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Valentim-Neto, Pedro Alexandre; Gesteira, Tereza Cristina Vasconcelos
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Health State, morphological characterization and biometric parameters in the mangrove crab *Ucides cordatus* Linnaeus, 1763 (Brachyura: Ocypodidae)

Pedro Alexandre Valentim-Neto* and Tereza Cristina Vasconcelos Gesteira

Centro de Diagnóstico de Enfermidades de Organismos Aquático, Instituto de Ciências do Mar, Universidade Federal do Ceará, Av. Abolição 3207, 60165-081, Cx. Postal 52756, Meireles, Fortaleza, Ceará, Brasil. *Author for correspondence. E-mail: pavneto@yahoo.com.br

ABSTRACT. The crab *Ucides cordatus* is an important feature of mangroves in the North and Northeast of Brazil. In 2002 a large mortality was reported on this species in the Jaguaribe River Estuary. In order to investigate the possible causes specimens were collected monthly between May 2002 and December 2003 at five sites: Fortim (E1), Fortim (E2), Cumbe (E3) Aracati (E4) and Guajiru (E5). The mangrove structure and environmental parameters (salinity, pH, temperature and DO) were also evaluated. Water was collected for suspended solids analysis, BOD and nutrient levels. Salinity decreased in the rainy season, with no significant variation of pH and temperature. Dissolved oxygen levels had a mean of 5.7 mg L⁻¹. Suspended solids increased at site E4, with BOD varying at E1 and E2 during the dry season. The same was observed at sites E1, E2 and E3 during the rainy season. Ammonia levels at E1 and E2 and phosphorus in all seasons, increased with rain events. Four lines of investigation were adopted: biometry, histology, hematology, and bioassay. There was no significant difference between animals in biometrics and histology showed no cellular alterations. However, hematology showed a significant difference between the E1 and E3 sites due to depletion in the number of hemocytes in E1, probably in response to environmental impacts. This can lead to poor immunity, leading to opportunistic pathogen infections such as viruses, bacteria and fungi. The bioassay showing no abnormal behavior or mortality. The structure of mangrove was developed in all areas, except in E5 which served as a control site, with significant environmental stress with high levels of herbivorous growth (> 50%) and a salinity of around 50 ‰. It is believed that the mortality of the crabs was an occasional occurrence, probably due to toxin production by some fungal organism.

Keywords: Biometric parameters; Crustacea; Histology; Bioassay.

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Introduction

The contribution of aquaculture to global supplies of fish, crustaceans, mollusks and other aquatic animals has subsequently increased, reached the mark of about 171 million tonnes in 2016, of which 88 percent was utilized for direct human consumption, thanks to relatively stable capture fisheries production, reduced wastage and continued aquaculture growth. The total first sale value of fish production and aquaculture in 2016 was estimated at USD 362 billion, of which USD 232 billion came from aquaculture production (Food and Agriculture Organization of the United Nations [FAO], 2018).

The activity of shrimp farming occupies a prominent position within the Brazilian fishing industry, in terms of production organization including the generation of employment, income and investments, it thus contributes positively to the establishment of new production structures along the coastline. Additionally, shrimp farming has created an important social inclusion, given that the participation of micro, small and medium producers account for 95% of all enterprises in the sector (FAO, 2016).

Aquatic organisms are subject to the influence of several environmental factors resulting from the daily and seasonal rhythms characteristic of coastal and estuarine ecosystems. Often, the environmental stress caused by human activities and intense exploitation pressure, since 1990 can be a determining factor for the onset or increase in the prevalence of diseases and decrease in crustacean populations (Duarte, Duran, Mendonça, & Pinheiro, 2014; Moullac & Haffner, 2000). The actual and possible environmental impacts of coastal aquaculture include nutrient enrichment; chemical pollution; habitat loss and change; impacts on

populations of aquatic organisms; and upstream effects related to the production of fishmeal used to feed farmed fish (FAO, 1991; 1992). The crab *Ucides cordatus* Linnaeus, 1763 is a typical estuarine species, found from Northern Florida to the coast of Santa Catarina, Southern Brazil (Melo, 1996).

