

# Health assessment of the oyster *Crassostrea rhizophorae* on the southern coast of Bahia, northeastern Brazil

Avaliação da saúde da ostra *Crassostrea rhizophorae* no Litoral Sul da Bahia, nordeste do Brasil

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## Abstract

This study investigated the health of natural stocks of the oyster *Crassostrea rhizophorae* on the southern coast of Bahia in northeastern Brazil, during summer and winter 2010, at three localities (sampling points) in the estuaries of the Maraú (Camamu Bay) and Graciosa rivers. A total of 180 oysters (30/sampling point/season) were examined macroscopically for the presence of pathogens and anatomical changes. The specimens were subsequently fixed in Davidson solution, processed for paraffin embedding, sectioned and stained with Harris' hematoxylin and eosin. Histological analysis revealed the presence of *Rickettsia*-like organisms (RLOs), *Ancistrocoma*, *Trichodina*, *Sphenophrya*, *Nematopsis*, *Urastoma*, *Bucephalus* in the sporocyst phase, a nonspecific metacercaria, and a metacestode of genus *Tylocephalum*. The prevalence of infection was low except for parasitism by *Nematopsis* sp. which also caused histopathological changes. The presence of *Bucephalus* sp. caused parasitic castration. These two pathogens significantly affect the health of *C. rhizophorae*.

**Keywords:** Oysters, parasitism, pathogen, *Bucephalus*, *Nematopsis*, Bahia - Brazil.

## Resumo

Este estudo investigou a saúde de ostras da espécie *Crassostrea rhizophorae* de estoques naturais do Litoral Sul do Estado da Bahia, Nordeste do Brasil, durante o verão e o inverno de 2010, em três pontos amostrais distribuídos nos estuários dos rios Maraú (Baía de Camamu) e Graciosa. Um total de 180 ostras (30/ponto amostral/período) foram examinadas macroscopicamente para a presença de patógenos e alterações anatômicas e posteriormente fixadas em solução de Davidson, processadas para inclusão em parafina, seccionadas e coradas com hematoxilina de Harris e eosina. A análise histológica evidenciou a presença de organismos com características similares a *Rickettsia* (RLOs), *Ancistrocoma*, *Trichodina*, *Sphenophrya*, *Nematopsis*, *Urastoma*, *Bucephalus* em fase esporocística, metacercária inespecífica e metacestóide de *Tylocephalum*. As prevalências de infecção foram baixas, com exceção do parasitismo por *Nematopsis* sp., o qual também causou alterações histopatológicas. A presença de *Bucephalus* sp. causou castração parasitária. Esses dois patógenos têm interferência significativa na saúde de *C. rhizophorae*.

**Palavras-chave:** Ostras, parasitismo, patógeno, *Bucephalus*, *Nematopsis*, Bahia - Brasil.

## Introduction

Diseases are one of the main limiting factors for the cultivation of marine bivalves (BOWER et al., 1994; VILLALBA et al., 1997). On the Brazilian coast, the main pathogens recorded in bivalve mollusks include bacteria, protozoans, fungi and others (MAGALHÃES; FERREIRA, 2006; BOEHS et al., 2012). The bacterial *Rickettsia*-like organisms (RLOs) occur in several bivalves (PONTINHA, 2009; BOEHS et al., 2010; DA SILVA et al., 2011; SABRY et al.,

2011; CEUTA; BOEHS, 2012). The review by Boehs et al. (2012) listed protozoans of phyla Ciliophora, Apicomplexa, Perkinsozoa and Microspora, and metazoan parasites of the groups Turbellaria, Digenea, Cestoda, Polychaeta, Pinnotheridae and Copepoda.

*Crassostrea rhizophorae* (Guilding, 1828) (Bivalvia: Ostreidae), popularly known as the mangrove oyster, is naturally distributed from the Caribbean to Uruguay. It settles on consolidated substrates, mainly rhizophores of the red mangrove *Rhizophora mangle* L. in coves, bays and estuaries (RIOS, 2009). This oyster is now being cultivated on the southern coast of Bahia; however, there is little information about potential diseases that can affect this bivalve, either farmed or in the natural environment. This study evaluated

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the health of natural populations of *C. rhizophorae* in this region, with the main goal of developing supporting information for oyster farming. The study included identification of pathogens, analyses of prevalence and intensity of infection, and characterization of the infection site and histopathological changes induced by each parasite.

