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Intravalvular oyster fluid as a innovation matrix for quantification of nitrite

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ABSTRACT

Among the organisms used as biomonitors, oysters stand out due to the large amounts of contaminants that accumulate in their tissues. The objective of this research was to determine the physicochemical parameters of the estuarine system at three points in São José Bay, Maranhão - Brazil, to perform the biometry of oysters, and to quantify nitrites (NO_2^-) in the intravalvular fluid of oysters. The physicochemical parameters of the brackish water showed that dissolved oxygen presented values below the levels recommended by Brazilian legislation. The pH and temperature remained within the established limits. In the biometric analysis, it was possible to observe that the oysters from harvesting areas are smaller in all dimensions, especially during the rainy season. The result of the quantification of NO_2^- in the oyster supernatant revealed significantly higher concentrations in the harvesting area, showing that this area is the most impacted when compared to the urban area and farm area (control group). The results indicate that the quantification of NO_2^- by the modified Griess reaction is a valid, simple, effective and low-cost method that can be used in environmental monitoring, in addition to the physicochemical parameters of the water and oyster biometric analysis. The use of oyster supernatant for NO_2^- quantification proved to be a new sampling unit that is easy to collect. The present study may help in biomonitoring studies through the quantification of NO_2^- in the oysters' supernatant.

Keywords: Biomonitoring; bivalve; aquatic toxicity.

1 Introduction

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Biomonitoring of water quality in aquatic ecosystems is extremely important for environmental conservation and human health. Traditionally, physicochemical analyses have been used to assess water quality, but the search for more sensitive methods that can be performed continuously has driven the development of new approaches (Silva et al., 2015; Lal, Jaywant, Arif, 2023). Aquatic organisms as biological indicators have been presented as fundamental for the evaluation and quantification of organic and inorganic contaminants in these ecosystems (Dalzochio et al., 2016).

Bivalve mollusks, such as oysters, due to their filtering habits and low mobility, are highly susceptible to the bioaccumulation of organic and inorganic contaminants. In this way, these organisms become excellent biomonitors, reflecting the environmental quality and the presence of contaminants in an ecosystem (Dos Santos et al., 2021). Most oyster species of *Crassostrea* gender are found in shallow coastal regions (Almeida et al., 2024). Bivalves from marine ecosystems have great economic importance, such as food and the use of the shell as a raw material in the manufacture of industrial, artisanal and medicinal products, especially in Brazil. It also provides indirect benefits by stabilizing coastlines and mitigating nutrient pollution, and there is a lot of bivalve-mediated research requiring collaboration across conventional disciplinary boundaries (Vaughn and Hoellein, 2018).

The main tissues of oysters used for biomonitoring studies are the soft tissues, mainly the gills and muscle. Any contaminants present in the water, which may be of biological and/or chemical origin, i.e., microorganisms, inorganics, organics (volatile and synthetic), and disinfectants products, are filtered and remain accumulated in their tissues (Inglezakis et al., 2016; Shen et al., 2019; Oliveira et al., 2020; Silva et al., 2020). However, the intravalvular fluid (oyster supernatant) is essential for the transport of nutrients and other substances in the oyster's body and significantly influences the chemical composition of the soft tissue (Hand and Stickle, 1977).

Among the various inorganic contaminants that can affect aquatic ecosystems, nitrites (NO_2^-) and nitrates (NO_2^-), mainly from anthropogenic activities such as domestic sewage, agriculture, and industry, stand out for their negative impacts on aquatic life. These nitrogenous compounds can cause eutrophication, formation of dead zones, and toxicity to several organisms, including bivalve mollusks (Camargo; Alonso; Salamanca, 2005). Although NO_3^- is a natural intermediate in the process of NO_2^- formation, it has high toxicity and can cause serious health problems such as methemoglobinemia. Known as blue baby syndrome, methemoglobinemia occurs when NO_2^- reacts with hemoglobin, preventing the proper transport of oxygen in the blood. This condition can lead to serious complications and

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even death, especially in infants. The NO_2^- can reacts with secondary amines, forming nitrosamines, substances proven to be carcinogenic (Fabris; John; Borges, 2020). In addition, an association between the presence of these ions in drinking water and the risk of gastric cancer has been observed (Picetti et al., 2022).

The use of the biological fluid of oysters in the quantification of NO_2^- offers advantages such as the practicality of collection and the absence of interference from other cellular components. Based on this sample unit, the aims of the present study were to determine the physicochemical parameters of water at three points in São José Bay, located in the Amazon Coast, Maranhão-Brazil, to perform the biometry of oysters of the genus *Crassostrea,* and to evaluate the NO_2^- using the Griess method. This approach will make it possible to assess the exposure of these organisms to NO_2^- and contribute to the knowledge about water quality in São José Bay.

2 Materials and methods

2.1 Study area

The collection points are located in São José Bay, on the north coast of the island of São Luís, Maranhão. São José Bay is of great importance for fishing in Maranhão, as there are several ports for unloading and selling fish in this region, and a large part of the community survives from the sale of oysters.

Collections were carried out at three points (figure 1): Oyster farm area (S1), cultivation area without anthropogenic interference, located in the municipality of Raposa – MA. Oysters that are grown directly in estuary water and are sold throughout the Metropolitan Region of Greater São Luís. Harvesting area (S2), mangrove ecosystem located in the municipality of Paço do Lumiar – MA. In this area, residents use oysters for their own consumption. The region does not have basic sanitation and many residents throw solid waste on the banks of the mangrove swamp; Urban area (S3), pier located in the municipality of São José de Ribamar – MA. At this point there are nearby residences and sport fishing activities.

2.2 Sampling design

Two collections were carried out, one in the dry period (October 2022) and the other in the rainy period (March 2023). At each point, 20 oysters were collected.

Adult oysters were collected manually from all three points during low tide. In the farm area, the oysters were randomly removed from the wooden banks. In the harvesting area, the

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oysters were taken directly from the mangrove trees. While in the urban area, the oysters were removed from the concrete wall of the pier, where they were encrusted.

Immediately after harvest, the oyters were cryoanesthetized on ice (0°C) and transported to the Biochemistry and Ecotoxicology Lab for biometric analysis and removal of the supernatant for NO_2^- analysis.

The physicalchemical parameters of the water were measured at the time of collection using the HANNA model multiparameter: salinity, pH, dissolved oxygen, conductivity and water temperature. At each point, 2ml of seawater was also collected for NO_2^-quantification.

2.3 Biometric analysis of oysters

In the laboratory, on the same day of harvesting, the animals were measured using a caliper (0.01 mm). The dimensions analyzed were the width of the valves in frontal view, the length and height of the valves (figure 2). The total weight of the animals was obtained using a semi-analytical scale (0.01g). All samples were registered in the Maranhão Fauna Tissue and DNA Collection (CoFauMA) located at the State University of Maranhão.

2.4 *Quantification of nitrites (curve 1.3.23 mg/L) modified Griess method*

In the laboratory, the supernatant from the oysters was removed using an automatic pipette, placed in eppendorf-type tubes and frozen at -20ºC for subsequent nitrite analysis. For biochemical quantification, 500 µl samples were thawed and centrifuged at 10,000 rpm for 10 min at a refrigerated centrifuge (DAIKI. Mod. DT 10KD). Subsequently, 250 µl of the supernatant was transferred to plastic cuvettes and 250 µl of Griess reagent was added. The samples were placed in a dark place at room temperature for 10 minutes for color development and the absorbance was determined by spectrophotometry (AJMICRONAL-AJX-1000) at a wavelength of 540 nm. All samples were tested in duplicate or triplicate.

