First record of parasitism in the mangrove oyster *Crassostrea rhizophorae* (Bivalvia: Ostreidae) at Jaguaribe River estuary – Ceará, Brazil

Sabry, RC.[a]*, Gesteira, TCV.*[a] *and Boehs, G.*[b]

[a]Instituto de Ciências do Mar – LABOMAR, Universidade Federal do Ceará, Av. Abolição, 3207, Meireles, CEP 60165-081, Fortaleza, CE, Brazil

[b]Departamento de Ciências Biológicas – DCB, Universidade Estadual de Santa Cruz – UESC, Rodovia Ilhéus-Itabuna, Km 16, CEP 45650-000, Ilhéus, BA, Brazil

*e-mail: rachelsabry@yahoo.com.br

Received February 22, 2006 – Accepted May 8, 2006 – Distributed November 30, 2007

(With 4 figures)

Abstract

Mangrove oysters *Crassostrea rhizophorae* were sampled monthly in the estuary of Jaguaribe River, on the east coast of Ceará State, Brazil, between August, 2000 and December, 2001, making up 170 individuals. The water temperature varied from 26 to 30 °C and salinity from 21 to 42‰. The animals' size ranged from 3.4 to 7.2 cm height. Macroscopical and histopathological analyses were carried out in the oysters' tissues. The histological exams showed protozoans and metazoans of genera *Nematopsis* and *Tylocephalum*, respectively. *Nematopsis* prevalence varied from 60 to 100% and it was higher in the gills and mantle. The oocysts presented a mean size of 11.5 µm (±1.32) length and 9.1 µm (±1.06) width (n = 30), up to 3 oocysts/phagocyte having been observed. Several animals presented focal hemocitical reaction. The percentage of *Tylocephalum* was 1.7%. In spite of the high infection prevalence by *Nematopsis*, infected animals did not have their reproductive cycle impaired.

Keywords: oyster, parasite, histopathology, *Nematopsis*; bivalve.

Primeiro registro de parasitismo na ostra do mangue *Crassostrea rhizophorae* (Bivalvia: Ostreidae) do Estuário do Rio Jaguaribe – Ceará, Brasil

Resumo

Ostras do gênero *Crassostrea rhizophorae* foram coletadas mensalmente no Estuário do Rio Jaguaribe, litoral Leste do Estado do Ceará, Brasil, entre agosto de 2000 e dezembro de 2001, totalizando 170 animais. A temperatura da água do local variou de 26 a 30 °C e a salinidade de 21 a 42‰. O tamanho dos animais variou de 3,4 a 7,2 cm de altura de concha. As ostras foram submetidas à análise macroscópica e histopatológica dos tecidos. Os exames histológicos evidenciaram protozoários e metazoários dos gêneros *Nematopsis* e *Tylocephalum*, respectivamente. A prevalência de *Nematopsis* variou de 60 a 100% e foi maior nas brânquias e manto. Os oocistos apresentaram tamanho médio de 11,5 µm (±1,32) e 9,1 µm (±1,06), (n = 30), sendo observados até 3 oocistos/fagócito. Alguns animais apresentaram reação hemocítica focal. A porcentagem de *Tylocephalum* foi de 1,7%. Apesar da elevada prevalência de infecção por Nematopsis, aparentemente os animais parasitados não tiveram o seu ciclo reprodutivo prejudicado.

Palavras-chave: ostra, parasita, histopatologia, *Nematopsis*, bivalve.

1. Introduction

The mangrove oyster, *Crassostrea rhizophorae* (Guilding, 1828), is an important fishery resource in the estuaries of Ceará State, Northeastern Brazil, and its rearing constitutes an alternative of revenue for riverine populations. The diseases can cause significant loss of bivalve mollusks, both in natural and reared areas. According to Figueras and Novoa (2004), studies on bivalve pathologies have been much focalized; however, there are still many questions to answer, i.e. the pathogenicity of new identified parasites and its impact on bivalves.

Protozoans of *Nematopsis genus* (Schneider, 1892) have been reported in many marine bivalves (Azevedo and Cachola, 1992; Carballal et al., 2001; Winstead

et al., 2004; Cremonte et al., 2005). Those parasites are often observed in the gills and mantle of mollusks and complete their life cycle in the gut of marine arthropods (Bower et al., 1994).

