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In vitro effects of a plastic additive (DEHP) on contraction dynamics of *Hymeniacidon heliophila* (Demospongiae: Halichondrida)

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A B S T R A C T

Microplastic impacts in marine ecosystems are a major topic in ocean pollution research. Besides the problems caused by the particles, additives used in its manufacture, such as phthalates, can leach from the particles. Once in the environment or inside the organisms, these compounds can bioaccumulate and cause endocrine disruption in several marine organisms. Although marine sponges are efficient filter feeders, limited research has explored the potential physiological impacts of these additives on these organisms. To verify possible effects of the additives in sponges, individuals of *Hymeniacidon heliophila* were exposed to different concentrations of di-(2-ethylhexyl)-phthalate (DEHP). Expansion-contraction cycles were monitored by time-lapse photography and parameters of *in vitro* cell cultures (primmorphs), such as number and size, were analyzed. Exposition to DEHP caused alterations in the contraction-expansion cycles and dose-dependent effects on the volume of the canals of the aquiferous system. Cell aggregation parameters in the primmorph cultures were not altered. However, the effects on the organisms were not permanent, with the sponges being able to recover their natural expansion-contraction cycle. Possible mechanisms involved in the effect of DEHP in the sponges and detoxification pathways are discussed.

1. Introduction

Microplastic impacts in marine ecosystem are a major topic in ocean pollution research (Horton, 2022). The extensive use of this material since 1950's, the lack of waste management and its persistence use were responsible for the increase of plastic debris in the environment, especially in the ocean, where plastic particles can accumulate in large "garbage patches" (Lebreton et al., 2018). In addition to this, the attention has been drawn in recent years to microplastics, particles with 1–5 mm in size, which were found to be ubiquitous in several ecosystems (Horn et al., 2019). Those called *primary microplastics* come either from deliberately manufactured small-sized particles, widely used as raw material by the industry in cosmetic abrasives or in the manufacture of several products, while the secondary microplastics are not intentionally manufactured, but come from environmental degradation of larger materials (Sharma and Chatterjee, n.d.). The estimation of the floating particles already present in the oceans is about 5.25 trillion particles, weighing 268,940 tons (Eriksen et al., 2014). The distribution of these microplastics in the environment is only beginning to be well documented and their ecological impact is still uncertain (Jahnke et al., 2017).

Microplastics can harm marine organisms by physical or chemical action. Currently, most information available is from its physical impact, the first one assessed when the topic raised political and scientific attention (Wright et al., 2013). In general, filter feeding organisms can assimilate microplastics in the same way that any suspension in the water is filtered (Wright et al., 2013). However, reports show that some ingested particles can translocate to the hemolymph (Scanes et al., 2019) and even to tissues such as muscles (Daniel et al., 2021; Zeytin et al., 2020). This can lead to changes in feed habits, decreasing food ingestion and consequently the fertility, growth, and hatching (Cole et al., 2015). In addition to this physical impact, the plastic polymers are blended into a wide variety of compounds known as plasticizers during the manufacture process. These additives are essential to give different characteristics to the materials, such as color, toughness, durability, and malleability to make plastic products manufacture easier (Al-Malaika et al., 2017). The interaction of these molecules with the polymer's structure is made by weak hydrogen bond, and as occurs with other additives, they can leach to environment or into one organism's tissues if conditions are favorable (Chen et al., 2019).

Among several different plasticizers used in plastic production, phthalates are the most used ones (Awuchi et al., 2019). Members of this large family of compounds are recognized as endocrine disruptors and considered priority pollutants by several national sanitary authorities worldwide (USEPA, 2012; EC, 2003). Among them, the bis (2-ethylhexyl) phthalate (DEHP) is a high-density phthalate applied

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especially in polyvinyl chloride (PVC) production and the most used plastic additive in medical devices such as blood and plasma bags and dialysis equipment (Tickner et al., 2001). It is one of the most found phthalates in marine environments (Biemann et al., 2012), with surface levels ranging from 0.33 to 97.87 μg/L (Hammad Khan and Jung, 2008). The ubiquitous DEHP presence in the sea led to investigations of possible endocrine disruption in marine organisms. The results indicate that this compound have multiple negative effects such as growth inhibition and reduction of immune and endocrine responses in different fishes, including *Danio rerio, Gobiocypris rarus*, and *Pelteobagrus fulvidraco* (Guo et al., 2015; Uren-Webster et al., 2010; Yuan et al., 2017). Filter feeding organisms can also be impacted, with alterations in lysosomal response and disruption of metabolic balance, such as changes in the expression of oxidative enzymes in the pearl oyster *Pinctada marensii* (Xiang et al., 2017) and the mussel *Mityllus galoprovinciallis* (Xu et al., 2021). The mussel also showed an increased presence of stress biomarkers such as HSP70, as well as the activation of energy storage compounds (Xu et al., 2021).

Among invertebrates, marine sponges (phylum Porifera) are important suspension-feeder organisms with several essential roles in the benthic fauna. These include primary production and nitrification by its microbiological symbionts, for the maintenance of the reef ecosystems and the bentho-pelagic coupling by acting on carbon, oxygen, and nitrogen cycles (Bell, 2008). Sponges can filter large volumes of water, retaining from dissolved material to particles around 50 μm (Reiswig, 1974). They can bioaccumulate several compounds and as such be used to assess the presence of heavy metals, polycyclic aromatic hydrocarbons, or rare earth elements (Venkateswara Rao et al., 2009; Batista et al., 2013; Orani et al., 2022). Similarly, to other filter-feeders' organisms, physiological mechanisms were developed in sponges to expel undesired material and control the feeding currents (Elliott and Leys, 2007). Despite having no developed organs or tissues as found in more derived animals, the sponges can effectively coordinate rhythmic body expansion-contraction cycles to face endogenous or external stimuli (Leys and Meech, 2006). Such processes have been studied in some species and used to investigate possible role of electrical signals in Demospongiae (Mackie, 1979), but were never used to assess the effects of pollutants on the sponge physiology.