The NO_2^- present in the supernatant and in the brackish water was quantified using the modified Griess method, based on the methodology described by Green et al. (1982) and Castro (2025). This reaction forms a chromophore during the reaction of nitrites with sulfanilamide and N-(1-naphthyl) ethylenediamine, forming a pink-colored compound, which was quantified in a spectrophotometer. Nitrite concentration was determined by comparison with a calibration curve, using a standard sodium nitrite solution in a concentration range of 0.2; 0.4; 0.6; 1.2 and 2 mg/L. Line equation: $y = 0.9292x - 0.0438$. $R^2 = 0.9981$.

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2.5 Statistical analysis

The normality and homogeneity of the data were tested with the Shapiro-Wilks and Levene tests, respectively. Analysis of variance (ANOVA - two way) with Tukey's post-hoc test was used to verify the significant differences in biometric parameters and NO_2^- concentrations in the supernatant between the points and the seasonal periods. For non-parametric data, analyses were performed using the Kruskal-Wallis test followed by the Nemenyi post-hoc test. The level of significance adopted was 5%. The analysis was performed using the Statistic® test.

3 Results

3.1 Physicochemical analysis of water

The results of the abiotic data are shown in table 1. The water temperature varied between 28.6ºC and 29.9ºC. The CONAMA legislation does not establish temperature values.

Regarding the pH parameter, the values varied between 6.93 and 8.04, in accordance with CONAMA legislation No. 357/2005 (Brazil, 2005).

We observed that, at the sampled points, Dissolved Oxygen (DO) concentrations are below the levels permitted by current legislation. Only point S1 (farm area) and the urban area (S3), during the dry period, are within acceptable values. The low values of DO in the areas studied indicate the presence of organic matter in the environment, possibly coming from untreated domestic sewage.

The salinity of the points varied between 16.1 g.Kg-1 and 36.7 g.Kg-1, and only the farm point (S1) in the dry period is in accordance with the legislation. The low salinity at all sampling points is below that recommended by legislation, this is due to the fact that they are brackish waters, which are probably heavily influenced by river water.

3.2 Oyster biometrics

The biometric data of the oysters are represented in figure 3. The results showed that the total weight, height, length and width of the oysters collected at the farm area (S1) presented significantly higher values in relation to oysters from the harvesting area (S2) and urban area (S3).

Oysters from area S2 presented the lowest values in all dimensions analyzed, when compared to points S1 and S3, except for length in the dry period.

The total weight of oysters at point S1 varied between 39.20 g ± 12.53 and 36.4 g ± 13.4, at S2 from 19.25 g ± 6.7 to 16.75 g ± 5.24 and at point S3 from 25.22 ± 7.5 to 20.26 ± 3.7. It

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was found that oysters in the dry period are heavier and consequently accumulate more supernatant. There was no statistically significant difference in the weight of oysters from the 3 points between the two seasonal periods (dry and rainy). However, when comparing the weight in the same period, the oysters collected at point S1 were heavier than oysters from points S2 and S3, both in the dry (p=0.00012, MS = 57,891, df = 106,00) and rainy (p=0.00012, MS = 57,891, df = 106,00) periods. When comparing oysters from points S2 and S3, it was found that there was no significant difference in both seasonal periods.

The oysters presented variable heights throughout the three collection areas (figure 3). The height of oysters at point S1 varied between 6.55 ± 0.7 and 5.61 ± 1 cm, at point S2 between 4.52 ± 0.8 and 4.45 ± 0.6 cm, and at point S3 between 5 .39 ± 0.7 and 4.65 ± 0.4 cm. There were significant differences in points S1 and S3 between different periods (p=0.00199 and p=0.03652 respectively / MS = ,16212, df = 108,00), with the oysters being taller in the dry period. A significant difference was also observed between all points in the dry period, with oysters from S1 being higher (between S1 and S2 p=0.000119, between S1 and S3 p=0.000223, and between S2 and S3 p=0.007561 / MS = ,16212, df = 108,00). During the rainy season, the height of oysters from S2 and S3 were statistically similar.

The length of oysters at point S1 varied between 4.015 ± 0.6 and 3.91 ± 0.8 cm, at point S2 between 3.87 ± 2.1 and 2.89 ± 0.6 cm, and at point S3 between 3 .68 ± 0.5 and 3.29 ± 0.3 cm. In the dry period, the oysters with the biggest length were from point S1, followed by S2 and finally S3, in this same period there was a significant difference between points S1 and S2 (p=0.035, MS = ,61048, df = 114,00). In the rainy season, oysters from S2 and S3 had statistically similar length dimensions. Oysters from point S1 had biggest length compared to oysters from S2 (p=0.00013, MS = ,61048, df = 114,00) and S3 (p=0.024874, MS = ,61048, df = 114,00).

The width values of the oysters analyzed in area S1 varied respectively between 1.79 ± 0.4 and 1.7 ± 0.7 cm. In area S2, this variation was between 1.38 ± 0.3 and 1.25 ± 0.3 cm. In area S3, oyster width values were between 1.83 ± 0.8 and 1.51 ± 0.3 cm (figure 3). No statistical differences were found in the width of oysters between the 3 points in the same period, nor between the points in the seasonal periods (dry and rainy).

3.3. Quantification of nitrites

The analysis of nitrites in the supernatant of oysters at the harvesting point (S2) showed a higher concentration of NO_2^- in both seasonal periods, as shown in figure 4.

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When comparing the two periods (dry and rainy), there was a significant difference in the concentration of NO_2^- at points S2 and S3 (p=0.000319 and p=0.002305, respectively / MS = ,00960, df = 104,00) (figure 4).

Regarding the results between areas in the dry period, there was a significant difference between points S1 and S2 (p=0.00012, MS = ,00960, df = 104,00) and between S2 and S3 (p=0.00012, MS = ,00960, df = 104,00). The rainy period also showed statistical differences between points S1 and S2 (p=0.00016, MS = ,00960, df = 104,00) and S2 with S3 (p=0.00012, MS = ,00960, df = 104,00).

The result of statistical analysis shows that nitrite concentration is higher in the dry period, in all areas studied (figure 4). Therefore, the data leads us to believe that the reason for the higher values during the dry period is due to the higher concentration of pollutants at this time, which allows organic matter to accumulate for a long time in the same location. We observed that in the dry period oysters tend to accumulate more supernatant, probably due to the fact that oysters are heavier in this period. The figure 4 shows the concentration of NO_2^- in mg/L in the oyster supernatant.

Regarding the nitrite reading in water, the results are represented in table 2. Point S2 presented the highest amount of NO_2^- in the dry period (0.262 mg/L) compared to the other areas in the same period. Point S1 presented the lowest amount of nitrite among the points in both periods (table 2).

4 Discussion

4.1 Physicochemical analysis of water

The Brazilian equatorial margin is characterized by being very dynamic and susceptible to seasonal changes (Oliveira et al., 2024). The area of this study covers São José Bay that is influenced by the bays of São José and São Marcos, both of which form the Gulf of Maranhão, in the north-northeast region of Brazil, with macrotidal dynamics with amplitudes of 6.0 to 7.0 meters, mangrove areas, and significant seasonal movements (Do Nascimento et al., 2020). This study evaluated, at three collection points, the physicochemical parameters of the water, the biometry of the oysters and was the first to quantify the NO_2^- in the oysters' supernatant by the modified Griess method.

