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Original Article

# Pathogens and microorganisms in the mangrove oyster *Crassostrea gasar* cultivated in an estuarine environment in Northeast Brazil

## Patógenos e microorganismos presentes na ostra nativa *C. gasar* cultivada em ambiente estuarino do Nordeste do Brasil

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**Abstract**

Estuaries are important ecosystems due to the ecological services they provide, acting as nurseries for many species of fish and invertebrates, and are also used as environments for the extraction and cultivation of mollusks. Oysters are animals that filter water to obtain oxygen and nutrients. In this process, they can bioaccumulate microorganisms and chemical substances in their tissues. The growth of mollusk culture in Northeastern Brazil requires the health identification of cultivated oysters through the quantification of the potentially harmful microbiota accumulated in the animals. Therefore, the present work aims to quantify and identify bacteria and possible pathogens found in the tissues of cultivated oysters and their culture waters. The Most Probable Number of Coliforms (MPN) in oysters and water were considered suitable according to the Brazilian current legislation, *Vibrio* sp. obtained low colonization and *Salmonella* sp. was not observed. The prevalence of microorganisms potentially pathogenic to oysters was 33.7%, highlighting metazoans and *Nematopsis* sp., however, the intensity of the infestation of these organisms was moderate. The low contamination of oysters demonstrates that this culture environment is promising for this activity. However, continuous environmental and sanitary monitoring is fundamental to guarantee the safety of the culture waters and the sustainability of aquaculture activities.

**Keywords:** environmental health, mollusk culture, parasites, coliforms.

**Resumo**

Os estuários são ecossistemas importantes devido a serviços ecológicos que fornecem, os quais conferem a função de berçário para muitas espécies de peixes e invertebrados, e também são utilizados como ambientes de extração e cultivo de moluscos. As ostras são animais que filtram a água para obtenção de oxigênio e nutrientes. Nesse processo podem bioacumular microorganismos e substâncias químicas em seus tecidos. O crescimento da malacocultura no Nordeste do Brasil fomenta a necessidade de identificar a sanidade das ostras cultivadas através da quantificação da microbiota potencialmente nociva acumulada nos animais. Portanto o presente trabalho visa quantificar e identificar bactérias e possíveis patógenos encontrados nos tecidos dos moluscos cultivados e nas suas águas de cultivo. O Número mais Provável de Coliformes (NMP) nas ostras e na água foram considerados próprios segundo as legislações vigentes, *Vibrio* sp. obteve baixa colonização e *Salmonella* sp. não foi observada. A prevalência de microorganismos potencialmente patógenos para as ostras foi de 33,7%, destacando como mais prevalentes os metazoários e *Nematopsis* sp., porém a intensidade da infestação desses organismos foi moderada. A baixa contaminação das ostras demonstra que este ambiente de cultivo é promissor para esta atividade. No entanto, o contínuo monitoramento ambiental e sanitário é fundamental para garantir a inocuidade das águas de cultivo e a sustentabilidade das atividades aquícolas.

**Palavras-chave:** sanidade ambiental, malacocultura, parasitas, coliformes.

## 1. Introduction

Mollusk farming is Brazil's third most important aquaculture activity, representing 15,000 t in 2019 (IBGE, 2021). This production is based on three species, two native mangrove oysters *Crassostrea rhizophorae* (Guilding, 1828) and *Crassostrea gasar* (Adanson, 1757), and one introduced species *Crassostrea gigas* (Thunberg, 1793) (Sampaio et al.,

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2017). In Northeast Brazil, the oyster culture is based on the native mangrove species and is a small-scale activity usually run by small associations or family-based.

Environmental quality is essential for oyster farming since it helps minimize production diseases, including infections caused by bacteria, protozoa, viruses, and fungi (Ingham and Schimdt, 2000; Zeidan et al., 2012). Diseases of the highest incidence are those of the gastrointestinal tract, associated with consumption of contaminated fish and seafood (Mignani et al., 2013).

Oysters are filter feeders as they pump water into their bodies. An oyster can retain up to 75% of the microorganisms present in the environment, characterizing it as a bioaccumulating animal (Instituto Adolfo Lutz, 2004; Pontual et al., 2006; Zhang et al., 2015).