Although the filtration capability makes them excellent bioindicators of ocean pollution, studies dealing with the presence of plastic debris and its effects in sponges are rare. Few works have investigated the presence of microplastic particles (Fallon and Freeman, 2021; Girard et al., 2021; Saliu et al., 2022; Soares et al., 2022) and one reported quantifiable level of DEHP in two species from Florida Keys (Giametti and Finelli, 2022). On Brazilian coast, one of the contaminated species of Florida Keys, *Hymeniacidon heliophila,* is found in chronically polluted environments all year long. This sponge occurs from North Carolina (USA) to Santa Catarina (Brazil). It has an orange color and presents several conical papillae with oscula on top surrounded by a thin perioscular membrane, which is visible when the organism is active filtering. Although some work is available on the microplastic presence in marine sponges (e.g. Fallon and Freeman, 2021), there are no studies about the possible effects of plastic-associated contaminants in this phylum at any biological level.

Therefore, the purpose of this study was to assess possible toxicological effects in sponges caused by DEHP exposure. To this end, we used the contraction patterns of the whole body of *Hymeniacidon heliophila,* in addition to histological observations, and experiments on cellular reaggregation to indicate possible response pathways to the observed changes in the sponge expansion-retraction cycles.

2. Material and methods

2.1. Organisms

Specimens of *Hymeniacidon heliophila (*Wilson, 1911) (Suberitida: Halichondriidae) were collected during low tides in two coastal cities of São Paulo state (Fig. 1A): São Sebastião (23°49′23.5″S, 45°25′00.6″W - Fig. 1B) and São Vicente (23°58′39.4″S, 46°22′09.0″W - Fig. 1C).” The animals were transported in seawater to the laboratory at the University of São Paulo on refrigerated containers (18 °C) and maintained in 80 L aquariums with natural water, 12h/12h dark-light cycles, biological filters, and constant aeration. Other laboratory's conditions were set to recreate local sampling conditions such as pH of 8.2, salinity of 32 ppm and temperature of 24 °C. During the experiments. pH, salinity and temperature of aquaria were maintained as the same marine environmental conditions of local sampling sites (pH of 8.2, salinity of 32 ppm and temperature of 24 °C) with 12/12h of light exposure cycles.

2.2. DEHP exposure

2.2.1. Initial effects assessment on contraction

The experimental setup consisted in a 2 L aquarium connected to another one with 80 L, biological filter, and aeration. Constant flow was maintained between the two aquaria and the sponges were acclimatized for four days before the start of the experiments. The acclimatation period was determined when all the sponges' papillae presented the perioscular membrane, indicating that their aquiferous system was functioning adequately. During the experiments. pH, salinity and temperature of aquaria were maintained as the same marine environmental conditions of local sampling sites (pH of 8,2, salinity of 35 g/kg and temperature of 24 °C) with 12/12h of light exposure cycles. For each experimental group, three papillae (ca. 1 cm height) from the sponges were cut and affixed in the bottom of the 2 L aquaria. After 24 h these fragments regenerate as small functional sponges, as shown by the presence of oscula and an inflated perioscular membrane indicating a functional aquiferous system (Fig. 2A)

For the experiments, the flow between the aquaria was interrupted and the sponges were exposed to 90 μL/L of bis(2-ethylhexyl) phthalate (DEHP, Quimesp), with other group maintained in natural seawater as a control. Pictures were taken in timelapse every 5 min under constant light for 72 h with a camera connected to a computer and analyzed with the ImageJ software (NIH). For this analysis, the series of images of each sponge was stacked, converted into binary mode (black and white; Fig. 2A–C) with the same threshold applied to all frames. The total area of the sponge was then measured for each one of the frames and plotted into line graphs to visualize the contraction patterns during the experiments.

2.2.2. Dose-response assessment

To test the dose-response effects of DEHP on *H. heliophila* contraction, we submitted sponges to three different concentrations of DEHP (0.9, 9 and 90 μL/L), using a control group exposed to natural seawater. During the experiments. pH, salinity and temperature of aquaria were maintained as the same marine environmental conditions of local sampling sites (pH of 8,2, salinity of 35 g/kg and temperature of 24 °C) with 12/12h of light exposure cycles. In these experiments, smaller glass chambers were used to maintain the sponges at the same level and focused on the camera (Fig. 3). These chambers were placed inside the 2 L aquariums where DEHP or seawater was added, and sequential images were captured during 72 h and analyzed as described before.

2.2.3. Histological analyses

Six organisms, three from the control group and three from the exposed group initially tested for the effect assessment, were removed from the aquaria 3h after exposure and fixed for 48 h in glutaraldehyde 2.5 % in sea water. After the fixation period, the samples were kept for 2 h in hydrofluoric acid 5 % for spicule removal, then dehydrated in a serial gradient of ethanol (50, 70, 90 and 100 %, 30 min each; Martoja and Martoja-Pierson, 1967) and cleared in xylene (2 baths, 30 min each). A sequence of histological paraffin infiltration was made, and the samples were cut in a microtome in 8 μm sections. The slides were

