BaA[a]	6.6	31.8	4.8	8.1	31.8	3.9	23.9	ND	ND	21.9	ND	ND
Ch[a]	7.1	34.3	4.8	6.8	34.3	5	21.9	34.6	1.6	19.1	34.6	1.8
C1Ch	4.9	52.7	10.7	8.1	52.7	6.5	10.9	7.3	0.7	12.7	7.3	0.6
C2Ch	3.8	73.2	19.4	9.3	73.2	7.9	4.2	10.7	2.6	5.7	10.7	1.9
BbFl[a]	8.3	67.2	8.1	12.6	67.2	5.3	28.2	5	0.2	20.2	5	0.2
BkFl[a]	2.1	19.9	9.3	5.3	19.9	3.8	12.5	2.7	0.2	7.9	2.7	0.3
BePy	5.7	30.9	5.4	6.2	30.9	5	17	2.2	0.1	12.4	2.2	0.2
BaPy[a]	6.3	35.6	5.6	9.7	35.6	3.7	27	1.9	0.1	20	1.9	0.1
Per	ND	7.8	ND	2.5	7.8	3.1	9	ND	ND	3.3	ND	ND
I-Py[a]	3.4	53.2	15.7	10.1	53.2	5.3	18.4	6.9	0.4	15.3	6.9	0.4
DBahA[a]	ND	19.3	ND	2.4	19.3	8.2	3.8	2	0.5	3.5	2	0.6
BghiPer[a]	6.4	43.5	6.8	11.3	43.5	3.9	18.6	5.9	0.3	14.6	5.9	0.4
***p*-terphd$_{14}$**	72.9	58.3	-	68.2	58.3	–	106.5	78.3	-	89.1	78.3	-
Σ16PAHs	72.8	474.8	6.5	108.7	474.8	4.4	219.5	91.4	0.4	231.8	91.4	0.4
ΣTotalPAHs	103.7	1676.2	16.2	300.1	1676.2	5.6	392.6	1395.1	3.6	718.2	1395.1	1.9

Water – mean concentrations (ng L^{-1}) in water samples (n=3 for each site) collected from Quadrado da Urca and Itaipu beach;
Sponge – mean concentrations (µg kg^{-1} dry weight) in sponge samples collected from Quadrado da Urca (n=3) and Itaipu beach (n=2);
BCF – bioconcentration factor = ratio between each PAH concentration in sponge and in water or suspended solids;
SS - mean concentrations (ng L^{-1}) in suspended solids samples (n=2 for each site), collected from Quadrado da Urca and Itaipu beach;

[a] 16 USEPA priority PAHs; ND: non-detected;

N**:** naphthalene; C2N, C2 naphthalenes; C3N, C3 naphthalenes; C4N, C4 naphthalenes; ACF: acenaphthylene; ACE: acenaphthene; F: fluorene; C1F, C1 fluorenes; C2F, C2 fluorenes; C3F, C3 fluorenes; DBT: dibenzothiophene; C1DBT, C1 dibenzothiophenes; C2DBT, C2 dibenzothiophenes; C3DBT, C3 dibenzothiophenes; Ph: phenanthrene; C1Ph, C1 phenanthrenes; C2Ph, C2 phenanthrenes; C3Ph, C3 phenanthrenes; C4Ph, C4 phenanthrenes; A: anthracene; Fl: fluoranthene; Py: pyrene; C1Py, C1 pyrenes; C2Py, C2 pyrenes; BaA: benzo[*a*]anthracene; Ch: chrysene; C1Ch, C1 chrysenes; C2Ch, C2 chrysenes; BbFl: benzo[*b*]fluoranthene; BkFl*: benzo[*k*]fluoranthene; BePy: benzo[*e*]pyrene; BaPy: benzo[*a*]pyrene; Per: perylene; I-Py: indeno[*1,2,3-cd*]pyrene; DBahA: dibenzo[*a,h*]anthracene; BghiPer: benzo[*ghi*]perylene.;
p-terphd$_{14}$: para-terphenyl d_{14} (% recovery);
Σ16PAHs: sum of the 16 USEPA PAHs; ΣTotalPAHs: sum of 38 PAHs.

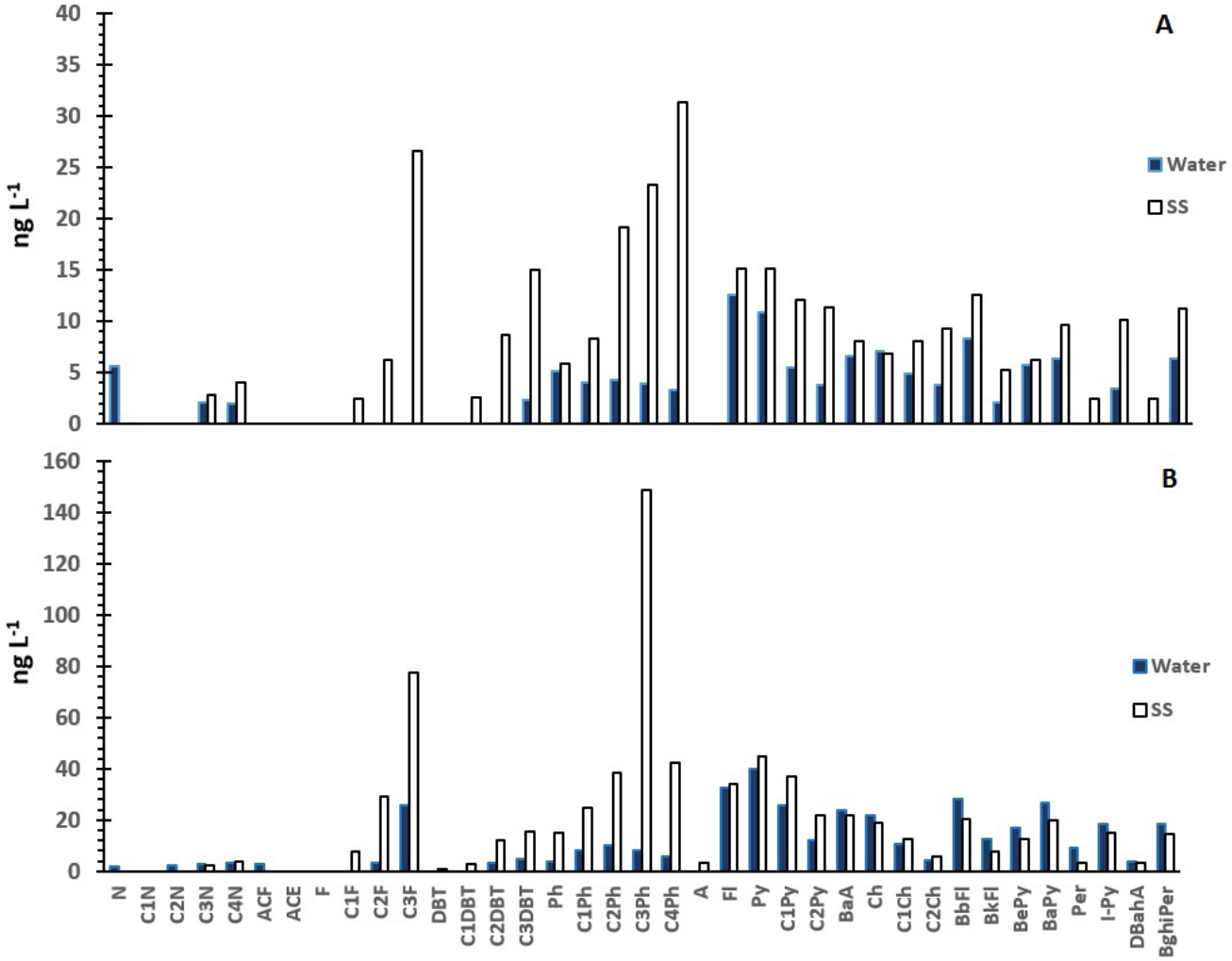

Figure 2. PAHs Profile in water and suspended solids (SS) field samples collected from Quadrado da Urca (A) and Itaipu beach (B). N: naphthalene; C2N, C2 naphthalenes; C3N, C3 naphthalenes; C4N, C4 naphthalenes; ACF: acenaphthylene; ACE: acenaphthene; F: fluorene; C1F, C1 fluorenes; C2F, C2 fluorenes; C3F, C3 fluorenes; DBT: dibenzothiophene; C1DBT, C1 dibenzothiophenes; C2DBT, C2 dibenzothiophenes; C3DBT, C3 dibenzothiophenes; Ph: phenanthrene; C1Ph, C1 phenanthrenes; C2Ph, C2 phenanthrenes; C3Ph, C3 phenanthrenes; C4Ph, C4 phenanthrenes; A: anthracene; Fl: fluoranthene; Py: pyrene; C1Py, C1 pyrenes; C2Py, C2 pyrenes; BaA: benzo[a]anthracene; Ch: chrysene; C1Ch, C1 chrysenes; C2Ch, C2 chrysenes; BbFl: benzo[b] fluoranthene; BkFl*: benzo[k]fluoranthene; BePy: benzo[e]pyrene; BaPy: benzo[a]pyrene; Per: perylene; I-Py: indeno[1,2,3-cd]pyrene; DBahA: dibenzo[a,h]anthracene; BghiPer: benzo[ghi]perylene.

of PAHs sources is their applicability in marine organisms. For this, the incorporation rate of the PAHs that make up each ratio must be the same. Organisms may preferentially incorporate a particular PAH over another, also used for a given source diagnostic ratio (Denton *et al*., 2006; Batista *et al*., 2013). Sponges are filtering organisms that incorporate particles, onto which the heavy PAHs are mainly adsorbed, while as discussed earlier, some sponge samples may display a higher relative amount of light PAHs because they are fixed in the intertidal region and filter surface water containing oil films. Therefore, the reasons for the PAHs origin in sponges are questionable because of the mixed sources that these organisms may be exposed to.