This crustacean is totally dependent on the marsh environment to breed and to protect themselves from predators. During the construction of their burrows, removal of sediment indirectly promotes the cycling of nutrients, playing a crucial role in several environmental processes of that ecosystem (Pinheiro & Fiscarelli, 2001; Schories et al., 2003). Based on previous reports of crab mortality (Schmidt, Theil, & Galli, 2008; Sinimbu, 2006) in northeastern states (BA, MA, PI and CE) a monitoring program was implemented in the state of Ceará, for nineteen months, to assess the possible impacts of shrimp aquaculture on the surrounding environment and its biota, as well as the histological description of some tissues and cell types.

Material and methods

Study area

The study area corresponded to the estuarine area of Jaguaribe River (Figure 1), and four locations:

E1 – Right side of the Jaguaribe estuary mouth, E2 – Jaguaribe River channel opposite the fishing port of Fortim, E3 – Canal outflow of effluent from shrimp farms in the regions of Cumbe and Canavieira, E4 – Canal outflow of effluents at the tidal creek bordering the Ilha dos Veados in Aracati ($4^{\circ} 26' 15''$ S) and ($37^{\circ} 48' 45''$ W). The aim was to encompass a large area of the estuary, in which the mortality of crabs was recorded. In addition, E5 – Estuary Pirangi in Guajiru, served as a control due to its remote location, outside the influence of the Jaguaribe River and the lack of any crab mortality.

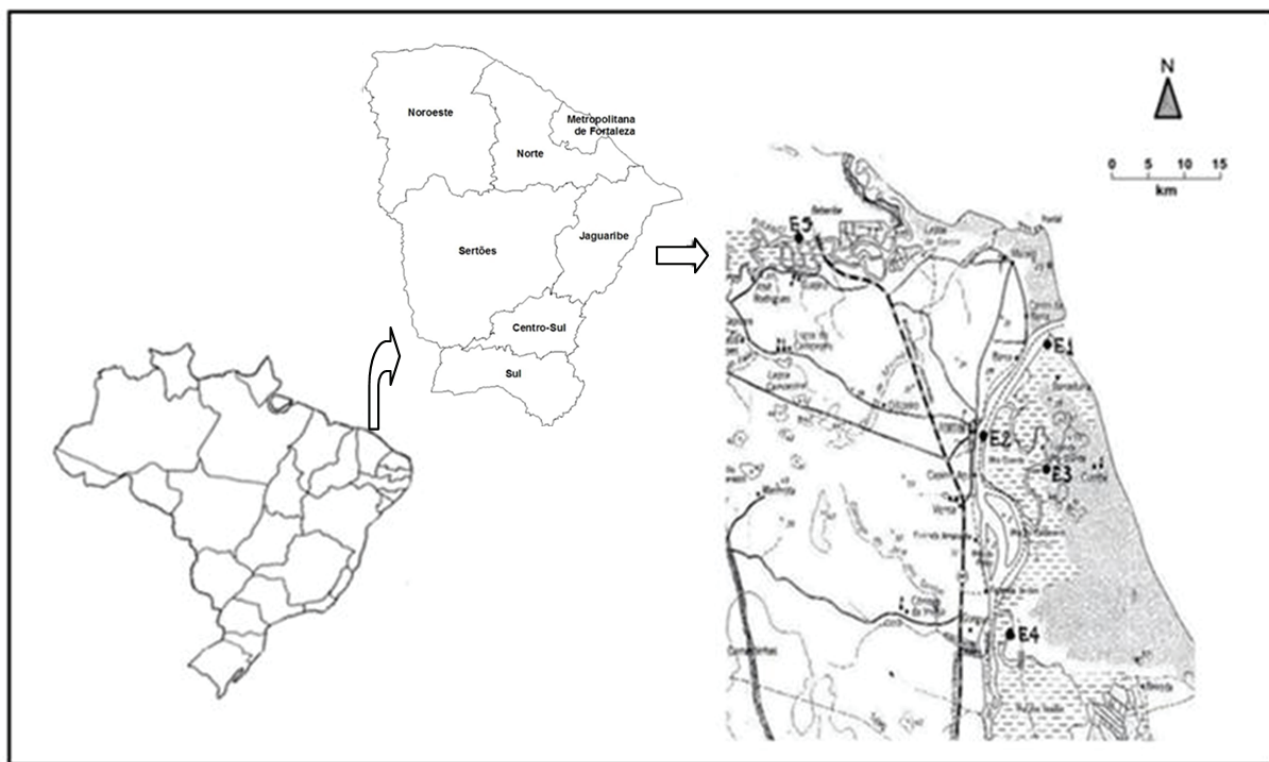


Figure 1. Map locating the Jaguaribe river and the collection points along the estuary. E1 – Right side of the Jaguaribe estuary mouth, E2 – Channel opposite the fishing port of Fortim, E3 – Channel outflow of effluent from shrimp farms in the regions of Cumbe and Canavieira, E4 – Channel outflow of effluents at the tidal creek bordering the Ilha dos Veados in Aracati and E5 – estuary Pirangi in Guajiru (control).