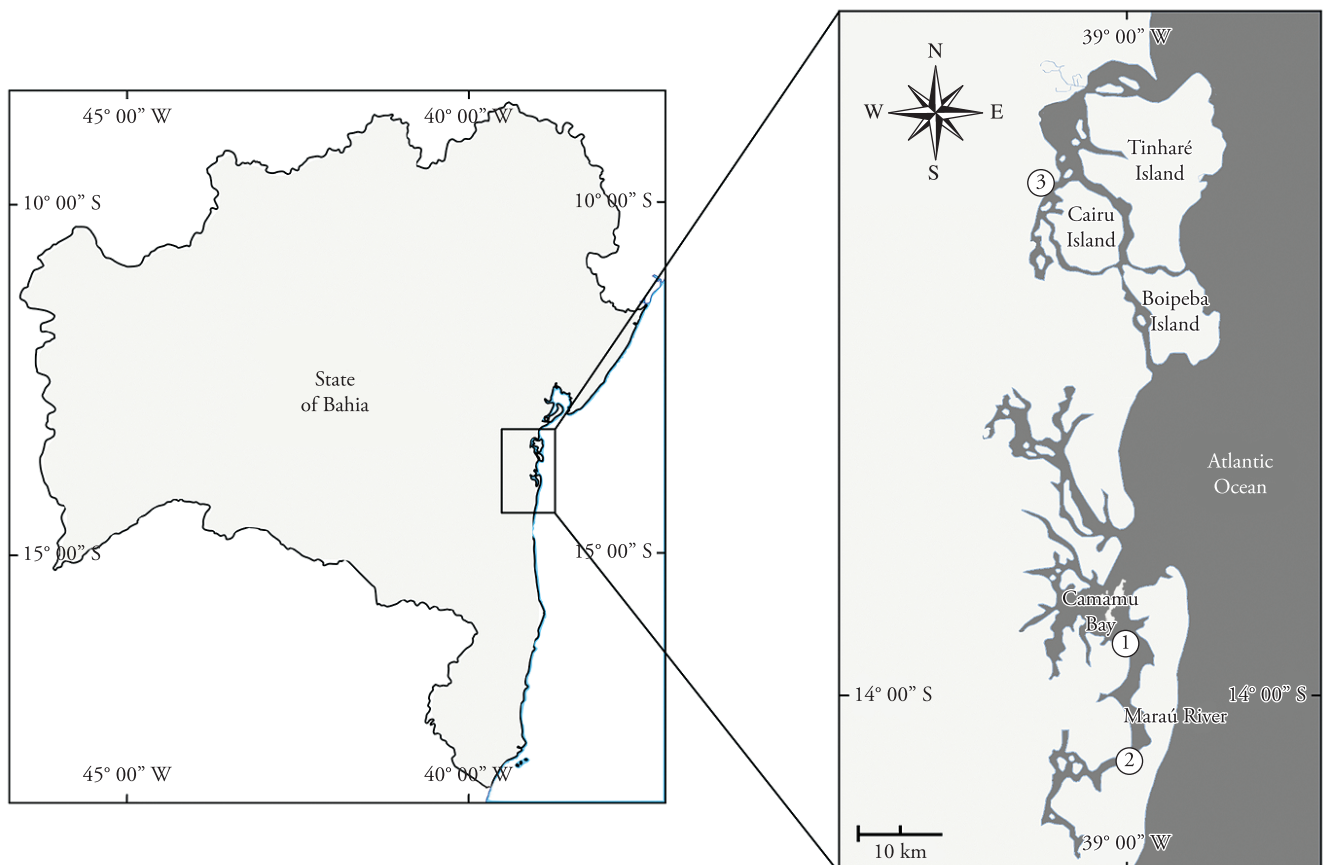
## Materials and Methods

The southern coast of Bahia State in northeastern Brazil is approximately 300 km long, extending from Valença to Canavieiras municipalities. Camamu Bay, approximately 384 km<sup>2</sup> in area, includes the Maráu River estuary of approximately 119 km<sup>2</sup> (SOUZA-LIMA et al., 2003); Graciosa River is located to the north (Figure 1). Small oyster farms culturing *C. rhizophorae* are located in both places.