Surface temperature is influenced by several factors, such as coordinates, season, time of day, flow and depth. Temperature can also influence the physiological activities of animals that live there, such as respiration, reproduction, and digestion (Vaughn and Hoellein, 2018). The surface distribution of water temperature showed a slight seasonal variation when comparing the 3 points for both dry and rainy seasons, ranging from 27.1ºC to 29.9ºC, which is

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characteristic of equatorial zones that have high temperatures throughout the year. These variations may be influenced by the time of collection, which in this research occurred in the afternoon from 2:00 p.m. to 4:00 p.m. Previous studies have shown a slight increase in temperature in the rainy season for the Raposa estuarine system, the author attributed this increase to the time of collection, which was from 10:00 a.m. to 1:00 p.m. (Do Nascimento et al., 2020). The lowest temperature recorded in the rainy season at point S2 (27.1ºC) occurred due to the large amount of rainwater that slightly decreased the temperatures in the water bodies. Studies show that the appropriate temperature for the aquatic environment is important, as it can influence the development of local organisms in terms of its morphology, physiology and ecology, and can bring future damage to humans (Do Nascimento et al., 2020).

Regarding pH, the averages ranged from 6.93 to 8.04 between the dry and rainy periods, within the standards of current environmental legislation for brackish waters class 3, CONAMA legislation No. 357/2005 (Brazil, 2005), as shown in table 1. These results are similar to those found by Torres et al. (2024), in which the pH of water samples in São Marcos Bay, Maranhão-Brazil, ranged between 6.7 and 7.2.

The pH is an extremely important factor for the aquatic environment, as it influences the chemical reactions and phenomena that occur in water. When the pH is more alkaline, there is a greater loss of nitrogen to the environment, as there is a greater transformation of the ammonium ion (NH_4) into free and gaseous ammonia (NH_3), which can influence the development of aquatic biota (Oliveira et al., 2024).

DO in water depends on different environmental factors, such as temperature, atmospheric pressure, ocean water input, and salinity (Do Nascimento et al., 2020), being vital to the life of fish, mollusks, plankton, bacteria, and aquatic plants (Oliveira et al., 2024). In the rainy season, DO presented low values in all three collection points, ranging between 4.1 and 4.6 mg/L, and point S2 presented values below those established by CONAMA (Brazil, 2005) during the dry period, which can be explained by the increase in the concentration of dissolved salts in the water through sea currents (Krumme; Herbeck; Wang, 2012). The low availability of DO during the rainy season was observed by Do Nascimento et al. (2020), the authors highlighted that this can cause damage to aquatic biota during this period. However, the environment improves its oxygenation during the dry season, reestablishing its chemical balance, due to the renewal of its waters from the sea (Do Nascimento et al., 2020).

Salinity did not vary between the collection sites. However, at point S1, in the dry season, high values were recorded (36.7%) where the influence of the sea current is more significant,

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contributing even more to the salinization process (Dos Santos et al., 2021). High salinity levels in the Raposa region have also been observed in previous studies and can be associated with the entry of the ocean into these waters characterizing the estuary as euhaline (Do Nascimento et al., 2020). Additionally, there may be variations in the abiotic variables depending on the environmental conditions and human factors (De Jesus et al., 2021).

4.2 *Oyster biometric*

It is likely that there are several factors contributing to a smaller size and total weight of oysters collected at point S2 (harvesting area), demonstrating that possibly the environmental conditions such as high amount of anthropogenic contaminants, observed at the point S2 and low levels of DO both in the dry and rainy seasons, have influenced the growth and survival of oysters. The highest biometric values recorded for animals in the farm area during the dry season, both in relation to the other location and in relation to the rainy season, reflect greater energetic and metabolic stability of the organisms in the face of increased salinity (Dos Santos et al., 2021).

In an experimental study carried out by Funo et al. (2015) on the influence of salinity on the growth and survival of oysters, it was found that the highest biometric values recorded were at a salinity of 25 g. kg^{-1}, for the height; at a salinity of 20 g. kg^{-1}, for length; salinity between 20 g. kg^{-1} to 25 g. kg^{-1} for width; and salinity from 20 g. kg^{-1} to 30 g. kg^{-1} for weight. The authors also found that the best salinity range for the survival of organisms was from 20 to 25 g. kg^{-1}. The salinity values recorded at points S2 and S3 are below 18.2 g.kg^{-1} and the oysters presented lower biometry values. Studies on fertilization of oyster *Crassostrea rhizophorae* in hatcheries have shown the significant influence of salinity (Lopes et al., 2024). According to Noleto et al. (2021), if the environment in which oysters are found is impacted by contaminants, their development may also be affected, despite being very tolerant organisms to certain concentrations of toxic compounds, such as pesticides, heavy metals, hydrocarbons, among others. Another factor that can contribute to the smaller size of the oysters at point S3 is the fact that they are located in the pier region, which is made of concrete and can significantly affect the fixation and development of the oysters.

It is known that oysters, like most aquatic mollusks, are osmoconformers organisms and, as they are typical of estuarine environments, they have a high capacity to control their cell volume. However, once subjected to situations of osmotic stress, they can reduce the energy directed to growth due to the cost of survival demands (Dos Santos et al., 2021). Previous

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studies carried out in the Raposa farm region, using DNA techniques on gills, showed that oysters of the genus Crassostrea sp. were contaminated with the parasite Cryptosporidium sp. The authors suggest that oysters should be thoroughly cooked to ensure the safety of consumers of this delicacy (Silva et al., 2023).

The redox relationship between nitric oxide (NO) and nitrogen oxides (NOx: NO_2^- + NO_3^-) is broad and sometimes controversial. When ingested, NO_3^- needs to be reduced into NO_2^- and then to NO, which is extensively studied in Medicine and Biological Sciences, but there is still a lack of comprehensive understanding about it. In bivalves, NO signaling is related to defense, environmental stress, motility control, among others (Shepherd et al., 2022).

4.3. Quantification of nitrites

The highlight of this research shows the importance of NO_2^- monitoring in estuaries, due to its relevance to both human health and the integrity of aquatic ecosystems. At sampling points S1 and S3, the concentrations of NO_2^- demonstrated compliance with the limits established by CONAMA legislation No. 357/2005 (Brazil, 2005) for brackish water class 3. However, the NO_2^- concentration at point S2 exceeded the maximum allowed limit, suggesting the presence of pollution or local processes that favor the accumulation of this compound.

These findings corroborate with the results of several national studies that point to the influence of factors such as season of the year and anthropogenic activity on NO_2^- concentration in aquatic environments. Biotic and abiotic studies carried out in the water of the Guajará-Mirim estuary, in the State of Pará-Brazil, showed that NO_2^- values ranged between 0.001 and 0.007 mg/L and indicate the need for frequent monitoring to assess abiotic contamination (Alencar et al., 2019). Similar results were reported by Oliveira et al. (2024) who found NO_2^- values in the mouth of the Anil River, Maranhão-Brazil, which ranged between 60.38 and 44.76 mg/L for the rainy and dry periods, respectively.