Material collection

Monthly collections of crabs were made at the five stations previously described. Due to the relationship between the organism investigated and the environment, measurements were made of salinity, pH, temperature, dissolved oxygen (DO) using a multiparameter probe YSI Model 55/12 FT Bernauer. Water was collected for analysis of suspended solids, biochemical oxygen demand (BOD) and nutrients (ammonia and phosphorus) in accordance with APHA (Eaton, Clesceri, & Greenberg, 1995).

A study of the structure of mangrove was made to better understand the nature of the environment where the crabs were collected, and involved the identification of the species, size and density of vegetation. Initially plots were selected of 100 m² according to Schaeffer-Novelli and Cintrón (1986), in an area parallel to the mangrove estuary. Trees were then counted to obtain the density, estimated height and measured perimeter. The tree species present in each area were identified and recorded. The number of crab burrows was also recorded, for each station sampled.

Four lines of research were adopted for the study of crabs: a. Biometric parameters, b. Histological c. Total hemocyte count (THC), and d. Bioassay. The collection of animals was carried out in dry and rainy seasons, with 25 individuals sampled per period in each season, totaling 50 individuals at each point. The crabs were collected by scavengers from the region by the method of "braceamento" which involves putting the arm in the hole to capture the crab by hand. Individuals were tagged and transported in cool boxes to the laboratory and acclimated in 150 L tanks containing water with salinity around 20 ‰ and constant aeration, for a period of 24 hours.

For biometric analysis, we measured the width and length of the carapace and chelas (mm) and took the total weight (g) of all animals collected at five stations. The analysis aimed to investigate whether there were significant differences between the parameters being tested in each variable in relation to carapace width, which was the independent variable used in linear regression equations for logarithmic transformation.

In each sub-collection 10 individuals per station were randomly sampled, between males and females for the THC. After examining the general appearance of the animals, hemolymph was withdrawn, using the technique employed by Johansson, Keyser, Sritunyalucksana, and Söderhäll (2000). The procedure consisted in the withdrawal of a 5µL aliquot of hemolymph in the pericardial sinus using a hypodermic syringe containing anticoagulant in a 1:1 ratio, the sample was transferred to an eppendorf tube and kept under refrigeration.

Statistical analysis

The THC was submitted to statistical analysis by the Tukey test for comparison between the respective mean values of the number of hemocytes, according to the null hypothesis (Ho) and alternative (Ha), for the significance level $\alpha = 0.05$.

Ho: The number of *U. cordatus* hemocytes does not differ between areas of Baixo Jaguaribe.

Ha: The number of *U. cordatus* hemocytes is different in at least one of the areas of Baixo Jaguaribe.

The THC was performed with the aid of a hemocytometer, noting the dilution of the hemolymph and subjected to statistical analysis by one-way ANOVA. After hemolymph collection, the animals were dissected to remove the gills, middle digestive tract, hepatopancreas (Castejón et al., 2019) and male gonads. The material was fixed with Davidson solution for 24 hours and then standard histological preparation was performed (Bell & Lightner, 1988). Cuts of 5µm were stained with hematoxylin and eosin and examined under a light microscope. For the bioassay, 20 crabs of *U. cordatus* were divided, into 4 and stored in PVC cages totaling 5 animals per cage. These were positioned in areas close to the source of effluent from two shrimp farms, located as follows: 2 in Cumbe (E3) and 2 at station Aracati (E4), for a period of 30 days. This test aimed to investigate the influence of effluents on this species.