Bioconcentration factor

The bioconcentration factor was evaluated to assess the ability of the sponges to accumulate PAHs and their level of incorporation. The partition of hydrophobic compounds between the particulate and dissolved form is influenced by their solubility in water. Each PAH is different, as is water solubility for each compound determining its fate in the environment in the water-particulate partition (Baird & Cann, 2012; Neff, 2002). The bioconcentration factor (BCF) in the present study is the ratio of the concentration of the compound present in the sponge tissue to the concentration of the same compound present in the main matrix of PAHs incorporation, water and suspended solids. Bioconcentration factors (BCF) were calculated for individual PAHs, for the sum of the priority PAHs (Σ16PAHs) and for the sum of 38 PAHs (ΣTotalPAHs) in field samples (0 h).

The bioconcentration factor for each PAH (Table 1) in sponges collected in Quadrado da Urca was higher for water than suspended solids samples, ranging from 0.5 to 54.6 and from 1.8 to 8.7, respectively. The accumulation in the sponges occurred through the suspended solids for heavy

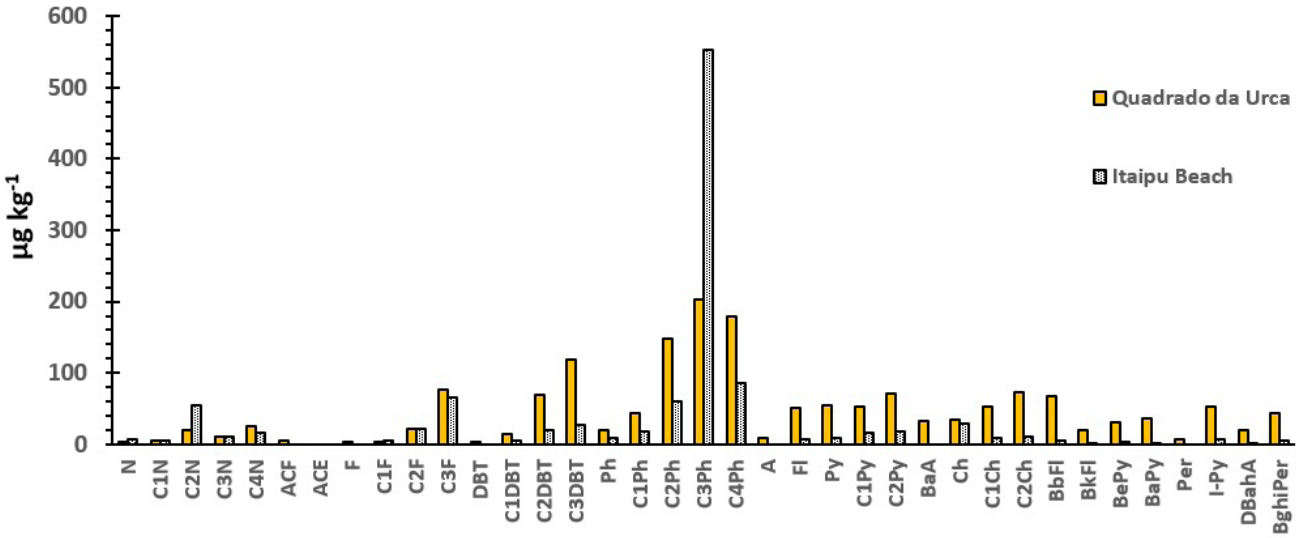

Figure 3. PAHs profile in sponge field samples from Quadrado da Urca and Itaipu beach. N: naphthalene; C2N, C2 naphthalenes; C3N, C3 naphthalenes; C4N, C4 naphthalenes; ACF: acenaphthylene; ACE: acenaphthene; F: fluorene; C1F, C1 fluorenes; C2F, C2 fluorenes; C3F, C3 fluorenes; DBT: dibenzothiophene; C1DBT, C1 dibenzothiophenes; C2DBT, C2 dibenzothiophenes; C3DBT, C3 dibenzothiophenes; Ph: phenanthrene; C1Ph, C1 phenanthrenes; C2Ph, C2 phenanthrenes; C3Ph, C3 phenanthrenes; C4Ph, C4 phenanthrenes; A: anthracene; Fl: fluoranthene; Py: pyrene; C1Py, C1 pyrenes; C2Py, C2 pyrenes; BaA: benzo[a]anthracene; Ch: chrysene; C1Ch, C1 chrysenes; C2Ch, C2 chrysenes; BbFl: benzo[b]fluoranthene; BkFl*: benzo[k] fluoranthene; BePy: benzo[e]pyrene; BaPy: benzo[a]pyrene; Per: perylene; I-Py: indeno[1,2,3-cd]pyrene; DBahA: dibenzo[a,h]anthracene; BghiPer: benzo[ghi]perylene.

compounds (4-6 rings), while accumulation by water occurred predominantly for light PAHs (2-3 rings) and alkylated compounds. Sponges from Itaipu beach presented BCF value for water (0.2 – 96.5) higher to that of suspended solids (0.2 – 5.5) (Table 1). Another interesting aspect is that the difference between BCF of Σ16PAHs and ΣTotalPAHs in the suspended solids (4.4 – 5.6 in Quadrado da Urca; 0.4 – 1.9 in Itaipu beach) is small, implying a large accumulation of priority and parental PAHs, since total PAHs are mostly composed of alkylated compounds. In general, BCF values are higher considering water, due to the lower concentrations found in this matrix, which interfere with the BCF calculation.

Depuration assay

PAHs levels

The sum of 16 priority PAHs (∑16PAHs) and the sum of 38 PAHs (∑TotalPAHs) ranged from 474.8 (0 h) to 264.8 (336 h) and from 1678.9 (0 h) to 928.9 µg kg^{-1} (336 h), respectively, resulting in a difference of 749.9 µg kg^{-1} between final and initial concentration, while the difference between the mean concentrations at 0-96 h and at 168-336 h was of 400 µg kg^{-1} (significant, p=0.032, Mann-Whitney). The results for individual compounds are reported in Table 2.

Variations in PAHs concentration over 96 h were not statistically significant, although a sharp decreasing trend was observed in the first 24 h. The variation of PAHs along the depuration period is displayed in Fig. 2, which highlights the significant decrease of these compounds from 168 h onwards. Assuming a filtration rate of 0.1 L $h^{-1}g^{-1}$ and taking into account the mass of the individuals in each aquarium it was possible to estimate that the whole water volume in the aquarium (70 L) was pumped through the samples from 2 (24 h) to 18 (336 h) times during the assay period. The depuration rate was estimated using the 24-96 h decrease and is equal to 0.5 µg $kg^{-1}h^{-1}$ over this time interval, while the depuration rate after 96 h was of 2.6 µg $kg^{-1}h^{-1}$. The linear adjustment was chosen to estimate the global depuration rate of 1.95 µg $kg^{-1}h^{-1}$, since the test for second order kinetics was not significant. Based on these responses, three possible mechanisms for elimination from the sponge body may be cited: (1) metabolization and excretion; (2) degradation by microorganisms living in symbiosis with the sponge mass; and (3) partition equilibrium between sorbed PAHs and the clean water.

The variation of the replicates at each time of the assay is illustrated in Fig. 6. The PAHs concentrations presented a high deviation at 0 h, which is reduced along the time points, reaching a minimum deviation between individuals at 336 h. This variation was expected, since different individuals were analyzed and their response is not equal. Total PAHs depurated almost 50 % from time 0 h to 336 h, and the most representative decreases were from 168 h to 240 h (15 %) and from 240 h to 336 h (27 %). The depuration rates for each PAH are displayed in Table 3.

The other PAHs, except the priority ones, were the major contributors to the depuration, which is most pronounced, considering high molecular weight alkylated compounds. The fact that sponges to preferentially eliminate alkylated PAHs may be due to the methylation of these compounds, since it is easier to break the bond between the methyl radical ($-CH_3$) and the aromatic ring than between the condensed rings themselves, which are more stable. The parent compound dibenzothiophene, and its homologous series (Fig. 7), showed greater concentration

Table 2. Mean PAHs concentrations (µg kg^{-1} dry weight) in sponges (n=3 for each depuration time point).

