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Zooplankton studies warn abnormal protrusions on the copepods from coastal environments under anthropic impacts

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A R T I C L E I N F O

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Copepod
Histology
Tissue protrusion

A B S T R A C T

Faced with a global scenario where the etiology of abnormal tissue protrusions in copepods and their ecological consequences are unclear, it is crucial to seek a better understanding about these abnormalities. In this study, supported by images confocal laser microscope, scanning electron microscopy and histological analysis, the levels and distribution of morphological abnormalities in copepods were analysed. The analysis of zooplankton samples from Todos-os-Santos Bay showed the occurrence of two types of abnormalities on the body surfaces of copepods. The images clearly showed that the abnormalities in the prosome were formed between the somites from a rupture of the intersomatic membrane, causing a process of herniation of the internal tissues, with part of the digestive tract projected outside the body. Abnormalities in the urosome observed by scanning electron microscopy showed continuity of the copepod intestine, forming a rectal prolapse. Overall, this study highlights a significant reduction in indices of zooplankton community structure and abnormal tissue protrusions, evidencing a pathological condition in copepods from coastal environments under anthropic impacts.

1. Introduction

Zooplankton play a keystone in the marine environment, being a fundamental link in the marine food web, a major actor in the global carbon cycle and the champion of the ocean largest migration (Ratnarajah et al., 2023).

In the last years, abnormal tissue protrusions in the cephalothorax and/or in the urosome have been observed among planktonic copepods from different parts of the world (Crisafi and Crescenti, 1977; Messick et al., 2004; Skovgaard, 2004; Bhandare and Ingole, 2008; Mantha et al., 2013). These abnormal protrusions usually appear as internal body tissue projections onto the carapace surface (Bridgeman et al., 2000). This irregular condition was first reported in copepods from Lake Michigan (USA), leading to great scientific community interest in the effects on the aquatic food web (Omair et al., 1999; Bridgeman et al., 2000; Messick et al., 2004).

There is no consensus on the primary causes that could trigger abnormal tissue protrusions development among copepods. However, morphological abnormalities in the group have been associated with environmental changes, water pollution, and parasitism (Crisafi, 1974; Crisafi and Crescenti, 1975; Silina and Khudolei, 1994; Vanderploeg et al., 1998; Dias, 1999; Bhandare and Ingole, 2008; Mantha et al., 2013). Indeed, the incidence of endoparasites, such as the dinoflagellate *Blastodinium sp.*, and infections from the ectoparasitic alveolate *Ellobiopsis sp.* can result in copepod tissue protrusions (Bridgeman et al., 2000; Manca et al., 2004; Skovgaard, 2004; Bhandare and Ingole, 2008). Although etiological aspects and ecological impacts remain poorly understood, Bridgeman et al. (2000) noted a decrease in the survival capacity of the affected individual.

Considering the species diversity, the interconnections of the food chains, and the anthropic impacts, ecosystems worldwide may undergo distinct environmental configurations (Ju et al., 2021). And this scenario may explain why the abnormal tissue protrusions of copepods have a high temporal and spatial variation (Omair et al., 1999; Bridgeman et al., 2000; Mantha et al., 2013).

Todos-os-Santos Bay (12°S/38°W) is the second largest navigable

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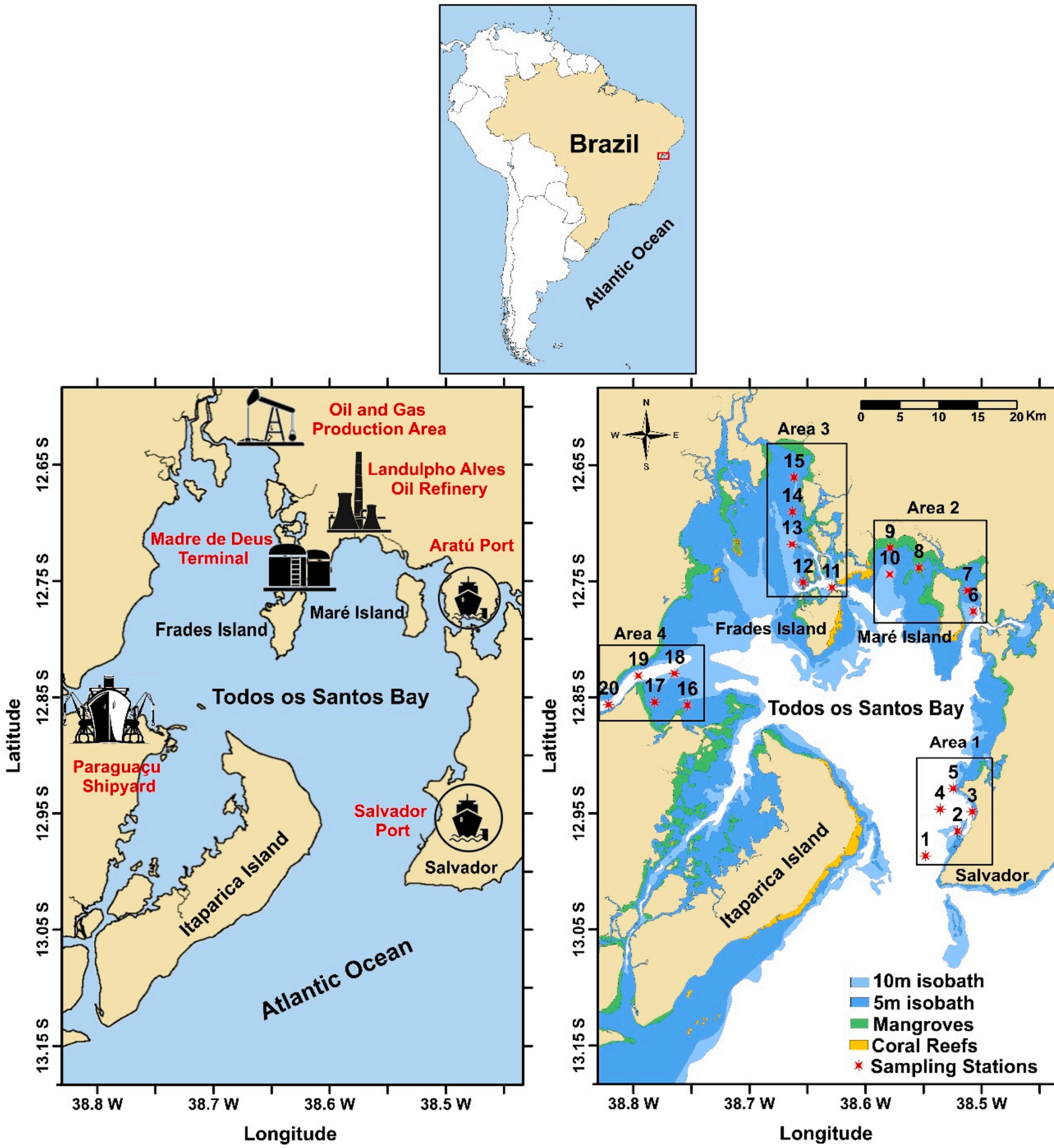

Fig. 1. Locations of sampling stations in the Todos-os-Santos Bay (Bahia, Brazil) and the main anthropogenic activities in the region.

bay on the Brazilian coast, harbouring the most productive marine ecosystems. Beyond the scenic beauty of its pristine environments, the bay has significant socioeconomic relevance – traditional fishing communities rely solely on its natural resources for survival. Furthermore, the bay has an oil refinery complex, public terminals, and commercial port areas, resulting in the daily heavy traffic of international vessels and oil spills.

For this section of the coast, data on the abundance, distribution, and taxonomy of zooplankton are available in the literature (see Maltez et al., 2014; Mafalda et al., 2008), but no studies have hitherto pointed out for abnormal tissue protrusions among planktonic copepods – a condition that brings concern on the impacts that are likely to affect healthy and the development of the organisms that form the bases of the marine food web.