The samples were collected at three localities (collection points): Caranguejo Island = Point 1 (13° 58' 36" S and 39° 00' 03" W) and Maráu = Point 2 (14° 06' 55" S and 39° 02' 08" W) in the Maráu River estuary (Camamu Bay); and Graciosa = Point 3 (13° 29' 16" S and 39° 05' 56" W) in the Graciosa River estuary (Figure 1). Together with the biological samples, the temperature and salinity of each locality were measured using, respectively, a standard mercury thermometer and an Atago S/Mill manual

optical refractometer. Thirty specimens of *C. rhizophorae* were collected at each sampling point in summer (February/March) and winter (July/August) 2010. The oysters were removed from natural beds in mangroves with the aid of a machete, and taken fresh to the Laboratory of Animal Histology of the Universidade Estadual de Santa Cruz (UESC), where they were immediately processed. Initially, with the aid of a digital caliper, the oysters were measured on the longest axis of the body (=height), as proposed by Galtsoff (1964), and then opened with a knife. Each specimen was inspected macroscopically for the presence of macroparasites or signs of tissue alterations. Then, with the aid of surgical scissors and a scalpel, a diagonal section about 5 mm thick was taken from each specimen, including the mantle, gills, gonad and digestive tract. The sections were immediately fixed in Davidson's solution (SHAW; BATTLE, 1957) and after 24 hours were transferred to 70% ethanol. The tissues were processed by routine histological techniques for paraffin embedding. Histological sections 7 µm thick were stained with Harris' hematoxylin and eosin (H&E) according to the protocol of Howard et al. (2004) and analyzed by light microscopy.

Pathogens were analyzed according to their prevalence (no. of infected individuals/no. of individuals analyzed) and sites of infection. The intensity of the infection by pathogens that occupied large areas of tissue was calculated using the stereology



**Figure 1.** Map of Bahia State and indicating the sampling localities on the southern coast (1 = Ilha do Caranguejo; 2 = Maráu; 3 = Graciosa).

technique of Lowe et al. (1994), using a Weibel morphometric scale. The tissue area occupied by pathogens (TP) was evaluated in five fields randomly chosen from each infection site (organ) of several specimens, and the mean number in each organ and among the total number of animals were calculated, resulting in *Nematopsis* sp.: N = 30; *Bucephalus* sp.: N = 1. The result was classified according to Lowe et al. (1994): I - light infection = <5% TP; II - moderate = 5-25%; III - high = 25-50%; and IV - very high = >50%. Less intense infections were counted and quantified as no. pathogens/histological section.

## Results

The temperature was similar in the sampling points, and slightly lower in winter (Table 1). Salinity was high (29 and 31) at Point 1 near the entrance to Camamu Bay; slightly lower (28 and 24) at Point 2, located farther upstream in the Marau River; and varied widely (0 and 29) at Point 3 (Table 1).

There was no macroscopic evidence of pathogens or signs of histopathological changes. Microscopic analysis revealed nine different pathogens including bacteria, protozoans and metazoans. *Rickettsia*-like organisms (RLOs) and the protozoan *Nematopsis* sp. were present in all samples from both estuaries. Specimens of three groups of ciliates, a metacercaria, and the cestode *Tylocephalum* sp. occurred only in oysters from Camamu Bay. *Urastoma* sp. (Turbellaria) and *Bucephalus* sp. (Digenea) were found only in oysters from Graciosa estuary (Table 1).

The RLOs were observed as individual colonies in the cytoplasm of epithelial cells of the digestive gland, in prevalence from 3.3% to 13.3%, with slightly higher frequencies at Point 2, especially in summer (Table 1). The round colonies measured between 8 and 10 µm in diameter and occurred in low intensity of infection (1-3

colonies/histological section) (Figure 2a). These bacteria did not cause a hemocyte response, but in all cases the epithelial host cells were hypertrophic, and some were in partial lysis.

Among protozoa, ciliates of the genera *Ancistrocoma*, *Trichodina* and *Sphenophrya* were observed in Camamu Bay, and gregarines of the genus *Nematopsis* (Apicomplexa) were observed in both estuaries, consisting of one morphotype for each genus (Table 1). *Ancistrocoma* sp. (Ciliophora: Ancistrocomidae) occurred in the lumen of the digestive tubules and between the gill filaments. These rounded to oval ciliates measured between 12 and 20 µm in length (Figure 2b) and occurred in low prevalence (3.3-10%); the highest frequency was recorded at Point 2 during summer (Table 1). The intensity of the infection was low (1-2 individuals/histological section) and caused no evident damage to the host.

*Trichodina* sp. (Ciliophora: Trichodinidae), was observed in prevalence from 3.3% to 13.3%, and similarly to *Ancistrocoma*, had the highest prevalence recorded at Point 2 during summer (Table 1). This ciliate measured between 5 and 40 µm in diameter, occurred near the mantle and in the lumen of the digestive-gland tubules, with low infection intensity (1-3 individuals/histological section) and caused no evident damage to the host (Figure 2c).