Sample	T0	T24	T48	T72	T96	T168	T240	T336
N[a]	2.79	2.08	2.67	4.99	3.14	3.13	3.86	4.94
C1N	5.02	3.01	4.56	6.32	7.40	5.15	5.02	4.79
C2N	20.66	12.28	19.80	27.68	38.29	30.45	13.50	18.88
C3N	10.85	7.18	7.99	9.27	11.24	10.93	8.97	5.85
C4N	24.84	17.37	18.88	21.23	31.12	25.57	16.69	12.23
ACF[a]	4.32	2.45	2.45	3.05	2.90	2.87	3.83	2.17
ACE[a]	ND	ND	ND	ND	ND	ND	ND	ND
F[a]	3.02	2.31	2.17	ND	2.58	1.86	2.38	ND
C1F	4.22	2.83	3.49	3.19	3.72	3.63	3.87	2.54
C2F	22.57	19.50	19.68	19.17	21.91	16.22	17.39	10.38
C3F	76.28	82.18	107.65	108.05	96.88	95.17	82.23	53.23
DBT	2.74	2.42	ND	ND	2.14	1.29	1.85	ND
C1DBT	15.19	14.27	14.56	11.75	12.01	11.67	9.45	5.83
C2DBT	68.43	71.19	68.32	68.21	57.06	55.02	43.55	28.09
C3DBT	119.25	133.52	118.99	131.62	109.11	102.88	48.42	51.95
Ph[a]	20.20	12.33	13.51	7.63	9.91	9.79	12.52	5.61
C1Ph	43.82	35.34	28.23	28.14	26.64	25.55	21.91	11.86
C2Ph	148.13	155.02	125.38	155.07	116.70	107.24	71.54	56.72
C3Ph	202.55	238.76	216.61	247.90	197.00	194.75	131.78	108.31
C4Ph	178.64	210.64	169.77	207.56	191.03	187.76	133.66	120.81
A[a]	7.97	4.51	4.15	3.40	3.61	3.63	4.48	2.62
Fl[a]	50.93	30.49	40.34	21.89	23.31	23.84	34.23	15.14
Py[a]	53.77	35.75	84.40	32.64	39.93	36.37	59.24	27.44
C1Py	53.17	44.21	34.90	41.28	48.88	43.30	38.18	25.69
C2Py	70.31	66.25	56.51	78.61	81.34	71.98	63.68	47.65
BaA[a]	31.83	18.17	16.81	14.59	18.29	17.55	18.10	9.15
Ch[a]	34.31	22.26	20.36	24.80	25.95	24.70	27.28	16.47
C1Ch	52.69	41.40	40.48	52.70	54.24	52.67	39.83	31.07
C2Ch	73.16	68.96	70.65	89.78	102.70	92.49	71.52	60.49
BbFl[a]	67.18	42.68	43.95	47.94	51.23	54.17	59.39	42.69
BkFl[a]	19.91	12.23	12.07	13.82	15.01	16.32	17.25	11.90
BePy	30.94	19.89	20.04	23.19	25.86	25.43	28.97	18.86
BaPy[a]	35.56	19.12	23.02	23.11	25.27	22.96	30.30	18.22
Per	7.76	4.63	5.72	6.09	6.74	7.63	6.87	4.06
I-Py[a]	53.20	38.41	37.92	39.68	42.07	45.47	58.59	40.83
DBahA[a]	19.32	14.93	16.84	17.35	16.65	17.43	19.89	16.81
BghiPer[a]	43.53	32.38	31.74	33.78	35.50	34.98	50.33	34.41
***p*-terphd$_{14}$**	58.32	54.77	61.58	51.00	59.74	54.01	55.11	51.16
Σ16PAHs	474.81	306.33	370.25	309.17	337.67	337.58	426.33	264.81
ΣTotalPAHs	1,676.19	1,537.21	1,503.13	1,622.79	1,542.88	1,479.33	1,257.70	923.99

[a] 16 USEPA priority PAHs; ND: non-detected;
N**:** naphthalene; C2N, C2 naphthalenes; C3N, C3 naphthalenes; C4N, C4 naphthalenes; ACF: acenaphthylene; ACE: acenaphthene; F: fluorene; C1F, C1 fluorenes; C2F, C2 fluorenes; C3F, C3 fluorenes; DBT: dibenzothiophene; C1DBT, C1 dibenzothiophenes; C2DBT, C2 dibenzothiophenes; C3DBT, C3 dibenzothiophenes; Ph: phenanthrene; C1Ph, C1 phenanthrenes; C2Ph, C2 phenanthrenes; C3Ph, C3 phenanthrenes; C4Ph, C4 phenanthrenes; A: anthracene; Fl: fluoranthene; Py: pyrene; C1Py, C1 pyrenes; C2Py, C2 pyrenes; BaA: benzo[*a*]anthracene; Ch: chrysene; C1Ch, C1 chrysenes; C2Ch, C2 chrysenes; BbFl: benzo[*b*]fluoranthene; BkFl*: benzo[*k*]fluoranthene; BePy: benzo[*e*]pyrene; BaPy: benzo[*a*]pyrene; Per: perylene; I-Py: indeno[*1,2,3-cd*] pyrene; DBahA: dibenzo[*a,h*]anthracene; BghiPer: benzo[*ghi]*perylene.;
p-TERPHd$_{14}$: para-terphenyl d$_{14}$ (% recovery);
Σ16PAHs: sum of the 16 USEPA PAHs; ΣTotalPAHs: sum of 38 PAHs.

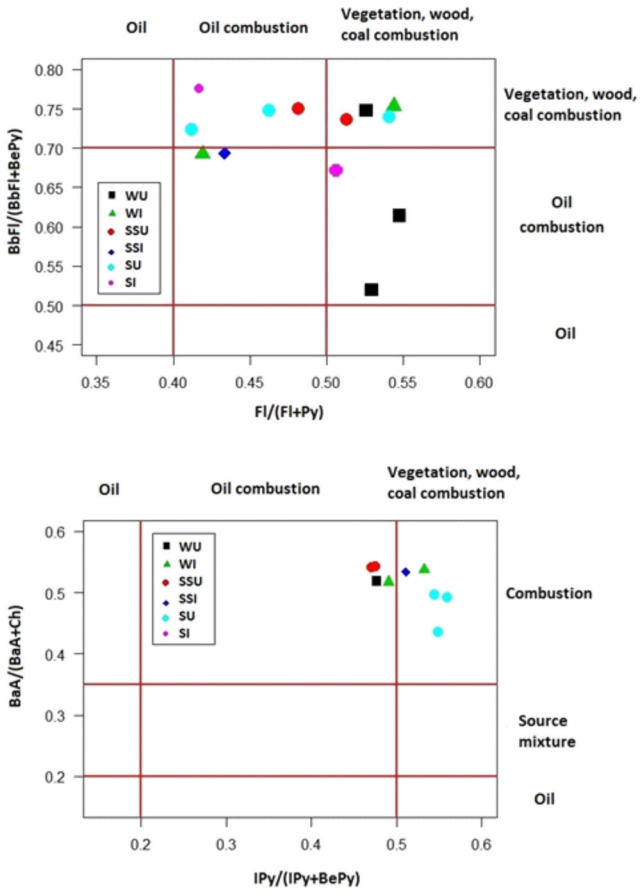

Figure 4. Cross-plots of diagnostic ratios applied for field samples. WU – Water Urca; WI – Water Itaipu; SSU – suspended solids Urca; SSI - suspended solids Itaipu; SU – Sponge Urca; SI – Sponge Itaipu. BbFl/(BbFl+BePy) – ratio between benzo(b)fluoranthene and the sum of benzo(b)fluoranthene and benzo(e)pyrene; Fl/(Fl+Py) - ratio between fluoranthene and the sum of fluoranthene and pyrene; BaA/(BaA+Ch) - ratio between benzo(a)anthracene and the sum of benzo(a)anthracene and chrysene; IPy/(IPy+BePy) - ratio between indeno[1,2,3-cd]pyrene and the sum of indeno[1,2,3-cd]pyrene and benzo(e)pyrene.

decreases with depuration time and molecular weight of these compounds. The depuration varied slowly and gradually in the first 96 h. The high correlation ($r2 = 0.921$, $p < 0.05$) between the sum of dibenzothiophene and its alkylates and depuration time shows the most significant depuration from 168 h.

The variation in the concentration of each PAH in sponge replicates at initial (0 h) and final time of assay (336 h) is displayed in Figure 8. The deviations decreased throughout the experiment, being much lower at 336 h when compared to the field samples (0 h). This indicates that sponges can thus purify the PAHs and that their concentration variability becomes lower, and, as such, the replicates are representative of the sponge population. Alkylated compounds were primarily responsible for reducing the concentration of low molecular weight PAHs during the depuration assay, while parental compounds contributed to the decrease of high molecular weight PAHs.

Biomarkers

The Neutral Red Retention Time (NRRT) remained at 120 min throughout the depuration assay. This is due to the fact that, even removing the sponge individuals from a considerably contaminated site and transferring them to aquaria containing clean water, in order to reduce the stress from their environment of origin, their cells remained in the same health status in which they were in the field. Thus, the transfer from a natural setting to the aquaria containing clean water, free of suspended solids, did not affect sponge health at the cellular level as indicated by the NRRT assay. In a healthy cell, the NRRT ranges from 150 to 180 min (Lowe *et al*., 1995) and is about 120 min (Pereira *et al*., 2014) in mussels from temperate and tropical zones, respectively. This corroborates with the results found in the present study.

Variations (non-significant) in GSH were small (1.6 ± 0.5 µmol g^{-1} sponge) and scattered over time. No correlation between GSH and decreases in PAH concentrations was observed. These variations may be explained by looking at both sides, increases and decreases: the decreases in GSH may be explained by the fact that sponges were kept in

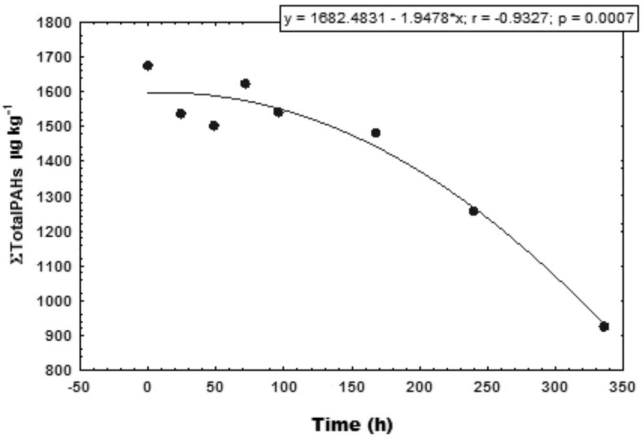

Figure 5. PAHs variations throughout the depuration assay.