When comparing the results with the international standards of the WHO and the EPA, it is observed that, for drinking water, the limits for nitrite are 3 mg/L (WHO, 2010) and 1 mg/L (EPA, 2012), respectively. However, for aquatic environments, guidelines vary according to water use (Inglezakis et al., 2016). The reference value is protective against nitrite-induced methemoglobinemia, from both endogenous and exogenous sources, in formula-fed infants and in the general population (Greer and Shannon, 2005). However, data from the EPA (1986) suggest that NO_2^- concentrations higher than 1 mg/L indicate highly polluted and probably bacteriologically infected water.

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High levels of NO_2^- found at point S2, 0.262 mg/L in the dry season and 0.109 mg/L in the rainy season, may be related to the water abiotic parameters, especially to the lower DO concentration at this point, in both seasons. According to Barbieri et al. (2014), factors such as temperature and DO significantly influence the biogeochemical processes related to NO_2^-. The lower concentration of DO at the S2 point may have contributed to the increase in NO_2^- levels, possibly due to the eutrophication process. Severe eutrophication promotes harmful algal blooms, reducing light penetration and affecting photosynthesis. Bacterial decomposition of dead algal biomass consumes large amounts of oxygen, resulting in hypoxic conditions and accumulation of NO_2^- and other nitrogenous substances in the environment (Laughinghouse et al., 2022).

The low DO concentration at point S2, associated with high levels of NO_2^-, may have negatively impacted the growth of the oysters collected at this site, in the dry and rainy season. Previous studies indicate that the reduction of DO limits the filtration rate and, consequently, the growth rate of Crassostrea sp. (Patterson; Boettcher; Carmichael, 2014). The combination of low oxygen availability and NO_2^- presence in high concentrations may have influenced the oysters' biometry.

This study showed that NO_2^- concentrations in the oysters were higher when compared to the estuary water, both in the dry and rainy seasons. This can be attributed to the presence of denitrifying bacteria in the digestive tract, gills, and shells of oysters, which convert nitrite into gaseous nitrogen (N_2). This habitat function for microorganisms contributes to significant denitrification rates on reefs, as suggested by Arfken et al. (2017).

In estuarine environments, nitrification and denitrification are biogeochemical processes that, although opposite, often coexist (figure 5). As organic matter present in the environment, originated, for example, from untreated domestic effluent discharge, is decomposed by microorganisms, ammonia (NH_4^+) is released. This NH_4^+ is oxidized by bacteria and archaea in a two-step process: first, NH_4^+ is converted into NO_2^-, and then nitrite is oxidized to NO_3^-. The NO_3^- and NO_2^- produced during nitrification can be use by denitrifying bacteria, present in oysters, as electron acceptors under anaerobic or microaerobic conditions. During this process, nitrate is converted into nitrogen gas (N_2), which is released into the atmosphere (Vaughn and Hoellein, 2018; Ray et al., 2021).

However, despite the presence of denitrifying microorganisms, high concentrations of NO_2^- can cause damage to mollusks, even leading to lethality. Epifanio and Sarna (1975) evaluated the tolerance of *Crassostrea virginica* to contaminant and observed that the average tolerance limits to NO_2^- range from 1.081 to 2.415 mg/L, indicating high tolerance.

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In another study, Smith and Williams (1974) conducted experiments with two species of fish, *Salmo gairdneri* and *Oncorhynchus tschawytscha,* exposed to nitrite concentrations of 0.15 mg/L and 0.55 mg/L for 48 and 24 hours. The authors found that both produce methemoglobin in response to nitrite, but mortality occurred only at a concentration of 55%. They also observed that nitrite toxicity is lower in marine waters, corroborating the results of the present study, where areas with higher salinity had lower amounts of nitrite in oysters and water.

Although nitrite is a common food additive, especially in meat products, Brazil still does not have specific regulations for acceptable levels of this compound in animals. This reinforces the need for further studies on the effects of NO_2^- on *Crassostrea* sp., in order to ensure the food safety of the population and prevent health risks associated with excessive consumption. It is important that NO_2^- levels in foods comply with the recommended daily intake of 0.06 mg/kg/day of NO_2^- as an ion, as established by Brazil and Mercosur (WHO, 1989). The EPA has set a limit of 0.33 mg of nitrite/kg of body weight per day (EPA, 2012).

Factors such as the rapid growth of the world's population, climate change and the salinization of coastal waters have been altering the world's water-related legislation. The United Nations 2030 Agenda defined the 17 Sustainable Development Goals (SDGs), among which it is worth mentioning the SDG 14 - protect marine life, which calls for better integrated management of water resources and covers broader environmental protection issues (*Nações Unidas Brasil*, 2015). Monitoring programs to allow a continuous evaluation of all activity involving the exploitation of natural resources and their effects on marine sustainability are fundamental for conservation and ecologically correct attitudes (Santos et al., 2023).

Finally, this research represents a first step towards a better understanding and management of the NO_2^- presence in oysters, using the supernatant as a new sampling unit. As a pilot study, it has limitations regarding temporal coverage and seasonal effects, which may influence the interpretation of the results regarding the presence of nitrite in oyster supernatant.

5 Conclusions

The results allowed us to conclude that DO and salinity in the three points studied presented values below the recommended by the legislation in the rainy season, suggesting decomposition of organic matter. Regarding the biometric analysis, it is possible to observe that the oysters from harvesting area are smaller in all dimensions, especially in the rainy

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season. The harvesting area presented statistically higher values for NO_2^- in the oysters' supernatant for both seasons.

Complementary studies such as biomarkers of oxidative stress, quantification of phosphate and chlorophyll-a, as well as studies of the physiological effects of nitrite on oysters will allow to obtain a better understanding of the high levels of NO_2^- observed at point S2.

Through this pilot study, it is possible to conclude that the physicochemical analysis of water added to biometry and NO_2^- quantification in oysters is an effective, fast and low-cost tool that can be used for biomonitoring studies.

During the preparation of this work the authors used Google translate® for writing process in some sentences. Aline S. Varela reviewed and edited the manuscript and the authors takes full responsibility for the content.

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REFERENCES

Alencar, V. E. S. A., Rocha, E. J. P., Souza-Júnior, J. A., Carneiro, B. S., 2019. Analysis of water quality parameters as a result of the effects of the rainfall on the Guajará bay - Belém - PA. Revista Brasileira de Geografia Física. 12, 661-680. https://doi.10.26848/rbgf.v12.2.p661-680

Almeida, J. B., Gonçalves, H. G. P., Silva, W. B. T., Pereira, L. P. L., Tchaicka, L., 2024. Identificação molecular e especificidade pelo substrato de ostras Crassostrea spp. do litoral do Maranhão, Brasil. Interfaces Científicas - Saúde e Ambiente. 9 (3), 329-341. https://doi.org/10.17564/2316-3798.2024v9n3p329-341

Arfken, A., B. Song, J. S. Bowman, M. Piehler., 2017. Denitrification potential of the eastern oyster microbiome using a 16S rRNA gene based metabolic inference approach. PLoS One. 12 (9), e0185071. https://doi.org/10.1371/journal.pone.0185071

Barbieri, E., de Almeida Marques, H. L., Bondioli, A. C. V., Campolim, M. B., Ferrarini, A. T., 2014. Concentrações do nitrogênio amoniacal, nitrito e nitrato em áreas de engorda de ostras no município de Cananeia-SP. O mundo da saúde. 38 (1), 105-115. https://doi.org/10.15343/0104-7809.20143801105115

SciELO Preprints - This document is a preprint and its current status is available at: https://doi.org/10.1590/SciELOPreprints.15698

BRAZIL, Conselho Nacional do Meio Ambiente (CONAMA). 2005. Resolução nº 357, de 17 de março de 2005. RESOLUÇÃO CONAMA Nº 357, de 17 de março de 2005 (cetesb.sp.gov.br). Accessed 19 Mar 2024.