Metazoan larvae of *Tylocephalum* genus Linton (1890) were observed infecting oysters of genera *Crassostrea* (Cheng, 1975; Winstead et al., 2004) and *Pinctada* (Hine and Thorne, 2000). Adult individuals of this parasite are found infecting the elasmobranchii's gut spiral valve (Lauckner, 1983). In South America, there is not enough knowledge about health status and parasites of bivalves (Bower et al., 1994). According to Cremonte et al. (2005), this occurs because the mollusks' rearing is poorly developed in the coasts of South Western Atlantic. In Brazil, studies on mollusk diseases are recent and scarce. However, they have been enhanced due to the expansion of cultivation systems. Parasitism by *Bucephalus* sp. was recorded in mussels *Perna perna* in Santa Catarina State, South Brazil (Silva et al., 2002) and in *Anomalocardia brasiliana* in Ceará State (Araújo and Rocha-Barreira, 2004). *Nematopsis* occurrence was recorded in *C. rhizophorae* (Nascimento et al., 1986), and in mussels *Mytella guyanensis* (Azevedo and Matos, 1999) and *Perna perna* (Lima et al., 2001). Metazoans of genus *Tylocephalum* were observed in *C. rhizophorae* (Nascimento et al., 1986) and in *A. brasiliana* (Boehs and Magalhães, 2004).

This study aims to investigate the presence of parasites in the mangrove oyster *C. rhizophorae* on the Jaguaribe River Estuary, in Fortim, Ceará, Brazil.

2. Material and Methods

2.1. Field procedures

Adult oysters were gathered monthly (n = 10) on the Jaguaribe River Estuary (04° 27' 07" and 37° 47' 50") from August, 2000 to December, 2001, totalling 170 animals. Water variables (temperature and salinity) of the collection site were measured monthly during the whole study period.

2.2. Laboratory procedures

The animals were analyzed in the laboratory of the Bivalve Mollusks Study Group (GEMB), installed in the Marine Science Institute of Federal University of Ceará. The oyster height was measured using a caliper rule according to Galtsoff (1964). After that, a macroscopic exam of the tissues and shells was carried out in order to evaluate the presence or signs of parasitism. For histological procedures, the tissues of each animal were fixed in "Bouin" during 24 hours under room temperature. After fixation, the material was transferred to a 70% ethanol solution and prepared according to the classical histological techniques (Howard and Smith, 1983), which included paraffin embedding, sectioning (5 μm) and hematoxilin-eosin (HE) staining. The slides were examined with an optical microscope, recorded and photomicrographed. All the histological sections of parasited oysters were kept by the authors.

3. Results

The water temperature varied from 26 to 30 °C and salinity from 21‰ (April) to 42‰ (November). Oyster size varied from 3.4 to 7.2 cm shell height. Macroscopical analyses of tissues and shells did not show any symptom or sign of parasitism. However, histopatological analyses detected the occurrence of *Nematopsis* sp. parasiting mantle, gonads, gills and digestive glands (Figure 1). The prevalence of infection varied from 60% to 100% and it was higher in the gills and mantle (Figure 2). Oocysts measured under light microscopy, presented a mean size of 11.5 μm (±1.32) length and 9.1 μm (±1.06) width (n = 30), with up to 3 oocysts/phagocyte being observed. Several animals presented focal hemocitical reaction (Figure 3). Metazoans larvae of genus *Tylocephalum* were found capsulated in the connective tissue around the digestive gland, appearing in 1.7% of the analyzed individuals (Figure 4).

4. Discussion

Oocysts occurrence of *Nematopsis* in the connective tissue of marine bivalves, mainly in the gills, is widely registered (Nascimento et al., 1986; Azevedo and Cachola, 1992; Bower et al., 1994; Azevedo and Matos, 1999; Carballal et al., 2001). Nevertheless, the pathogeny of this gregarine is very doubtful. Azevedo and Cachola (1992), studying the *Nematopsis* effect on *Ruditapes decussatus* and *Cerastoderma edule*, observed destruction of gill cells infected with oocysts and linked it to animal mortality, mainly in *C. edule*. According to Bower et al. (1994), infections caused by those protozoans are usually associated with a focal hemocyte infiltration, without measurable effects on health. In this study, it was observed that infected animals did not present damage in tissues and/or organs, and apparently they did not have their reproductive cycle impaired.