Bacterial microbiota naturally occurring in the environment can accumulate in oysters and thus become potentially pathogenic to human health, mainly if consumed raw or only lightly cooked (Vásquéz-García et al., 2019). Several studies record contamination by pathogenic microorganisms in oysters, from poor environmental quality, such as *Escherichia coli* (Miotto et al., 2019; Oliveira et al., 2020; Souza et al., 2023), *Aeromonas* sp. (Figueras et al., 2017; Ribeiro et al., 2020; Souza et al., 2023), Coagulase positive *Staphylococcus* (Silva et al., 2020; Souza et al., 2023), *Salmonella* sp. (Fang et al., 2015; Cabral et al., 2017; Souza et al., 2023) and *Vibrio* sp. (Audemard et al., 2018; Shen et al., 2019), highlighting the risk to public health. These bacteria are studied worldwide because of their prevalence and pathogenicity (Zhang et al., 2015).

In addition to environmental quality, knowledge of the organisms that parasitize these mollusks is necessary to assist aquaculture management since this microbiota influences the health of animals planned for commercialization. Among the parasites most known for attacking bivalves are the bacteria of the genus *Rickettsia* Rocha Lima, 1916, protozoa such as *Ancistrocoma* sp. Chatton and Lwoff, 1926, *Tylocephalum* sp. Linton, 1890, *Nematopsis* sp Schneider, 1892, *Sphenophrya* sp. Chatton and Lwoff, 1921, *Trichodina* sp. Ehrenbeg, 1830, *Perkinsus* sp. Levine, 1978, *Steinhausia mytilovum* Field, 1924, *Urastoma* sp. Dörler, 1900; the polychaetes *Polydora websteri* Hartman in Loosanoff & Engle, 1943, *Neanthes succinea* Leuckart, 1847; the digenetic trematodes *Bucephalus* sp. Baer, 1827; the copepod *Pseudomyicola spinosus* Raffaele and Monticelli, 1885, and unidentified fungi (Sabry and Magalhães, 2005; Boehs et al., 2009; Boehs et al., 2012; Scardua et al., 2017).

Studies addressing cultivated oysters' health through integrated evaluation of the presence and quantity of microorganisms are scarce in Brazil, particularly in the Northeast, a region with significant potential for aquaculture. This study evaluated the sanity of the *C. gasar* cultivated in Maranhão, Northeast Brazil, by identifying and quantifying microorganisms and parasites in cultivated oysters and surrounding water, supporting commercial oyster farming implementation in the region.

## 2. Materials and Methods

### *2.1. Study area and sample collection*

Samplings were performed in an experimental fixed rack and bag oyster cultivation unit located in an estuarine area (2º25'15.9 "S 43º26'5.1 "W) in Primeira Cruz municipality, Maranhão state, Northeast Brazil (Figure 1).

During November and December 2017 (dry season) and March and May 2018 (rainy season), water and oyster samples were collected at low tide to evaluate microbiological aspects and parasites. In each month mentioned, one liter of water was collected in an amber bottle, and 30 specimens of oysters were obtained from the experimental culture unit. Samples were stored on ice

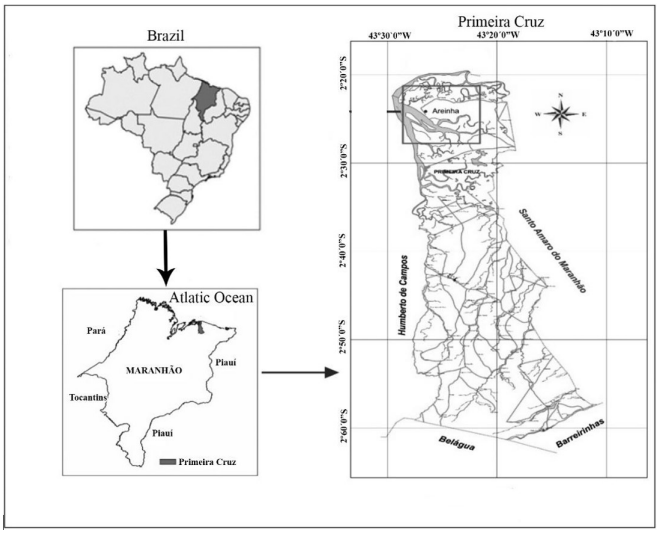


**Figure 1.** Location of the oyster cultivation unit in Primeira Cruz municipality, Maranhão state, Northeast Brazil. Source: Geoenvironmental Nucleus of the State University of Maranhão, 2018.

in an isothermal box and transported to the laboratory to conduct the microbiological analyses within 24 h.