*Sphenophrya* sp. (Ciliophora: Sphenophryidae) was found in the gills of only two oysters from Point 1 (Table 1) and in both it formed a xenoma (Figure 2d), a hypertrophy of the epithelial cells and their nuclei. In both cases only one xenoma/histological section was observed. Apart from the effect at the cell level, apparently the xenomas did not cause significant damage to the host.

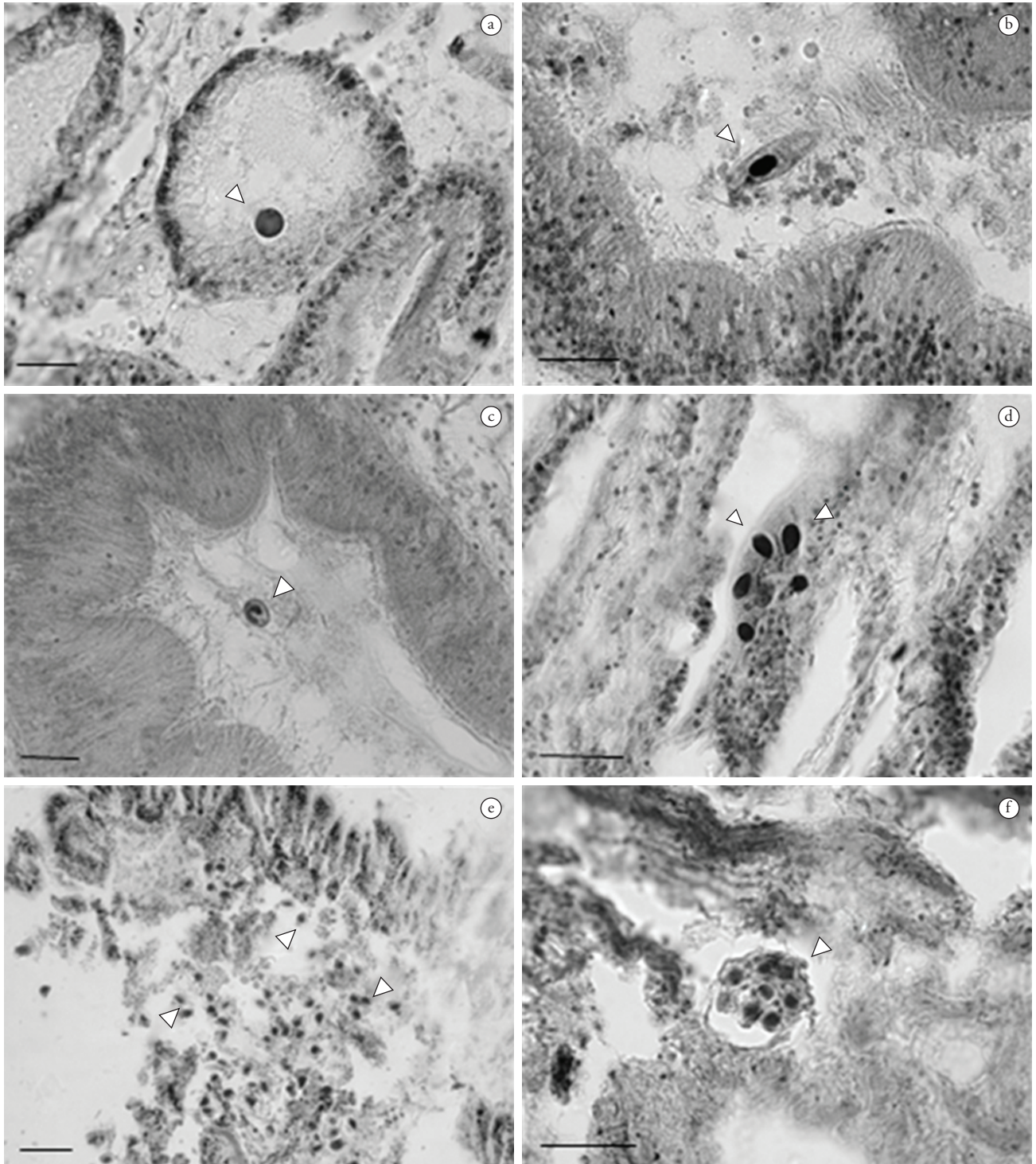
Intrahemocytic oocysts of *Nematopsis* sp. (Eugregarinida: Porosporidae) (Figures 2e, f) were observed at all three sampling points and in both seasons, in prevalences from 20 to 100%; the highest prevalence occurred at Point 3 (Table 1). The highest frequency of these pathogens was observed in the mantle and

**Table 1.** Temperature (T) and salinity (S) values and pathogens associated with *Crassostrea rhizophorae*, with respective prevalences (%) in Camamu Bay (points 1 and 2) and Graciosa River (Point 3) in February/March (summer) and July/August (winter) 2010. NI = non-identified; RLOs = *Rickettsia*-like organisms; N = Number.