Only oysters with a high number of oocysts presented histopathological signs of focal host reaction. This effect and small alterations of gill filaments, as a result of lesions, were also observed by Carballal et al. (2001) in *C. edule* with a high infection degree. Pearls production or calcareous sediment in the internal shell surface was associated with *Nematopsis* oocysts presence in the mantle tissue of *Mytillus edulis* (Lauckner, 1983). Lima et al. (2001) and Winstead et al. (2004) verified the absence of pathologic processes linked to *Nematopsis* presence in the mussel *Perna perna* and in the oyster *C. virginica*, respectively. Cremonte et al. (2005) relate also the absence of host response to parasitism by *Nematopsis* in the gut epithelial cells of *Pitar rostrata*. Nascimento et al. (1986), observed equally with this study, high prevalence of *Nematopsis* in oysters *C. rhizophorae* from Bahia State, Brazil, but it had very low to moderate levels of infection. Those authors concluded that the presence of this protozoan alone is not enough to cause mortality in oysters.

In this work, the slightly lower prevalence of *Nematopsis* was related to the rainy season, a period

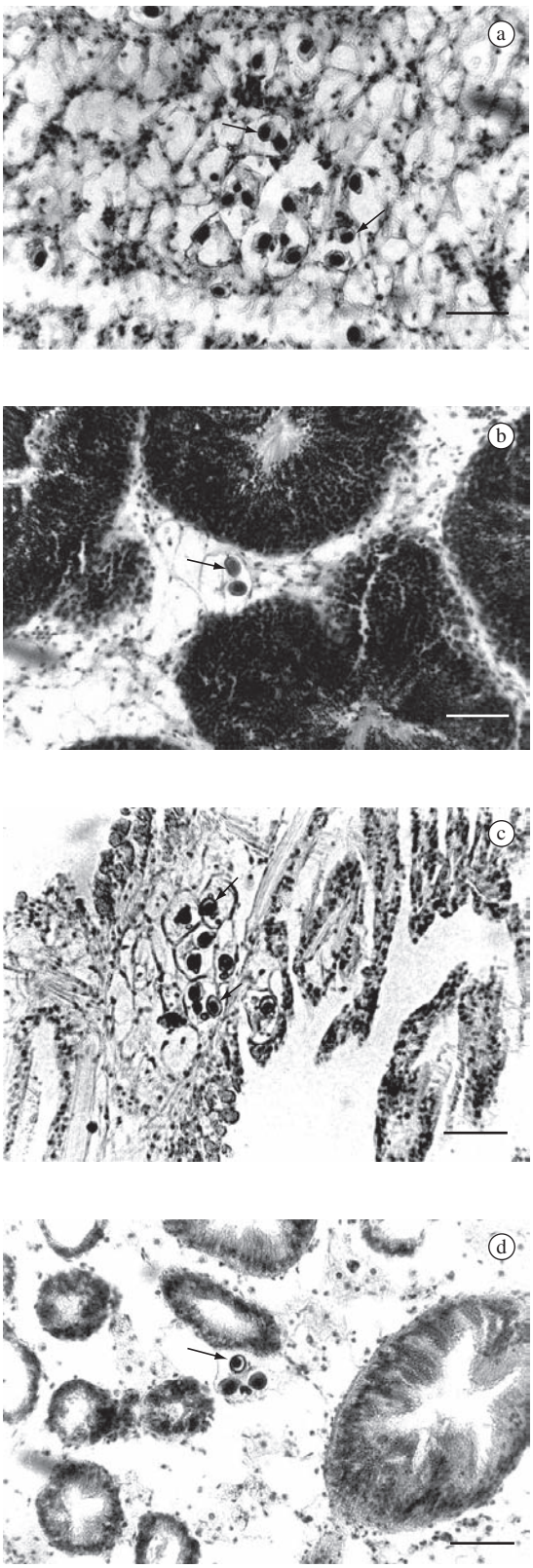

Figure 1. Photomicrographs of *Nematopsis* sp. in *Crassostrea rhizophorae*. a) mantle, b) male gonad, c) gills, and d) digestive gland. Staining: HE. Bar = 20 µm.