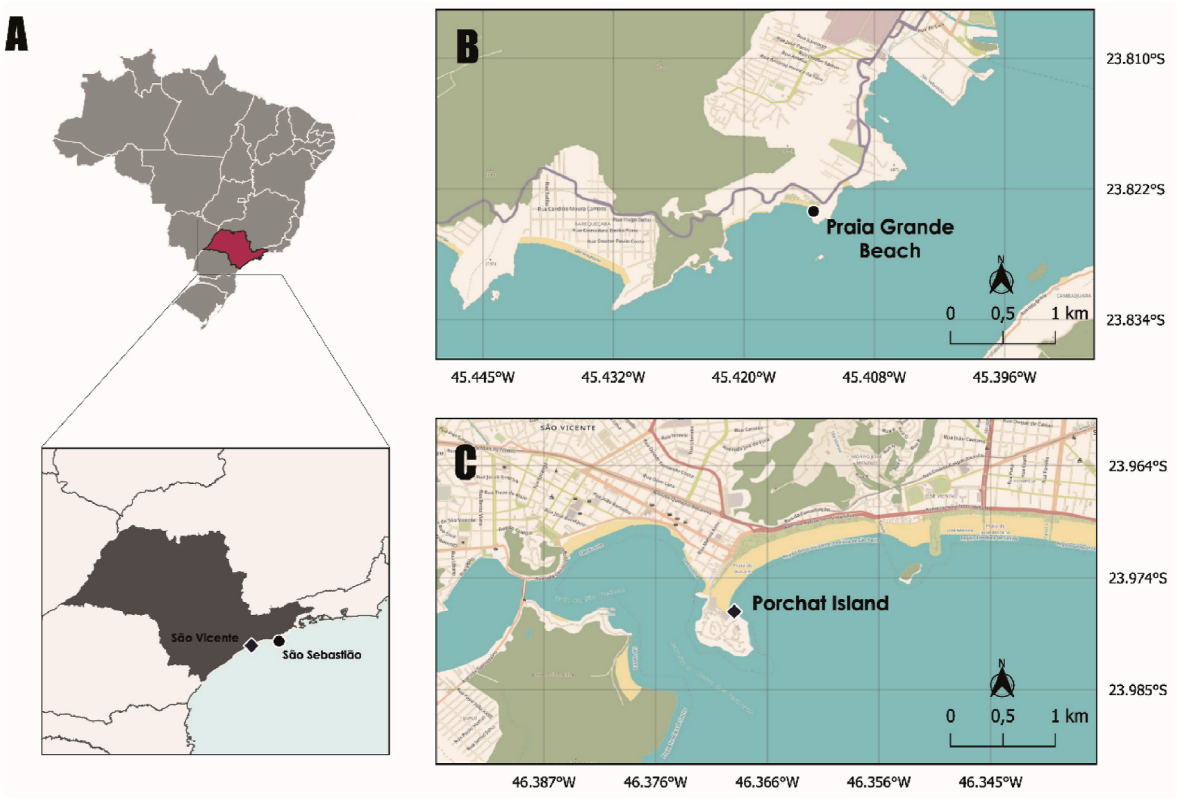

Fig. 1. Sampling areas of this study. **A:** Brazil and São Paulo State (highlighted in red) at the southeast Atlantic coast. **B:** Praia Grande beach. **C:** Porchat Island. Both sites are located near to port access channels (Santos and São Sebastião ports) and exposed to polluted seawater. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

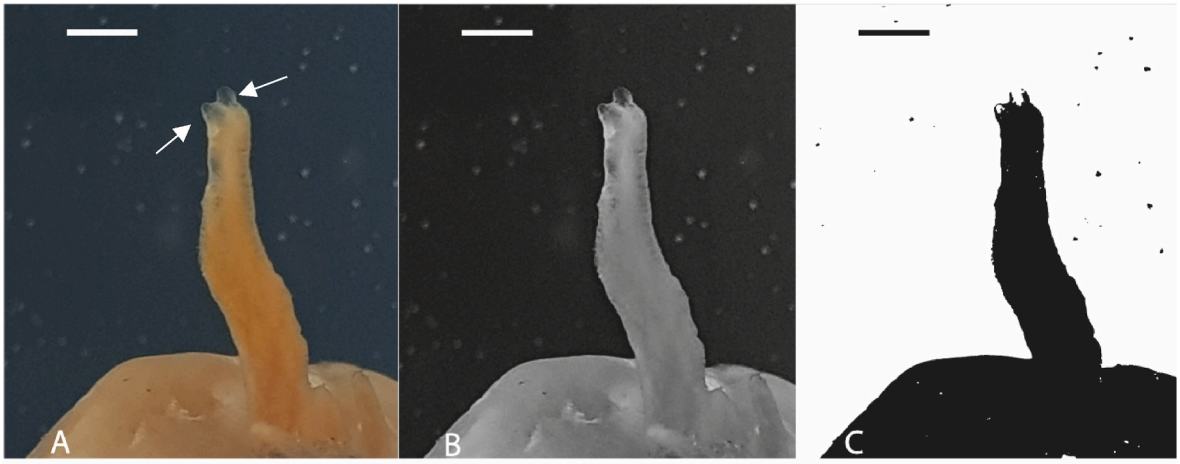

Fig. 2. Image transformations for timelapse analysis. **A:** original image presenting an *Hymeniacidon heliophila* functional papilla with two oscula with inflated perioscular membranes (arrow). **B:** The same image after a grayscale (8-bit) transformation. **C:** Result of the threshold transformation to obtain a black and white image and measure the variation of the area with the software ImageJ. The smaller black or white dots are discarded in the analysis (scale bars: 1 cm).

rehydrated and stained with Mallory's Trichrome. Several adjacent pictures of each histological section were taken with a D5100 digital camera (Nikon) in a TE300 inverted microscope (Nikon). The images were then assembled to visualize the entire section and the areas analyzed by ImageJ software using the same procedure described before.