Abiotic parameters (temperature, salinity, and dissolved oxygen) were also measured using a multiparameter and manual optical refractometer. Rainfall data were obtained from the Geoenvironmental Nucleus of the State University of Maranhão-UEMA.

*2.2. Processing and microbiological analysis*

In the laboratory, ten oysters were washed, dried, opened, and then aseptically eviscerated at each sampling. The soft parts and intervalvar liquor were removed and weighed on a precision scale. Five sets of four tubes were organized to count coliforms in the water and oysters, each with 9 mL of sterilized culture medium enriched with Lauryl Sulfate Broth (APHA, 2001). Aliquots of 1mL of crushed soft tissues and water were transferred to the five-tube series ($10^{-1}$ to $10^{-4}$ for the water samples and $10^{-1}$ to $10^{-3}$ for the oyster samples). Subsequently, they were incubated at 35ºC/48 h. Aliquots were removed and transferred to EC Broth and Bile Brilliant Green 2% tubes. From the EC, aliquots were removed and incubated at 45°C/24 h, and the tubes containing inoculum in Bile Brilliant Green were incubated at 35°C/24 h. The test results were determined by turbidity in the medium and gas production. The Most Probable Number (MPN) was calculated by consulting the MPN table in the Bacteriological Analytical Manual of the Division of Microbiology (U.S. Food & Drug Administration, 1998).

For the analysis of *Vibrio* sp., 25 g of meat samples were weighed together with intervalvar liquor, grounded, and homogenized in 225 mL of peptone water for 30 min. From the initial dilution ($10^{-1}$), a series of dilutions ($10^{-2}$ to $10^{-4}$) were prepared. After the incubation period, turbidity was verified to confirm the presence of *Vibrio* sp. From the positive tubes, 0.2 mL aliquots of each dilution were removed and spread on the surface of thiosulfate-citrate-bile-sucrose agar (TCBS) using the spread plate technique. Plates with colony growth (positive and negative sucrose) (Baron et al., 1994) were counted by observing the interval between 25 and 250 colonies (Downes and Ito, 2001). Finally, the standard plate count was measured in the Colony Forming Unit per g (CFU/ g).

To detect the presence of *Salmonella* sp., 25g of oyster and 225 mL of Difco Lactosate Broth (Difco-CL) were crushed and then incubated at 37ºC/24 h. Aliquots of 0.1 mL and 1.0 mL were then collected from the CL broth and inoculated into tubes containing 10 mL of Rappaport Broth (RV-Difco), Selenite Broth, and incubated at 42°C and 43°C, respectively, for 24 h in a water bath. From the microbial growth in both tubes, aliquots of each medium were plated using the surface plating method on Hektoen (HE) agar and Xylose Lysine Deoxycholate Agar (XLD). The colonies that presented growth characteristics of *Salmonell*a sp. (blackened colonies) were inoculated on Triple Iron Agar (TSI-Difco) and Lysine Iron Agar (LIA- Difco) and incubated at 37ºC/24 h. The growth on TSI agar (acidic butt and alkaline slant) and in the LIA agar (alkaline slant and butt, with or without production of $H_2S$) were considered to indicate the presence of the bacteria. The results were expressed as presence or absence in 25 g of the sample.

*2.3. Analysis of parasites*

For the microscopic analysis of parasites in the tissue of the cultivated oysters, 20 organisms were evaluated in each sampling. Oysters were sent to the laboratory, where the height measurement of the shells, with the aid of a manual pachymeter, and subsequently, the evisceration and cutting of the tissue through transverse sections were carried out.

The gills, mantle, digestive gland, and gonads were sampled, as Howard and Smith (1983) described. The tissues were fixed in saline Davidson solution for approximately 24 h and then stored in vials containing 70% alcohol.

In the laboratory, the samples were dehydrated in a series of increased alcohol and then underwent diaphanization in xylene, clarification, and inclusion in paraffin. The prepared sections were cut to 5 µm in a microtome. The resulting sections were laid on slides stained on Harris Hematoxylin and Eosin (HE), and then the sections were mounted and sealed in Entellan® medium.

The slides were analyzed with an optical microscope to identify and quantify the number of parasites and infestation intensity, if prevalent in the animal. The prevalence of infection was calculated as a function of the number of animals infected by the number of animals analyzed, according to Bush et al. (1997), and the results were expressed as percentages.