Due to the ecological importance of the studied area and zooplankton populations, the present study had the objectives to examine the zooplankton community structure, as well as, supported by images from a laser confocal microscope, scanning electron microscopy and histological analysis, to evaluate the levels and distribution of abnormal tissue protrusions in copepods, under anthropic impacts influences in Todos-os-Santos Bay.

Table 1
Comparison of environmental variables and community structure of the four areas in the Todos-os-Santos Bay (Bahia, Brazil).

		Area 1	Area 2	Area 3	Area 4	P-value
Environmental variables	Temperature (°C)	25.91 ± 0.04	25.86 ± 0,17	28.24 ± 0,16	29.14 ± 0,47	0.0010
	Salinity (psu)	35.15 ± 0.19	33.91 ± 0.36	35.34 ± 0.35	32.41 ± 3.15	0.0020
	Oxygen (mg.L^{-1})	6.31 ± 0.75	5.23 ± 0.70	4,51 ± 0.21	6.50 ± 0.35	0.0032
	pH	8.18 ± 0.11	8.27 ± 0.15	8.15 ± 0.31	8.11 ± 0.18	0.1274
	Phytoplankton Biovolume (ml.L^{-1})	9.71 ± 2.06	2.79 ± 0.54	1.93 ± 0.98	11.53 ± 2.31	0.0019
Community structure	Zooplankton Density	130.49 ± 5.59	90.13 ± 4.49	69.35 ± 2.56	230.62 ± 366.63	0.0061
	Shannon-Wiener index	2.91 ± 0.23	2.51 ± 0.11	2.44 ± 0.15	3.04 ± 0.23	0.0085
	Pielou evenness index	0.79 ± 0.06	0.69 ± 0.03	0.76 ± 0.04	0.79 ± 0.07	0.1338
	Taxon number	45 ± 4.27	31 ± 7.13	26 ± 4.12	48 ± 3.77	0.0031

Values are median ± standard deviation. Differences among the Areas were tested by Kruskal Wallis test

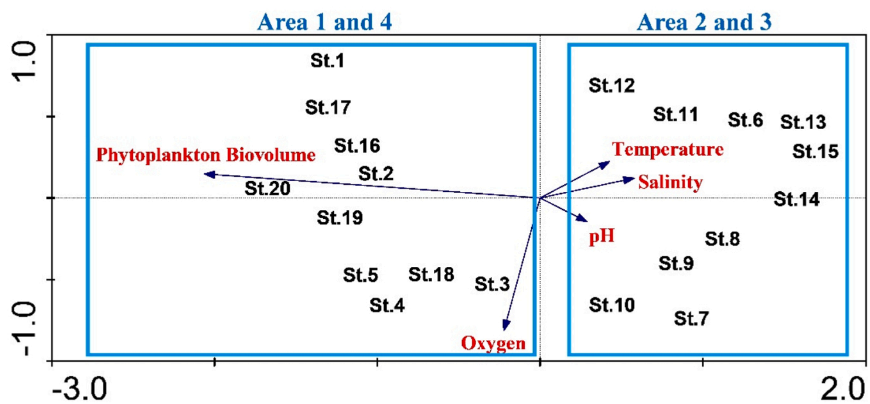

Fig. 2. PCA biplot for relationships between environmental variables (Temperature, Salinity, pH, Oxygen and Phytoplankton biovolume) and all sampling stations in the first two axis.

2. Materials and methods

2.1. Study area

Todos-os-Santos Bay (TSB) (12°35'30″–13°07'30″ S and 38°29'00″–38°48'00″ W) is located on a tropical coastline with a humid climate (Lessa et al., 2018) (Fig. 1). It has 1112 km^2 of total area, being considered the second largest bay in Brazil (Hatje and Andrade, 2009). The TSB has several pristine, well-preserved ecosystems, including coral reefs, mangroves and estuaries, being a source of livelihood for the local communities. Despite of its ecological and socio-economic relevance, this section of the Bahia littoral has been intensively used for commercial traffic of international vessels, and gas and oil exploration, being all activities sources of PAHs contamination along the last seven decades (e. g., ports of Salvador and Aratú, Paraguaçú shipyard, Madre de Deus Terminal and the Landhulpho Alves Petroleum Refinery – founded in the early 50 s) (Venturini et al., 2004; Nascimento et al., 2017). Furthermore, the TSB have been impacted by discharges of domestic sewage (Wagener et al., 2010). The strong tourist and anthropic activity, and rapid urbanization bring many threats and it is important to develop studies aimed at interpreting the functioning of biological communities throughout the entire bay.

2.2. Sampling

Plankton samples were collected from 4 areas in Todos-os-Santos Bay (Fig. 1), each area containing 5 sampling stations. All stations were located in areas with anthropic activities. Zooplankton and phytoplankton were collected through horizontal subsurface trawls with a conical plankton net (300 μm mesh and 50 μm mesh, respectively), during five minutes at a speed of 2 to 3 knots, with a flowmeter coupled to the mouth of the net, to obtain the volume of filtered water. The samples were preserved in 4% formalin solution (with sea water).

2.3. Plankton processing

In the laboratory, the samples were subjected to sorting, identification, counting and data processing. To zooplankton processing, one aliquots of 20 ml of the samples were analysed using a stereomicroscope (Nikon SMZ 745 T). Taxa identification was carried out at the lowest possible taxonomic level supported by the specialized zooplankton literature (Boltovskoy, 2005; Bonecker, 2006; Neumann-Leitão et al., 2017). Data were represented as the number of individuals per cubic meter (ind. m^{-3}).

During sorting, copepods that showed abnormal protrusions were separated. These copepods were then analysed and photographed using a stereomicroscope (Nikon SMZ 745 T) equipped with an INFINITY3–1UR camera using an image analysis system (INFINITY ANALYZE Software v6.1.0 Release).

2.4. Scanning electron microscopy (SEM)

For SEM image analyses, the copepods were prepared as the following four-step protocol: (1) hydration in distilled water (2) rinsing in a buffer solution of sodium cacodylate 0,1 M (3 baths during 10 min each), (3) dehydration in ethanol graded series (30%, 50%, 70%, 90%, 100%) (10 min each) and (4) critical point-dried. Then, the copepods were mounted on aluminium pin stubs, and coated with a thin layer of gold, 10/20 Å, for 2 min. Images were captured using the JOEL JSM-6390LV equipment (Oswaldo Cruz Foundation, FIOCRUZ-BA).

2.5. Histological analysis with historesin

The histological protocol was based on Kmecick et al. (2023), with modifications, and the sections for light microscope were stained with Mallory's triple stain (Neves and Silveira, 2003). The copepods were dehydrated in ethanol graded series (70%, 80%, 90% and 100%)

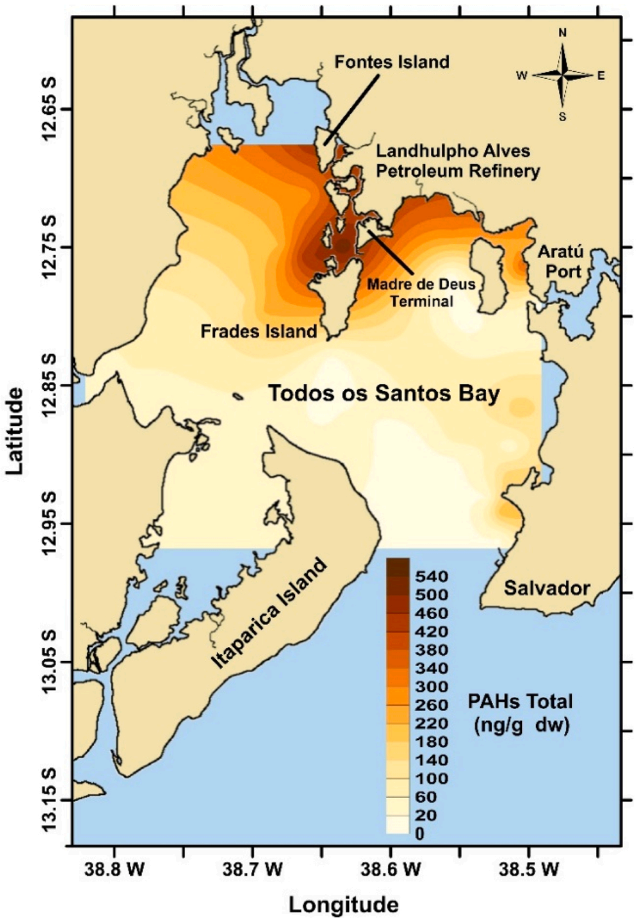

Fig. 3. Distribution of PAHs total (ng/g dw) concentrations in the surface sediments in the Todos-os-Santos Bay (Bahia, Brazil).
(Modified from Almeida et al., 2018).