Camargo, J. A., Alonso, A., Salamanca, A., 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere. 58 (9), 1255-1267. https://doi.org/10.1016/j.chemosphere.2004.10.044

Castro, G. S., Pinheiro A. M. C., Carvalho, W. L., Teixeira, E. N. S., Silva, W. B. T., Figueiredo, I. V., Tchaick, L., Nunes, S. F. L. C., 2025. Nitrite quantification in intravalvular fluids of oyters Crassostrea sp. (Bivalvia, ostreidae): a preliminary study. Revista Observatório de La Economia Latinoamericana. 23 (7), e107790. https://doi.org/10.55905/oelv23n7-140

Dalzochio, T., Rodrigues, G. Z. P., Petry, I. E., Gehlen, G., Da Silva, L. B., 2016. The use of biomarkers to assess the health of aquatic ecosystems in Brazil: a review. International Aquatic Research. 8, 283-298. https://doi.org/10.1007/s40071-016-0147-9

De Jesus, W. B., Andrade, T. De S. O. M., Soares, S. H., Pinheiro-Sousa, D. B., De Oliveira, S. R. S., Torres, H. S., Protazio, G. S., Da Silva, D. S., Santos, D. M. S., De Carvalho Neta, A. V., Benjamin, L. Dos A., Carvalho Neta, R. N. F., 2021. Biomarkers and occurrences of heavy metals in sediment and the bioaccumulation of metals in crabs (Ucides cordatus) in impacted mangroves on the Amazon coast, Brazil. Chemosphere. 271, 129444. https://doi.org/10.1016/j.chemosphere.2020.129444

Do Nascimento, J. E. F., do Nascimento Farias, C. M., Pires, M. L. T., Eschrique, S. A., da Silveira, P. C. A., 2020. Variação sazonal de parâmetros físico-químicos na porção estuarina do município de Raposa–MA. Interfaces Científicas-Saúde e Ambiente. 8 (2), 257-271. https://doi.org/10.17564/2316-3798.2020v8n2p257-271

Dos Santos, C. C. M., Ferreira, J. A., Dos Santos, C. R. M., Amado, L. L., 2021. Seasonal modulation of oxidative stress biomarkers in mangrove oyster (Crassostrea gasar) from an Amazon estuary. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 257, 110953. https://doi.org/10.1016/j.cbpa.2021.110953

EPA, US Environmental Protection Agency., 1986.Quality criteria for water. Office of water. Regulations and standards, Washington, DC 20460. https://www.epa.gov/sites/default/files/2018-10/documents/quality-criteria-water-1986.pdf

EPA, US Environmental Protection Agency., 2012. Basic Information about Nitrate in Drinking Water. Washington DC [updated 2012 May; accessed 2024 Oct]. Available from: http://water.epa.gov/drink/contaminants/basicinforon/nitrate.cfm.

Epifanio, C. E., Sarna, R. F., 1975. Toxicity of ammonia, nitrite ion, nitrate ion, and orthophosphate to Mercenaria mercenaria and Crassostrea virginica. Marine Biology. 33, 241-246. https://doi.org/10.1007/BF00390928

SciELO Preprints - This document is a preprint and its current status is available at: https://doi.org/10.1590/SciELOPreprints.15698

Fabris, B. T., João, J. J., Borges, E. M., 2020. Quantificação de nitrito em água utilizando um scanner de mesa. Revista Virtual de Química, 12, 569. https://doi.org/ 10.21577/1984-6835.20200045

Funo, I. C. S. A., Antonio, I. G, Marinho, Y. F., Gálvez, A. O., 2015. Influence of salinity on survival and growth of *Crassostrea gasar.* Bol. Inst. Pesca. 41 (4), 837-847.
Greer, F. R., Shannon, M., 2005. American Academy of Pediatrics Committee on Nutrition. Infant methemoglobinemia: the role of dietary nitrate in food and water. Pediatrics. 116 (3), 784-786. https://doi.org/10.1542/peds.2005-1497

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Tannenbaum, S. R., 1982. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem. 126 (1), 131-138. https://doi.org/10.1016/0003-2697(82)90118-x

Hand, S. C., Stickle, W. B., 1977. Effects of tidal fluctuations of salinity on pericardial fluid composition of the American oyster Crassostrea virginica. Marine Biology, 42, 259-271. https://doi.org/10.1007/BF00397750

Inglezakis, V. J., Poulopoulos, S. G., Arkhangelsky, E., Zorpas, A. A., Menegaki, A. N., 2016. Aquatic environment. In Environment and development. Chapter 3, p. 137-212. Elsevier. https://doi.org/10.1016/B978-0-444-62733-9.00003-4

Krumme, U., Herbeck, L.S., Wang, T., 2012. Tide-and rainfall-induced variations of physical and chemical parameters in a mangrove-depleted estuary of East Hainan (South China Sea). Mar. Environ. Res. 82, 28–39. https://doi.org/10.1016/j.marenvres.2012.09.002

Lal, K., Jaywant, S. A., Arif, K. M., 2023. Electrochemical and optical sensors for real-time detection of nitrate in water. Sensors. 23 (16), 7099. https://doi.org/10.3390/s23167099

Laughinghouse, H., Smyth, A., Havens, K., Frazer, T., 2022. Repensando el papel del nitrógeno y fósforo en la eutrofización de los ecosistemas acuáticos. EDIS. 2, 1-5. https://doi.org/10.32473/edis-SG191-2022

Lopes, R. G. P. S., Antonio, I. G., Rego, A. P., Gomes, S. M. J., Freire, T. B., Coimbra, M. R. M., 2024. Effects of salinity on pre- and post-fertilization developmental events in the mangrove oyster *Crassostrea rhizophorae* (GUILDING, 1828). Theriogenology. 218, 62-68. https://doi.org/10.1016/j.theriogenology.2024.01.033

Nações Unidas Brasil. 2015. Agenda 20230 para o desenvolvimento Sustentável. Agenda 2030 para o Desenvolvimento Sustentável | As Nações Unidas no Brasil https://brasil.un.org/pt-br/91863-agenda-2023-para-o-desenvolvimento-sustentável

Noleto, K. S., De Oliveira, S. R. S., Lima, I. M. A., de Jesus, W. B., da Silva Castro, J., de Santana, T. C., Cardoso, L. M., Jorge, M. B., Santos, D. M. S., Torres, J. R. S., Fortes Carvalho Neta, R. N., 2021. Biochemical and histological biomarkers in Crassostrea sp. (Bivalvia, Ostreidae) for environmental monitoring of a neotropical Estuarine Area (Sao Jose Bay, Northeastern Brazil). Bulletin of Environmental Contamination and Toxicology. 106, 614-621. https://doi.org/10.1007/s00128-021-03149-z

SciELO Preprints - This document is a preprint and its current status is available at: https://doi.org/10.1590/SciELOPreprints.15698

Oliveira, A. M., Barauna, R. A., Marcon, D. J., Lago, L. A., Silva, A., Lusio, J., Tavares, R.D.S., Tacão, M., Henriques, I., Schneider, M. P., 2020. Occurrence, antibiotic-resistance and virulence of E. coli strains isolated from mangrove oysters (Crassostrea gasar) farmed in estuaries of Amazonia. Marine Pollution Bulletin, 157, 111302. https://doi.org/10.1016/j.marpolbul.2020.111302