Results and discussion

The observations of mangrove structure (Table 1), allowed an evaluation point at each station: E1 - the mangrove swamp at the mouth of the estuary consists of 10 specimens of *Avicennia germinans*, *Laguncularia racemosa* (5 of each) and *Rhizophora mangle* with an average height of 5.0 m, and 1.0 m average perimeter per plot of 100 m². A total of 35 crab burrows were counted. E2 - the Jaguaribe channel showed characteristics of coastal mangroves, with the same species in number and height similar to those observed in E1, with the only difference in mean circumference that was 0.8 m. The number of crab burrows found - totaled 40. E3 - a density of 10 specimens of *Avicennia germinans*, 3 of *Laguncularia racemosa* with average height of 5.0 m and 0.8 m perimeter. Forty-two burrows were found. E4 - coastal mangrove presenting the same density, variety of species and mean circumference found in the previous location, with a height that averaged 4.0 m. The count of burrows reached a total of 46. E5 - characterized by being a highly degraded mangrove only composed of shrubs and intensive grazing (> 50% leaf area), indicating significant environmental stress. The species *Avicennia germinans* is dominant with 170 plants, with an average height of 1.8 m and a perimeter of 0.08 m, followed by 30 specimens of *Laguncularia racemosa* with average height

of 3.0 m and perimeter of 0.1 m and 6 of *Rhizophora mangle* with an average 1.8 m in height and 0.1 m in diameter. A total of 40 crab burrows were found at this location.

Table 1. The mangrove structure allowed an evaluation point at each station.

Sampling Station	<i>Avicennia germinans</i>	<i>Laguncularia racemosa</i>	<i>Rhizophora mangle</i>	Burrows
	Density (trees 100 m ⁻²) Height ⁻¹ (m) PBH ⁻¹ (m)	Density (trees 100 m ⁻²) Height ⁻¹ (m) PBH ⁻¹ (m)	Density (trees 100 m ⁻²) Height ⁻¹ (m) PBH ⁻¹ (m)	Density
E1	10 5 ⁻¹ 1 ⁻¹	5 5 ⁻¹ 1 ⁻¹	5 5 ⁻¹ 1 ⁻¹	35
E2	10 5 ⁻¹ 0.8 ⁻¹	5 5 ⁻¹ 0.8 ⁻¹	5 5 ⁻¹ 0.8 ⁻¹	40
E3	10 5 ⁻¹ 0.8 ⁻¹	3 5 ⁻¹ 0.8 ⁻¹	ND	42
E4	10 4 ⁻¹ 0.8 ⁻¹	3 4 ⁻¹ 0.8 ⁻¹	ND	46
E5	170 1.8 ⁻¹ 0.08 ⁻¹	30 3.0 ⁻¹ 0.1 ⁻¹	6 1.8 ⁻¹ 0.1 ⁻¹	40

ND: not detected. PBH: perimeter at breast height

The results of measurements of environmental variables during the trials (Table 1), although isolated, showed different levels of salinity between the stations studied, both in the dry and rainy seasons. The pH did not vary much and remained alkaline, with the average around 8.4, justified by the buffering action of sea water in estuaries. The temperature throughout the year remained at around 29°C characteristic of the regional climate. With respect to nutrients, the release of soluble inorganic nutrients (nitrogen and phosphorus) has the potential to cause nutrient enrichment (hypertrophication) possibly followed by eutrophication (increase of primary production) of a waterbody. Related changes in phytoplankton ecology may result in algal blooms, which can be harmful to wild and farmed organisms (FAO, 1992). It was observed that dissolved ammonia tended to increase at stations E1 and E2 in the rainy season, probably due to the input of land runoff from storm drains. These stations are located at the mouth of the estuary, where there is a higher input. The levels of phosphorus in the rainy season were higher than those observed in the dry season. In both seasons, levels were well above expected levels of natural waters. According to Lacerda (2006) shrimp production in the Jaguaribe Estuary, generates a significant amount of phosphorus. Moreover, the waste arising from agricultural practices and other activities common to the area, may also be responsible for the found level. The BOD, at stations E1 and E2 during the dry season and the E1, E2 and E3 in the rainy season, were lower than that of *Conselho Nacional do Meio Ambiente* (CONAMA, 2005) which defines for brackish and saltwater, levels no lower than 4 and 5 mg L⁻¹, respectively. This is probably due to the significant accumulation of organic matter in the estuary.

The amount of suspended solids was higher at E4 in the dry season, probably due to the effluents from shrimp farming in the area, as well as the enrichment of nutrients in the water which agrees with studies by Fuchs, Martin, and An (1999) and Pérez-Osuna (2001). These works state that the water from prawn cultivation has a greater flow of suspended solids and nutrients. Statistical analysis performed on the biometric studies of individuals of *U. cordatus* population collected in different seasons, showed no significant difference between the populations of crabs from the Low Jaguaribe, with respect to their morphology, suggesting that the growth of the individuals does not differ within the studied areas (Figure 2).