Pathogens	Point 1		Point 2		Point 3	
	Summer	Winter	Summer	Winter	Summer	Winter
	N = 30	N = 30	N = 30	N = 30	N = 30	N = 30
Average height of the oysters ± SD (mm)	54.1 ± 5.6	57.7 ± 7.7	50.7 ± 6.2	52 ± 4.9	57.5 ± 10.2	71.1 ± 7.6
T (°C)	31.2	23	30	24	30.1	23
S	29	31	28	24	29	0
<b>Bacteria</b>						
RLOs	3.3	3.3	13.3	6.7	3.3	3.3
<b>Protozoa</b>						
<i>Ancistrocoma</i> sp.	3.3	6.7	10			
<i>Trichodina</i> sp.		3.3	13.3			
<i>Sphenophrya</i> sp.	6.7					
<i>Nematopsis</i> sp.	66.7	20	63.3	70	93.3	100
<b>Metazoan</b>						
<i>Urastoma</i> sp.					6.7	16.6
<i>Bucephalus</i> sp.						3.3
Metacercariae NI				6.7		
<i>Tylocephalum</i> sp.		3.3	13.3	13.3		

digestive gland, but they also occurred in the gills and gonads. The number of oocysts/phagocytes varied between 1 and 8 (Figures 2e, f). The intensity of the infection was usually light (<5% TP). In most cases this protozoan did not cause a hemocytic

reaction or tissue damage. However, one oyster with high infection intensity in the mantle (TP = 47.6%) and gills (TP = 50.5%) showed extensive hemocyte infiltration and tissue destruction, especially in the gills (Figure 2e).



**Figure 2.** Bacteria and protozoa (indicated by arrows) associated with *Crassostrea rhizophorae* at three points in two estuaries (Marau = Camamu Bay and Graciosa) on the southern coast of Bahia. a) Intracellular colony of *Rickettsia*-like organisms (RLOs) in a cell that is separating from the digestive tubule epithelium. Bar = 20  $\mu$ m. b) *Ancistrocoma* sp. in the lumen of the secondary digestive duct. Bar = 20  $\mu$ m. c) *Trichodina* sp. in the lumen of a secondary digestive duct. Bar = 10  $\mu$ m. d) *Sphenophrya* sp. in the gills, with initial xenoma. Bar = 20  $\mu$ m. e) Intrahemocytic oocysts of *Nematopsis* sp. in high infection intensity in gills. Bar: 10  $\mu$ m. f) Phagocyte containing eight oocysts of *Nematopsis* sp. Bar = 20  $\mu$ m.

Among metazoans, only representatives of the phylum Platyhelminthes were observed: helminthes of genus *Urastoma* (Turbellaria), a trematode in the sporocyst phase of the genus *Bucephalus*, an unidentified metacercaria, and a cestode of the genus *Tylocephalum*. *Urastoma* sp. (Turbellaria: Urastomidae) (Figure 3a) was present only in oysters from Point 3, with prevalences of 6.7% in summer and 16.6% in winter (Table 1). This turbellarian was found in the gills and mantle connective tissue, with 1-2 individuals/histological section, measuring between 150 and 200  $\mu\text{m}$  in length. No tissue immunological response or apparent histological damage was observed.

The digenetic trematode *Bucephalus* sp. (Digenea: Bucephalidae), in the sporocyst phase – with germ masses and cercariae (identifiable by the forked tail with a very short and wide base) was observed in only one oyster from Point 3, during winter. The infection intensity was high in the gills (TP = 36.2%) and very high in the gonads (TP = 86.7%); the parasite caused castration, with complete destruction of follicles and germ cells, and made it impossible to determine the sex (Figures 3b, c).

Organisms with morphological characteristics of metacercariae (15 and 20  $\mu\text{m}$  in diameter) were observed; however, taxonomic identification was not possible (Figure 3d). Metacercariae were observed in the mantle of one oyster and in the digestive gland of another, both at Point 2, in low infection intensity (1-2 individuals/histological section), in winter. Histopathological changes were not observed.

Metacestodes of *Tylocephalum* sp. (Cestoda: Tetragonocephalidae), measuring between 120 and 200  $\mu\text{m}$  diameter, including the encapsulation reaction, were seen in oysters at points 1 and 2 in prevalence from 3.3% to 13.3%, with low infection intensity (1-2 individuals/histological section). The larvae were observed in the connective tissue between the acini of the digestive gland. All oysters parasitized by this cestode showed an intense hemocytic reaction and fibrosis of the connective tissue, encapsulation of the parasite and in some cases its reabsorption (Figure 3e).