Oliveira, A. V. G. D., Azevedo-Cutrim, A. C. G. D., Cutrim, M. V. J., Cruz, Q. S. D., Rosas, R. S., Sá, A. K. D. D. S., 2024. Assessment of the trophic status and water quality in an urbanised tropical estuary, Brazil. Chemistry and Ecology. 1-17. https://doi.org/10.1080/02757540.2024.2396839

Patterson, H. K., Boettcher, A., Carmichael, R. H., 2014. Biomarkers of dissolved oxygen stress in oysters: a tool for restoration and management efforts. PloSone. 9 (8), e104440.https://doi.org/10.1371/journal.pone.0104440

Picetti, R., Deeney, M., Pastorino, S., Miller, M. R., Shah, A., Leon, D. A., Dangour, A.D., Green, R., 2022. Nitrate and nitrite contamination in drinking water and cancer risk: A systematic review with meta-analysis. Environmental Research. 210, 112988. https://doi.org/10.1016/j.envres.2022.112988

Ray, N. E., Hancock, B., Brush, M. J., Colden, A., Cornwell, J., Labrie, M. S., Maguire, T.J., Maxwell,T., Rogers, D., Stevick, R.J., Unruh, A., Kellogg, M.L., Smyth,A.R., Fulweiler, R. W., 2021. A review of how we assess denitrification in oyster habitats and proposed guidelines for future studies. Limnology and Oceanography: Methods. 19 (10), 714-731. https://doi.org/10.1002/lom3.10456

Santana, L. L. S., Medeiros, C., 2020. Características biométricas e Índice de condição da ostra-do-mangue Crassostrea rhizophorae (Guilding, 1828) do estuário do rio Ipojuca, PE, Brasil. Tropical Oceanography. 48 (1), 20-38. https://doi.org/10.5914/tropocean.v48i1.247361, 2020.

Santos, J. P., Guimarães, E. C., Garciov-Filho, E. B., Brito, P. S., Lopes, D. F. C., Andrade, M. C., Ottoni, F. P., Dias, L. J. B. S, Dos Anjos, M. R. Carvalho-Neta, R. N. F., Rodrigues, L. R. R., Nogueira, M. A. M. P., Pelicice, F. M., Agostino, A. A., Fearnside, P. M., 2023. Fisheries monitoring in Brazil: How can the 2030 agenda be met without fisheries statistics?. Biota Neotropica. 23, 1-15. https://doi.org/10.1590/1676-0611-BN-2022-1439

Shen, X., Su, Y. C., Liu, C., Oscar, T., De Paola, A., 2019. Efficacy of Vibrio parahaemolyticus depuration in oysters (Crassostrea gigas). Food microbiology, 79, 35-40. https://doi.org/10.1016/j.fm.2018.10.005

Shepherd, M., Giordano, D., Verde, C., Poole, R. K., 2022. The evolution of nitric oxide function: From reactivity in the prebiotic earth to examples of biological roles and therapeutic applications. Antioxidants. 11 (7), 1222. https://doi.org/10.3390/antiox11071222

Silva, J. D. S., Rocha, I. K. B. D. S., Freitas, L. C. D., Pereira, N. J., Carvalho Neta, R. N. F., 2015. Princípios bioéticos aplicados a los estudos ecotoxicológicos aquáticos. Revista Bioética. 23, 409-418. https://doi.org/10.1590/1983-80422015232079

SciELO Preprints - This document is a preprint and its current status is available at: https://doi.org/10.1590/SciELOPreprints.15698

Silva, O. L. L., Veríssimo, S. M. M., da Rosa, A. M. B. P., Iguchi, Y. B., de Lima, E. D. S. C., de Moraes, C. M., Cordeiro, C.A.M., Xavier, D.A., Pinto, A. S. O., Joele, M. R. S. P., Brito, J. S., Juen, L., da Rocha, R. M., 2020. Effect of environmental factors on microbiological quality of oyster farming in Amazon estuaries. Aquaculture Reports, 18, 100437. https://doi.org/10.1016/j.aqrep.2020.100437

Silva, C. M., Silva, A. L. P., Watanabe, K. F. C., Porto, R. D. B., Bezerra, D. C. Santos, L. S., Coimbra, V. C. S., Santos, H. P., Bezerra, N. P. C., 2023. Detection of Cryptosporidium sp. in cultivated oysters and the natural oyster stock of the state of Maranhão, Brazil. Ciência Rural. 53 (1), e20210014. https://doi.org/10.1590/0103-8478cr20210014

Smith, C. E., Williams, W. G., 1974. Experimental nitrite toxicity in rainbow trout and chinook salmon. Transactions of the American Fisheries Society. 103 (2), 389-390.

Torres, H. S., de Jesus, W. B., Ribeiro, E. B., Pinheiro-Sousa, D. B., Costa Filho, R. N. D., Neta, R. N. F. C., 2024. Trace elements and multibiomarkers in Sciades herzbergii (Pisces, Ariidae) for monitoring port areas on the north coast of the Amazon, Brazil. Regional Studies in Marine Science. 77, 103656. https://doi.org/10.1016/j.rsma.2024.103656

Vaughn, C. C., Hoellein, T. J., 2018. Bivalve impacts in freshwater and marine ecosystems. Annual review of ecology, evolution, and systematics. 49 (1), 183-208. https://doi.org/10.1146/annurev-ecolsys-110617-062703

WHO, World Health Organization., 1989. Evaluation of certain food additives and contaminants. Thirty-third Report of the Joint FAO/WHO Expert Committee on Food Additives, 776(2), 1. EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS - ProQuest

WHO,World Health Organization., 2010. Hardness in drinking-water: background document for development of WHO guidelines for drinking-water quality (No. WHO/HSE/WSH/10.01/10). World Health Organization. https://apps.who.int/iris/bitstream/handle/10665/70168/WHO_HSE_WSH_10.01_10_Rev1_eng.pdf

Declaration of conflict of interest

The authors declare that there is no conflict of interest.

Research data availability statement

The entire dataset supporting the results of this study was published in the article itself.

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Tables and figures

Table 1. Abiotic data recorded at three points in São José Bay-MA, in the dry and rainy seasons.

Parameters	Dry (October/2022)			Rainy (March/2023)			Recommended values
	S1	**S2**	**S3**	**S1**	**S2**	**S3**	**CONAMA N°357**
Temperature (°C)	29.3	29.7	28.6	29.9	27.1	28.4	-
pH	7.81	7.33	8.04	7.34	6.93	7.22	6.5 - 8.0
O2 dissolved (mg/L)	5.3	**4.8**	5.1	**4.2**	**4.1**	**4.6**	>5 mg/L
Salinity ($g.kg^{-1}$)	36.7	16.7	18.2	28.2	16.1	16.2	>30%

S1: Farm area, Raposa-MA; S2: Extractivism, Paço do Lumiar-MA; S3: Urban Area, São José de Ribamar-MA.

Table 2. Reading of nitrite quantification in brackish water in São José Bay-MA in the dry season (October 2022) and in the rainy season (March 2023).

	Dry	Rainy
S1	0.047 ± 0.001 mg / L	0.013 ± 0.001 mg / L
S2	0.262 ± 0,005 mg / L	0.109 ± 0.006 mg / L
S3	0.060 ± 0,002 mg / L	0.048 ± 0.002 mg / L

S1) Farm area; S2) Extractivism area; S3) Urban area.