The intensity of infection was calculated using the stereology technique proposed by Lowe et al. (1994), using the Weibel graticule, where the tissue area of the parasite was calculated in five fields of the mantle of each animal with parasitizes. The results were analyzed according to the classification of Lowe et al. (1994): I - Low infestation = <5%; II - moderate = 5-25%; III - high = 25-50%, and IV - very high = over 50%.

Shapiro-Wilk and Levene tests were used to verify the distribution of data. To compare the mean height of the oysters between the dry and rainy seasons, the Student-*t-test* was used. To demonstrate differences in prevalence between the months, the Chi-Square and Mann-Whitney tests were used at the 95% significance level, and the results were obtained with the STATISTICA and PAST programs.

## 3. Results

*3.1. Abiotic variables*

The cultivation water temperature varied from 29.1 to 31.2°C, the salinity from 24 to 41, and the precipitation from 40 to 303.8 $mm^3$. The dissolved oxygen values varied from 5.3 to 5.7 mg / L (Table 1).

*3.2. Biometric variables*

The mean height of the oysters was 43.25±6.8 mm, ranging from 57.2±9.7 mm in November to 35.1±6.5 mm in March. There was a significant difference in the average height of oysters between the dry and rainy seasons ($t= 4.2$; $p<0.05$).

### 3.3. Water and oyster microbiology

The MPN results for thermotolerant and total coliforms for the cultivation water were similar in all months tested (<1.8 / 100 mL). The oysters showed total MPN from <3.0 / g to 210 / g and variance in thermotolerants from <3.0 / g to 23 / g. The highest results for both were recorded in November.

The CFU for *Vibrio* sp. was higher than 2.6 $10^3$ CFU / g (Table 2). Most colonies were sucrose fermenters, presenting smooth and opaque colonies with thin yellow borders. The results showed the absence of *Salmonella* sp. in oyster samples in all months of the study.

### 3.4. Identification and quantification of parasites

Microscopic analyses revealed the presence of bacteria, protozoa, and metazoan. Bacteria of the genus *Rickettsia*, protozoa *Nematopsis* sp. (Apicomplexa:Eugregarinida: Porosporidae), trematodes *Bucephalus* sp. (Digenea: Bucephalidae), unidentified Turbellaria *Tylocephalum* sp. (Cestoda: Tetragonocephalidae), and unidentified metazoans were observed. The prevalence of parasitized individuals was 33.7%.

Metazoans were the most prevalent parasites in the mantle, digestive gland, gonads, connective tissue, and gills. The mantle and connective tissue had the highest number of parasites. May was the month with the highest occurrence of metazoan in oysters (Table 3). A mild infestation (<5%) was predominant, occurring in 80% of the individuals with the parasite (Figure 2). There was no significant difference between the number of

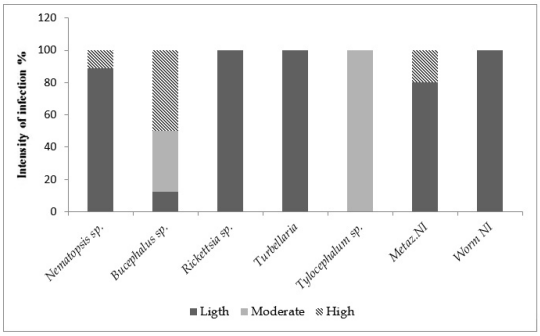


**Figure 2.** Percentage of infestation intensity in *C. gasar* cultivated in Maranhão, NE Brazil. Metaz NI = Unidentified metazoan; Worm NI = Unidentified worm.

**Table 1.** Abiotic variables in cultivation water of *C. gasar* in Maranhão state, Brazil, during the dry and rainy seasons.

| Season | Month/Year | Temperature ºC | Salinity | Oxygen mg / L | Rainfall mm³ |
|---|---|---|---|---|---|
| Dry | November/17 | 30 | 40 | 5.7 | 40 |
| | December/17 | 29.1 | 41 | 5.7 | 57.6 |
| Rainy | March/18 | 31.2 | 30 | 5.3 | 114.6 |
| | May/18 | 30 | 24 | 5.3 | 303.8 |

**Table 2.** Quantification of total and thermotolerant coliforms and *Vibrio* sp. in samples of oysters in Maranhão, Northeast Brazil.

| Season | Month/Year | Total coliforms* (MPN / g) | Thermotolerant coliforms (MPN / g) | *Vibrio* sp.+ (CFU / g) |
|---|---|---|---|---|
| Dry | November/2017 | 210 | 23 | 3.5 $10^3$ |
| | December/2017 | 15 | <3.0 | 3.5 $10^3$ |
| Rainy | March/2018 | 14 | <3.0 | 2.6 $10^3$ |
| | May/2018 | <3.0 | <3.0 | 2.6 $10^3$ |

*Maximum value of total coliforms allowed by ANVISA norm nº 12/2001 (Brasil, 2001) = 1000 coliforms / g. +There is no minimum regulation for bacterial concentrations in food.

