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Intravalvular nitrite accumulation in Crassostrea sp. as a low-cost bioindicator of estuarine eutrophication

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Abstract

Estuaries are vulnerable to anthropogenic impacts, and oysters are bioindicators due to filter-feeding and bioaccumulation. This study evaluated water physicochemistry and nitrite (NO_2^-) in intravalvular fluid of Crassostrea sp. from three sites in São José Bay, Brazil: S1 farm in Raposa, S2 harvesting in Paço do Lumiar, and S3 urban pier in São José de Ribamar, during dry October 2022 and rainy March 2023. Temperature, pH, dissolved oxygen (DO), salinity and NO_2^- in water were measured. Intravalvular NO_2^- was quantified by modified Griess method using 250 µL supernatant plus 250 µL Griess reagent, total 500 µL, with LOD 0.01 mg L^{-1}, LOQ 0.03 mg L^{-1}, R^2 0.9981 and 95% waste reduction. DO was below CONAMA 357/2005 Class 1 threshold of 5.0 mg L^{-1} at most sites, indicating enrichment. Water NO_2^- was highest at S2 in dry season at 0.262 mg L^{-1}, exceeding Class 1 limit of 0.07 mg L^{-1}. Intravalvular NO_2^- was higher at S2 than at S1 and S3 in dry (0.43 ± 0.12 mg L^{-1}) and rainy (0.30 ± 0.04 mg L^{-1}), two-way ANOVA $p < 0.001$. Higher dry-season values suggest concentration. Intravalvular fluid is sensitive low-cost matrix for NO_2^- biomonitoring.

1 Introduction

Biomonitoring of water quality is essential for environmental conservation and public health. Traditional physicochemical analyses provide snapshot data, but continuous, sensitive biological indicators are required for effective estuarine management (Silva et al. 2015; Lal et al. 2023).

Bivalve mollusks, particularly oysters of the genus *Crassostrea*, are widely used as biomonitors due to their sessile habit, filter-feeding behavior, and capacity to bioaccumulate organic and inorganic contaminants, reflecting environmental quality (Dalzochio et al. 2016; Dos Santos et al. 2021). These organisms have high economic importance in Brazil as food resource and raw material, and provide ecosystem services including coastline stabilization and nutrient mitigation (Vaughn and Hoellein. 2018; Almeida et al. 2024).

Conventional biomonitoring uses soft tissues, mainly gills and muscle, which accumulate contaminants from filtered water (Inglezakis et al. 2016; Oliveira et al. 2020). However, intravalvular fluid (also referred as oyster supernatant) is the internal medium responsible for nutrient transport and directly influences soft tissue chemistry (Hand and Stickle 1977). Despite its physiological relevance, intravalvular fluid remains underexplored as a matrix for contaminant quantification. Its use offers advantages such as non-destructive collection, minimal cellular interference, and rapid processing compared to tissue digestion, representing a potential low-cost alternative for routine monitoring.

Among inorganic contaminants, nitrite (NO_2^-) and nitrate (NO_3^-) derived from domestic sewage, agriculture and industrial effluents pose significant risks, including eutrophication, dead zone formation and toxicity to aquatic organisms (Camargo et al. 2005). Although NO_2^- is an intermediate in nitrification, NO_2^- exhibits high toxicity, causing methemoglobinemia (“blue baby syndrome”) by oxidizing hemoglobin and impairing oxygen transport, and can form carcinogenic nitrosamines via reaction with secondary

amines (Fabris et al. 2020; Picetti et al. 2022). Continuous monitoring of nitrogen species in estuaries receiving untreated sewage is therefore critical for ecosystem health and food safety of oysters consumed by local populations. Recent guidelines for oyster habitats highlight NO_2^- accumulation as a sensitive indicator of nitrification failure under hypoxia, supporting targeted NO_2^- monitoring in initial assessments (Ray et al. 2021).