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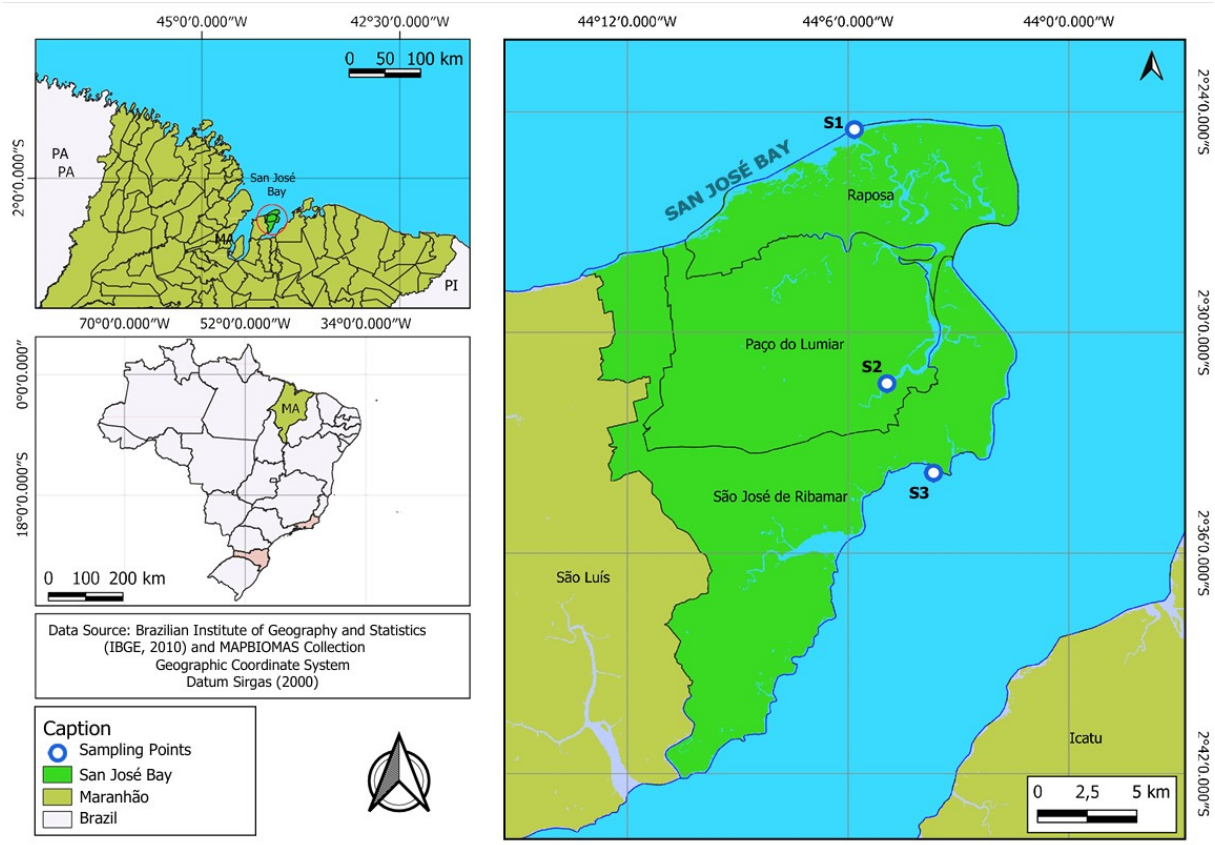


Fig. 1. Collection points of oysters of the genus *Crassostrea* sp. and brackish water in São José Bay, Maranhão: S1) Farm area; S2) Harvesting area; S3) Urban area.

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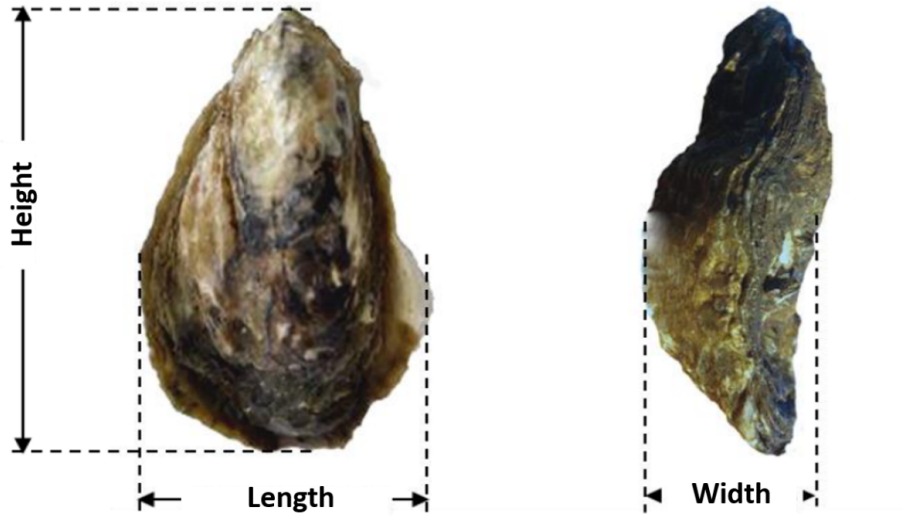


Fig. 2. Biometric measurement scheme for oysters of the genus *Crassostrea* sp. (height, length and width). Fonte: Santana (2020).

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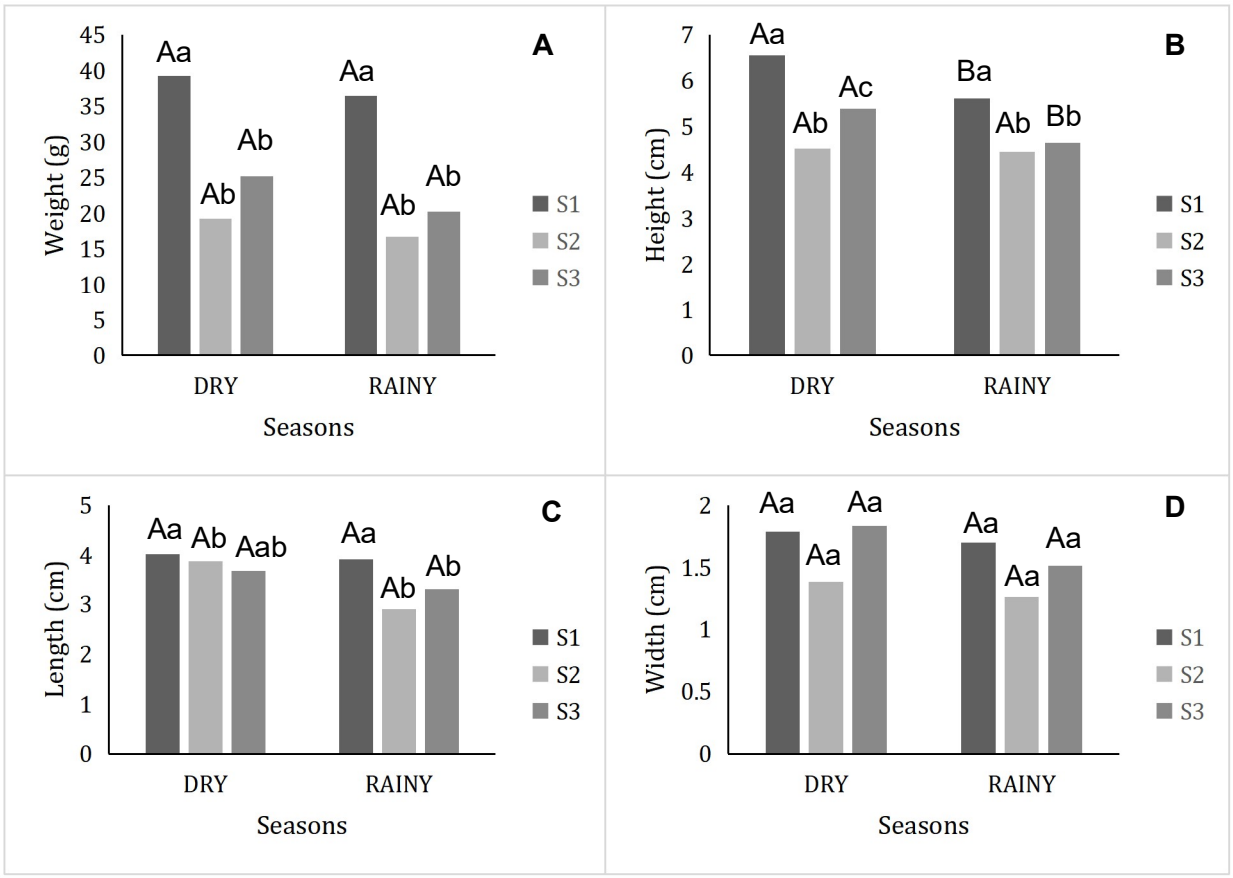


