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ECOSYSTEMS

Morphological abnormalities in *Acartia lilljeborgii* Giesbrecht (1889) (Copepoda, Calanoida) in a tropical estuary under industrial development

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Abstract: Morphological abnormalities in crustaceans have been registered and several are attributed to pollution and others anthropogenic activities. This study reports for the first time a temporal record of the amount and variety of morphological abnormalities in *Acartia lilljeborgii*, in an impacted neotropical estuary. The specimens were obtained from Suape port area, Northeast Brazil, between May 2009 and September 2010 using a 300 µm plankton net. Seven types of abnormalities were observed in one of the terminal spines of the prosome, but no temporal variation of abnormalities was found in our study. The deformities were registered in 85.7% of samples and they were found in up to 10% of the individuals (3.2 ± 2.9%). The proportion of females with abnormalities was greater than for males, in opposite to most previous reports. Due to its high distribution and abundance in part of the neotropical Atlantic coastal area, *A. lilljeborgii* has the potential to be used as a bioindicator of environmental conditions, although the reasons of the abnormality occurrences should be accurately investigated.

Key words: Brazil, contamination, malformation, Polycyclic Aromatic Hydrocarbons, Suape port.

INTRODUCTION

Deformities and abnormalities in crustaceans have been attributed to genetic factors (Zou & Finger, to parasites and failures in the moulting process (Moncada & Gómez 1980, Purohit & Vachhrajani 2016), to poor wound healing after moulting (Rajkumar et al. 2016, Purohit & Vachhrajani 2016), nutritional deficiencies (Guillaume et al. 2001), extreme temperatures during ontogeny (García-Guerrero et al. 2003) or, even to environmental pollutants (Fransozo et al. 2012, Melo et al. 2017) and exposition to antibiotics during larval stages (Pates Jr et al. 2017). Negative impacts of mid-ultraviolet (UVB) radiation have been demonstrated to cause planktonic copepod deformations as well (Naganuma et al. 1997, Kouwenberg et al. 1999, Lacuna & Uye 2000, Lee et al. 2014). Borutzky et al. (1991) found morphological deformities in calanoid copepods from continental waters, but did not explain their potential causes, meanwhile some authors suggested that these effects may result from genetic variation, mutation due to environmental pollutants or even sampling or fixation artifacts (Pombo & Martinelli-Filho 2012).

Copepods of the family Acartidae have been found with morphological abnormalities in several coastal ecosystems (Montú & Gloeden 1982, Loureiro Fernandes et al. 1998, Dias 1999, Lacuna & Uye 2001, Pombo & Martinelli-Filho 2012). A morphological deformity of the 5th pair of legs in three species of *Acartia* seems to be most likely a natural phenomenon for this genus (Behrends et al. 1997). On the other hand, Lacuna & Uye (2001) showed morphological alterations on *Acartia omorii* nauplii submitted to different levels of UVB radiation. The first report of prosomal spine bifurcation and growth of a smaller spine from the base of the main structure for a species of *Acartia* was reported by Pombo & Martinelli-Filho (2012), in specimens found in stomach contents of a common carcinophagus fish from the Brazilian coast (*Stellifer rastrifer* Jordan, 1889).

Acartia (Odontacartia) lilljeborgii Giesbrecht (1889) is a calanoid copepod found in tropical coastal areas in the world (Razouls et al. 2005-2019). This species is very common in Brazilian coast, being more frequent and abundant in estuarine, mangrove and bay ecosystems (Björnberg 1981, Silva et al. 2003, Magalhães et al. 2009, Magalhães et al. 2011). According to Giesbrecht (1889) description, A. lilljeborgii female has a couple of spines on the last prosome segment, being one in each side, extending approximately 3/4 of way along the genital segment. These lateral spines on the last somite are the synapomorphy for the thirteen species of the subgenus *Odontacartia* (Steuer 1915) and Pombo & Martinelli-Filho (2012) suggest that non-sexual anomalies may also occur in other species of this subgenus.

This study reports for the first time a temporal occurrence and a relatively high frequency of morphological abnormalities in *Acartia lilljeborgii* from Suape port area, which is a neotropical impacted estuary.

MATERIALS AND METHODS

The studied area is approximately 40 km south of Recife City (Pernambuco State capital) in tropical Brazil (8°15' to 8°30'S and 34°55' to 35°05'W) (Figure 1). Two rivers (Massangana, 2-4 m deep, and the marine channel of Tatuoca, 4-5 m deep) drain into Suape Bay, which is 1-2 m deep (Neumann et al. 1998), except the channel



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area near the internal port of Suape, which is up to 16 m deep. The current during flood tide has a West direction and during ebb tide the direction is East, with maximum velocities of 0.5 to 0.6 m.s⁻¹ between the North and South reef openings. Climate is warm-humid with a mean annual temperature of 24°C and a rainfall of 1500-2000 mm yr⁻¹, concentrated from March to August. The average salinity in the rainy period is 30.33±2.74, and during the dry period is 35.58±4.14.