(30 min each). Then, they were immersed in a pre-infiltration solution (100% ethanol plus activated resin, 1:1) (30 min), being transferred into an infiltration solution (activated resin) and stored at 4 ∘C per 12 h, at least.

For the historesin embedding, each copepod was placed in 0.4 ml of inclusion solution (activated resin and hardening compound, 15:1), laid down in polyethylene molds (6 mm x 8 mm) to obtain longitudinal sections, being, as long as necessary, transferred into an oven (at about 45ºC) for hardening. To avoid humidity, the resin blocks were removed from the molds and stored in a container with silica.

Histological sections, 4 - 5 μm in thickness, were obtained with support of a semi-automatic microtome (LEICA, HistoCore Multicut). The stained slides were analysed under a light microscope (ZEISS Axio Scope A1 supplied with a Axioncam 305 color), while non-stained slides were examined with a laser confocal microscope (ZEISS LSM 900). Confocal images were acquired with fluorescence contrast method, 0.89 AU/35 μm pinhole and 561 nm laser wavelength.

2.6. Environmental data

Oxygen (mg. L^{-1}), pH, temperature (°C) and salinity (ppt) were determined using multiparameter meters (AKSO, Model AK88). Samples collected from the phytoplankton trawl were used to measure phytoplankton biovolume (ml. L^{-1}). A set of environmental variables was compared among sampled areas, to explore the possibility that variations in zooplankton assemblage structure may have varied in response to natural environmental variations.

2.7. Data analysis

Non-parametric Kruskal Wallis test with a confidence level of 95% was used to evaluate variations in terms of environmental variables (temperature, salinity, oxygen, pH, phytoplankton biovolume) and community structure (taxa number, zooplankton density, Shannon-Wiener index, Pielou uniformity index), among sampled areas. Zooplankton density data were log(x + 1) transformed before analysis to reduce the weight of dominant taxa relative to rare ones. Differences were considered significant at $p < 0.05$. Non-parametric Kruskal Wallis test by program Biostat version 5.3.

Principal Component Analysis (PCA) was used to explore the spatial trends of environmental variables (temperature, salinity, pH, oxygen and phytoplanktonic biovolume) in relation to the sampled areas. Analysis was employed for this study using the CANOCO program, version 4.5.

Similarity Analysis (ANOSIM) was used to verify possible differences in the composition of zooplankton assemblages among the sampled areas. To assess the strength of the differences, R statistics were used, on a scale of − 1 to 1, value 0 indicating completely random grouping (Clarke, 1993). The taxa responsible for differences in assemblage composition were identified using Contribution to Dissimilarity Analyses (SIMPER). Analyses ANOSIM and SIMPER was performed by Paleontological Statistics Software (*PAST*), version 3.25.

3. Results and discussion

3.1. Environmental conditions

The Todos-os-Santos Bay (TSB) was characterized by a coastal water mass of tropical origin, with temperature varying between 25.67 and 29.82 °C, and salinity varying between 27.12 and 35.85 PSU. The lowest temperatures were recorded at stations 8, 9 and 10 (Area 2), and the highest at stations 16, 18, 20 (Area 4). Sampling in area 2 was carried out in July 2022, while in area 4 it was carried out in December 2022. According to Lessa et al. (2018), the maximum temperatures in the TSB are observed between December and March, around 30 °C, and the minimum temperatures occur in the months of July, August and September, between 21 °C and 22 °C. The lowest salinity value (27.12 PSU) was observed at station 20, located on the Paraguaçu River. The TSB receives discharge from a drainage area corresponding to 61, 110 km^2, of which 92.1% (56,300 km^2) are associated with the river (Lima and Lessa, 2002).

The non-parametric Kruskal Wallis test showed that the environmental variables in TSB (temperature, salinity, oxygen, phytoplankton biovolume) differed significantly among the sampled areas ($p < 0.05$) (Table 1).

Principal Component Analysis (PCA) revealed difference between areas 1 and 4 and areas 2 and 3 (Fig. 2). The stations in areas 1 and 4 (Salvador Port and Paraguaçu estuary) were mainly characterized by higher values of phytoplankton biovolume, while the stations in areas 2 and 3 (Landhulpho Alves Petroleum Refinery, Madre de Deus Terminal, Aratú Port) were characterized by low values of oxygen and phytoplankton biovolume.

These results reflected spatial changes in environmental conditions among areas, demonstrating that areas 2 and 3 seem to show greater action of anthropic influence. In the study by Mafalda et al. (2008), they observed that the area adjacent to Madre de Deus had low values of chlorophyll a and high values of oil and greases.

The total PAHs concentrations in the surface sediments of the Todos-os-Santos Bay increase towards the shores of the bay, where the Landhulfo Alves Petroleum Refinery, Madre de Deus Terminal and Aratú Port are located, which carry out various anthropic activities and are sources of PAHs. Values of the total PAHs in the TSB range from below the limit of detection of the method (<DL) to 540 ng/g (dry weight) (Fig. 3) (Almeida et al., 2018).