Fig. 3. Biometrics of oysters collected in São José Bay in the dry season (October 2022) and in the rainy season (March 2023). A) Average total weight. B) Average height. C) Average length. D). Average width: S1) Farm area; S2) Harvesting area; S3) Urban area. A and B indicate statistical differences between stations, while a, b and c indicate statistical difference between points in the same station.

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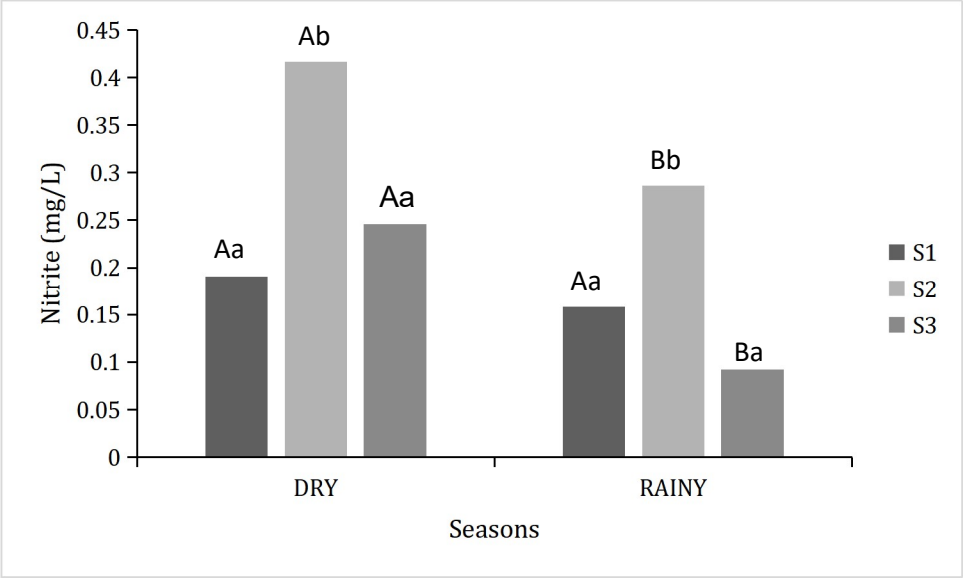


Fig. 4. Average nitrite concentration in the supernatant of oysters collected in São José Bay in the dry season (October 2022) and in the rainy season (March 2023): S1) Farm area; S2) Harvesting area; S3) Urban area. A and B indicate statistical differences between stations, while a and b indicate statistical difference between points in the same station.

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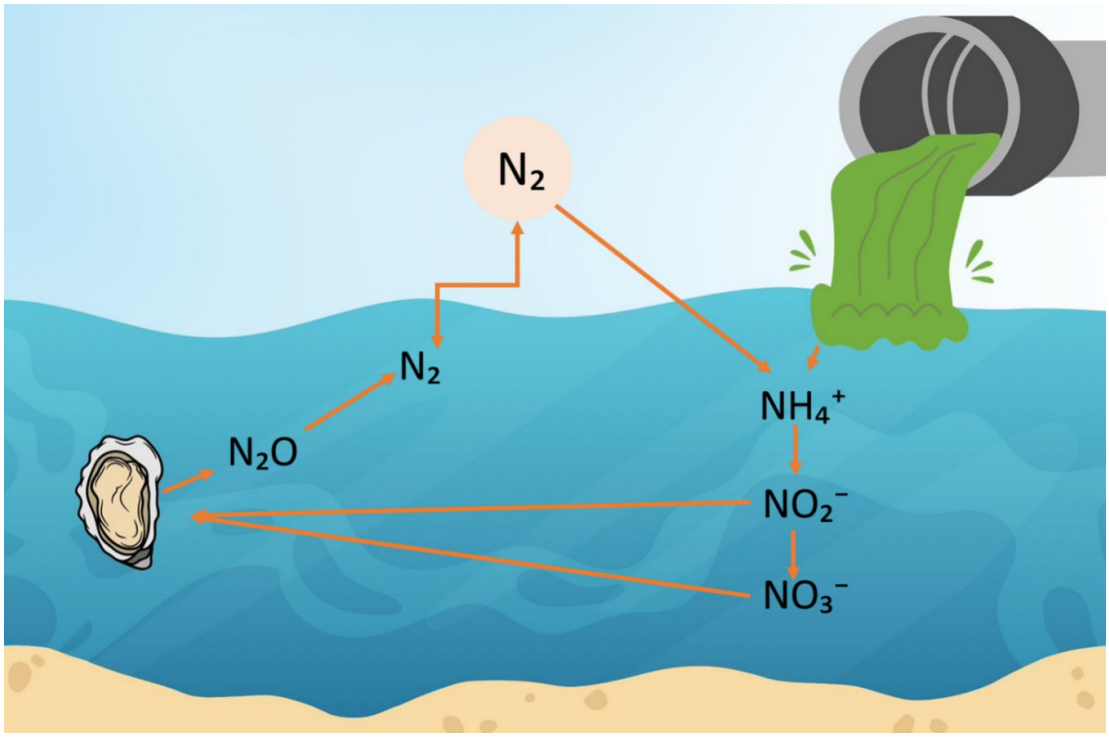


Fig. 5. Proposal of a model for the conversion of NO_2^- into N_2 in oysters, as a deposition result of domestic effluents.

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Contribuição de autoria

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Elaine Nunes Teixeira. CRedit: Data curation, Investigation, Writing - original draft.

Lina Clara Moreno. CRedit: Data curation, Writing - original draft, Validation.

Isabel Vitória Figueiredo. CRedit: Validation, Writing - review and editing.

Raimunda Fortes Carvalho-Neta. CRedit: Validation, Writing - review and editing.

Ligia Tchaicka. CRedit: Validation, Writing - review and editing.

Sandra Fernanda Loureiro de Castro Nunes. CRedit: Supervision, project administration, Writing - review and editing.

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