The estuarine Suape port area has suffered several impacts since the port activities began, such as sediment suspension after dredging, mangrove suppression, and changes in hydrodynamics and in phyto- and zooplanktonic communities (Braga et al. 1989, Neumann et al. 1998, Neumann-Leitão & Matsumura-Tundisi 1998, Koening et al. 2003, Silva et al. 2004, Muniz et al. 2005). The original Atlantic rainforest has been largely replaced by sugar cane culture. Formerly mangrove forests covered large areas, however more than 600 ha have been logged to accommodate industrial expansion since 1980. Suape Port Industrial complex holds around 70 business industries that can cause considerable impacts on the environment due to their waste that has a potential to cause toxicity to the local biota (Souza-Santos & Araújo 2013). In addition, the intense shipping traffic and industrial activities also contribute to the increased contamination level and degradation of the area (Lemos et al. 2014, Zanardi-Lamardo et al. 2018).

The specimens were obtained from plankton samples collected bimonthly in the Suape port area (Figure 1), between May 2009 and September 2010, during an assessment study. Seven campaigns were conducted in two fixed stations (St.1 and St.2) (Table I), at diurnal high and low tides, during spring and neap tides, totalizing 28 samples (one sample at each occasion). Stations were chosen in order to verify the influence of the most impacted river by industries that would reach Suape bay: one nearby the river mouth (St. 1; 8°23'01"S and 34°58'14"W) and another more

Date	Tide	Period	Tidal Stage	Abundance (ind. m ⁻³)	Proportional Abundance (%)	Anomaly types
May/09	Spring tide	Rainy	Low	33.4	23.4	,
			High	87.0	35.5	II, IV, VII
Jul/09	Neap tide	Rainy	Low	22.8	29.2	I
			High	77.1	82.9	II
Nov/09	Spring tide	Dry	Low	212.5	59.0	V, VI
			High	158.3	49.6	I, II
Dec/09	Spring tide	Dry	Low	144.2	51.2	I, II
			High	55.1	38.6	IV
Mar/10	Spring tide	Rainy	Low	322.5	32.1	IV, V
			High	526.1	52.9	I
Jul/10	Spring tide	Rainy	Low	145.8	58.9	V
			High	155.4	62.7	-
Sep/10	Spring tide	Dry	Low	8.2	30.9	III, VI, VII
			High	1.8	11.7	-

Table I. Acartia lilljeborgii abundance (ind. m⁻³), proportional abundance (%; in relation to zooplankton community) and anomaly types (according to Figure 2) found between May/09 and Sep/10, in Suape area (northeastern Brazil).

distant (port, St. 2; 8°23'17"S and 34°58'05"W). The stations positions were determined based on local currents circulation data, provided by Physical Oceanography team of the Department of Oceanography of UFPE.

Mesozooplankton was sampled with standard plankton net (mesh size 300 µm; net mouth diameter 0.30 m), with a coupled flowmeter. Sub-surface hauls were conducted for 3 minutes at a speed of 2 to 3 knots. The samples were fixed in 4% buffered formalin according to the procedure described by Harris et al. (2000). In the laboratory, each sample was placed in a beaker, diluted with distilled water and homogenized. Three aliquots of 5 mL were analyzed under a binocular compound microscope from a sample between 100-500 mL. Density was estimated according to Boltovskoy (1981). All zooplankton community (data not shown in this article) was identified and the Acartia lilljeborgii specimens (adults) were observed for potential anomalies. If no individuals with morphological abnormalities occurred in the three aliguots, the entire sample would then be analyzed.

The proportion of anomalous copepods data was Arcsin square root transformed in accordance with Zar (2010) to percentages data. The data was checked for normality (Shapiro-Wilk test) and homoscedasticity (Levene median test). T-test (parametric) was performed to detect tides (low and high) or seasonal differences (rainy and dry periods) (5% significance level). Data from the different sampling stations were considered as replicates, since no differences were detected (paired t-test; t = -0.540; df=13; p = 0.598) and the short distance between them (800 m). The statistical tests were conducted using the Sigmaplot software version 11 (Jandel Scientific, Erkrath, Germany).

RESULTS AND DISCUSSION

Acartia lilljeborgii abundances varied from 1.8 to 526 ind. m⁻³ and reached up to 82.9% of the zooplankton community (Table I) and a total of seven types of anomalies were observed in this study (Figure 2). The females were the majority of anomalous specimens (89.3%) and presented six different abnormality types (Figure 2, type 1 to 6). On the other hand, just one type of anomaly was observed in males (Figure 2, type 7), which represented 10.7% of the abnormal individuals. Dominance of females with abnormalities was also observed in a Brazilian southeastern estuarine bay (Dias 1999). Paradoxically, Pombo & Martinelli-Filho (2012) reported an equally occurrence among males and females. These two studies analyzed different kind of samples (plankton and fish stomachs, respectively), but there is no clear reason for this variation. However, both areas are under crescent anthropogenic activities and frequent industrial and domestic wastes input.