**Table 3.** Monthly and total prevalence (%) of parasites in *C. gasar* cultivated in an experimental fixed rack and bag oyster cultivation unit located in Maranhão, NE Brazil (total N = 80 and N monthly = 20).

| | Ntps | Bucp | RLOs | MNI | Turb | Tyl | VNI |
|---|---|---|---|---|---|---|---|
| November | 30 | 40 | 10 | 10 | - | - | - |
| December | 10 | - | - | - | - | - | - |
| March | - | - | 5 | 10 | 5 | - | - |
| May | 5 | - | 5 | 25 | 5 | 10 | 10 |
| Total prevalence | 11.2 | 10 | 5 | 11.2 | 2.5 | 2.5 | 2.5 |

Ntps = *Nematopsis* sp.; Bucp = *Bucephalus* sp.; RLOs = *Rickettsia* sp.; MNI = Unidentified metazoan; Turb = Unidentified Turbellaria; Tyl = *Tylocephalum* sp.; VNI = Unidentified worm.

animals infected and not infected by parasites during the sampling period (p>0.05, Mann-Whitney). A significant difference ($\chi^2$= 14.6; p= 0.00) was observed between the most prevalent parasites (Metazoan and *Nematopsis* sp.) in the dry (November and December) and rainy (March and May) seasons. The same was not observed for the least prevalent (*Rickettsia* sp. and *Bucephalus* sp.) ($\chi^2$= 0.14; p= 0.7).

*Nematopsis* sp. was the second most prevalent parasite and was in greater quantity in November (Table 3). The number of oocysts per phagocyte varied from one to two and was observed in the digestive gland, mantle, connective tissue, gonads, gills, and labial palps (Figure 3D-3E). The digestive gland was the tissue where the protozoa were most prevalent among these. The predominant infestation intensity was low in 88.8% of the cases due to the small number of phagocytes in the tissues analyzed.

All the organisms affected by *Tylocephalum* sp. had moderate infections (Figure 2). This parasite was in the connective tissue presenting a thick fibrous capsule, and no hemocyte infiltration was observed around the metacestode (Figure 3B). In the lumen of the digestive tract of two individuals, an unidentified worm was found that did not provoke any apparent defense response from the host (Figure 3C).

Sporocysts and cercariae of the digenetic trematode *Bucephalus* sp., with a bifid tail and a short and broad base, were observed in germinative masses (Figure 3F-3G). These parasites were recorded only in November and were most prevalent in the gonads and digestive glands (Figure 3F). Infestation intensity was high in 50% of infected animals, which presented intense occupation of the gonadal follicles by sporocysts and cercariae.

Colonies of *Rickettsia* sp. (RLOs) were found in the mantle, labial palps, and digestive glands (Figure 3H), with low prevalence in November, March, and May. No tissue damage was observed due to bacterial presence. The infestation intensity was classified as mild in 100% of infected individuals.

Unidentified Turbellaria was observed in March and May (Table 3), causing apparent infiltration of hemocytes at the infestation site but at low intensity (Figure 3A).

## 4. Discussion

Aquatic ecosystems can be considered brackish when the salinity is between 0.5 and 30 and saline when higher than 30 (Wetzel, 2001). On the northeast coast of Maranhão, the salinity of estuarine waters varies between 10 and 30 (Funo et al., 2015). However, this study observed higher values in the dry season (41). The salinity of the estuary studied decreased in the months considered rainy, March and May, and increased in the dry months, November and December, which is frequently observed in the region due to the influence of the freshwater that flows from the rivers to the estuary in the rainy season (Vilanova and Chaves, 1988).

The water temperature in the environment studied did not vary considerably but is considered high, which is characteristic of the estuarine habitats of the Northeastern coast of Brazil, where temperatures are high and oscillate little during the year (Silva et al., 2003; Martins et al., 2009).