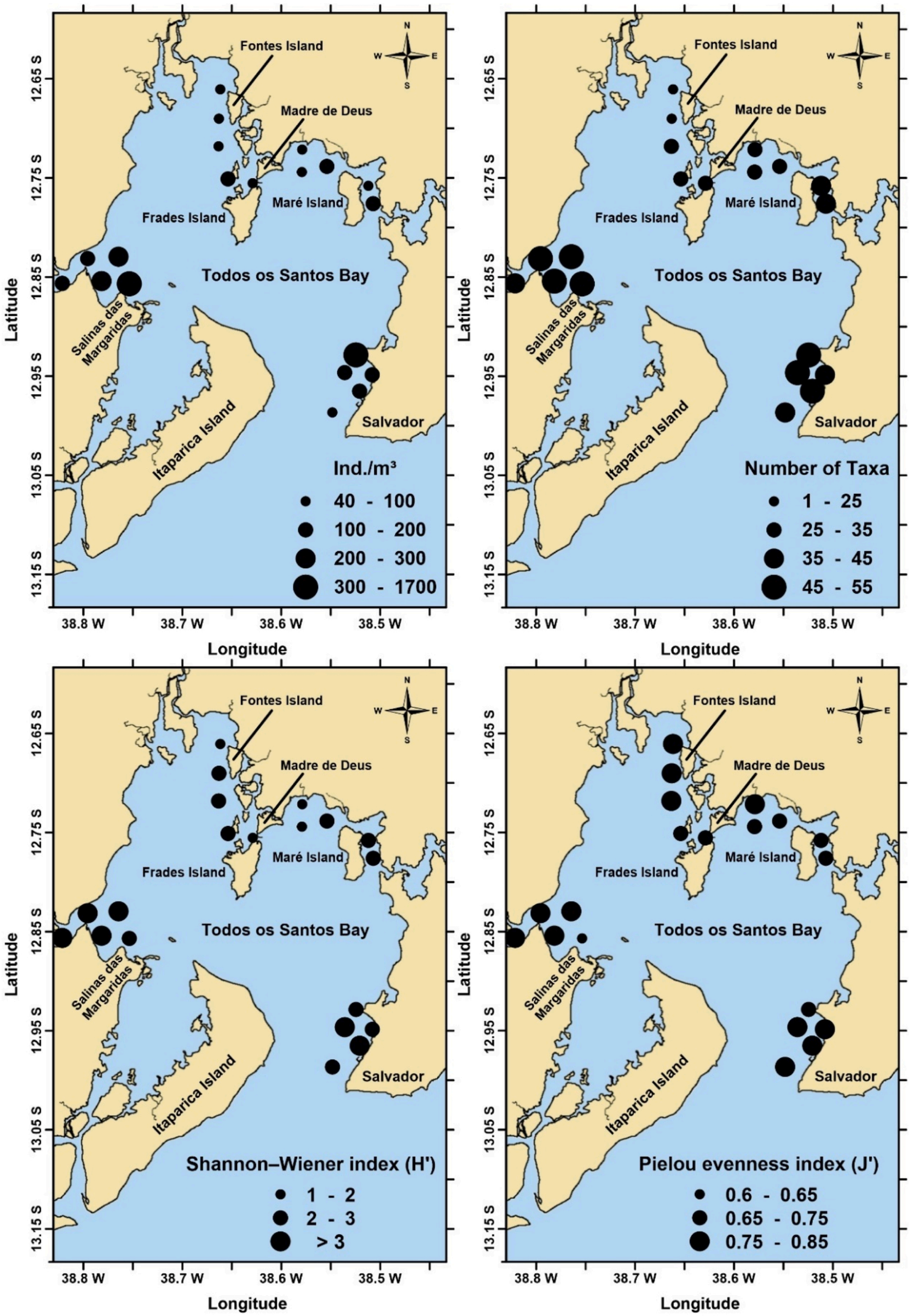

Fig. 4. Spatial variation of zooplankton density (ind/m^3), Number of taxa, Shannon–Wiener index and Pielou evenness index in the Todos-os-Santos Bay (Bahia, Brazil).

Table 2
Analyses of similarity (ANOSIM) and Analyses of Contribution to the Dissimilarity (SIMPER) comparing the composition of zooplankton community observed in the areas in the Todos-os-Santos Bay (Bahia, Brazil).

	ANOSIM		SIMPER	
	R	*p*	Taxa	Contrib (%)
Areas	0,1597	0,0132	*Paracalanus sp.*	44,46
			Paracalanus cf *parvus*	23,97
			Paracalanus quasimodo	14,62
			Paracalanus aculeatus	6845
			Temora turbinata	2981
			Centropages velificatus	2632
			Centropages tipicus	1895
			Farranula gracilis	1232

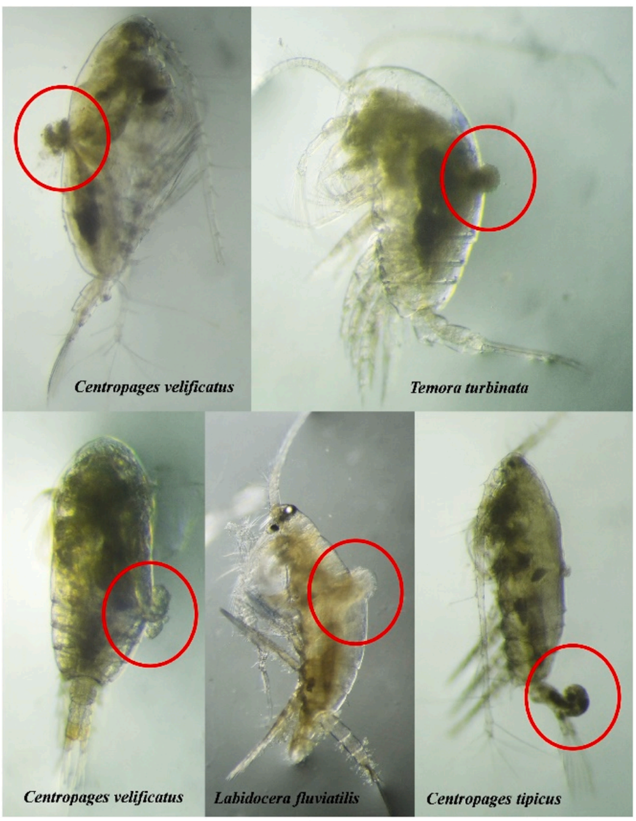

Fig. 5. Abnormal protrusions observed in the prosome and urosome of copepods (red circle) in the in the Todos-os-Santos Bay (Bahia, Brazil).

3.2. Community structure of zooplankton

Community structure indices (total density, number of the taxa and Shannon-Wiener index) showed a similar spatial variability (Fig. 4), the highest values were observed in areas 1 and 4 (Area 4 characterized by high values of phytoplankton biovolume) and the lowest values were observed in areas 2 and 3 (characterized by high total PAHs concentrations in the sediments, low values of oxygen and phytoplankton biovolume). Significant difference was observed between the sampled areas ($p < 0.05$) (Table 1). The non-parametric Kruskal Wallis test showed that Pielou evenness index did not differ significantly among the sampled areas ($p > 0.05$) (Table 1).

Coastal zones are areas affected by a variety of oceanographic processes, and these processes are influenced by several anthropogenic impacts related to continental sources (Conceição et al., 2021; Thirunavukkarasu et al., 2020). The degree of water pollution influences the structure of planktonic communities, in addition, different predictors may be interrelated (Souza et al., 2023).

The species composition showed a significant difference between the sampled areas. The highest copepod densities were observed in areas 1 and 3, mainly species of the genus *Paracalanus* (size: 0.5 – 1.5 mm) that had densities above 300 ind.m^{-3} in area 3 (Paraguaçú estuary).

Areas 2 and 3 were associated with a smaller number of copepod species such as *Temora turbinata*, *Centropages velifcatus*, *Centropages tipicus* and *Labidocera fuviatilis*, species that vary in size from medium to large (size: 2.5 – 4.5 mm). Similarity analysis (ANOSIM) confirmed significant variations ($p < 0.05$) in the composition of the zooplankton community between the sampled areas (Table 2). Analyzes of Contribution to the Dissimilarity (SIMPER) showed that four species of the genus Paracalanus (Paracalanus sp., Paracalanus cf parvus, Paracalanus quasimodo, Paracalanus aculeatus) were responsible for the observed differences. These species contributed 89.9%. differences observed in the structure of the zooplankton community between the areas studied (Table 2).

Zooplankton composition can vary significantly based on several factors, including geographic location, seasonality, water temperature, nutrient availability and anthropic influence (Souza et al., 2005; Souza et al., 2022). Different species have adapted to specific environmental conditions and ecosystems (Rezai et al., 2005).

Studies have shown that anthropic influences (such as the impact caused by oil spills and heavy metals) promote an immediate loss of diversity and peak abundance of some species, characterized as opportunistic (Fernandes et al., 2020; Souza et al., 2023).

The stability of zooplankton community structure depends on species diversity, reduction in plankton species diversity can lead to a decline in community stability and functions in marine ecosystems (Neumann Leitão et al., 2019).

3.3. Abnormal protrusions on the copepods

The analysis of zooplankton samples showed the occurrence of abnormal protrusions in copepods. We observed two types of abnormalities on the body surfaces of copepods: abnormality in the prosome (inter somital regions between the last prosome and the first metasomal segment), which corroborated various earlier reports (Al-Aidaroos and Mantha, 2018; Messick et al., 2004; Manca et al., 2004) and abnormality in the urosome (also observed by Crisafi, 1974; Dias, 1999; Jagadeesan and Jyothibabu, 2016; Souissi and Souissi, 2020) (Fig. 5).

The abnormal protrusions observed in the prosoma were classified into 3 levels of abnormality (Fig. 6). At level 1 of abnormality, the organism presents the intersomial region with a darker color (as if the region had some type of lesion) but without hermination of internal tissues. At level 2, the organism presents hermination of internal tissues and at level 3, it is possible to observe the projection of internal structures (such as the digestive tract) outside the body (Fig. 6). Al-Aidaroos and Mantha (2018) observed different levels and variations of abnormal protrusions, which exhibited various morphological structures such as, round/oval, transparent and non-granular structure and round, dark and granular structure.