The abnormal individuals abundance ranged from 0 to 14.4 ind. m^{-3} (4.1 ± 4.6 ind. m^{-3}) with no variation in the proportion of anomalous copepods between sampling periods (t-test;



Figure 2. Schematic drawing of the anomaly on the terminal spine of the prosome in *Acartia lilljeborgii* Giesbrecht, 1889 found in Suape area (northeastern Brazil). Modified from Pombo & Martinelli-Filho (2012).

t = -1.315: df = 12: p = 0.213). The occurrence of abnormal individuals was observed in 85.7% of the samples, and the mean percentage of copepods with anomalies was 3.2 ± 2.9%, varying between 0 and 10% (Figure 3). In some occasions (n=6) the abnormality percentage was higher than this mean, mainly in low tides (n=5) when resident individuals are expected to be more concentrated. During high tides the marine coastal waters might transport or disperse the estuarine abnormal subpopulations, justifying the absence of abnormal individuals in two high tides occasions (July and September 2010) (Table I). These results are reinforced by the higher proportion of anomalous organisms sampled during low tide in comparison to high tide (t-test; t = 2.486; df = 12; p = 0.0286). The maximum types of anomalies were found in May 2009 (high tide) and September 2010 (low tide) with no pattern associated to tides.

Our study did not measure chemicals, but a concomitant technical study was conducted

from September 2008 to May 2010 (PETROBRAS 2009), investigating the 16 priorities Polycyclic Aromatic Hydrocarbons (PAH) in water (USEPA 2014). Such research included the stations of the present study, but not all sampling periods were simultaneous. The highest total PAHs concentrations were registered in December 2009 (4.29 μ g L⁻¹ at St.1 and 5.58 μ g L^{-1} at St.2) and were coincident with the highest abundance anomalies observed in the area. Even though it is not possible to prove that the observed anomalies were related to that PAH concentrations, previous studies have already associated genetic effects on organisms exposed to these compounds (Hansen et al. 2008, Rocha et al. 2015). Some other studies have reported sediments and water contamination in Suape area (Souza-Santos & Araújo 2013, Araújo & Souza-Santos 2013, Lemos et al. 2014, Zanardi-Lamardo et al. 2018), which could contribute to the expression or development of the observed anomalies. Some studies have



Figure 3. Occurrence of anomalous abundance and percentage in *Acartia lilljeborgii* Giesbrecht, 1889 between May/09 and Sep/10 in Suape area (northeastern Brazil). The dashed line is the mean anomalous percentage (3.2%).

shown positive correlations between pollutant concentrations in impacted ecosystems and percentage of anomalous copepods (Krupa 2005) and cladocerans (Melo et al. 2017). Particularly to Acartidae species, individuals with morphological abnormalities have been recorded in some polluted environments in Brazilian coast (Table II). No other studies on morphological abnormalities in this family were found elsewhere.

Due to the occurrence and/or population maintenance of *Acartia* species in polluted

areas, they can be considered as potential indicators of estuaries and bays health (Montú & Gloeden 1982, Loureiro Fernandes et al. 1998, Dias 1999). That is the case of the resistant and abundant *Acartia lilljeborgii* which may be used to assess the extent of impacts on the zooplankton community in Brazilian waters. Toxicological studies with *A. lilljeborgii* exposed to isolated pollutants and to synergistic effects are prominent to certify and encourage the use of such species in monitoring programs.

Species	Stage	Features	Probable associated cause	Locality	Authority
Acartia lilljeborgii Giesbrecht, 1889	Adults	Morphological alterations, intestinal prolapse and protoplasmatic extrusion	Pollution (heavy metals and domestic sewage)	Espírito Santo bay, Southeastern Brazil	Loureiro- Fernandes et al. 1998
	Juveniles and Adults	Intestinal prolapse and partial rupture of the chitinous carapace	Pollution (domestic sewage)	Espírito Santo bay, Southeastern Brazil	Dias 1999
	Juveniles and Adults	Morphological abnormality in terminal spines of the prosome	Antropogenic disturbance or teratogenic origin	Caraguatatuba bay, Southeastern Brazil	Pombo & Martinelli- Filho 2012
	Adults	Morphological abnormality in a terminal spine of the prosome	Pollution	Suape area, Northeastern Brazil	Present record
Acartia tonsa (Dana, 1849)	Adults	Female with juvenil toracic apendages (CIV and CV), intersexuality and rupture of the chitinous carapace	Pollution	Patos lagoon, South Brazil	Montú & Gloeden 1982

Table II. Studies on morphologica	l abnormalities in Acartid	ae species in Brazilian coast
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Pedro A.M. de Castro Melo, Sigrid Neumann-Leitão and Mauro de Melo Júnior Conceptualization, collected the data, designed the data analysis, and wrote the paper. Eliete Zanardi-Lamardo and Manuel J. Flores-Montes designed the data analysis and wrote the paper.