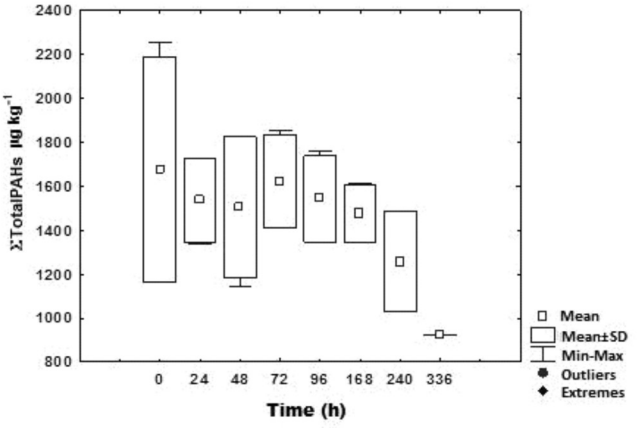

Figure 6. PAHs concentration variations in replicates throughout the depuration assay.

Table 3. PAH depuration (%) between the assay time points.

Sample	0-24	24-48	48-72	72-96	96-168	168-240	240-336	0-336
N[a]	25.4	-28.4	-86.9	37.1	0.3	-23.3	-28.0	-77.1
C1N	40.0	-51.5	-38.6	-17.1	30.4	2.5	4.6	4.6
C2N	40.6	-61.2	-39.8	-38.3	20.5	55.7	-39.9	8.6
C3N	33.8	-11.3	-16.0	-21.3	2.8	17.9	34.8	46.1
C4N	30.1	-8.7	-12.4	-46.6	17.8	34.7	26.7	50.8
ACF[a]	43.3	0.0	-24.5	4.9	1.0	-33.4	43.3	49.8
ACE[a]	ND	ND	ND	ND	ND	ND	ND	ND
F[a]	23.5	6.1	ND	ND	27.9	-28.0	ND	ND
C1F	32.9	-23.3	8.6	-16.6	2.4	-6.6	34.4	39.8
C2F	13.6	-0.9	2.6	-14.3	26.0	-7.2	40.3	54.0
C3F	-7.7	-31.0	-0.4	10.3	1.8	13.6	35.3	30.2
DBT	11.7	ND	ND	ND	39.7	-43.4	ND	ND
C1DBT	6.1	-2.0	19.3	-2.2	2.8	19.0	38.3	61.6
C2DBT	-4.0	4.0	0.2	16.3	3.6	20.8	35.5	59.0
C3DBT	-12.0	10.9	-10.6	17.1	5.7	52.9	-7.3	56.4
Ph[a]	39.0	-9.6	43.5	-29.9	1.2	-27.9	55.2	72.2
C1Ph	19.4	20.1	0.3	5.3	4.1	14.2	45.9	72.9
C2Ph	-4.7	19.1	-23.7	24.7	8.1	33.3	20.7	61.7
C3Ph	-17.9	9.3	-14.4	20.5	1.1	32.3	17.8	46.5
C4Ph	-17.9	19.4	-22.3	8.0	1.7	28.8	9.6	32.4
A[a]	43.4	8.0	18.1	-6.2	-0.6	-23.4	41.5	67.1
Fl[a]	40.1	-32.3	45.7	-6.5	-2.3	-43.6	55.8	70.3
Py[a]	33.5	-136.1	61.3	-22.3	8.9	-62.9	53.7	49.0
C1Py	16.9	21.1	-18.3	-18.4	11.4	11.8	32.7	51.7
C2Py	5.8	14.7	-39.1	-3.5	11.5	11.5	25.2	32.2
BaA[a]	42.9	7.5	13.2	-25.4	4.0	-3.1	49.4	71.3
Ch[a]	35.1	8.5	-21.8	-4.6	4.8	-10.4	39.6	52.0
C1Ch	21.4	2.2	-30.2	-2.9	2.9	24.4	22.0	41.0
C2Ch	5.7	-2.5	-27.1	-14.4	9.9	22.7	15.4	17.3
BbFl[a]	36.5	-3.0	-9.1	-6.9	-5.7	-9.6	28.1	36.5
BkFl[a]	38.6	1.3	-14.5	-8.6	-8.7	-5.7	31.0	40.2
BePy	35.7	-0.8	-15.7	-11.5	1.7	-13.9	34.9	39.0
BaPy[a]	46.2	-20.4	-0.4	-9.3	9.1	-32.0	39.9	48.8
Per	40.3	-23.5	-6.5	-10.7	-13.2	10.0	40.9	47.7
I-Py[a]	27.8	1.3	-4.6	-6.0	-8.1	-28.9	30.3	23.3
DBahA[a]	22.7	-12.8	-3.0	4.0	-4.7	-14.1	15.5	13.0
BghiPer[a]	25.6	2.0	-6.4	-5.1	1.5	-43.9	31.6	21.0
Σ16PAHs	35.5	-20.9	16.5	-9.2	0.0	-26.3	37.9	44.2
Σ38PAHs	8.3	2.2	-8.0	4.9	4.1	15.0	26.5	44.9

[a] 16 USEPA priority PAHs; ND: non-detected;
N**:** naphthalene; C2N, C2 naphthalenes; C3N, C3 naphthalenes; C4N, C4 naphthalenes; ACF: acenaphthylene; ACE: acenaphthene; F: fluorene; C1F, C1 fluorenes; C2F, C2 fluorenes; C3F, C3 fluorenes; DBT: dibenzothiophene; C1DBT, C1 dibenzothiophenes; C2DBT, C2 dibenzothiophenes; C3DBT, C3 dibenzothiophenes; Ph: phenanthrene; C1Ph, C1 phenanthrenes; C2Ph, C2 phenanthrenes; C3Ph, C3 phenanthrenes; C4Ph, C4 phenanthrenes; A: anthracene; Fl: fluoranthene; Py: pyrene; C1Py, C1 pyrenes; C2Py, C2 pyrenes; BaA: benzo[*a*]anthracene; Ch: chrysene; C1Ch, C1 chrysenes; C2Ch, C2 chrysenes; BbFl: benzo[*b*]fluoranthene; BkFl*: benzo[*k*]fluoranthene; BePy: benzo[*e*]pyrene; BaPy: benzo[*a*]pyrene; Per: perylene; I-Py: indeno[*1,2,3-cd*] pyrene; DBahA: dibenzo[*a,h*]anthracene; BghiPer: benzo[*ghi*]perylene.;
Σ16PAHs: sum of the 16 USEPA PAHs; ΣTotalPAHs: sum of 38 PAHs.

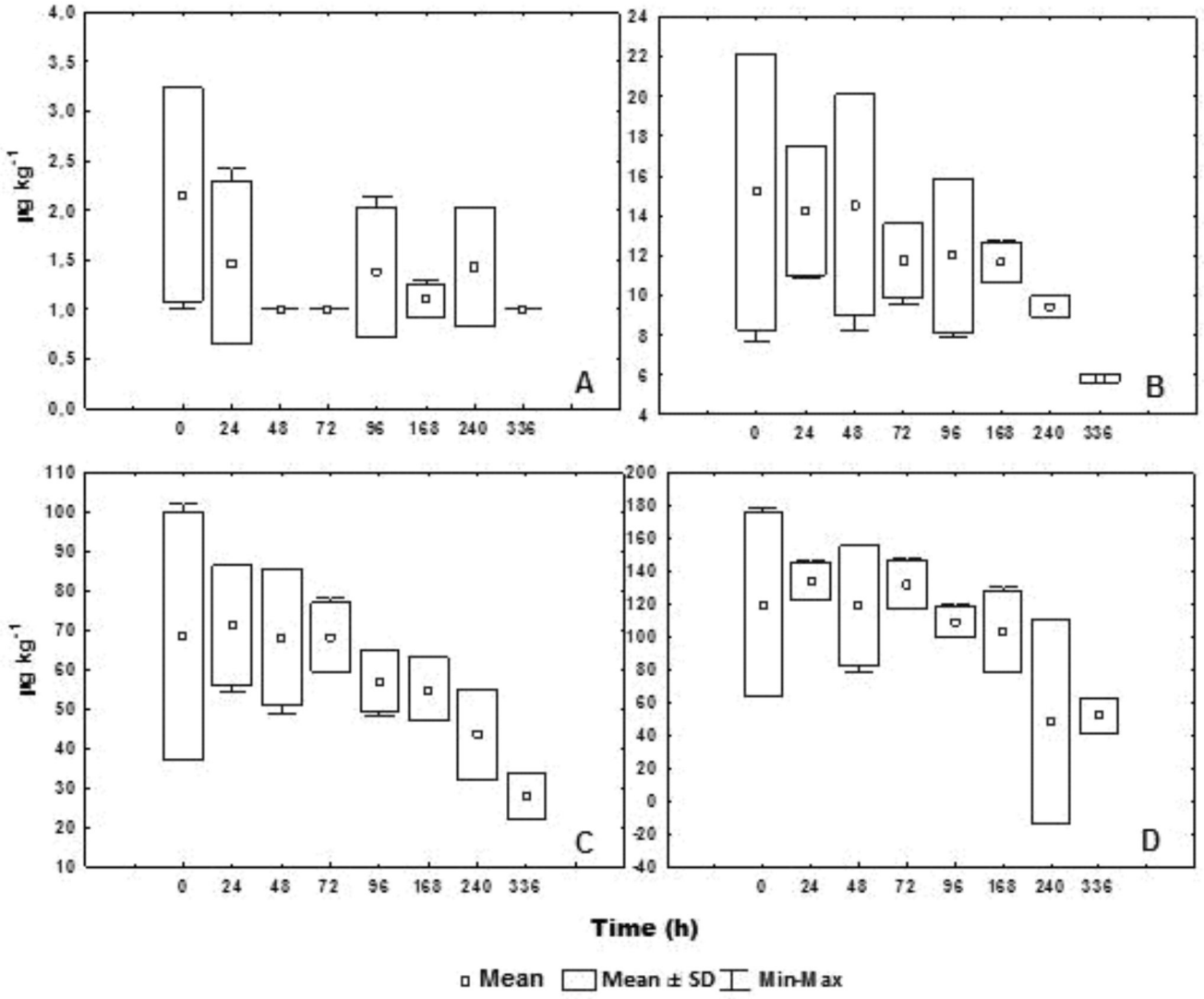

Figure 7. Concentration variations (µg kg^{-1}) of dibenzothiophenes (DBT) in sponge replicates along depuration time. A – DBT; B – C1DBT; C – C2DBT; D – C3DBT.

aquaria without contaminants that could have induced GSH synthesis, while the slight increases may be explained by the restoration of oxidized glutathione to GSH by glutathione reductase, thus being less consumed by the biotransformation reactions involving GPx and GST, or by the fact that no food was available throughout the experiments, possibly leading to stress. In all cases, sponges were able to depurate PAHs.