Abnormal protrusions showed to occur on the dorsal, lateral, and ventral surfaces of the body, being more frequent on the dorsal surface of the body. In the first report of abnormal protrusions on the copepods in the Jeddah Coast, Central Red Sea, Al-Aidaroos and Mantha (2018) observed that abnormalities were more frequent in the dorsal region than in the ventral region of copepods. In the Clyde Sea area, Jepps (1937) observed this same pattern.

Abnormal protrusion in urosome was recorded mainly in *Centropages tipicus*. These abnormalities showed a round and dark morphological structure. Souissi and Souissi (2020) observed protuberance in the last somite of the urosome of ovigerous females (*Eurytemora affinis*) from field samples, however no abnormality was observed in the prosome.

According to the study carried out in Indian marine waters by

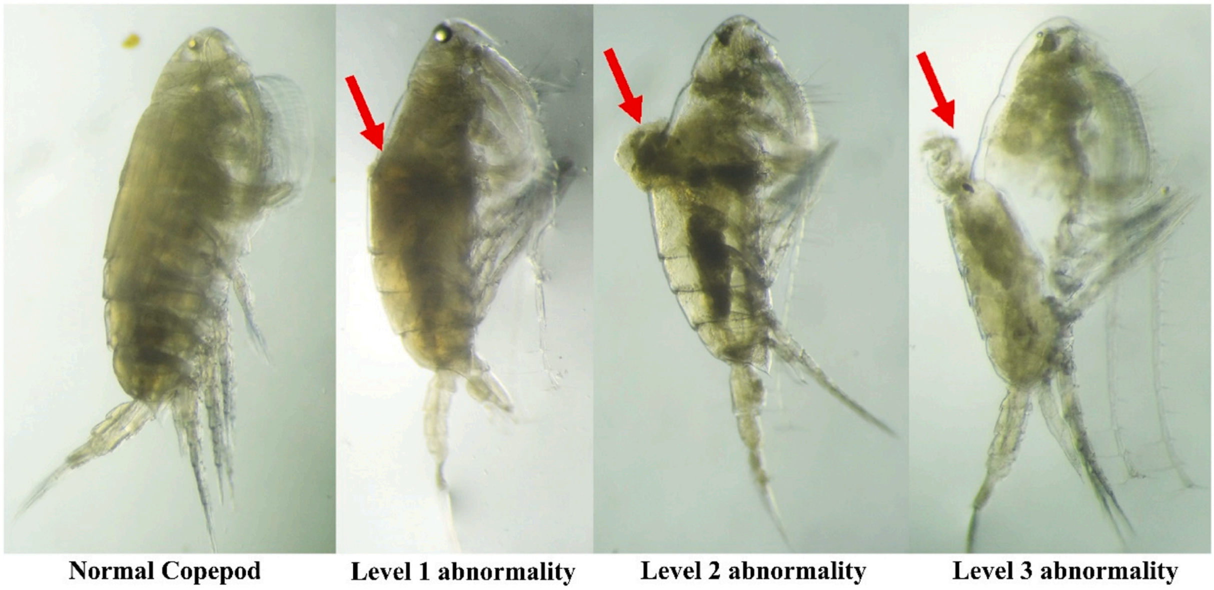

Fig. 6. Classification of levels of abnormal protrusions observed in copepods (red arrow) in the in the Todos-os-Santos Bay (Bahia, Brazil).

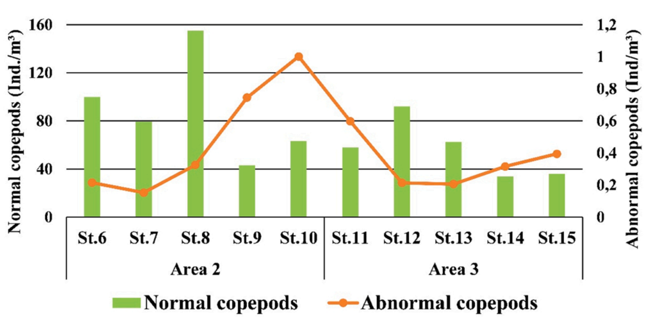

Fig. 7. Variation of Normal and Abnormal copepods density (ind/m^3) in the 2 and 3 areas in the Todos-os-Santos Bay (Bahia, Brazil).

Jagadeesan and Jyothibabu (2016), occurrence of urosome abnormalities was observed in 10 to 30% of copepods. These abnormalities varied in appearance (dark granular, multi-lobed, muff shaped with a stalk, small and slightly curled, transparent and elongated and stalk-like extension) and was recorded mostly in *Acartia erythraea*, *Acartia danae*, *Temora turbinata*, *Paracalanus parvus* and *Pseudodiaptomus serricaudatus*.

All abnormalities were observed in adult copepods, no protrusions were recorded in copepodites and nauplius. This result corroborates Al-Aidaroos and Mantha (2018) who found a significantly higher prevalence of abnormalities in adult copepods than copepodites or nauplii. However, Messick et al. (2004) found an opposite result, with protrusions occurring more frequently in nauplius than copepodites or adults.

Protrusions were observed in areas 2 and 3 and the incidences of these protrusions varied between sampling stations (Fig. 7). The highest densities of copepods with protrusions were observed at stations 9, 10 and 11, which are under greater influence from the oil refinery and Madre de Deus terminal.

In general, the stations that had lower normal copepod density values had higher abnormal copepod density values, suggesting an increase in mortality of organisms due to the abnormality (Fig. 7). Several studies have reported a reduction in the swimming efficiency of copepods due to the presence of abnormal protrusions (Bridgeman et al., 2000; Souissi et al., 2013; Jagadeesan and Jyothibabu, 2016), which may increase the copepod vulnerability to predation by other organisms,

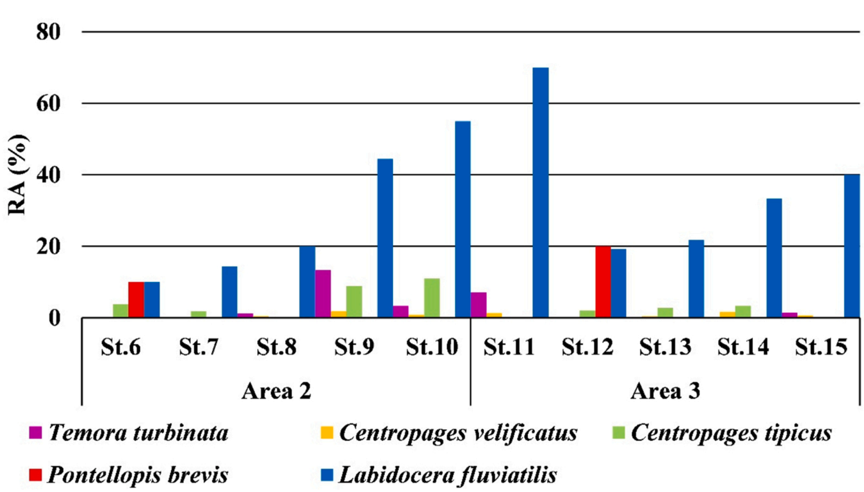

Fig. 8. Variations in the percentage of Abnormal Protrusions incidences in copepods species in the 2 and 3 areas in the Todos-os-Santos Bay (Bahia, Brazil).