The microbiological analyses of water and oysters presented low total and thermotolerant coliforms during the studied period. The four samplings indicated that low coliform densities are most probable in the environment's water and conformed with the National Environmental Council - CONAMA (Brasil, 2005) that establishes a maximum value for cultivating bivalve mollusks intended for human consumption of 43 coliforms per 100 milliliters. Sousa et al. (2023) analyzing the MPN of coliforms in water samples from oyster farms in the state of Maranhão, observed its low quality with a variation from 1,587 to 2,302 MPN/100 mL. Figueiredo et al. (2015), carrying out a similar study, identified high values of coliforms, on average ranging from 450 to 25,000 MPN/100 mL and thermotolerants from 1.7 to 7.1 MPN/100 mL in the mangrove oyster (*Crassostrea rhizophorae*) cultured in the estuarine region of the municipality of Salinópolis – Pará. Silva et al. (2021) evaluating the density of the same group in the region of Espirito Santo, detected total coliforms in a range of < 2 to > 1,600 NMP/100 mL and thermotolerants < 2 to 375 NMP/100 mL.

Coliform bacteria are directly related to unsatisfactory hygienic-sanitary conditions, and their presence suggests probable contamination by pathogenic microorganisms in water and food (Sousa et al., 2023). Thus, the results suggest that this area is suitable for oyster farming since the range in concentration of coliforms indicates low contamination by domestic sewage.

Related to the oysters evaluated, although the samples in November had the highest concentration of thermotolerant coliforms, they did not reach ten percent of the value stipulated by the current sanitary resolution in Brazil (1,000 coliforms / g) (Brasil, 2001). Thus, it can indicate that the animals analyzed in the study fit consumption based on these characteristics.

The results of the microbiological analyses are probably due to the low demographic occupation and the absence of high-impact activities near the oyster farming region, suggesting a low concentration of the bacteria analyzed in the growing area. Similar results were observed by Doi et al. (2015) with *Crassostrea* sp. in Cananéia-São Paulo, Freitas et al. (2017) in the Baia do Iguape in Bahia state and by Vieira et al. (2008) in Ceará state, who associated the low density of bacteria with the low disposal of effluents in the water.

Otherwise, studies carried out in other Brazilian estuaries, verified high MPN coliform values in the tissues of cultured mollusks. Silva et al. (2021) defined values from 28 to > 1,600 MPN/g of total coliforms and from < 2 to 21.5 MPN/g of thermotolerant coliforms, and Sousa et al. (2023) observed in oyster samples a variation of total coliforms from 5.9 to 71.9 MPN/g and from 2.7 to 12.4 MPN/g for thermotolerants.

Ballesteros et al. (2016), in their research with *Crassostrea* sp. in Cananéia - SP, found a density of thermotolerant coliforms up to 981 MPN/g of oyster meat. Freitas et al. (2017), in research with native oysters carried out in the Baía do Iguape Marine Extractive Reserve - BA,