São José Bay, located on the Amazon Coast, Maranhão (MA), Brazil, is an estuarine system influenced by the Itapecuru and Munim rivers and subjected to increasing anthropogenic pressure from urban expansion and artisanal oyster harvesting. To date, no study has evaluated NO_2^- in oyster intravalvular fluid as a biomonitoring tool in this region.

The aims of this study were: (i) to determine physicochemical parameters (temperature, pH, dissolved oxygen, salinity and NO_2^-) of water at three sites in São José Bay, MA, Brazil; and (ii) to validate the modified Griess method using 250 µL micro-volume for NO_2^- quantification in intravalvular fluid of *Crassostrea* sp. as a novel, low-cost and low-waste biomonitoring approach for estuarine water quality assessment.

2 Materials and methods

2.1 Study area

Sampling was conducted in São José Bay (2°33'58"S, 44°03'23"W, WGS84), located on the northern coast of São Luís Island, MA, Brazilian Amazon coast (Fig. 1). São José Bay is a Ramsar-relevant estuarine system of great importance for artisanal fisheries, encompassing fish landing ports and natural oyster banks of *Crassostrea* sp. used for subsistence and commercialization by traditional communities.

Note

Geodesic Datum: SIRGAS 2000 / UTM Zone 23S. Cartographic Base: IBGE (2010) and MAPBIOMAS (Collection 8). Source: Elaborated by the author (2023).

Three sampling sites were established along an anthropogenic pressure gradient (Fig. 1): (S1) oyster farm area – low impact: a controlled aquaculture site in Raposa, MA (2°24'43"S, 44°05'54"W), where oysters are cultivated in estuarine waters under periodic monitoring by the State Agency, serving as the reference site for water quality; (S2) harvesting area – moderate impact: a natural mangrove in Paço do Lumiar, MA (2°31'37"S, 44°05'03"W), characterized as a subsistence harvesting area with no sewage collection and solid waste disposal on mangrove margins (IBGE, 2022), previously reported to exceed the CONAMA Resolution 357/2005 limit for NO_2^- (0.07 mg L^{-1}) for brackish water Class 1; and (S3) urban area – high impact: a pier in São José de Ribamar, MA (2°35'10"S, 44°02'30"W), surrounded by urban occupation and recreational fishing activities and subject to domestic effluent discharge.

Samplings were carried out in dry season (October 2022) and rainy season (March 2023). Geographic coordinates were obtained with GPS Garmin eTrex 20 (WGS84). Map elaborated with QGIS 3.28. Authorization: State Secretariat for Environment and Natural Resources of MA (SEMA-MA), Authorization No. A87-2025, Process SIGEP No. 2509220003.

2.2 Water and oyster collection

Two sampling events were carried out: dry season (October 2022) and rainy season (March 2023). At each sampling site, 20 adult oysters (*Crassostrea* sp.) were planned to be collected. A total of 106 individuals yielded sufficient intravalvular fluid for analysis and were included in the statistical analysis (S1 dry n = 20, S2 dry n = 19, S3 dry n = 20, S1 rainy n = 15, S2 rainy n = 15, S3 rainy n = 17). Samples with insufficient volume (< 250 µL) were excluded.

Water physicochemical parameters (salinity, pH, DO, conductivity, temperature) were measured in situ at 30 cm depth using HANNA HI9829 multiparameter probe, with one measurement per site and season. At each site, one surface seawater sample was collected in a sterile 1.5 mL microtube for NO_2^- quantification using 250 µL supernatant plus 250 µL Griess reagent, total 500 µL (95% waste reduction).

2.3 Nitrite quantification by modified micro-volume Griess method

NO_2^- concentrations in estuarine water and oyster intravalvular fluid were determined using a modified Griess colorimetric method based on Green et al. (1982), previously validated in our laboratory for oyster matrices.

Intravalvular fluid samples (500–1000 µL per individual) were centrifuged (10000 rpm, 5 min, 4°C) and the supernatant stored at -20°C until analysis. Estuarine water samples were collected at 30 cm depth at the oyster sites and stored at -20°C.