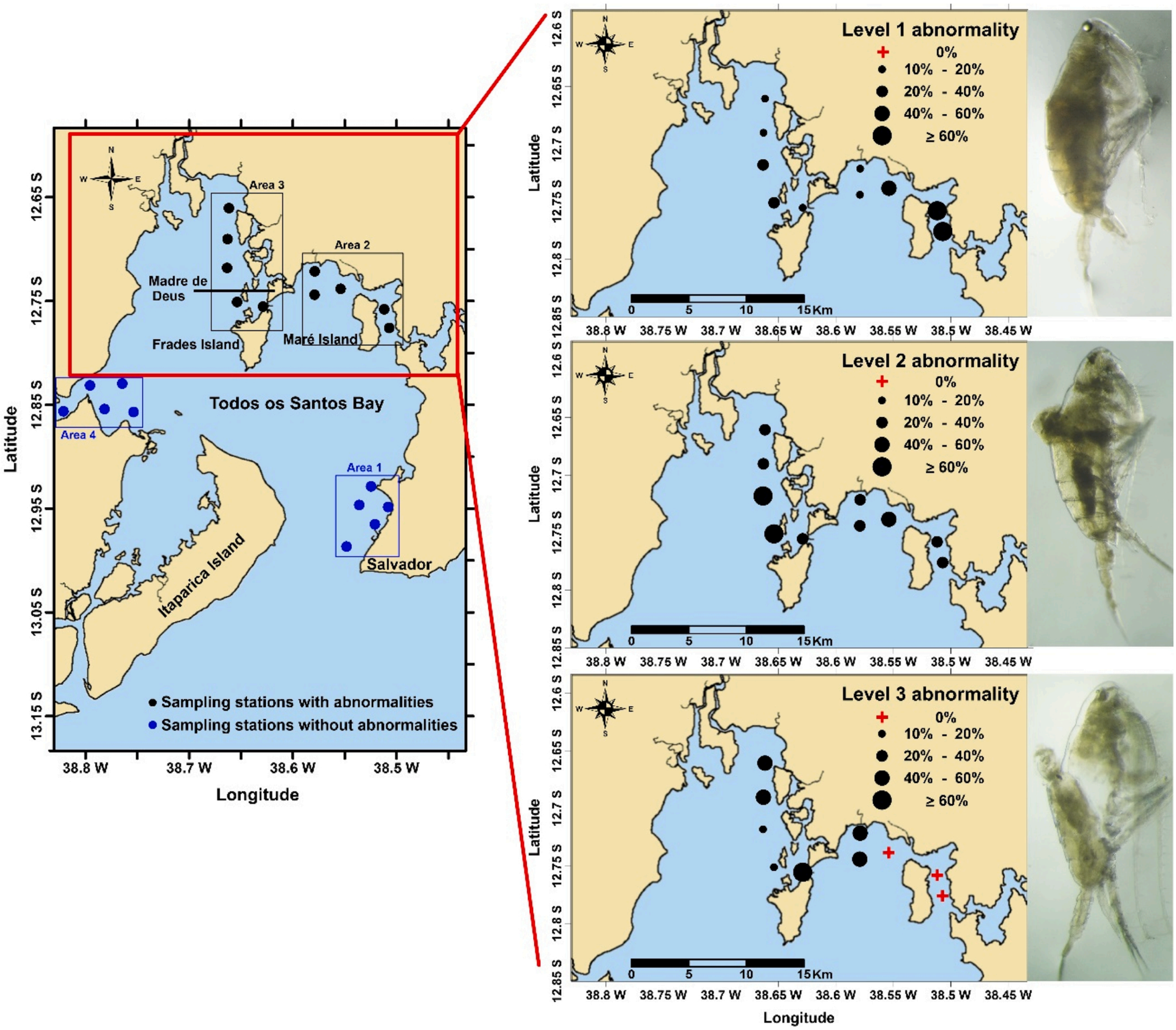

Fig. 9. Percentage of abnormalities levels in copepods in the 2 and 3 areas in the Todos-os-Santos Bay (Bahia, Brazil).

decreasing copepod life expectancy. Reduced swimming efficiency can also affect vertical migration (Souissi and Souissi, 2020).

Abnormalities were observed in five species of copepods (*Temora turbinata*, *Pontellopis brevis*, *Centropages velificatus*, *Centropages tipicus* and *Labidocera fluviatilis*), All belonged to the order Calanoida (Fig. 8).

Species of the genus *Paracalanus* were dominant in the study area, however, *Labidocera fluviatilis* was the species that showed the highest percentage of organisms with abnormal protrusions (Fig. 8). At stations 10 and 11 more than 50% of *Labidocera fluviatilis* showed abnormal protrusions (Fig. 8).

Abnormal protrusions can affect 50%– 70% of copepods of certain species, the calanoids have been shown to be especially vulnerable to this abnormality that present temporal and spatial variations (Bridgeman et al., 2000; Mantha et al., 2013). Omair et al. (2001) observed protrusions on 46% of the 28 common species of zooplankton and on 53% of the 32 taxa collected from the Great Lakes and contiguous water.

Abnormalities observed by Al-Aidaroos and Mantha (2018) were more frequent in dominant copepods in the coastal waters of the Saudi Red Sea, particularly among Calanoida. Most prominent abnormalities were observed on calanoid, *Labidocera sp*.

Level 1 abnormalities dominated at stations 6, 7 and 8 in area 2 and these stations did not show level 3 abnormalities. Organisms with higher levels of abnormalities were associated with stations 9 and 10 in area 2 and stations 11, 14 and 15 in area 3. These results demonstrate that at stations 9, 10, 11, 14 and 15 the organisms present a higher degree of degradation, and station 11, located in the Madre de Deus channel, presented more than 60% of copepods with protrusions with level 3 abnormalities (Fig. 9).

3.4. Scanning electron microscopy

Fourteen organisms of the species *Labidocera fluviatilis* with abnormality in the prosome and ten organisms of the species *Centropages tipicus* with abnormality in the urosome were studied by scanning electron microscopy (SEM) (Figs. 10 and 11).

In the images with reflected illumination (Fig. 11), it is possible to observe the presence of a protuberance formed by light tissues (translucent) (blue arrow) with a darker structure in the center (red arrow). The lighter tissue appears to be part of the longitudinal musculature projecting out of the body while the darker central part appears to have a

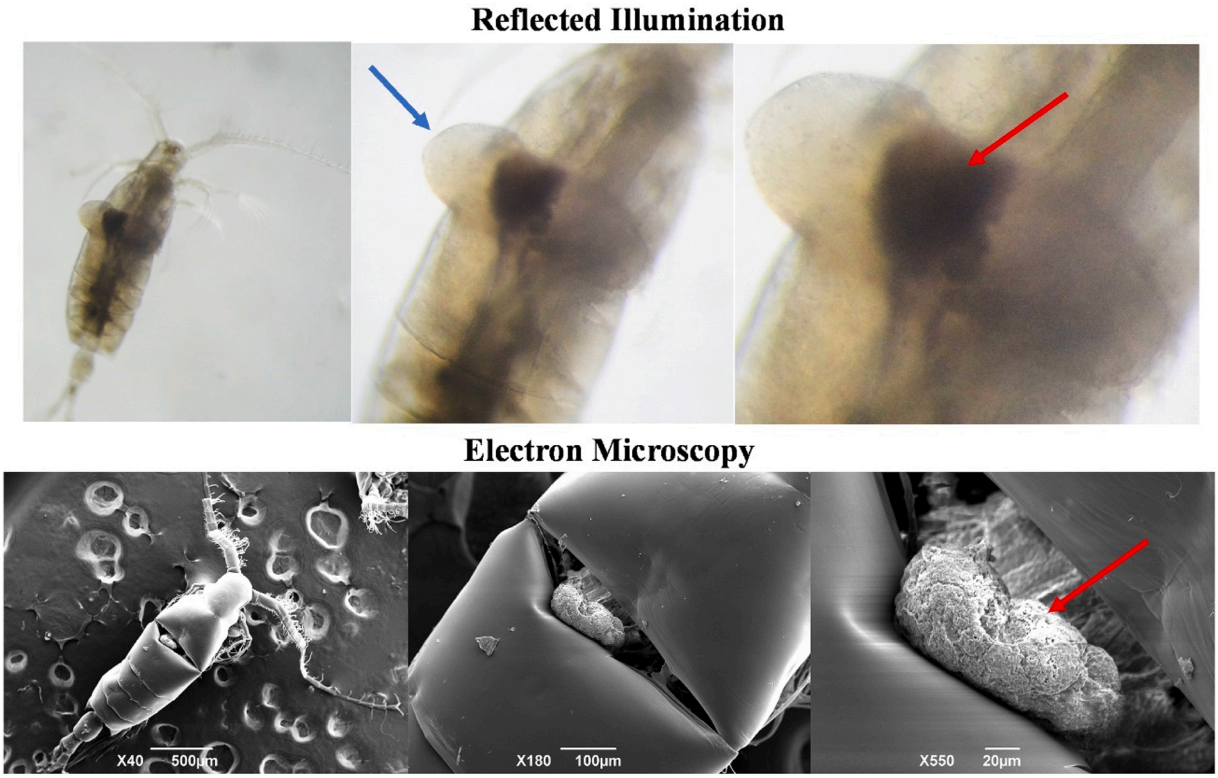

Fig. 10. Images of abnormal protrusions on the prosome of *Labidocera fluviatilis* observed under reflected illumination and electron microscopy.