The relative amount of sponge cavities (aquifer channels and choanocyte chambers) decreased during the bioassay, from 80.4 % to 65.7 % (Fig. 9). However, no correlation between the relative amount of cavities and PAHs concentrations was observed. Sponge tissue contraction may have disorganized the cavities, since the tissue becomes denser and thicker due to this contraction, possibly caused by stress (e.g. lack of feeding, artificial water, and transport) under the bioassay conditions, even not causing any effect on cell levels.

Collagen levels decreased during the depuration assay. The linear fit ($y= 5.28 – 0.01$; $r^2 = 0.83$) of the collagen decrease with time was significant at $p<0.05$ and indicated a reduction rate of 0.01 % h^{-1} (Fig. 10 suppl. mat.). This was probably also due to stress under the assay conditions, as mentioned before. No correlation between collagen and PAHs concentration was found. The amount of collagen and changes in tissue structure throughout the assay are displayed in Figure 11. In field organisms, collagen is fully concentrated on the edges of the tissue and around the aquifer channels. Over the course of the depuration, disorganization of cavities and consequent disruption of aquifer channels resulted in an internal concentration of collagen in the tissue.

Accumulation assay

Chrysene levels

Chrysene concentrations in *H. heliophila* in the course of the accumulation bioassay ranged from 10,330 µg kg^{-1} to 16,027 µg kg^{-1} (n=8, 24-96 h). Concentrations in field samples (0 h) ranged from 22.55 to 34.60 µg kg^{-1}. Chrysene

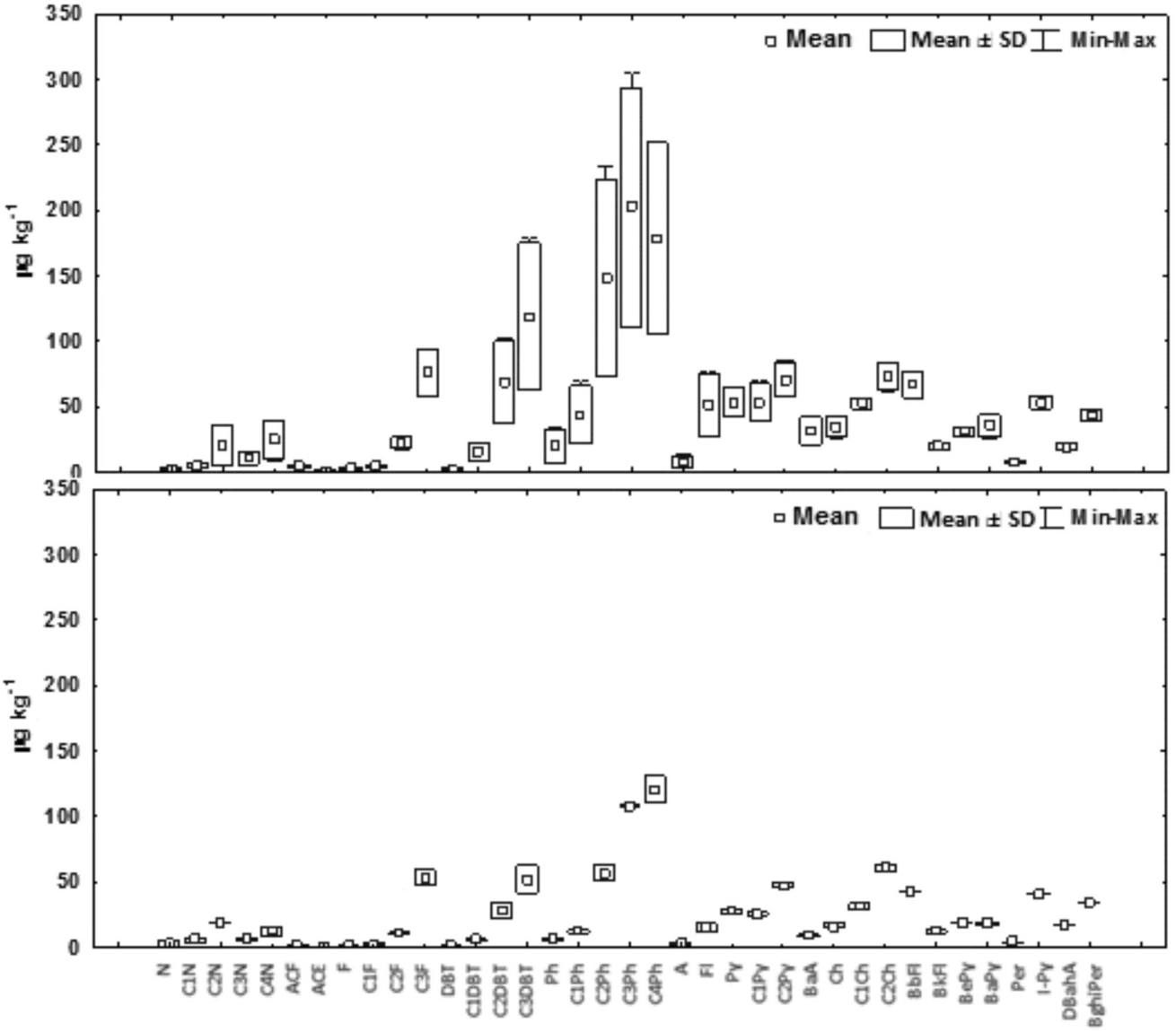

Figure 8. PAHs typology and variation in sponge replicates at 0 h (A) and at 336 h (B).

concentrations in sponge tissues increased, with a rapid and intense accumulation in the first 24 h (accumulation rate of ≈ 250 µg $kg^{-1}h^{-1}$). Chrysene accumulated 99.7% in 24 h and almost 40% from the time 24 h to 96 h, and decreased at 96 h after a significant uptake (Fig. 12), resulting in a negative percentage for accumulation. This decrease may be due to variations in accumulation responses between individuals.

Glyzina *et al.* (2002) exposed the sponge *Lubomirskia baicalensis* to anthracene and pyrene to a final concentration of 80 µg L^{-1} in the aquarium water. This species showed the ability to accumulate these PAHs in 36 h. Furthermore, the decrease of these compounds in water was proportional to the increase of sponge biomass. In the present study, sponges in the accumulation assay showed the capacity to accumulate high concentrations of chrysene, while in the depuration assay losses were significant after 96 h, and biomass decreased on the average 2%, as expected, due to lack of feeding, collagen loss and tissue alterations.

A test to verify possible losses in chrysene concentrations during the accumulation assay, without the sponges in the aquariums, was carried out, and demonstrated that 30% and 70% of the initial chrysene concentrations are lost by volatilization or adhesion to the aquarium wall in 24 h and 96 h, respectively.

Biomarkers

The Neutral Red Retention Time (NRRT) decreased throughout the experiment, from 120 min to 30 min, with the neutral red dye retained in less time in the lysosomes, showing a negative and significant correlation (Spearman -0.949, $p < 0.05$) with exposure time and indicating decreased sponge health status. The significant sponge health decline during the 96 h of the bioassay may have also contributed to the observed NRRT decreases. According to Francioni *et al.* (2007), the lower retention time at 60 minutes indicates