2.2.4. Cell culture

Cell cultures were made according to Custodio et al. (1998) to observe possible effects of DEHP on cellular reaggregation, primmorphs formation, number, and size. Briefly, the organisms were cleaned from debris and cut in fragments of approximately 2 mm and placed in 50 mL tubes with artificial seawater without calcium and magnesium with EDTA (CMFSW + E: 460 mM NaCl, 7 mM Na_2SO_4, 10 mM KCl, 10 mM, HEPES, 2.5 mM EDTA, pH 8.2; Dunham and Weissmann, 1986). The tubes were gentle shaken for 30 min and after that, the supernatant was discarded, 50 mL of fresh CMFSW + E added, and the tubes maintained in agitation for 1 h. In the end, the dissociated cells in the supernatant were filtered through a nylon mesh (100 μm) and centrifuged at 250×*g* for 10 min. The pellet was resuspended in sterile-filtered (0.22 μm)

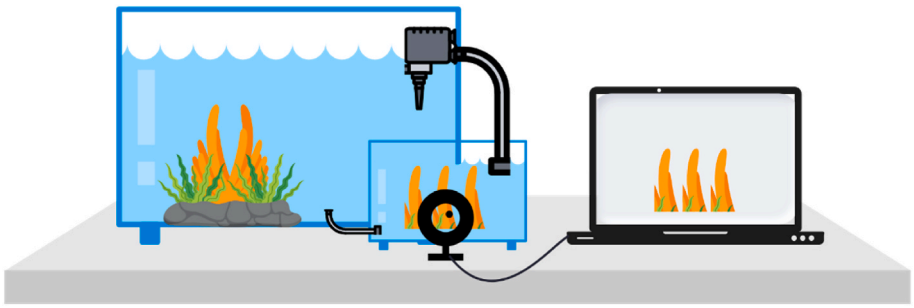

Fig. 3. Experimental setup for initial assessment of DEHP's effect on *Hymeniacidon heliophila* contraction-expansion cycles.

natural seawater supplemented with penicillin-streptomycin (0.2 mg/mL). The cells were counted in a Neubauer chamber and 1.5–2 x10^5 cells/mL (Müller et al., 1999) plated in 60 mm cell culture Petri dishes with 5 mL of sterile seawater containing either 9, 90 μL/L of DEHP, or only seawater as control. After five days, the cultures were photographed and the images analyzed for the number of primmorphs, occupied area, perimeter, and size using the same ImageJ protocol described before.

2.2.5. Statistical analysis

Identification of significant shifts in the mean sponge area during experiments to assess DEHP effects in the contraction pattern of *H. heliophila* were carried out by changepoint determination using segmentation of time-series analysis (Harguess and Aggarwal, 2009). Briefly, the aim of the analysis is to search for times steps where there is a statistical difference in a continuous time-series parameter such as mean or variance. Each changepoint divides the time series into different segments, each one clustering similar parameter results. Every time that the meaning of the series has a statistically significant shift (up or down), the analysis indicates a changepoint. All the statistical parameters for the analysis are provided by the user, especially the number of possible changepoints. The statistical analysis was part of the ChangePoint package (Killick and Eckley, 2014) , and was performed in RStudio version 3.6.3 (R Core Team, 2022)

Other statistical analysis such ANOVA for the mean sponge area before, during and after the first DEHP exposure and the T-test for control and exposed histological and culture parameter comparisons were carried out in Origin (2020) software as well as the graphics presented.

3. Results

3.1. Contraction patterns - control

The sponge *H. heliophila* presented rhythmic body contractions when in aquarium with continuous seawater flow (Fig. 4). Each contraction cycle is composed of an expansion phase, represented by a constant increase of the sponge area. During this phase, the animal canals and chambers are filled with water. The sponge then reaches a maximum area, variable for each organism, which is immediately followed by a drastic retraction phase. The water is expelled, and the sponge area decreases rapidly (Fig. 4). All sponges used in our experiments presented this pattern, but the timing and the amplitude of each contraction cycle was variable, ranging from few minutes to 4 h and increasing area from 50 to 400 %. When in closed seawater flow, sponges present an acclimatization period observed in the plateau before the first changepoint (Fig. 5A–B). Before exposure of seawater or DEHP, the sponge area increases significantly, leading to changepoints 2, 3 and 4. Exposure to seawater did not affect sponge area and until the end of the experiment the papilla went through two more changepoints (Fig. 5B)

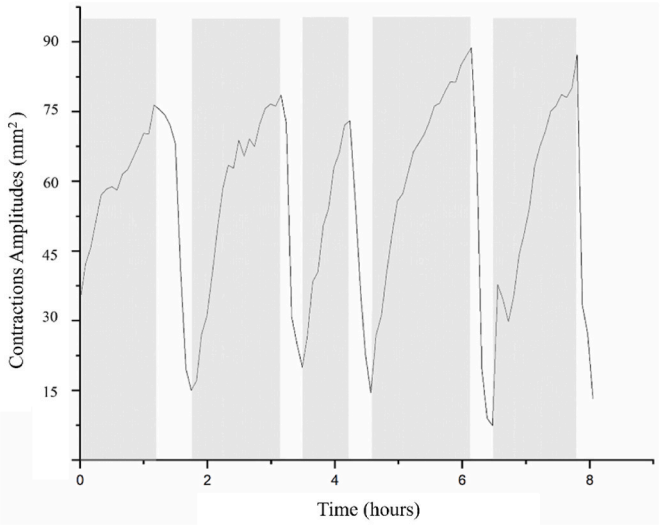

Fig. 4. Contraction patterns of a single *Hymeniacidon heliophila* individual. The shaded area indicates each expansion phase.

3.2. Contraction patterns - treatment

The sponges showed a dramatic change in their volumes right after being exposed to DEHP inducing the 5th changepoint (Fig. 5 A), represented by an increase ($F = 6{,}72$, $p < 0{,}05$) in the papillae total area in all analyzed organisms (Fig. 6; Table 1). The time that the effects of the exposure lasted were variable on each organism, from 20 to 260 min, but each time represented by a change in the area corresponding to 10 % in average. These effects were not related to the size of the animals since the same behavior was observed for all tested organisms. At the end of this expansion, the area decreased, returning to values close to those before exposure, and the sponges returned to their normal contraction pattern (Fig. 5). The papilla exposed to DEHP underwent through two more changepoints after DEHP exposure, as does the control ones.