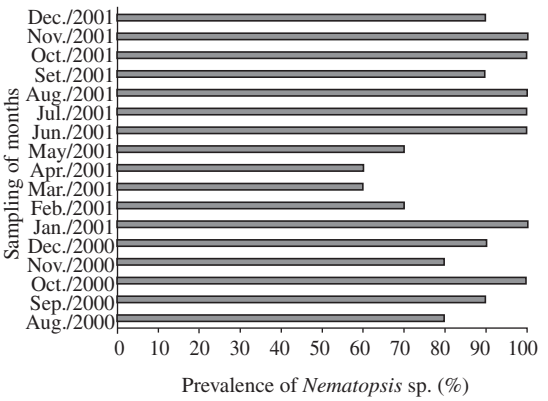

Figure 2. *Nematopsis* prevalence in *Crassostrea rhizophorae* during the studied period (August, 2000 to December, 2001).

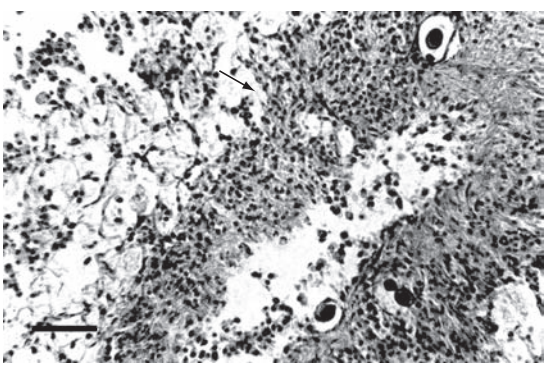

Figure 3. Histological section of the mantle in *Crassostrea rhizophorae* parasitised by *Nematopsis* sp., showing hemocitical reaction (arrow). Staining: HE. Bar. = 20 µm.

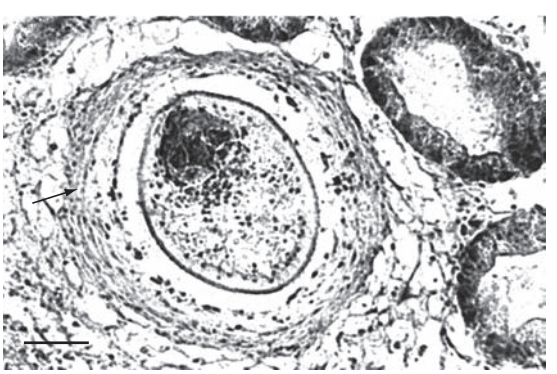

Figure 4. *Tylocephalum* larva around digestive gland of *Crassostrea rhizophorae* Staining: HE. Bar = 20 µm.

when both temperature and salinity decrease in the area. However, we cannot establish a relationship between the results and the distribution pattern of the parasite during that period.

The presence of a variable number of *Nematopsis* oocysts per host cell (phagocyte), usually 1 to 2, with each oocyst containing a single uninucleate sporozoite, coincides with observations made by Azevedo and Matos (1999) and Carballal et al. (2001).

In spite of low presence (1.76%) of the metazoan *Tylocephalum* found in *C. rhizophorae* during this study, its occurrence seems to be correlated with the higher water temperature, since those organisms are more easily found in tropical and subtropical waters. In relation to its pathogeny, except for the site where the parasite has lodged, it has been concluded that the larvae do not cause damage to the host. Nascimento et al. (1986), also concluded that damages caused by *Tylocephalum* in the oyster *C. rhizophorae* from Bahia State had only mechanical effects, caused by the entrance of this cestode into oyster tissues. According to the present study, larvae capsulation of *Tylocephalum* by fiber cells of the oysters (revealing a response of the host) is recorded in a number of papers (Cheng, 1975; Lauckner, 1983; Hine and Thorne, 2000; Boehs and Magalhães, 2004).

In short, low infection intensity by the parasites found in this work suggests that it is still not interfering in natural stocks or in oyster farming in Ceará State. However, more studies are required in order to evaluate the occurrence and pathogenicity of these and other oyster parasites in the studied region.

References

ARAÚJO, MLR. and ROCHA-BARREIRA, CA., 2004. Occurrence of *Bucephalus* sp. (Trematoda, Bucephalidae) in *Anomalocardia brasiliana* (Gmelin, 1791) (Mollusca, Veneridae) at Canto da Barra Beach, Fortim, Ceará State, Brazil. *Arq. Ciên. Mar*., vol. 37, p. 35-38.