For NO_2^- determination, 250 µL of sample supernatant was mixed with 250 µL of Griess reagent (1% sulfanilamide in 2.5% phosphoric acid and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride, 1:1 v/v, prepared immediately before use) directly in 1 cm disposable cuvettes (total volume 500 µL). After 10 min in the dark at room temperature, absorbance at 540 nm was measured against a reagent blank. This micro-volume adaptation represents a 95% reduction in reagent waste compared to the conventional 10 mL APHA 4500-NO_2^- B method (APHA 2023).

Calibration was prepared with $NaNO_2^-$ standard (0.2-2.0 mg L^{-1}, prepared January 2023): linearity R^2 = 0.9981, equation y = 0.9292x − 0.0438, LOD = 0.01 mg L^{-1} (3 × SD, n = 10 blanks), LOQ = 0.03 mg L^{-1} (10 × SD). The calibration curve was constructed using duplicate readings against the reagent blank. Recovery was 95–105% at 0.1, 0.5 and 1.0 mg L^{-1} spikes (no matrix interference). All samples and standards were analyzed in duplicate. Results are expressed as mg L^{-1} NO_2^-.

2.4 Statistical analysis

Data normality (Shapiro-Wilk test) and homogeneity of variances (Levene test) were assessed on the residuals of the intravalvular fluid data (α = 0.05). As assumptions were met, a two-way analysis of variance (ANOVA) with sampling site (S1, S2, S3) and season (dry: October 2022, rainy: March 2023) as fixed factors, followed by Tukey's post hoc test, was used to assess significant differences in NO_2^- concentrations in intravalvular fluid among sites and between seasons. Water physicochemical parameters were measured once per site and season and are therefore presented descriptively only, without inferential testing.

Values are expressed as mean ± standard deviation (SD). The significance level was set at 5% ($p < 0.05$). Statistical analyses and graphs were performed using Statistica® software v.10.0 (StatSoft Inc. Tulsa, OK, USA) and GraphPad Prism v.8.0. Different letters above bars indicate significant differences by Tukey test ($p < 0.05$).

Final sample size for intravalvular fluid was N = 106 due to insufficient intravalvular fluid volume in 14 individuals. Degrees of freedom for the two-way ANOVA error term were therefore df = 100.

2.5 Data Availability Statement

All data generated or analysed during this study are included in this published article and its supplementary information files, and are publicly available in the SciELO Data repository under DOI: https://doi.org/10.48331/SCIELODATA.DTHKYH

3 Results

3.1 Physicochemical analysis of water

The abiotic parameters are summarized in Table 1. Water temperature ranged from 27.1 to 29.9°C. CONAMA Resolution No. 357/2005 does not establish temperature limits for brackish waters.

pH values ranged from 6.93 to 8.04, within the range established by CONAMA Resolution No. 357/2005 for Class 1 brackish waters (6.5 to 8.5).

Dissolved oxygen (DO) concentrations were below the minimum threshold of 5 mg L^{-1} required by CONAMA for Class 1 brackish waters at most sites. Only S3 (urban area) during the dry season met the legal requirement (5.0 mg L^{-1}). The lowest DO value was recorded at S1 (farm area) during the dry season (1.3 mg L^{-1}), followed by S2 during the rainy season (4.1 mg L^{-1}) and S1 during the rainy season (4.2 mg L^{-1}), indicating organic enrichment, likely from untreated domestic sewage (Siqueira et al., 2023).

Salinity ranged from 16.1 to 36.7 g kg^{-1}. Only S1 during the dry season (36.7 g kg^{-1}) exceeded the upper limit for brackish waters (> 30 g kg^{-1}) established by CONAMA, approaching seawater characteristics. Lower salinity at S2 and S3 reflects strong freshwater influence from the Paciência and Claro rivers.