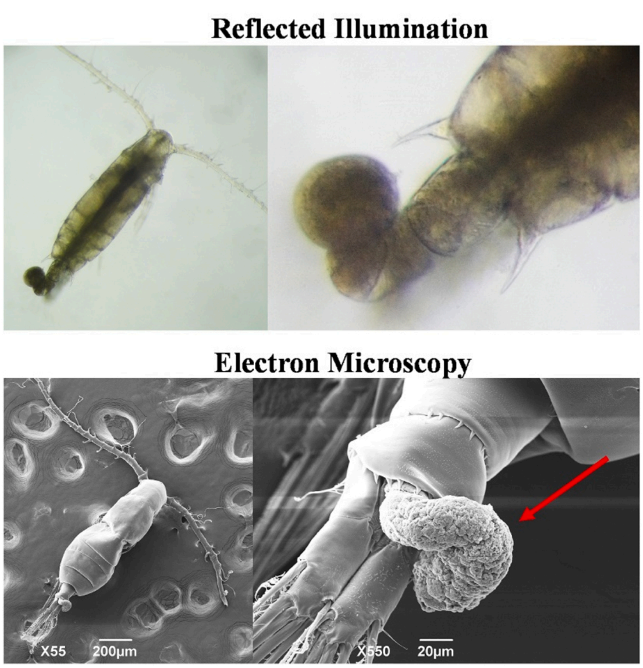

Fig. 11. Images of abnormal protrusions on the urosome of *Centropages tipicus* observed under reflected illumination and electron microscopy.

connection with the digestive tract of organism.

In the SEM images (Fig. 10) it is possible to observe that part of the digestive tract (red arrow) arises from a fissure in the membrane that connects the somite's exoskeleton. In this SEM image, it is not possible to observe the muscle tissue, this absence of muscle tissue may be related to the dehydration and drying process performed before the SEM, during the critical point process there seems to be a retraction of the softer tissues (muscle tissue).

According to Skovgaard (2004), Jagadeesan and Jyothibabu (2016), parasites or external predators can cause localized lesions and perforations in the organism's carapace that can cause a process of internal tissues herniation. Although this theory has some plausibility, our observations with scanning electron microscopy demonstrated that these abnormalities, observed in copepods in Todos-os-Santos Bay, were formed between the somites from a rupture of the intersomatic membrane and not from a perforation in the carapace. Omair et al. (2001) studying protrusion in *Epischura lacustrine* by scanning electron microscopy also observed that the protrusion arises from a fissure in the membrane that connects the hard plates to each other. For Messick et al. (2004), it is unlikely that these protrusions are due to fixation artifacts or caused by diatoms puncturing copepods in the collection devices.

According to Messick et al. (2004), intersomite regions are more vulnerable to rupture than other body parts of copepods. Omair et al. (2001) observed that the membranous intersomal region ruptures allowing the development of a hernia protrusion and this intersomatic membrane is not part of the hermination.

In Fig. 11, the protrusion in the urosome shown by SEM has a rough surface, which, at higher magnification, shows continuity of the affected copepods intestine (red arrow). Crisafi (1974), studying some responses of planktonic organisms to environmental pollution, observed intestinal prolapse induced by water pollution. Other authors observed that copepods infected by endoparasites carry structures in their anus, through which parasite gonospores are expelled (Skovgaard and Daugbjerg, 2008). However, in the present study, these structures were not detected.

In Indian marine waters, Jagadeesan and Jyothibabu (2016) observed anal protrusions in several species of copepods (*Acartia erythraea, Acartia danae, Temora turbinata, Paracalanus parvus, Pseudodiaptomus serricaudatus*) and all these protrusions showed continuity with the digestive tract of the affected copepod.

In Espírito Santo Bay (Brazil), about 23% of copepods of the species *Acartia lilljeborgi* presented different degrees of intestinal prolapse at

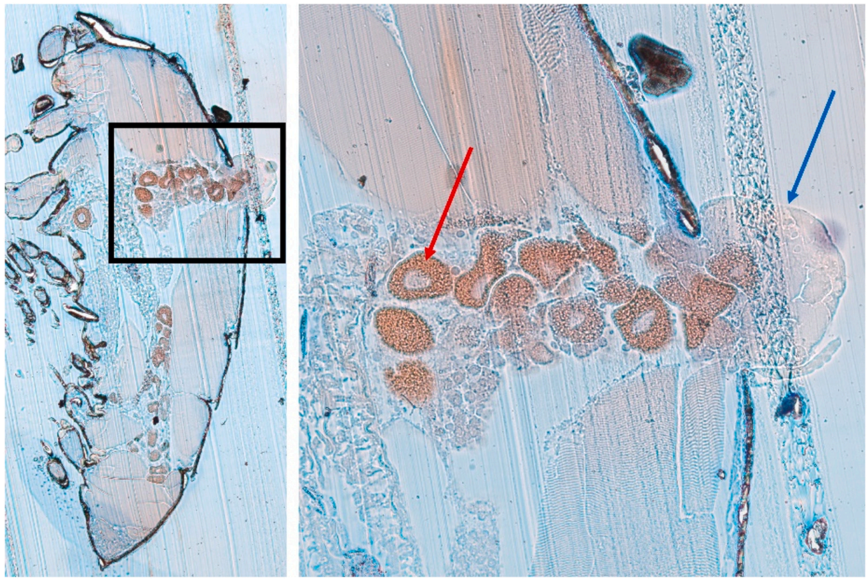

Fig. 12. Longitudinal histological sections of a copepod (*Labidocera fluviatilis*, female) stained with Acid Fuchsin and Mallory's solution.

different stages of development (Dias, 1999). According to Dias (1999), this morphological abnormality may be related to the stress caused by industrial and domestic waste pollution in the bay.

Rectal prolapse in copepods is a condition in which the rectum of a copepod protrudes or extends outside its body. This abnormality is a relatively rare occurrence and demonstrates a weakening of the rectal muscles. Rectal prolapse can have negative consequences for copepods, as it can hinder their ability to feed, reproduce, or perform other essential functions. It can also increase their vulnerability to predation or other environmental pressures.

3.5. Histological sections

Twenty organisms (*Labidocera fluviatilis*) were processed to obtain histological sections. Longitudinal histological sections were performed to better demonstrate the characteristics of copepod protrusion.

Microscopic analysis of the histological section of a female *Labidocera fluviatilis* revealed that the herniated protrusion leaving the organism seems to be delimited by a thin membrane that is in continuity with the internal membrane that covers the ovary (Fig. 12, blue arrow) and it is possible to observe large ovarian cells toward the point of extrusion. (Fig. 12, red arrow).

Omair et al. (2001) analyzing a sagittal histological section of *Epischura lacustrine* also observed that the protrusion was delimited by a membrane that seemed to be in continuity with an internal membrane on the surface of the ovary.

In Fig. 13, it is possible to observe through analysis in laser confocal microscope, that the intersomatic membrane was ruptured and the protrusions are composed of the same tissue observed inside the organism, part of the longitudinal musculature is projected outside the body, however, this herminated tissue does not have the same clarity of the internal tissue, presenting a hyaline colouring which indicates apparently degraded or necrotic tissue (Fig. 13, blue arrow).