3.3. Canals and chambers perimeter alterations

The histological analysis of the sponges during DEHP exposure (Fig. 7) indicated that there was no difference in the total canals and chambers area (Fig. SI 1A). However, their overall perimeter increased significantly (Fig. 8 $T = -4{,}5$, $p < 0{,}001$) (Fig. 8A). To understand how total area was maintained while the perimeter increased, the distribution density of this parameter from different canals and chambers was analyzed, revealing an important effect on their perimeters: Canals and chambers with perimeters ranging from 0.21 to 0.26 cm were not observed after exposure, while larger canals and chambers, with perimeters from 0.56 to 0.61 cm were present only in exposed organisms (Fig. 8B). The density distribution of total area, in contrast, shows that

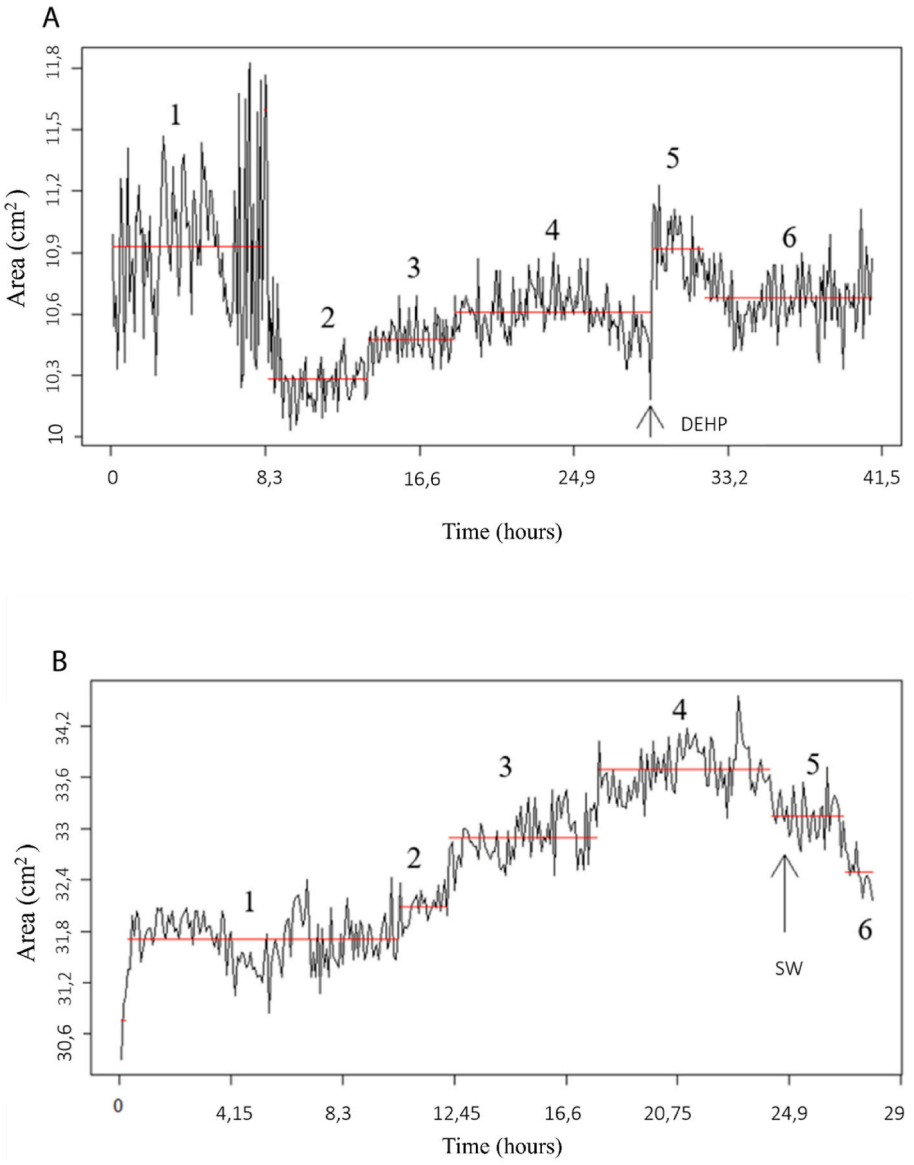

Fig. 5. Significant changes in the sponge area. **A)** DEHP; **B)** Control. Numbers indicates statistically different changes in the areas and the red lines the duration. Arrow indicates introduction of DEHP or seawater (SW) in the chambers. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the area occupied by all canals and chambers did not changed significantly (Fig. SI 1B).

3.4. Dose-response effects in contraction patterns and cell cultures

The sponges exposed to 0.9 μL/L DEHP did not show detectable responses before and after exposure. With 9 and 90 DEHP μL/L exposure, the average papilla area increased after exposure (Fig. 9) and the sponges followed the pattern observed in the initial experiments. The highest concentration showed a stronger response, with the area changing immediately after exposure, while with 9 DEHP μL/L, this happened only after a refractory period (Fig. 9).

The formation of primmorphs in cell cultures showed no statistically significant variations in all tested concentrations of DEHP after three days of experiment. However, their sizes and areas showed a tendency to increase when exposed to 90 μL/L (Fig. SI 2).

4. Discussion

Sponges performs contractions phenomena to help pumping seawater through their canal system, maintaining their levels of oxygen and food and get rid of unwanted substances (Kumala et al., 2017; Kornder et al., 2022). This process may be regular, with well-established intervals between expansion-contraction events, or irregular, showing no patterns at all. (Nickel, 2004; Kumala and Canfield, 2018). When regular contractions are present, analysis of the changes in these patterns can be a powerful tool to understand how environmental conditions, contaminants or compounds interfere in the organism physiology.