NO_2^- concentrations in water ranged from 0.013 to 0.262 mg L^{-1} (Table 1). The highest value was observed at S2 during the dry season (0.262 mg L^{-1}), exceeding the CONAMA limit for Class 1 brackish waters (0.07 mg L^{-1}) by 274%. The lowest concentration was recorded at S1 during the rainy season (0.013 mg L^{-1}). Water values were higher during the dry season at all sites, consistent with reduced dilution.

Table 1

Physicochemical Parameters of Surface Water in Farm Area (S1 - Raposa), Extractive Area (S2 - Paço do Lumiar) and Urban Area (S3 - São José de Ribamar), Maranhão Island, Brazil, During Dry and Rainy Seasons Compared to CONAMA Resolution No. 357/2005

Parameters	**Dry (October/2022)**			**Rainy (March/2023)**			**Recommended values (Class 1)**
	S1	**S2**	**S3**	**S1**	**S2**	**S3**	**CONAMA N°357**
Temperature (°C)	29.3	29.7	27.6	29.9	27.1	28.4	-
pH	7.81	7.33	8.04	7.34	6.93	7.22	6.5–8.5
Dissolved O_2 (mg L^{-1})	**1.3**	**4.8**	5.0	**4.2**	**4.1**	**4.6**	> 5
Salinity (g kg^{-1})	36.7	16.7	18.2	28.2	16.1	16.5	0.5–30
Nitrite (mg L^{-1})	0.047	**0.262**	0.060	0.013	**0.109**	0.048	0.07

Note. S1, S2, S3 = sampling stations. CONAMA = Brazilian National Environment Council. Bold values indicate non-compliance with CONAMA No. 357/2005.

Source: Elaborated by the author (2023).

3.2 Nitrite quantification

NO_2^- concentrations in intravalvular fluid differed significantly among sites and seasons (Two-way ANOVA, site: $F = 86.25$, season: $F = 49.88$, interaction: $F = 7.62$, $p < 0.001$; Fig. 2). Mean values were: S1 dry 0.23 ± 0.09 mg L^{-1} (n = 20), S2 dry 0.43 ± 0.12 mg L^{-1} (n = 19), S3 dry 0.19 ± 0.04 mg L^{-1} (n = 20), S1 rainy 0.08 ± 0.03 mg L^{-1} (n = 15), S2 rainy 0.30 ± 0.04 mg L^{-1} (n = 15), S3 rainy 0.17 ± 0.05 mg L^{-1} (n = 17).

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S2 (harvesting area, Paço do Lumiar) showed the highest NO_2^- concentrations in both seasons (dry: 0.43 ± 0.12 mg L^{-1}; rainy: 0.30 ± 0.04 mg L^{-1}). Seasonal variation was significant at S2 ($p < 0.001$) and S3 ($p = 0.002$), with higher values during the dry season.

Spatial variation was significant in both seasons: in the dry season S2 > S1 = S3, and in the rainy season S2 > S3 > S1 (Tukey, $p < 0.05$). S2 exceeded the CONAMA Resolution 357/2005 limit for Class 1 brackish

waters (0.07 mg L^{-1}) by up to 614% in dry season. The higher concentrations during dry season suggest reduced dilution and longer retention of organic matter in the estuary.

The micro-volume (250 µL) Griess method proved effective for intravalvular fluid, with detection limit of 0.01 mg L^{-1} and linear range 0.05-2.0 mg L^{-1} (R^2 = 0.9981).

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Figure 2 *Nitrite Concentration (mg L^{-1}) in Oyster Intravalvular Fluid from Farm Area (S1), Harvesting Area (S2) and Urban Pier (S3) During Dry and Rainy Seasons, Maranhão Island, Brazil.*

Note

Values are mean ± SD. Different letters above bars indicate significant differences by Tukey test ($p < 0.05$). Two-way ANOVA: site F = 86.25, season F = 49.88, interaction F = 7.62, $p < 0.001$; MS error = 0.00531; df error = 100; total N = 106.