Other histological studies in copepods also concluded that the protrusions are composed of apparently necrotic or degenerating tissues (Omair et al., 2001; Messick et al., 2004). Tissue necrosis can be caused by loss or restriction of blood supply to the tissue, exposure to the external environment, injury due to disease or other types of local injury (Messick et al., 2004).

It is also possible to observe in Fig. 13, that there is a canalization of the digestive tract between the protrusion and the copepod body, with part of the digestive tract projected outside the body (Fig. 13, red arrow). These results were also observed by Dias (1999) in the Espirito Santo Bay (Brazil), Jagadesan and Jyothibabu (2016) in the Indian marine waters and Skovgaard (2004) in the North west Mediterranean.

Abnormal protrusion in copepods is a significant problem that has been observed in various aquatic ecosystems around the world. Although the exact etiology is not confirmed, studies have shown that this abnormality is more prevalent in areas with high levels of pollution, such as in urbanized coastal areas (Vanderploeg, 1998; Dias, 1999), and in areas where there have been significant changes in water temperature or salinity (Crisafi and Crescenti, 1977). Additionally, some studies have suggested that parasitism can also be a contributing factor to abnormal protrusion in copepods (Bridgeman et al., 2000; Skovgaard, 2004).

Mantha et al. (2013) believe the potentially high levels of toxic minerals emerging from hydrothermal vents on Kueishantao Island, Taiwan weaken the exoskeleton of these crustaceans and thus make them more susceptible to infection. Other studies have observed that shallower environments have higher percentages of abnormalities compared to deeper waters (Silina and Khudolei, 1994).

In our study, morphological abnormalities were observed in copepods sampled in areas impacted by petrochemical activities in Baía de Todos-os-Santos. The northeast of the TSB, where Landhulpho Alves Petroleum Refinery and Madre de Deus Terminal are located, is indicated by several authors (Venturini et al., 2004; Nascimento et al., 2017; Wagener et al., 2010) with a high degree of contamination. Low values of oxygen and phytoplankton biovolume were also recorded in this area, which may indicate greater action of anthropic impacts.

The impacts of abnormal protrusion in copepods can be significant. The deformity can make the copepod more vulnerable to predation, reducing the population size and affecting the food web dynamics in aquatic ecosystems (Souissi and Souissi, 2020). Furthermore, the reduced swimming ability of copepods with this abnormality can result in reduced nutrient cycling and decreased primary productivity, ultimately affecting the overall health and resilience of the ecosystem (Al-Aidaroos and Mantha, 2018).

4. Conclusion

It has been demonstrated a significant reduction in the zooplankton community structure indices in the Northeast area of TSB (areas 2 and 3), and in these same areas, the occurrence of abnormal tissue protrusions was observed majorly among Calanoida copepods.

Analysis based on histology and images from SEM and laser confocal

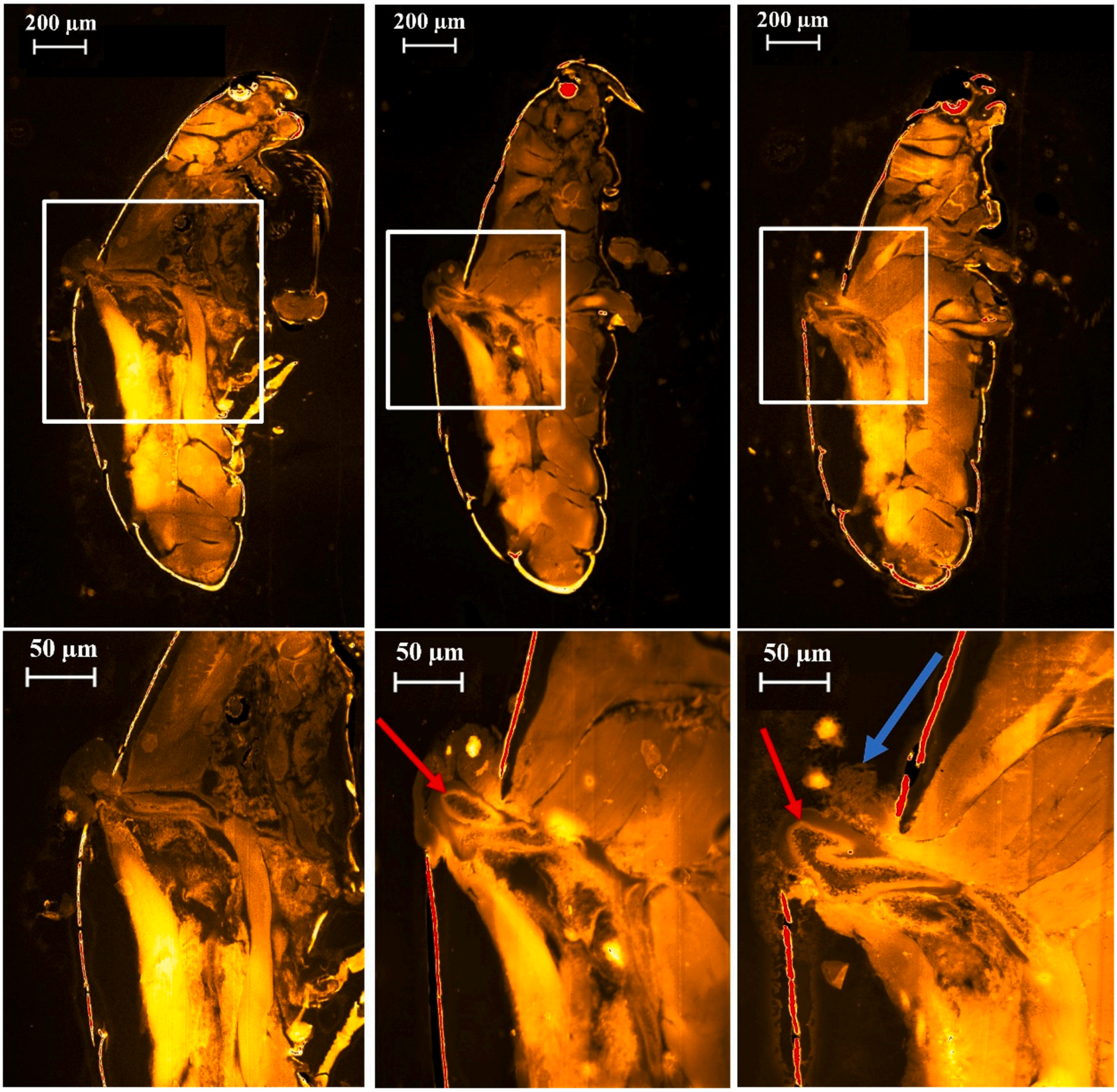

Fig. 13. Longitudinal histological sections of a copepod (*Labidocera fluviatilis*, male) analyzed in laser confocal microscope.

microscopy provided a better understanding of the irregular tissue projections, evidencing a pathological condition – which has never been described to the zooplankton from the Todos-os-Santos Bay.

The occurrence of abnormal protrusion in copepods can be used as an indicator of environmental stress in aquatic ecosystems. It is essential to monitor the occurrence and frequency of abnormal protrusion in copepods and identify potential stressors and disturbances in aquatic ecosystems. Management strategies, such as reducing pollution and controlling water temperature and salinity, can help mitigate the impact of environmental stressors on copepods.

CRediT authorship contribution statement

Souza Christiane Sampaio: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Farias Amilcar:** Formal analysis, Methodology, Writing – review & editing. **Johnsson Rodrigo:** Funding acquisition, Project administration, Resources, Writing – review & editing. **Mafalda Júnior Paulo:** Formal analysis, Methodology, Writing – review & editing. **Neumann-Leitão Sigrid:** Formal analysis, Methodology, Writing – review & editing. **Neves Elizabeth:** Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

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.

Data Availability

Data will be made available on request.

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