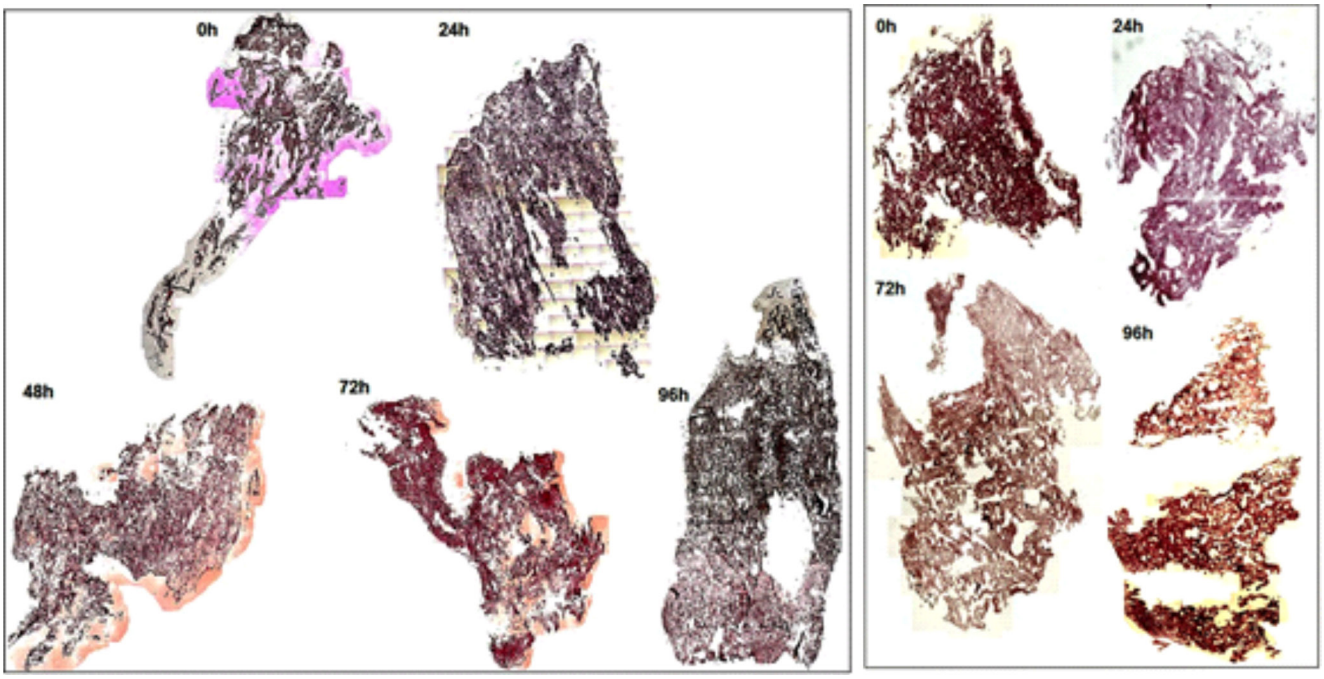

Figure 9. Sponge tissue sections colored by hematoxylin/eosin dye for relative quantification of cavities (aquifer channels and choanocyte chambers) in depuration (A) and accumulation (B) assays.

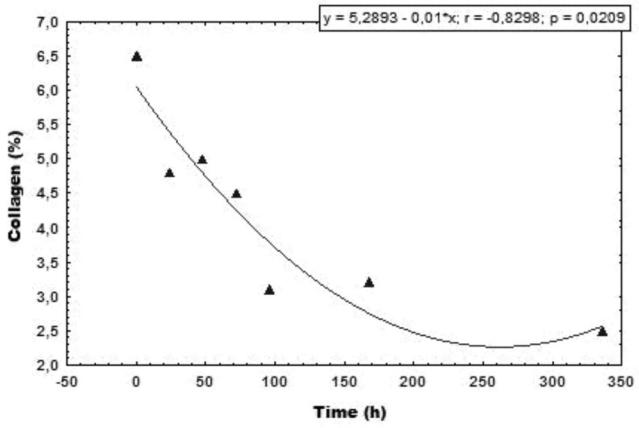

Figure 10. Collagen reduction of in sponge tissue throughout the depuration assay.

severe hemocyte damage. However, no significant correlation between retention time and chrysene concentration ($y = 121 – 0.004$; $r^2 = 0.59$, $P = 0.1$) was observed, since NRRT decreased constantly over the 96 h while chrysene concentrations, firstly increased (0-72 h) and then decreased at 96 h. Also, individual variation could be an influence. Despite this, it is a biomarker in which high correlations with exposure are rarely obtained due to individual variability.

The amount of GSH was directly proportional to chrysene exposure time (r^2=0.95, $p<0.05$). The high amounts of GSH at 0 h are possibly due to the influence of other contaminants in the environment, since chrysene concentrations in field samples were much lower than the concentration used in the bioassay. In addition, sampling and transport to the lab may have also been the cause for this increase. This seems to be confirmed due to the significant decrease in GSH from 0 h to 24 h exposure, alongside a rapid and intense accumulation of chrysene. This tripeptide indicates the level of oxidative stress caused by a determining factor that induces its expression (Hayes *et al*., 2005), and it is able to conjugate to certain xenobiotics to enable depuration by increasing pollutant solubility in water. This process is spontaneous or catalyzed by enzymes, such as glutathione-S-transferase (GST) (Hayes et al., 2005). GST oxidizes GSH, which is reduced by glutathione reductase (GR), thus, leading to increases in GSH levels. After 24 h, chrysene accumulation was accompanied by a gradual increase in GSH levels, possibly induced by chrysene exposure. The high GSH levels observed at the end of the experiment may explain the decreases in chrysene concentrations and suggests that a certain GSH threshold is required to stimulate depuration through conjugation mechanisms, thereby reducing accumulation.

The relative amount of sponge cavities increased from 42.2 % to 56.3 % throughout the experimental period. The increase at 96 h can be interpreted as a regression process resulting in large gaps. In a representative sponge section for the initial condition (0 h), a homogeneous distribution is observed with quite abundant choanocyte chambers of normal, relatively small, size (Fig. 5b suppl. Mat.). At 96 h, two types of morphology became evident: (1) the tissue was contracted, and condensed regions showed altered morphology characterized by cellular disorganization, with fewer choanocyte chambers; (2) the tissue presented relatively larger aquifer channels, as well as structures with large openings. This is, to the best of our knowledge, the first time the type (2) morphology has been described. This may be explained by the fact that the sponge was probably still in a contraction transition. Another possibility is that this tissue morphology change allows sponges to increase their

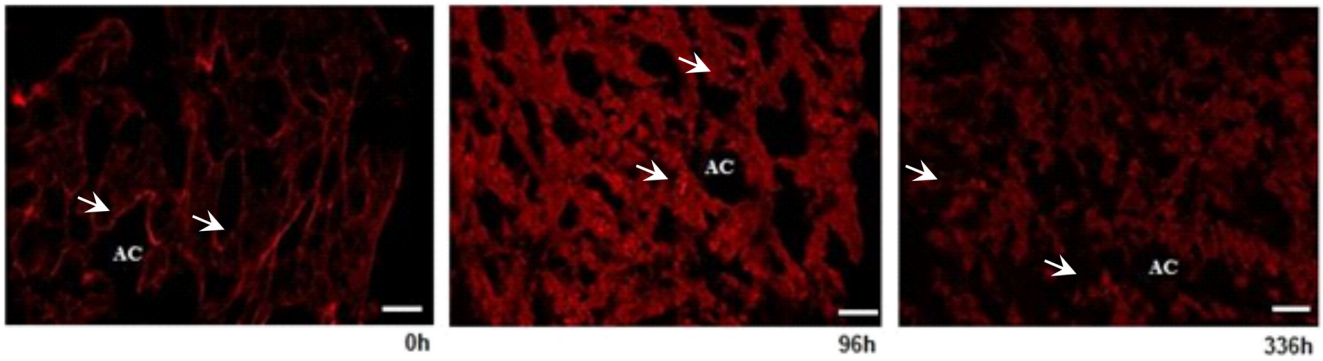

Figure 11. Collagen reduction in sponge tissue throughout the depuration assay and changes in tissue structure. Collagen is perfectly distributed around the aquifer channel in the beginning of the assay, becoming diffuse in the end of the assay. Staining of tissue with picrosirius red. AC – aquifer channels. Arrows indicate collagen distribution. Bars 100 µm.

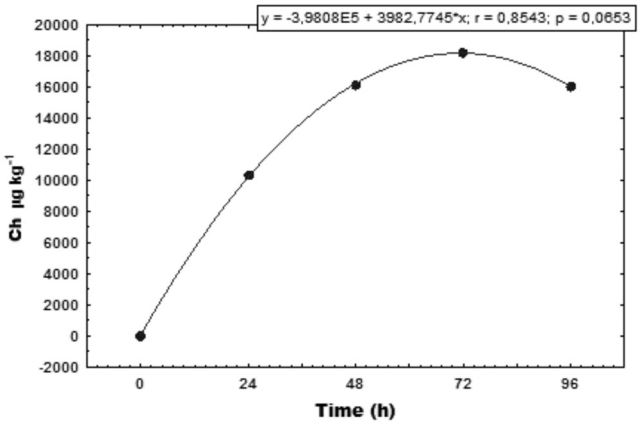

Figure 12. Chrysene concentrations during the accumulation assay.

filtering capacity by increasing the size of their channels and the number of choanocyte chambers. Lastly, it is possible that the choanocyte loss may have caused these alterations on tissue morphology. Combined with the increases in GSH levels, the size modifications of the aquifer channels may have also contributed for the chrysene concentration decreases observed at 96 h. There was, however, no correlation between the relative amount of cavities and chrysene concentrations.

Collagen also decreased during the assay, possibly due to tissue degradation, contributing to the disorganization of the aquifer channels and formation of large cavities. While at 0 h collagen distribution was perfectly concentrated around these cavities, as observed in Figure 13, during the course of experiment, as tissue contraction advanced, collagen was progressively dispersed with a significant decrease in the choanocyte chambers. These are strong evidences of a relationship between these structures.

According to Muricy & Hajdu (2006), H. *heliophila* has a dense mesohyl rich in collagen, which may favor the higher accumulation of certain pollutants, as described for heavy metals (Verdenal *et al*., 1990). In the present study, the amount of collagen and cavities distributed in the tissue decreased during both bioassays, without correlation with chrysene concentrations. Histological alterations observed in the accumulation assay were of the same level as those in the depuration bioassay, which may be a response to stress conditions inherent to the assays, such as sampling, transport and maintenance in the artificial environment, causing alterations which are, thus, not related to PAH exposure. Even so, a collagen-rich mesohyl may have play a role in pollutant accumulation in sponges and may have contributed to chrysene accumulation in the beginning of the assay. Overwall, it is difficult to connect biological responses to a certain exposure and also take into account individual variability. Sponges are organisms with high microbial activity, particular cellular characteristics, and an intricate secondary metabolism (Vogel, 1977; Muricy & Hajdu, 2006; Ribeiro *et al*., 2010; Turque *et al*., 2010), which, added to their tissue structure, result in high complexity, which poses an additional obstacle to the understanding of sponge processes and their association to chemical contamination.