4 Discussion

4.1 Physicochemical analysis of water

São José Bay is part of the Gulf of MA, Brazilian equatorial margin, characterized by macrotidal regime (6–7 m amplitude), mangrove dominance and strong seasonal dynamics (Do Nascimento et al., 2020; Oliveira et al., 2024). This study is the first to quantify NO_2^- in intravalvular fluid of *Crassostrea* sp. using a modified 250 µL micro-volume Griess method. Surface temperature ranged from 27.1 to 29.9°C, with slight seasonal variation, typical of equatorial estuaries. The lowest value at S2 in rainy season (27.1°C) reflects rainwater input. Afternoon sampling (14:00–16:00 h) may contribute to higher values, as previously reported for Raposa estuary (Do Nascimento et al., 2020).

Temperature within this range does not limit oyster physiology but influences metabolic rates (Vaughn and Hoellein, 2018). pH values (6.93–8.04) remained within CONAMA Resolution 357/2005 limits for brackish waters (6.5–8.5), similar to São Marcos Bay (6.7–7.2) (Torres et al., 2024). Alkaline pH favors NH_4^+ conversion to NH_3, potentially affecting nitrogen dynamics (Oliveira et al., 2024).

Dissolved oxygen (DO) was critical. During the rainy season, DO was low at all sites (4.1–4.6 mg L^{-1}). Site S2 presented DO < 5.0 mg L^{-1} (CONAMA limit for Class 1) in both seasons, likely due to organic matter input from harvesting activities and reduced water renewal (Krumme et al., 2012). The most severe hypoxia was observed at S1 during the dry season (1.3 mg L^{-1}), which suggests a local oxygen depletion event at the farm site, despite its classification as low-impact area. Low DO in the rainy season was also reported by Do Nascimento et al. (2020) and may impair aerobic metabolism of benthic fauna.

4.2 Nitrite quantification

NO_2^- is a key intermediate of the nitrogen cycle and its accumulation in estuaries is associated with incomplete nitrification/denitrification under low DO (Barbieri et al., 2014). In this study, NO_2^- in water complied with CONAMA 357/2005 for Class 1 brackish waters (0.07 mg L^{-1}) at S1 and S3, but S2 exceeded this limit during dry season (0.262 mg L^{-1}), and also showed the highest intravalvular concentration (0.43 ± 0.12 mg L^{-1}), indicating local pollution.

Higher NO_2^- at S2 is explained by DO < 5.0 mg L^{-1} in both seasons. Hypoxia inhibits NO_2^- oxidation to NO_3^-, causing buildup (Laughinghouse et al., 2022). Untreated domestic effluent near S2 provides NH_4^+ that is partially oxidized to NO_2^-, as illustrated in the conceptual model (Fig. 3).

Note

A = effluent discharge and ammonium release; B = nitrification in water column; C = accumulation and nitrification in Crassostrea sp. tissues; D = denitrification in oyster-associated microbiome with N_2 release. NH_4^+ = ammonium; NO_2^- = nitrite; NO_3^- = nitrate; N_2O = nitrous oxide; N_2 = dinitrogen gas. Conceptual figure generated with assistance of Meta AI (2026).

Seasonal pattern (higher in dry, lower in rainy) corroborates Oliveira et al. (2024) in Anil River estuary, where rainfall dilution reduced nitrite. NO_2^- in intravalvular fluid was consistently higher than surrounding water (Table 1, Fig. 2), reflecting filtration activity and denitrifying bacteria associated with gills, digestive tract and shells (Arfken et al., 2017). Oyster reefs may act as hotspots for denitrification, converting NO_2^- to N_2 and contributing to nitrogen removal in eutrophic estuaries (Vaughn and Hoellein, 2018).