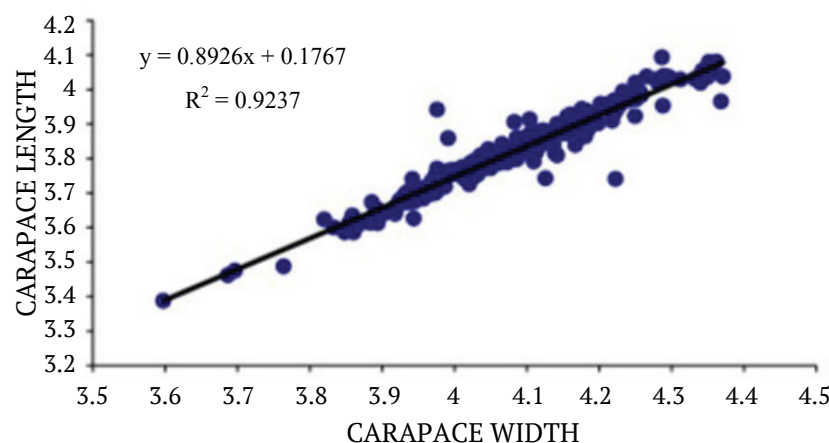


Figure 2. Regression line of the length x width carapace in *Ucidés cordatus* populations. Individuals collected in the five stations.

When the THC data was evaluated by one-way ANOVA, the null hypothesis was rejected ($F = 3.24$, $p < 0.05$), with respect to a difference in the number of hemocytes in the crab at one of the stations studied. A Tukey's test, with a value of $HSD = 0.358$, was used for comparison of means indicating the presence of a significant difference ($HSD = 0.391$) between the averages of individuals caught in Cumbe E3 and Fortim (E1 and E2) (Rotllant et al., 2010). The THC is one of the hemato-immunological parameters commonly used to assess the health of crustaceans. However, changes in the immune reaction in invertebrates, including depletion or increase in the number of hemocytes may result from the action of external factors including temperature, salinity and pollutants. These variations depend on the type and duration of exposure to causative agents (Moullac & Haffner, 2000; Rodríguez & Moullac, 2000). A decrease of THC, anti-bacterial and bacteriolytic activity was seen soon after a temperature change was observed in the shrimp *Litopenaeus vannamei* by Lu-Qing, Bo, Ling Xu, and Jing (2007). In this study the Fortim and Cumbe stations showed increase in ammonia and nitrite quantity respectively, which may explain differences in THC observed and influence the immune response of these animals. These changes can be momentary or punctual. However, in degraded environments they may persist for long periods. The authors suggested that after such a period, individuals tend to establish an immune adaptation.

The bioassay, which was used to assess the quality of the environment near the shrimp farms, showed no recorded mortality during the exposure period. This demonstrates that the environmental conditions were compatible with the survival of the individuals suggesting the absence of some pollutant that might reasonably cause harm to the health of animals exposed to farm effluents.

The spatial distribution of plant species in the mangrove agrees with their adaptation to the sediment and tolerance of each species to salinity. The genus *Rhizophora* is less tolerant to salt, performing better in places where the interstitial water salinity was lower than 50 ‰; *Avicennia* is a more tolerant genus and *Laguncularia* average tolerance is situated in the interior of the swamp where it undergoes less tidal influence (Schaeffer-Novelli, 1995). The environmental conditions encountered (Table 2) explain why the mangrove in E5 is undeveloped, when compared to other areas studied. Dwarf mangrove forests are common in areas of high salinity. However, depending on the region either species may be less abundant or even absent. This is due to the size of the estuary and its environmental characteristics. For example, artificial barrier along the river basin is identified as a major cause of the change in the estuarine circulation patterns due to the retention of fresh water and sediment, resulting in changes in the distribution of mangroves (Lacerda & Marins, 2002). This is the case in the Jaguaribe Estuary where, due to the construction of the Itaíçaba barrier it has decreased considerably. Another major modifier of environmental conditions is the coastal sediment dynamics, particularly affected by global and regional changes, which also results in a change in the distribution and composition of mangroves (Marins, Freire, Maia, Lima, & Lacerda, 2002).