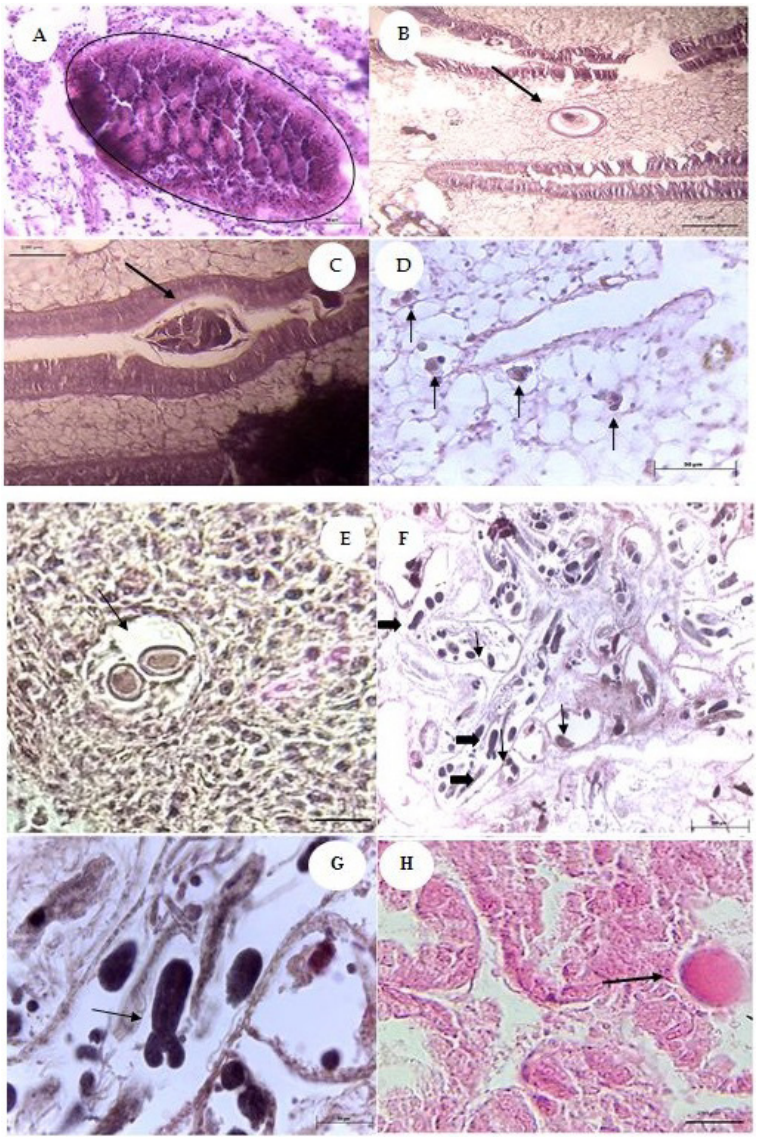


**Figure 3.** Parasites in the oyster *C. gasar* cultivated in Maranhão, Northeast Brazil. A. Unidentified Turbellaria in connective tissue (circle), with evident hemocyte infiltration. Bar = 50µm. B. *Tylocephalum* sp. in the connective tissue (arrow). C. Unidentified worm in the digestive lumen (arrow), Bar = 200 µm. D. *Nematopsis* sp. (arrows) in the connective tissue. E. Oematysts of *Nematopsis* sp. Bar = 200 µm. F. Sporocysts (fine arrows) and cercariae (broad arrows) of *Bucephalus* sp. in the digestive gland. Bar = 200 µm. G. Cercaria of *Bucephalus* sp. (arrow) in the digestive gland. Bar = 50µm. H. Colony of *Rickettsia* sp. in the digestive gland (arrows). Bar = 200 µm.

obtained a maximum thermotolerant coliform density of 350 MPN/g in oyster meat samples.

Related to *Vibrio* sp. presence in farmed mollusks, Silva et al. (2021) observed positive colonies, as registered in the present study. However, Sousa et al. (2023) studying oysters captured in São Luís – MA did not find *Vibrio* sp. in the samples. No legislation in Brazil regulates tolerable levels of *Vibrio* in mollusks intended for food or in waters used for aquaculture, making it difficult to establish standards for this genus in bivalves.

The highest values of CFU of *Vibrio sp.* were found in the dry season when rainfall was low and temperature and salinity were high. West (1989) states that *Vibrio* sp. may appear in high concentrations when water temperatures rise. Although they are present in water throughout the year, their concentration increases in the warmer months and accumulates in filtering mollusks and other aquatic animals (Tall et al., 2013).

*Salmonella* sp. was absent in all samples of this study. Brazilian legislation determines the absence of *Salmonella* sp. in 25g of samples of bivalve mollusks, crab meat, and similar cooked, seasoned, non-processed, cold, or frozen (Brasil, 2001). This absence may have been influenced by characteristics of the culture water, which had a salinity above 20, a level not tolerated by the bacterium. The same situation was observed in the cultivated *C. gigas*

in Florianópolis - SC (Pereira et al., 2006) and *C. rhizophorae* in Taperoá – BA (Santos et al., 2015) where the presence of coliforms was confirmed but *Salmonella* sp. was absent. However, the presence of the bacterium was registered in some natural environments used for oyster growth and cultivation in Brazil (Silva et al., 2003; Vieira et al., 2004; Santos et al., 2015; Ballesteros et al., 2016), which may indicate water contamination by domestic and industrial effluents.

Thus, from a microbiological perspective, the results demonstrated that the environment in the region evaluated was favorable for oyster culture. The low microbial load found in the water and oysters may be related to the high salinity of this estuary, which inhibits bacteria, and the distance of the cultivation region from urban agglomerates, which contaminate and impact the water bodies (Lewis et al., 2011; Bayen, 2012).

The parasite analyses were held to complement the sanitary aspects of oysters farmed in Maranhão, Northeast Brazil, and found that metazoan was the most prevalent (11.2%). Infiltration by hemocytes was also observed around some parasites as a defense response. Sabry et al. (2011) observed an unidentified metazoan causing damage to the digestive gland in *C. gigas* and intense hemocyte infiltration as a defense reaction to the parasite; however, it is not always possible to observe damage caused by metazoan to their hosts.