## Discussion

With the exception of *Bucephalus* sp. and *Nematopsis* sp., all pathogens found in this study caused no evident histopathological changes in the oysters. The high prevalence of *Nematopsis* coincided with observations by Jones (1975) in *Perna canaliculus* (Gmelin, 1791) from New Zealand; Cáceres-Martínez et al. (2010) in *Crassostrea corteziensis* (Hertlein, 1951) from Mexico; Nascimento et al. (1986) (Bahia, Brazil), Sabry et al. (2007) (Ceará, northeastern Brazil) and Sabry et al. (2011) (Santa Catarina, southern Brazil) in *C. rhizophorae*; and by Pinto and Boehs (2008) and Ceuta and Boehs (2012) in *Mytella guyanensis* (Lamarck, 1819) from the Cachoeira River in Bahia and from Camamu Bay, respectively. Azevedo and Cachola (1992) observed a high prevalence of *Nematopsis* sp. in *Cerastoderma edule* (Linnaeus, 1758) from Portugal, and related it to high mortality of this bivalve. According to Bower et al. (1994), when present in low infection rates in the mollusk, this protozoan causes only hemocytic infiltration; however, at high levels it may cause gill destruction, tissue degeneration and death. In this study, one

heavily infected oyster showed histopathological changes, consisting of destruction of gill filaments and connective tissue, which may have compromised the physiology of the gills. Ceuta and Boehs (2012) observed tissue alteration in the labial palps of heavily infected *M. guyanensis*, suggesting functional impairment of this organ. In this study, due to obvious histopathological changes in at least one specimen of *C. rhizophorae* and its high prevalence in the region, we suggest that this pathogen should be monitored, especially in oyster farms.

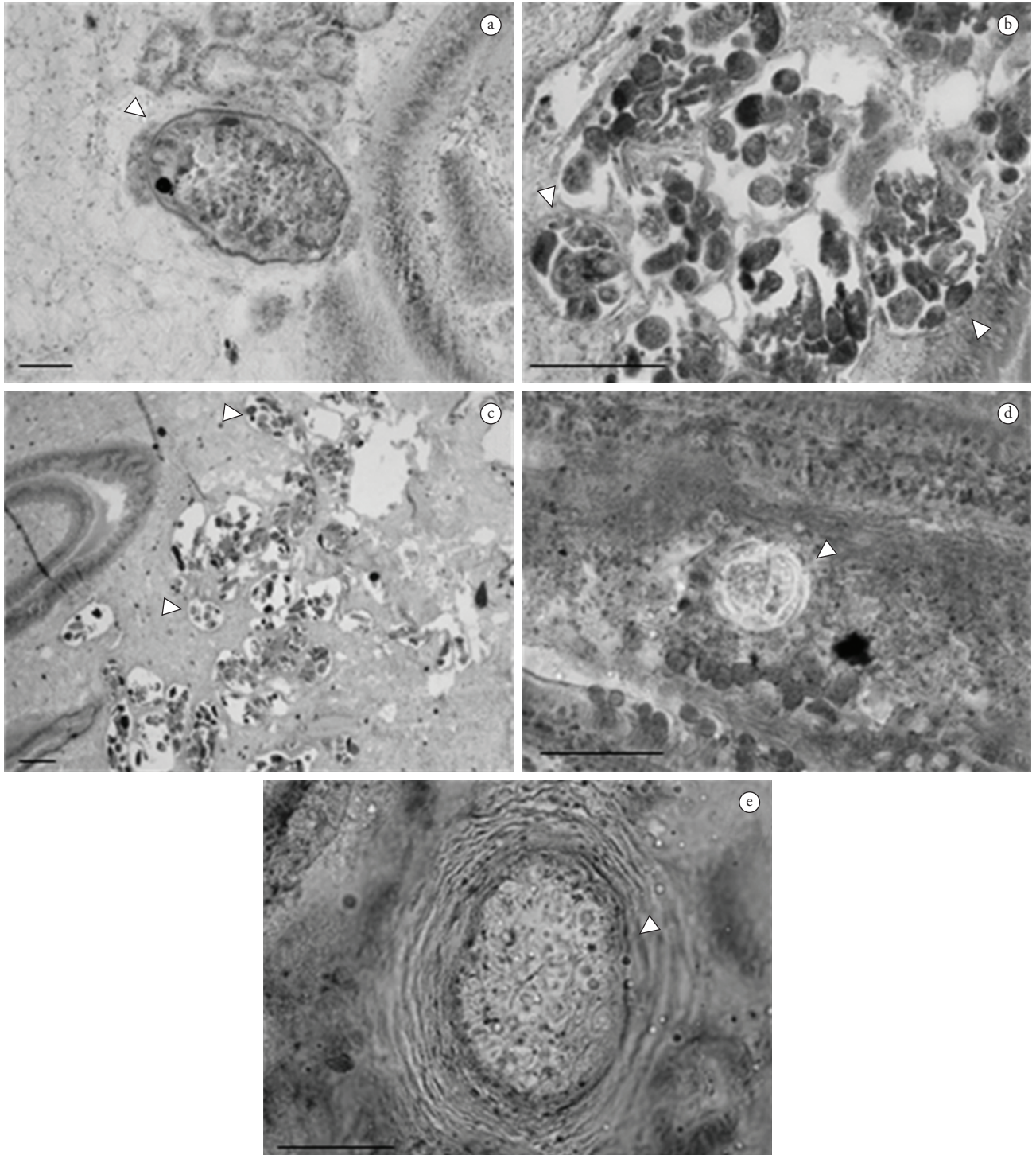
Although the digenetic *Bucephalus* sp. showed low prevalence, the oyster infected by this pathogen showed destruction of gonad tissue, causing parasitic castration. Castration caused by this parasite was also observed in *C. rhizophorae* (NASCIMENTO et al., 1986; ZEIDAN et al., 2012), *M. guyanensis* (BOEHS et al., 2010; CEUTA; BOEHS, 2012; ZEIDAN et al., 2012), *Anomalocardia brasiliiana* (Gmelin, 1791) (BOEHS et al., 2010) and *Perna perna* (Linnaeus, 1758) (DA SILVA et al., 2002, 2011; GARCIA; MAGALHÃES, 2008). These last authors observed moderate (TP = 5-50%) to high (TP > 50%) infection intensity.