There is no evidence that the contraction mechanism in Porifera is controlled by electrical signals, with exception of Hexactinellida (Leys and Meech, 2006). This correlates with the apparent absence of Ca-dependent channels and GAP junctions (Leys, 2015), important components to the formation of action potential and the transmission of electrical signals. It seems, otherwise, that signal transmission could be done by slower mechanisms, using nonselective ion channels and/or pumps coupled to ligand-dependent receptors such as G protein coupled

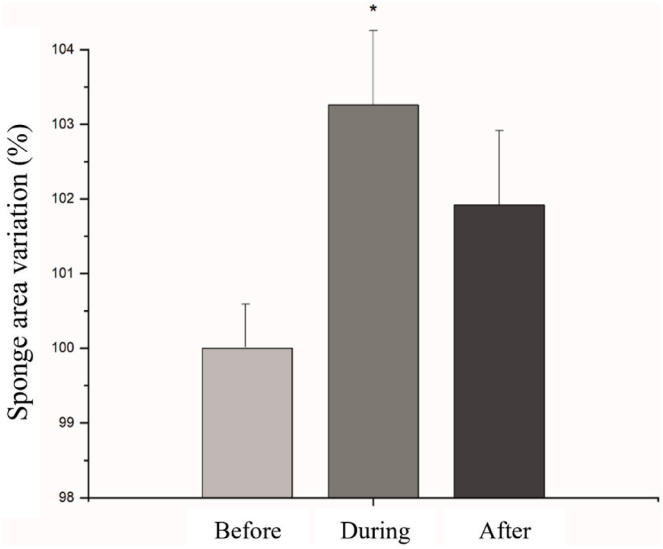

Fig. 6. Variation of the area of the *H. helophila* papillae related to the DEHP exposure (90 μL/L of DEHP). There was an increase in the area during DEHP exposure, followed by a partial recovery after the compound is removed (*: significant differences between groups. ANOVA, $F = 6{,}72$, $p < 0{,}05$).

Table 1
Changepoints location during control (A) and exposed (B) experiments. Significant changes in mean area locations for each sponge analyzed. **A:** control group, **B:** exposed group. Grey columns indicate changepoints location before (4), during (5) and after (6) DEHP exposure (at 1750).

A	Changepoints locations in control group (time in minutes)					
	1	2	3	4	5	6
Pap. 1	125	375	750	1060	1190	1400
Pap. 2	215	390	735	905	1150	1400
Pap. 3	15	625	735	1070	1455	1620
B	Changepoints locations in exposed group (time in minutes)					
	1	2	3	4	5	6
Pap. 1	120	535	675	750	1760	1780
Pap. 2	490	505	830	1110	1750	1920
Pap. 3	150	290	380	950	1750	2010

receptors (Billington and Penn, 2003). This is similar to what occurs in some slow contractions of actin/myosin filaments in vertebrates (Somlyo and Somlyo, 2003) and was also observed in *Amphimedon queenslandica* (Krishnan et al., 2014). On the other hand, contraction in sponges is related to the presence of calcium in the environment (Lorenz et al., 1996). Calcium is primarily regulated by plasma membrane channels and could act as a second messenger activating kinases which in turn catalyze myosin phosphorylation and ultimately contraction of cells (Allen and Walsh, 1994)

In vertebrates, it was observed that DEPH interferes with the calcium metabolism at a cellular level, suppressing calcium signaling of nicotinic acetylcholine receptors in bovine adrenal chromaffin cells (Liu and Lin, 2002). In human, stem cells derived cardiomyocytes, DEHP exposure led to a deficiency in contraction (Gillum Posnack et al., 2015). This effect is caused by a perturbation of the calcium load and release from the sarcoplasmic reticulum and the decrease of intercellular connectivity, which reduces the contractile response of these cells to external stimuli. Ecotoxicological studies also demonstrate that DEHP has negative effects in the mobility of some invertebrates (Herrero et al., 2017). However, the exact physiological mechanisms underlying the observed effects are not well established, especially in marine organisms.

All *H. heliophila* individuals used in this study showed contraction with regular intervals after the acclimatization. This pattern was