Table 2. Environmental parameters measured occasionally in dry and rainy seasons in five collection points

Parameters	Dry season					Rainy season					Mean \pm SD
	E1	E2	E3	E4	E5	E1	E2	E3	E4	E5	
Ammonia (mg L ⁻¹)	0.10	0.07	1.50	2.40	2.03	1.04	2.32	0.42	0.36	0.56	1.08 \pm 0.92
Nitrite (mg L ⁻¹)	0.01	0.00	0.02	0.01	0.01	0.00	0.00	0.02	0.02	0.00	0.009 \pm 0.01
Nitrate (mg L ⁻¹)	0.88	1.03	0.21	0.44	0.89	1.45	1.41	0.29	0.25	0.23	0.708 \pm 0.49
Phosphorus (mg L ⁻¹)	0.00	0.00	0.09	0.01	0.01	0.12	0.12	0.21	0.27	0.30	0.113 \pm 0.11
Suspended Solids (mg L ⁻¹)	25.80	37.20	9.40	166.4	52.40	7.70	6.50	50.00	43.00	43.20	44.16 \pm 46.41
DO (mg L ⁻¹)	7.40	8.50	5.60	7.60	6.50	4.10	4.00	6.00	3.54	4.30	5.754 \pm 1.74
BOD (mg L ⁻¹)	3.50	2.60	4.38	6.50	5.70	5.90	6.30	5.70	4.29	5.52	5.039 \pm 1.29
pH	8.60	8.80	9.70	8.50	8.10	8.10	7.90	8.50	8.50	8.00	8.47 \pm 0.52
Salinity (‰)	40.00	40.00	26.60	37.00	53.00	36.70	38.00	12.30	16.60	28.60	32.88 \pm 12.07
Temperature (°C)	28.80	28.40	30.00	30.00	29.80	29.40	29.80	30.50	27.80	28.70	29.32 \pm 0.86

SD: standard deviation

The state of cytological preservation in all the structures examined by histology showed subjects were able to perform their normal physiological functions such as breathing, digestion, excretion and reproduction, since all the tissues showed normal structures. Gills presents filaments with pillar cells (Martinez, Alvares, Harris, & Santos, 1999). Medium digestive tract, Hepatopancreas (MGL) with B-cells (blister-like), characterized by a very large supranuclear vacuole and R-cells (resorptive), homogeneously stained and contains few lipid vacuoles (Castejón et al., 2019; Icely & Nott, 1992) and structure of the anterior testis showing germ line cells (Figure 3).