CONCLUSIONS

The present study demonstrated the potential of *Hymeniacidon heliophila* as a water quality biondicator in estuarine systems, with a special focus on PAH contamination (biomarkers of exposure). The bioconcentration factor showed the ability of the sponges to accumulate PAHs and their level of incorporation.

The depuration experiment demonstrated that PAH elimination proceeds within the first 24 h of exposure to clean water, an amount of time sufficient for the whole water volume of aquaria to be filtered twice by the sponge. Biomarkers of effect did not differ with PAH depuration.

The ability of this species to accumulate chrysene at a rate of 250 µg $kg^{-1}h^{-1}$, that far exceeds the depuration rate, makes it a suitable PAH bioindicator. Despite of this species in accumulating PAHs at elevated rates, the biomarkers of effect for histological damages did not show a significant response, only for experimental conditions. Biomarkers of effect for cell evaluation showed significant relation to chrysene exposure.

The use of biomarkers provided a sensitive indication of exposure to contaminants and to stressing conditions, allowing for understanding the species response, as well as its resilience.

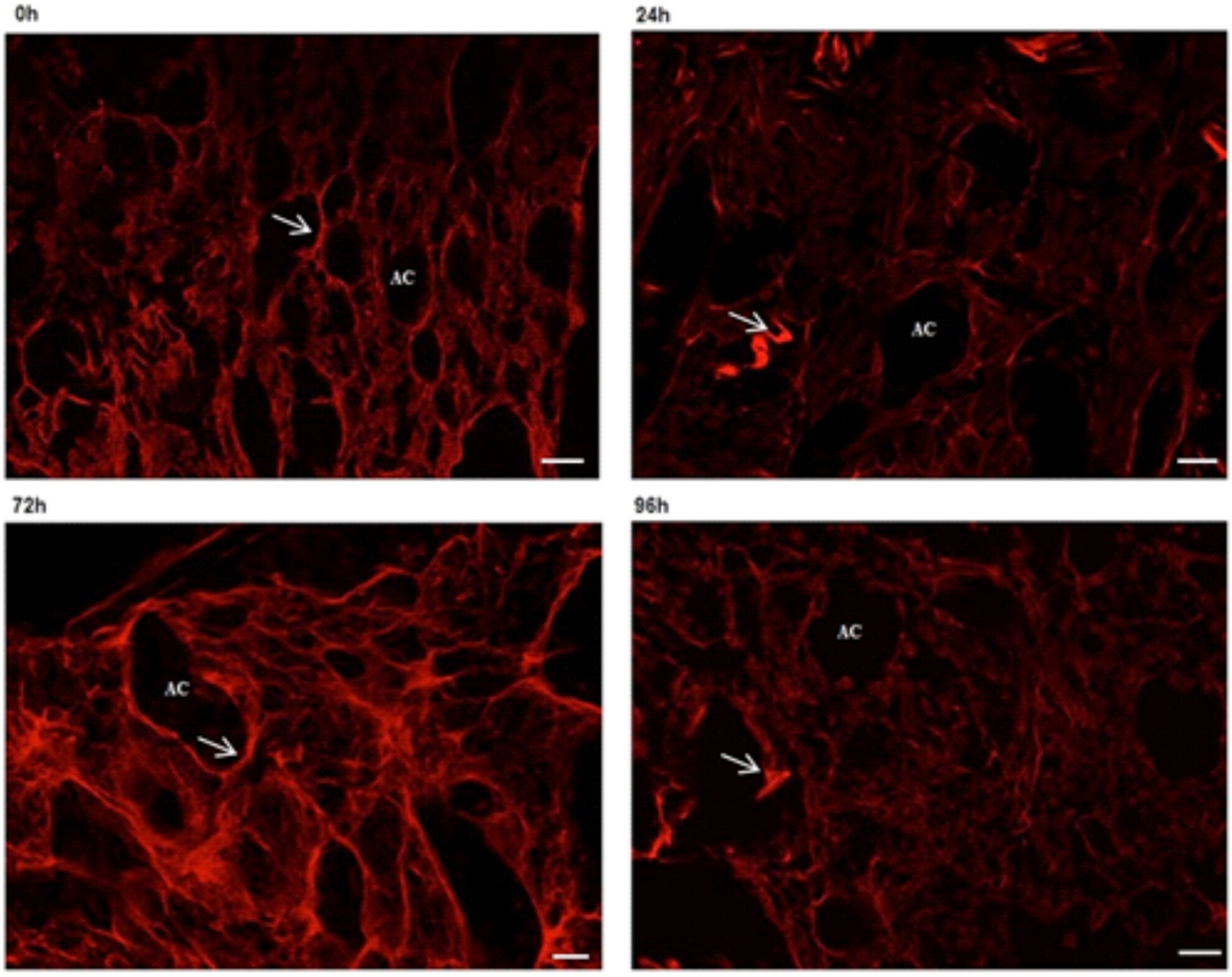

Figure 13. Collagen reduction in sponge tissue throughout the accumulation assay. Collagen is perfectly distributed around the aquifer channel at 0h. Staining of tissue with picrosirius red. AC – aquifer channels. Arrows indicate collagen distribution. Bars 100 µm.

ACKNOWLEDGEMENTS

This study was carried out with financial support from National Council for Scientific and Technological Development - CNPq (Master's grant – process no 133421/2011-2).

REFERENCES

ATSDR - Agency for Toxic Substances and Disease Registry. Toxicological profile for chrysene. Atlanta: Clement Assoc, Inc. Publication No. ATSDR/TP-88/11, 1990.

BACHINSKI, N., KOZIOL, C., BATEL, R., LABURA, Z., SCHRÖDER, H.C. & MÜLLER, W.E.G. 1997. Immediate early response of the marine sponge *Suberites domuncula* to heat stress: reduction of trehalose and glutathione concentrations and glutathione S-transferase activity. J. Exp. Mar. Biol. Ecol., 210: 129-141. https://doi.org/10.1016/S0022-0981(96)02705-0

BAIRD, C. & CANN, M. 2012. Other toxic organic compounds of environmental concern. In: Baird, C. & Cann, M. (eds.), Environmental Chemistry. New York: W.H. Freeman, pp. 663-694.

BATISTA, D., MURICY, G., ROCHA, R.C. & MIEKELEY, N.F. 2014. Marine sponges with contrasting life histories can be complementary biomonitors of heavy metal pollution in coastal ecosystems. Environ. Sci. Pollut. R., 21: 5785-5794. http://dx.doi.org/10.1007/s11356-014-2530-7

BATISTA, D., TELLINI, K., NUDI, A.H., MASSONE, T.P., SCOFIELD, A.L. & WAGENER, A.L.R. 2013. Marine sponges as bioindicators of oil and combustion derived PAH in coastal waters. Mar. Environ. Res., 92: 234-243. http://dx.doi.org/10.1016/j.marenvres.2013.09.022

BEHMER, O.A., TOLOSA, E.M.C. & FREITAS NETO, A.G. 1976. Manual de técnicas de histologia normal e patológica. EDART São Paulo Livraria Editora, São Paulo, SP. 241p.

BEUTLER, E., DURON, O. & KELLY, B.M. 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888. PMID: 13967893.

BOEHM, P.D. & FARRINGTON, J.W. 1984. Aspects of the polycyclic aromatic hydrocarbon geochemistry of recent sediments in the Georges Bank Region. Environ. Sci. Technol., 18 (11): 840-845. http://dx.doi.org/10.1021/es00129a007

BREVES-RAMOS, A., LAVRADO, H.P., JUNQUEIRA, A.O.R. & SILVA, S.H.G. 2005. Succession in rocky intertidal benthic communities in areas with different pollution levels at Guanabara Bay (RJ-Brazil). Braz. Arch. Biol. Technol., 48 (6): 951-965. http://dx.doi.org/10.1590/S1516-89132005000800012

BUCHMAN, M.F. 2008. NOAA Screening Quick Reference Tables, NOAA OR&R Report 08-1, Seattle WA, Office of Response and Restoriation Division, National Oceanic and Atmospheric Administration, 34 p.

CEBRIAN, E., MARTÍ, R., URIZ, J.M. & TURON, X. 2003. Sublethal effects of contamination on the Mediterranean sponge *Crambe crambe*: Metal accumulation and biological responses. Mar. Poll. Bull., 46: 1273–1284. https://doi.org/10.1016/S0025-326X(03)00190-5

CONAMA Nº 357/2005 - "Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências."- Legislation date: DOU 17/03/2005.

DENTON, G.R.W., CONCEPCION, L.P., WOOD, H.R. & MORRISON, R.J. 2006. Polycyclic aromatic hydrocarbons (PAHs) in small island coastal environments: A case study from harbours in Guam, Micronesia. Mar. Pollut. Bull., 52: 1090-1117. http://dx.doi.org/10.1016/j.marpolbul.2006.05.002.

DOLBER, P. & SPACH, M. 1993. Conventional and confocal fluorescence microscopy of collagen fibers in the heart. J. Histoch. Cytoch., 41: 465-469. https://doi.org/10.1177/41.3.7679127.