*Rickettsia*-like organisms (RLOs) have been previously observed in the digestive epithelial cells of several species of bivalves from the Brazilian coast: the mussel *M. guyanensis* (BOEHS et al., 2010; CEUTA; BOEHS, 2012) and the oysters *C. rhizophorae* (DA SILVA et al., 2011; SABRY et al., 2011; ZEIDAN et al., 2012) and *Crassostrea gigas* (Thunberg, 1793) (PONTINHA, 2009; DA SILVA et al., 2011), with similar low prevalences (except for SABRY et al., 2011, who observed a prevalence of up to 30%) and low infection intensities, without causing disease, as reported in a review by Bower et al. (1994) for oysters in general. These organisms can also be found, apart from digestive tubules (primary and secondary, as recorded in the present study) in the epithelium of the intestine and stomach (CÁCERES-MARTÍNEZ et al., 2010), as reported by Sabry et al. (2011), who observed changes in the gastric epithelium affected. The results of this study also resemble those found by Boehs et al. (2010) in *M. guyanensis*, with the occurrence of these bacteria in the same anatomical region and low prevalence and degree of infection; and by Pontinha (2009), Da Silva et al. (2011) and Ceuta and Boehs (2012), in *C. gigas*, *C. rhizophorae* and *M. guyanensis*, respectively, with the occurrence of hypertrophy of cells infected by RLOs.

With respect to the ciliates observed in this study (*Sphenophrya*, *Trichodina* and *Ancistrocoma*), the proximity between points 1 and 2 and the similarity of the abiotic conditions of temperature and salinity explain their presence in both locations. No histopathological alteration caused by them was noticeable, and it is possible that these are, for mollusks, closer to commensals than parasites, as suggested by Lauckner (1983). However, if present in large quantity, these ciliates possibly may affect the functioning of the gills, causing changes in the respiratory and feeding functions. Specifically, *Sphenophrya* can cause a tumor called xenoma, i.e., a hypertrophy of the infected cell, including its nucleus (BOWER et al., 1994). The first report of xenoma formation in *C. rhizophorae* was by Boehs et al. (2009) in Camamu Bay in an area close to points 1 and 2 of this study, and was associated with this ciliate by the authors. As in this study, the authors reported a low prevalence. *Sphenophrya* sp. was previously recorded in Todos os Santos Bay (Bahia) in *C. rhizophorae*, by Nascimento et al. (1986), in low

prevalence and intensity of infection, but these authors did not report the formation of xenomas. In *C. rhizophorae* and *C. gigas* at the Island of Santa Catarina, even at sites with a high prevalence (70%), there were no reports of defense reaction or formation of xenomas (DA SILVA et al., 2011; SABRY et al., 2011).