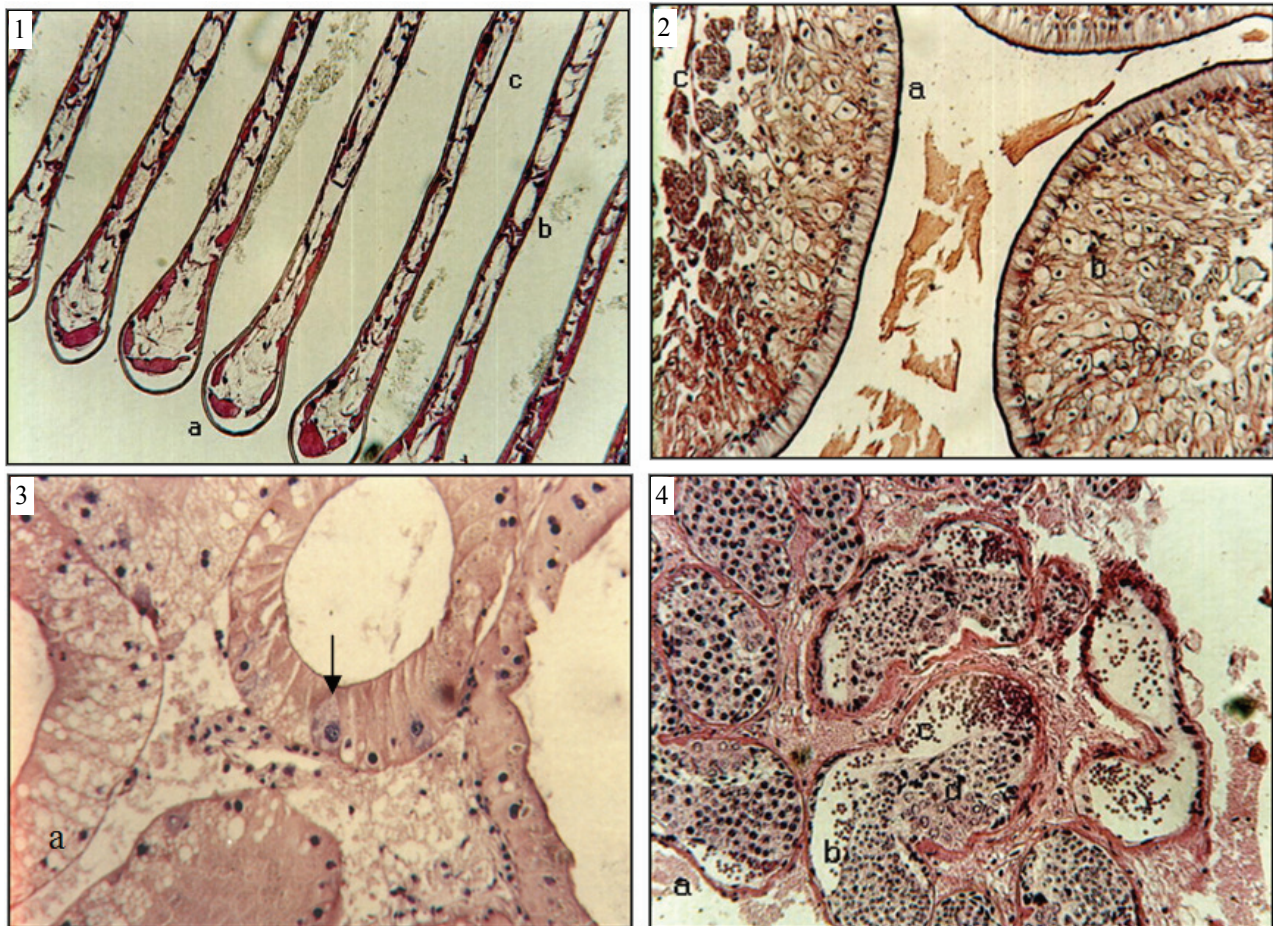


Figure 3. 1-Structure of gill filaments of *Ucides cordatus* in cross section stained with Hematoxylin and Eosin (HE). (a) cuticle coating all filaments; (b) pillar cell that support gill filaments; and (c) hemolymph circulation. 2-Structure of the medium digestive tract in cross section stained with Periodic Acid-Schiff (PAS). The arrow shows the food debris in lumen, (a) simple columnar epithelial tissue; (b) connective tissue; and (c) muscle tissue. 3-Hepatopancreas tubule cross section (HE), the arrow shows a R-cell (resorptive) and (a) B-cell (blister-like). 4-Cross section stained with (HE), structure of the anterior testis showing germ line cells at different stages of development. (a) indicates spermatocytes; (b) spermatids; (c) spermatozoa; and (d) spermatogonia. Scale bars: 1, 2, 4 = 200 μ m; 3 = 400 μ m.

Considering that during the period of this study, no mortality or occurrences such as slow movements and rapid death after capture were observed, it is assumed that this was in fact a single event caused by the input of a toxic organism. These events are common in natural environments and may be related to phytoplankton blooms resulting in high toxin levels and anoxia (Cardoso, 2012). A long-term monitoring of the planktonic structure of the estuary may clarify some points that were not clarified in this study. At the same time, it can be said that the results obtained during the exposure of crabs to shrimp farm effluent suggests that they are not responsible for the direct mortality of the species.

Boeger et al. (2005) performed the histopathological analysis on crabs collected in the state of Sergipe and Bahia, with symptoms similar to those previously reported in animals from Ceará and other Northeast states. The authors named the disease lethargic crab disease (LCD) and attributed its cause to a fungus belonging to the phylum Ascomycota that affects the heart tissue, nerve ganglia and hemolymph, and is responsible for changes in behavior and mortality of these animals. This fungus is a species of *Exophiala* and dispersion occurs via the hemal crab system (Boeger et al., 2007; Vicente et al., 2012). At the time of analysis undertaken in this study, these organs were not investigated, and it is therefore not possible to detect the presence of this agent in the material obtained. It is noteworthy that the activity of shrimp farming in the region continues and yet no new events of crab mass mortality have been recorded.

Conclusion

The crab *Ucides cordatus* is an animal typical to mangrove areas and is well adapted to these environmental conditions. However, human activities and exploitation are contributing to the population

decrease, especially with regarding to the indiscriminate harvesting of this resource. It is necessary therefore, to undertake a constant monitoring of habitat areas and to generate sustainable management aware of this important mangrove ecosystem component.

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