EFREMOVA, S.M., MARGULIS, B.A., GUZHOVA, I.V., ITSKOVICH, V.B., LAUENROTH, S., MÜLLER, W.E.G. & SCHRÖDER, H.C. 2002. Heat shock protein Hsp70 expression and DNA damage in Baikalian sponges exposed to model pollutants and waste water from Baikalsk Pulp and Paper Plant. Aquat. Toxicol., 57: 267-280. https://doi.org/10.1016/S0166-445X(01)00209-0

GLYZINA, O.Y., BELYKH, L.I., LATYSHEV, N.A., GLYZIN, A.V. & GORSHKOV, A.G. 2002. Study of mutual influence of polycyclic aromatic hydrocarbons (anthracene, pyrene) and Baikalian sponges in model experiments. Chem. Sustain. Develop., 10: 409–412. ISSN: 1023-8603

HAYES, J.D., FLANAGAN, J.U. & JOWSEY, I.R. 2005. Glutathione transferases. Annu. Rev. Pharmacol. Toxicol., 45: 51–88. http://dx.doi.org/10.1146/annurev.pharmtox.45.120403.095857

HELM, I., JALUKSE, L. & LEITO I. 2012. A highly accurate method for determination of dissolved oxygen: Gravimetric Winkler method. Anal. Chim. Acta, 741: 21-31. http://dx.doi.org/10.1016/j.aca.2012.06.049

LOWE, D.M., SOVERCHIA, C. & MOORE, M.N. 1995. Lysosomal membrane responses in the blood and digestive cells of mussels experimentally exposed to fluoranthene. Aquat, Toxicol., 33: 135-140. http://dx.doi.org/10.1016/0166-445X(95)00015-V

MAHAUT, M.L., BASUYAUX, O., BAUDINIERE, E., CHATAIGNIER, C., PAIN, J. & CAPLAT, C. 2013 The porifera *Hymeniacidon perlevis* (Montagu, 1818) as a bioindicator for water quality monitoring. Environ. Sci. Pollut. Res., 20(5): 2984-2992. http://dx.doi.org/10.1007/s11356-012-1211-7

MARQUES-JUNIOR, A.N., MORAES, R.B.C. & MAURAT, M.C. 2009. Poluição marinha. In: Pereira, R.C. & Soares-Gomes, A. (eds.), Biologia Marinha. Rio de Janeiro: Interciência, pp. 505-528.

MESTRE, C., MAHER, W., ROBERTS, D., BROAD, A., KRIKOWA, F. & DAVIS, A.R. 2012. Sponges as sentinels: patterns of spatial and intra-individual variation in trace metal concentration. Mar. Pollut. Bull., 64: 80–89. http://dx.doi.org/10.1016/j.marpolbul.2011.10.020

MICHALANY, J. 1998. Técnicas histológicas em anatomia patológica. São Paulo: Artes Médicas, 3 ed, 295 p.

MOORE, M.N., ALLEN, J.I. & MCVEIGH, A. 2006. Environmental prognostics: An integrated model supporting lysosomal stress responses as predictive biomarkers of animal health status. Mar. Environ. Res., 61: 278-304. http://dx.doi.org/10.1016/j.marenvres.2005.10.005

MURICY, G. & HAJDU, E. 2006. Porifera brasilis: guia de identificação das esponjas mais comuns do Sudeste. Museu Nacional – UFRJ, Rio de Janeiro, RJ. 104p.

NEFF, J.M. 2002. Bioaccumulation in marine organisms – Effect of contaminants from oil well produced water. Oxford: Elsevier Science Ltda., 472 p.

PDBG – Programa de Despoluição da Baía de Guanabara. PETROBRAS, CENPES e SEGEN. 2000. Caracterização Preliminar do Impacto do Vazamento Acidental de Óleo Ocorrido em 18/01/00 na Baía de Guanabara. Rio de Janeiro: Instituto de Biologia e Instituto de Química – UFRJ/. Departamento de Oceanografia e Hidrologia – UERJ/ Departamento de Química – PUC - Rio/ Instituto de Geociências – LAGEMAR - UFF, 51 p.

PEREIRA, C.D.S., ABESSA, D.M.S., CHOUERI, R.B., ALMAGRO-PASTOR, V., CESAR, A., MARANHO, L.A., MARTÍN-DÍAZ, M.L., TORRESA, R.J., GUSSO-CHOUERI, P.K., ALMEIDA, J.E., CORTEZ, F.S., MOZETO, A.A., SILBIGER, H.L.N., SOUSA, E.C.P.M., VALLS, T.A.D. & BAINY, A.C.D. 2014. Ecological relevance of sentinels' biomarker responses: A multi-level approach. Mar. Environ. Res., 96: 118-126. http://dx.doi.org/10.1016/j.marenvres.2013.11.002

PÉREZ, T., WAFO, E., FOURT, M. & VACELET, J. 2003. Marine sponges as biomonitor of polychlorobiphenyl contamination: concentration and fate of 24 congeners. Environ. Sci. Technol., 37: 2152-2158. http://dx.doi.org/10.1021/es026234v

REGOLI, F. & GIULIANI, M.E. 2014. Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Mar. Environ. Res., 93: 106-117. http://dx.doi.org/10.1016/j.marenvres.2013.07.006

RESENDE, L.L., PEDRETE, T.A., NUDI, A.H. & WAGENER, A.L.R. 2017. Nível de contaminação por hidrocarbonetos na baía de Guanabara, Rio de Janeiro – RJ. Rev. Bras. Inic. Cient., 1: 90-117. ISSN 2359-232X.

REY-SALGUEIRO, L., MARTÍNEZ-CARBALLO, E., GARCÍA-FALCÓN, M.S. & SIMAL-GÁNDARA. 2009. Survey of polycyclic aromatic hydrocarbons in canned bivalves and investigation of their potential sources. Food Res. Int., 42: 983–988. http://dx.doi.org/10.1007/978-3-319-19276-5_5

RIBEIRO, S.M., BIANCO, E.M., ROGERS, R., TEIXEIRA, V.L. & PEREIRA, R.C. 2010. Chemical defense of *Hymeniacidon heliophila* (Porifera: Halichondrida) against tropical predators. Braz. J. Oceanogr., 58(4): 315-321. http://dx.doi.org/10.1590/S1679-87592010000400006

TUBINO, R.A., MONTEIRO-NETO, C., MORAES, L.E.S. & PAES, E.T. 2007. Artisanal Fisheries production in the coastal zone of Itaipu, Niterói, RJ, Brazil. Braz. J. Oceanogr., 55 (3): 187-197. http://dx.doi.org/10.1590/S1679-87592007000300003

TURQUE, A.S., BATISTA, D., SILVEIRA, C.B., CARDOSO, A.M., VIEIRA, R.P., MORAES, F.C., CLEMENTINO, M.M., ALBANO, R.M., PARANHOS, R., MARTINS, O.B. & MURICY, G. 2010. Environmental shaping of sponge associated archeal communities. PLoS One, 5(12):e15774. http://dx.doi.org/10.1371/journal.pone.0015774.

USEPA (Environmental Protection Agency). 1996a. EPA Method 3510C - Separatory funnel liquid-liquid extraction. Test methods for evaluation solid waste physical/chemical methods, Washington DC.

USEPA (Environmental Protection Agency). 1996b. EPA Method 3540C - Soxhlet extraction. Test methods for evaluation solid waste physical/chemical methods, Washington DC.

USEPA (Environmental Protection Agency). 2014. EPA Method 8270D - Gas chromatography/mass spectrometry for semi volatile organics capillary column technique. Test methods for

evaluation solid waste physical/chemical methods, Washington DC.

VERDENAL, B., DIANA, C., ARNOUX, A. & VACELET, J. 1990. Pollutant levels in Mediterranean commercial sponges. In: Rützler, K. (ed), New perspectives in sponge biology. Washington: Smithsonian Institution Press, pp. 516–524.

VOGEL, S. 1977. Current-induced flow through living sponges in nature. Proc. Natl. Acad. Sci. USA, 74(5): 2069-2071. http://dx.doi.org/10.1073/pnas.74.5.2069

WAGNER, C., STEFFEN, R., KOZIOL, C., BATEL, R., LACORN, M., STEINHART, H., SIMAT, T. & MÜLLER, W.E.G. 1998. Apoptosis in marine sponges: a biomarker for environmental stress (cadmium and bacteria). Mar. Biol., 131:411-421. https://doi.org/10.1007/s002270050334.

WANG, Z., FINGAS, M. & PAGE, D.S. 1999. Oil spill identification. J. Chromatogr. A, 843: 369-411. http://dx.doi.org/10.1016/S0021-9673(99)00120-X

WEYERMANN, J., LOCHMANN, D. & ZIMMER, A. 2005. A practical note on the use of cytotoxicity assays. Int. J. Pharm., 288: 369-376. http://dx.doi.org/10.1016/j.ijpharm.2004.09.018

WILHELM-FILHO, D., TORRES, M.A., ZANIBONI-FILHO, E. & PEDROSA, R.C. 2005. Effect of different oxygen tensions on weight gain, feed conversion and antioxidant status in piapara, *Leporinus elongatus* (Valenciennes, 1847). Aquac., 224: 349-357. http://dx.doi.org/10.1016/j.aquaculture.2004.11.024

YUNKER, M.B., MACDONALD, R.W., VINGARZAN, R., MITCHELL, R.H., GOYETTE, D. & SYLVESTRE, S. 2002. PAHs in the Fraser river basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. Org. Geochem., 33: 489-515. https://doi.org/10.1016/S0146-6380(02)00002-5

ZAHN, R.K., ZAHN, G., MÜLLER, W.E.G., MICHAELIS, M.L., KURELEC, B., RIJAVEC, M., BATEL, R. & BIHARI, N. 1983. DNA damage by PAH and repair in a marine sponge. Sci. Total Environ., 26: 137-142. http://dx.doi.org/10.1016/0048-9697(83)90107-9