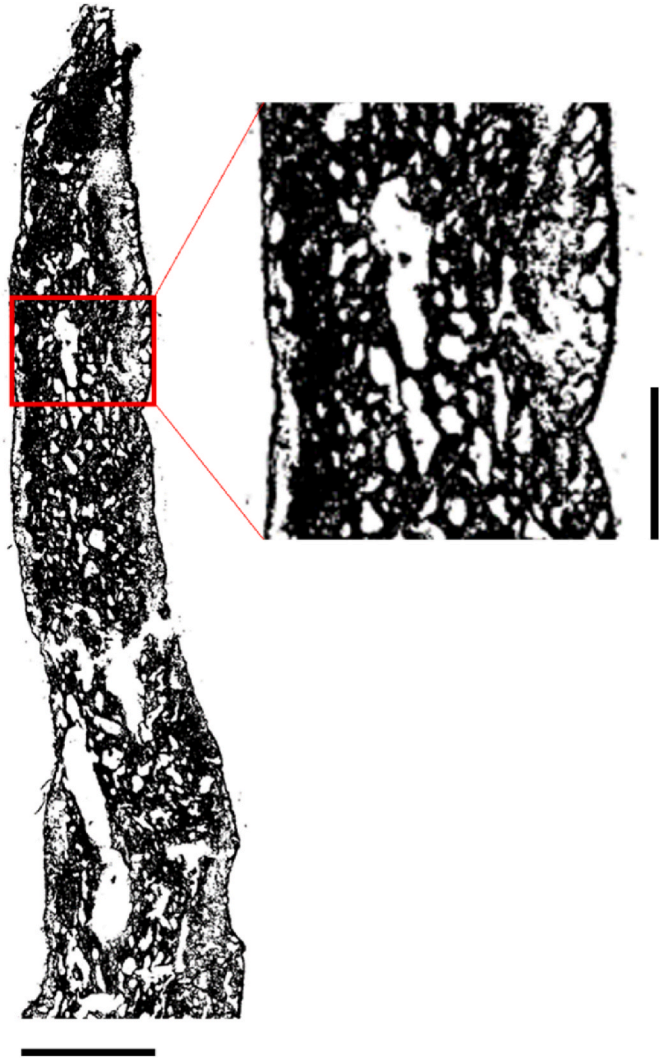

Fig. 7. *Hymeniacidon heliophila* histological slide example. Slides from exposed and control organisms were analyzed by ImageJ and white areas were considered to obtain perimeter and area of canals and chambers. Scale Bar: Papilla: 0.5 cm, Insert: 0.2 cm.

affected by DEHP, as observed by the arrest of the contraction cycles in all individuals after exposure in a dose-dependent manner. Later, the contraction returns to normal patterns, demonstrating that the effect is not definitive and that *H. heliophila* can recover. In addition, the histological analysis showed that the perimeter distribution density of the canals in the aquiferous system was altered. The smallest and largest canals perimeters increased after the exposure, while those with mean size were not changed, indicating effects on specific portions of the aquiferous system. One alternative explanation to the changes elicited by the DEPH exposure would be an effect in cell adhesion, which in sponges is also heavily dependent on calcium. In these animals, the mechanism is based on the interaction of a soluble proteoglycan complex, the aggregation factor, and its receptors in the cell membranes and extracellular matrix whose association is calcium dependent (Varner, 1995). With the changes in the calcium metabolism caused by DEHP, the intercellular adhesion could be compromised, especially in the exo/endopinacoderm exposed to the compound. Since during all experiments the perioscular membranes were inflated, indicating functional choanocyte chambers and therefore positive pressure inside the sponge body, the whole-body structure would not be affected, but with weaker cellular adhesion, the pinacoderm could be stretched, loosening the cell layer. The changes in the perimeter in larger and smaller canals

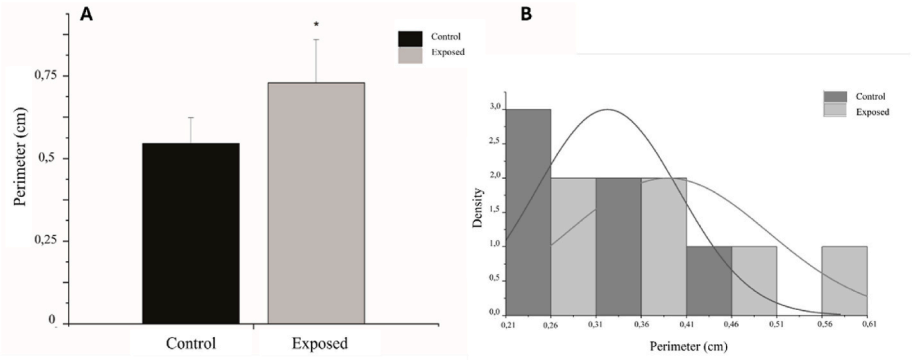

Fig. 8. A: Perimeter of canals of *H. heliophila* measured in histological sections during the experiments exposing their papillae to 90 μL/L μL/L of DEHP (T-test T = −4,5, p < 0,001). B: Perimeter density distribution from H. heliophila papillae control and exposed to DEHP (90 μL/L), indicating the differences between groups.

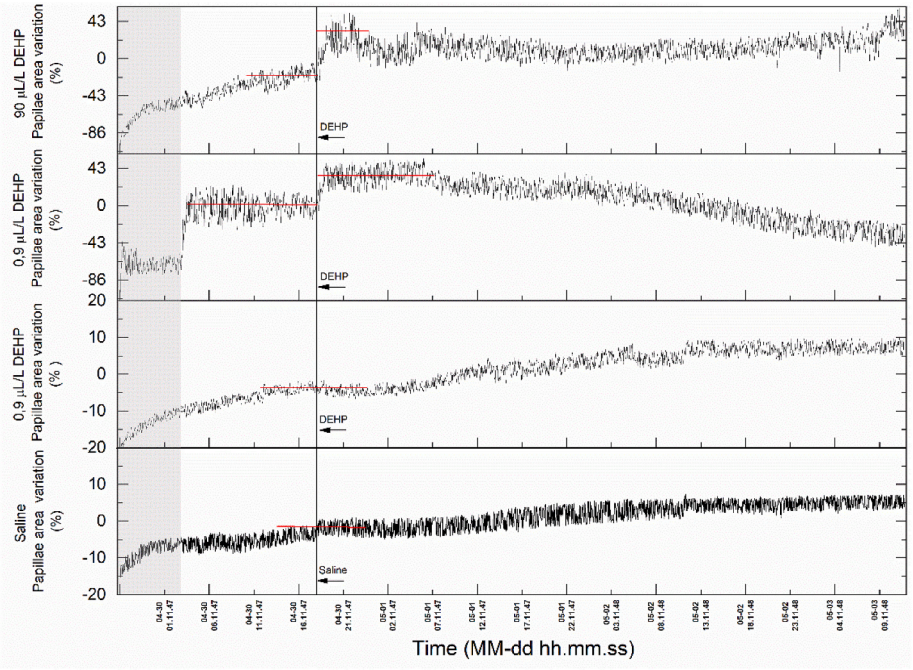

Fig. 9. Dose-response effect on *Hymeniacidon heliophila* contractions pattern. Red lines represent significant changes in average means before, during and after DEHP/Saline exposure. Shaded area indicates acclimatation period. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

pointed by the analysis corroborate this hypothesis, as both inhalant (smaller) and exhalant (larger) canals had their perimeter increased, suggesting some intercellular adhesion impairment. Despite the results showing that no significant differences were detected in the formation of primmorphs, the fact that they are 3D structures and area is a quadratic measure may have influenced those observations since cubic values would have increased differences between them. Possibly, if the volume of primmorphs were significantly different, the hypothesis of DEHP negative effects in cellular adhesion would be maintained as other results pointed to cell adhesion impairment.