The genus *Nematopsis* sp. was observed in higher prevalence in the dry season. Despite this, the infestation intensity was considered low and did not cause morphological alterations in the affected tissues or an evident defense response, typical for this degree of infection (Sabry et al., 2007; Boehs et al., 2010).

The trematode *Bucephalus* sp. was observed only in November, with a prevalence of 40%. In the gonadal follicles, sporocysts and cercariae were observed, which occupied large areas, making it difficult to determine the sex, which could impede gametogenesis performance (Lauckner, 1983). High concentrations of sporocysts and cercariae were also responsible for infection of the gonads and also affected the gonadal follicles in *Crassostrea rhizophorae* up to 100% of prevalence (Brandão, et al., 2013). Studies of *Bucephalus* in bivalves on the Brazilian coast confirmed that when present at high intensities, sex determination is impossible (Garcia and Magalhães, 2008; Ceuta and Boehs, 2012; Ribeiro et al., 2018).

Bucephalids have a complex life cycle, using bivalve mollusks as intermediate hosts and some bony fish as definitive hosts (Lauckner, 1983). When the cercariae mature, they rupture the tissues and emerge to look for a second intermediate host, which can cause the death of the first host and is therefore considered an essential parasite for aquaculture (Lauckner, 1983; Magalhães, 1998). However, despite the harm these parasites cause to the hosts, the prevalence found in this study did not seem to influence the development of the oyster cultivated.

The metazoan *Tylocephalum* sp. in the larval stage was observed in low prevalence and with a mild degree of infestation. Brandão et al. (2013), Sabry et al. (2007) and Cova et al. (2015) observed a similar situation in *C. rhizophorae*.

At the degree of infestation observed, parasitosis did not appear to cause damage to the vital organs of the hosts. The formation of the capsule that surrounds the *Tylocephalum* sp. constitutes a natural defense reaction to the parasite and is reported in other species of bivalve mollusks such as *Anomalocardia brasiliana* (Gmelin, 1791), *Iphigenia brasiliensis* (Lamarck, 1818), *Crassostrea gigas* (Thunberg, 1793), and *Mytella guyanensis* (Lamarck, 1819) (Sabry and Magalhães, 2005; Boehs et al., 2010; Ceuta and Boehs, 2012). This parasite-host relationship has not been found to cause death in mollusks.

Turbellaria was recorded in low prevalence (2.5%), most commonly in connective tissue. Infiltration of hemocytes around the parasite was observed as a defense response. Similar results were obtained by Ceuta and Boehs (2012), who observed an unidentified Turbellaria in the gills of *M. guyanensis*, and by Zeidan et al. (2012); Brandão et al. (2013) and Cova et al. (2015), who observed the Turbellaria *Urastoma* sp. in the gills and mantle of *C. rhizophorae*.

Organisms from the Turbellaria group were found in the mantle, gills, and digestive cavities of bivalve mollusks. These animals cause no significant damage in low degrees of infestation (Lauckner, 1983; Bower, 1992; Francisco et al., 2010).

The percentage of *Rickettsia* sp. was low throughout the sampling period, and the degree of infection was mild throughout the study. No severe lesions were observed in oyster tissues because of their presence. In a low degree of infestation, this parasite does not affect host physiology and does not cause severe tissue damage (Cremonte et al., 2005). It corroborates with Sabry et al. (2011), Boehs et al. (2012), and Boehs et al. (2010), who emphasize that *Rickettsia* sp. does not cause significant lesions in the host at low infection levels and those of Figueras et al. (1991) and Carballal et al. (2001), who describe these bacteria as not causing a defense response. Thus, this microorganism can be characterized as not harmful to mollusks.

## 5. Conclusion

The water used in oyster cultivation in the municipality of Primeira Cruz, Maranhão, Northeastern Brazil, had a satisfactory microbiological quality, and could be used in the production chain of this mollusk. As for sanitary standards, oysters were considered suitable for consumption according to current Brazilian legislation.

The prevalence of parasites in oysters was low, and although they are potentially harmful to cultivated animals, the population was not afflicted by parasitosis. Sometimes, the parasite/host relationships were not responsible for severe organ damage. Therefore, the environment in question is promising for the development and expansion of mariculture and shellfish extraction activities.

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