AZEVEDO, C. and CACHOLA, R., 1992. Fine structure of the apicomplexa oocyst of *Nematopsis* sp. of two marine bivalve molluscs. *Dis. Aquat. Org*., vol. 14, p. 69-73.

AZEVEDO, C. and MATOS, E., 1999. Description of *Nematopsis mytella* sp. (Apicomplexa), parasite of the mussel *Mytella guyanensis* (Mytilidae) from the Amazon Estuary and description of its oocytes. *Europ. J. Protistol*., vol. 35, p. 427-433.

BOEHS, G. and MAGALHÃES, ARM., 2004. Simbiontes associados com *Anomalocardia brasiliana* (Gmelin) (Mollusca, Bivalvia, Veneridae) na Ilha de Santa Catarina e região continental adjacente, Santa Catarina, Brasil. *Rev. Bras. Zool*., vol. 21 no. 4, p. 865-869.

BOWER, SM., MCGLADDERY, SE. and PRICE, IM., 1994. Synopsis of infectious diseases and parasites of commercially exploited shellfish. Annu. *Rev. Fish Dis*., vol. 4, p. 1-199.

CARBALLAL, MJ, IGLESIAS, D., SANTAMARINA, J., FERRO-SOTO, B. and VILLALBA, A., 2001. Parasites and pathologic conditions of the cockle *Cerastoderma edule* populations of the coast of Galicia (NW Spain). *J. Invertebr. Pathol*. vol. 78 no. 2, p. 87-89.

CHENG, TC., 1975. New geographic records for *Tylocephalum* metacestodes. *J. Invertebr. Pathol*., vol. 26, p. 395-396.

CREMONTE, F., BALSEIRO, P. and FIGUERAS, A., 2005. Occurrence of *Perkinsus olseni* (Protozoa: Apicomplexa) and other parasites in the venerid commercial clam *Pitar rostrata* from Uruguay, southwestern Atlantic Coast. *Dis. Aquat. Org.*, vol. 64 no.1, p. 85-90.

FIGUERAS, A. and NOVOA, B., 2004. What has been going on in Europe in bivalve pathology? Bull. Europ. Assoc. *Fish Pathol*., vol. 24 no. 1, p. 16-21.

GALTSOFF, PS., 1964. The American oyster *Crassostrea virginica*. Fish. Bull., vol. 64, p. 1-480.

HINE, PM. and THORNE, T., 2000. A survey of some parasites and diseases of several species of bivalve mollusk in northern Western Australia. *Dis. Aquat. Org*., vol. 40, p. 67-68.

HOWARD, DW. and SMITH, CS., 1983. Histological techniques for marine bivalve mollusks. Woods Hole, Massachusetts, NOAA Technical Memorandum, 97p.

LAUCKNER, G., 1983. Diseases of Mollusca: Bivalvia. In KINNE, O. (ed.), *Diseases of Marine Animals, Introduction Bivalvia to Scaphopoda*, vol. 2. Biologische Anstalt Helgoland, Hamburg, p. 477-879.

LIMA, FC., ABREU, MG. and MESQUITA, EFM., 2001. Monitoramento histopatológico de mexilhão *Perna perna* da Lagoa de Itaipu, Niterói, RJ. *Arq. Bras. Med. Zootec*., vol. 53, no. 2, p. 203-206.

NASCIMENTO, IA., SMITH, DH., KERN II, F. and PEREIRA, SA., 1986. Pathological findings in *Crassostrea rhizophorae* from Todos os Santos Bay, Bahia, Brazil. *J. Invertebr. Pathol*., vol. 47 no. 3, p. 340-349.

SILVA, PM., MAGALHÃES, ARM. and BARRACCO, MA., 2002. Effects of *Bucephalus* sp. (Trematoda: Bucephalidae) on Perna perna mussels from a culture station in Ratones Grande Island, Brazil. *J. Invertebr. Pathol*., vol. 79, no. 3, p. 154-162.

WINSTEAD JT., VOLETY, AK. and TOLLEY SG., 2004. Parasitic and symbiotic fauna in oyster (*Crassostrea virginica*) collected from the Caloosahatchee River and Estuary in Florida. *J. Shellf. Res*., vol. 23, no. 3, p. 831-840.