With respect to *Trichodina*, although it has been associated with mortality of marine animals (BOWER et al., 1994), this ciliate did not cause damage in the oysters analyzed here. The horseshoe-shaped basophile nucleus was similar to previous observations for other host species, with varying prevalences (BOEHS; MAGALHÃES, 2004;



**Figure 3.** Metazoans (indicated by arrows) associated with *Crassostrea rhizophorae* at three localities (sampling points) in two estuaries (Maraú = Camamu Bay and Graciosa) on the southern coast of Bahia. a) *Urastoma* sp. in connective tissue surrounding the digestive gland. Bar = 50  $\mu$ m. b) Sporocyst of *Bucephalus* sp. containing germ masses and cercariae. Bar = 50  $\mu$ m. c) *Bucephalus* sp. in high infection intensity, with destruction of reproductive follicles and connective tissue of the mantle. Bar = 50  $\mu$ m. d) Nonspecific metacercaria in the mantle. Bar = 20  $\mu$ m. e) *Tylocephalum* sp. reabsorbed between acini of the digestive gland. Bar = 50  $\mu$ m.

PONTINHA, 2009; DA SILVA et al., 2011; SABRY et al., 2011). There are indications that localities with high levels of organic pollution have increased the abundance of this ciliate, and accordingly some investigators believe that it has potential for use as a bioindicator (MARCOGLIESE; CONE, 1997; PALM; DOBBERSTEIN, 1999; OGUT; PALM, 2005; AKŞIT et al., 2008). The low prevalence of this protozoan in the study region may suggest a low environmental impact by domestic sewage and other pollutants. The absence of damage and host defense response were also observed with respect to the ciliate *Ancistrocoma*, according to studies by Pontinha (2009) and Sabry et al. (2011) in *C. gigas*; and for *C. rhizophorae* by Sabry et al. (2011) and Zeidan et al. (2012).

Metacercariae, observed in low prevalence in oysters only at Point 2, were present in the gills and digestive gland. Boehs et al. (2010) observed unidentified metacercariae in the digestive gland of *A. brasiliiana* and in the gonad of *Iphigenia brasiliiana* (Lamarck, 1818), and Da Silva et al. (2011) in the kidney of *A. brasiliiana*, in low prevalences. In the bivalve *Tagelus plebeius* (Lightfoot, 1786) studied at the Island of Santa Catarina, metacercariae of the genus *Parvatrema* (Cable, 1953) were observed in the mantle and labial palps, with high prevalence (up to 100%) and induced hyperplasia; metacercariae of the family Echinostomatidae were observed with lower prevalence, infecting the gonad connective tissue (DA SILVA et al., 2009). Tegumentary spines were seen on the metacercariae in this study, as reported elsewhere (CREMONTE et al., 2005; BOEHS et al., 2010), but it was not possible to identify this trematode.

Regarding the cestode *Tylocephalum*, the pathogen encapsulation response by the host was observed in all oysters parasitized. Ceuta and Boehs (2012) observed the same response in *M. guyanensis* from Camamu Bay, but no other histopathological damage to the host. These same authors also found that the parasite occurred in the mantle connective tissue. In addition to the mantle, in the present study the metacestode was also found in the connective tissue between the acini of the digestive gland, as observed by Sabry et al. (2007) in *C. rhizophorae*, Boehs et al. (2010) in *A. brasiliiana* and *I. brasiliiana*, Da Silva et al. (2011) in *Perna perna* and Sabry et al. (2011) in *C. gigas*.

In this study, although certain pathogens occurred at only some of the sampling points and estuaries, it was not possible to make inferences about the existence of a spatial-temporal pattern, or to establish correlations with temperature and salinity, given the specific character of the sampling. The ecology of parasitic infections of mollusks in Brazil is still in an initial phase, and many aspects need to be further researched for a better understanding, not only of the relationship of pathogens to the environment, but also to the host.

## Conclusions

The low prevalence and infection intensity found in this study indicate that the natural population of *C. rhizophorae* is in good general health on the southern coast of Bahia. This is most likely associated with the good environmental quality in this region. However, we believe that the digenetic trematode

*Bucephalus* sp. and the protozoan *Nematopsis* sp., when at high levels of infection, may threaten the health of *C. rhizophorae*. Constant health monitoring can promote the sustainability of local oyster farming. Studies are needed to clarify the ecological relationships between these pathogens and this host; seek possible control methods; and evaluate possible zoonotic aspects associated with the consumption of this oyster.

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