However, our results suggest that DEPH interferes with the contraction mechanism in the sponges, possibly by altering the intracellular calcium metabolism. Basically, there are two different types of contractions in organisms with fully formed muscle tissues: fast, present in voluntary movement muscles; and slow, typically found in visceral myocytes (Squire, 2019). Both depend on the association of actin and myosin filaments, this one formed by a heavy and a light regulatory chain. In vertebrates, a non-muscle myosin light chain (nmMyLC) is found in slow contraction smooth muscles and in some non-muscle contractile scenarios involving cell motility (Vicente-Manzanares et al., 2009). The activation of the nmMyLC is associated with the myosin light chain kinase pathway (MLCK) and is part of a more ancient mechanism, where the intracellular calcium binds to calmodulin and initiates the contraction. Elements of this MLCK pathway were already observed in sponges (Colgren and Nichols, 2022) and the contraction in these animals apparently depends on calcium under experimental conditions (Colgren and Nichols, 2022). In addition, it has been shown that rat cardiomyocytes exposed to DEHP lose their contraction abilities (Posnack et al., 2011). This is due to changes in cellular calcium signaling during contraction, specifically in the recapture and release of calcium from the sarcoplasmic reticulum as well as in the functioning of important enzymes such as calcium ATPase. In sponges, DEHP exposure might be altering calcium metabolism, interfering in the MLCK contractile pathway leading to a blockage of calcium dependent contraction of specific cells in the pinacoderm.

Our observations reinforce the hypothesis that DEHP effect is intracellular. This phthalate is a known endocrine disruptor, with its biological effects first described in humans (Kavlock et al., 2006). It was

found that it could have toxic potential in MCF-7 cells increasing caspase and affecting specially newborns and children by the use of plastic devices such as pacifiers and baby bottles (Simoneau et al., 2012). The exposure to DEHP led to epigenetic transgenerational inheritance of diseases such as obesity and reproductive organs malformations (Manikkam et al., 2013). In invertebrates, DEHP exposure led to enhanced stress, endocrine and housekeeping genetic pathways in *Chironomus riparius* larvae (Herrero et al., 2015), damage the immune function of haemocytes in the giant freshwater prawn *Macrobrachium rosenbergii* (Sung et al., 2003), and modifications in the expression pattern of proteins associated with detoxification, oxidative stress and hormone-regulating, cellular metabolism of the abalone *Haliotis diversicolor supertexta* (Zhou et al., 2010).

Some endocrine disruptors were tested in freshwater sponges but only with an ecotoxicological and developmental approach (Hill et al., 2002). Low concentrations of Nonylphenol, Ethylbenzene, and Bisphenol A were found to have no effect on germination rates. However, higher concentrations hindered the formation of the aquiferous system in *Heteromeyenia* sp. and *Eunapius fragilis*, ultimately disrupting the initial tissue organization following germination (Hill et al., 2002). Other chemical messengers, such as neurotransmitters, can modulate the speed and amplitude of expansion-contraction phases in sponges, indicating that even without a nervous system, some elements may play a role in signaling information between their cells (Goldstein et al., 2020). Glutamate and GABA were tested to understand possible nervous stimuli in the contraction of *Tethya wilhelma* and *Halichondria panicea* (Ellwanger et al., 2007; Goldstein et al., 2020). Both studies suggest that there is a coordination system based on chemical messengers and after our findings, DEHP could have a similar inhibitory effect in *H. heliophila* contractions.

The recovery of contractions after DEHP exposure may be linked to a possible cyclic compound detoxification pathway present in the sponge. Not much is known about detoxification features in sponges (Perez et al., 2002). Nevertheless, the important association of these organisms with prokaryotic communities can increase sponge resistance to changing environmental parameters (Pita et al., 2018). The holobiont, term used to define this important interaction between sponges and their microbiomes, and the possible shifts in its community structure after pollutant exposure is the center of recent research (Batista et al., 2013; Stévenne et al., 2021). Once again, our results could indicate the presence of possible DEHP degrading microbiota helping the sponge to get rid of the pollutant.

5. Conclusions

The effects of endocrine disruptors in contraction-expansion of sponges were never assessed until this moment. The data obtained using this approach showed the effect of environmental concentrations of DEHP, a common plasticizer used in polymer production, on contraction-expansion cycles in *H. heliophila*. This substance, whose interference in calcium metabolism is already known, seems to be acting in the intracellular contraction pathways in the sponge cells, a mechanism associated with intracellular calcium concentration. This interference led to a temporary body volume increase, followed by its normalization. The sponge response to DEHP is dose-dependent, as changes in area were not significant when exposed to the lower dose tested. The main findings in this study show new possible paths to study effects of plastic-bound chemicals in sponges. Effects on different pinacoderm cells should be investigated to understand differential response of inhalant and exhalant canals to DEHP and the influx of calcium should be analyzed to understand the importance of this ion in contraction pathways and its relation to DEHP. Finally, the recovery of contractions after exposure may be connected to a detoxification mechanism to be discovered and possibly linked to the microbiome activity.

CRediT authorship contribution statement

Liv Ascer: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Emilio Lanna:** Writing – review & editing, Visualization, Validation, Supervision, Funding acquisition, Formal analysis. **Márcio Reis Custódio:** Writing – review & editing, Validation, Supervision, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2025.107268.

Data availability

No data was used for the research described in the article.

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