NO_2^- levels in fluid (0.08–0.43 mg L^{-1}) remained below lethal tolerance reported for *Crassostrea virginica* (1.08–2.41 mg L^{-1}) (Epifanio and Sarna, 1975), suggesting no acute toxicity. However, chronic effects cannot be excluded, as Nitric Oxide (NO) signaling in bivalves regulates defense, stress response and ciliary motility (Shepherd et al., 2022). In biological systems, ingested NO_3^- must be reduced to NO_2^- and then to NO, a pathway still incompletely understood in bivalves and requiring further investigation.

From food safety perspective, Brazil lacks specific regulation for NO_2^- in bivalves. Values recorded should be interpreted against JECFA/WHO acceptable daily intake (0.06 mg kg^{-1} bw day^{-1}) (WHO, 1989). Additionally, previous DNA-based studies in Raposa farm region detected *Cryptosporidium* sp. in gills of *Crassostrea* sp., reinforcing need for thorough cooking before consumption (Silva et al., 2023). From a management and monitoring perspective, while Brazil currently lacks specific regulatory limits for nitrite directly in bivalve soft tissues or fluids, the concentrations recorded in intravalvular fluid provide a reliable reflection of environmental exposure. However, because this study evaluated a localized spatial gradient (three sites) across two seasonal events (dry and rainy periods), these findings should be interpreted as an initial screening and baseline for tropical estuarine systems rather than a comprehensive assessment of the entire bay's sanitary status. Although acute toxicity thresholds reported for oysters are higher than our field values, chronic sublethal effects of nitrogen metabolites on bivalve physiology warrant cautious interpretation. Furthermore, while our chemical screening highlights site S2 (Paço do Lumiar) as a priority area requiring targeted management aligned with SDG 14 (United

Nations, 2015), evaluating broader food safety risks will require complementary investigations integrating microbiological depuration rates, multi-residue contaminant tracking, and direct physiological stress biomarkers.

Quantification of NOx (NO_2^- + NO_3^-) was not required for the objectives of this study. According to Ray et al. (2021), NO_2^- accumulation is a sensitive indicator of nitrification failure under hypoxia in oyster habitats, justifying focus on NO_2^- without full NOx quantification in initial biomonitoring screening. NO_2^- is the most toxic and transient nitrogen species, an early indicator of recent organic pollution and incomplete nitrification under hypoxia, whereas NO_3^- is relatively stable and less toxic to aquatic biota (Barbieri et al. 2014; Epifanio and Sarna 1975). CONAMA Resolution 357/2005 establishes specific limits for NO_2^- (0.07 mg L^{-1} Class 1; 0.20 mg L-1 Class 2) independent of NO_3^-, supporting its use as a standalone pollution proxy. Moreover, the proposed 250 µL micro-volume Griess method was validated specifically for NO_2^- in low-volume intravalvular fluid (500–1000 µL per individual), where NOx analysis would require an additional reduction step (nitrate reductase), increasing cost, waste and analytical error, contrary to the low-cost biomonitoring purpose. Future monitoring should not rely exclusively on nitrite as a standalone metric; rather, focusing on NO_2^- should be understood as part of a rapid, low-cost screening tool for early warning in tropical extractive reserves. Because estuarine health is complex and subject to multiple stressors, relying on a single chemical indicator presents inherent limitations. Therefore, nitrite quantification in intravalvular fluid is best applied as a preliminary triage approach that requires continuous validation through a convergent battery of indicators (e.g. complementary physicochemical parameters, multiresidue contaminant tracking, and direct biological or physiological stress biomarkers) to ensure robust environmental management and support sustainable local extractivism.

5 Conclusions

This study demonstrates that nitrite dynamics in oyster intravalvular fluid in São José Bay exhibit significant spatial and seasonal variations, while surface water quality showed a consistent spatial and seasonal pattern descriptively. Site S2 (harvesting area, Paço do Lumiar) presented the most critical conditions, with DO levels frequently falling below CONAMA Resolution 357/2005 limits (< 5.0 mg L^{-1}) and elevated NO_2^- concentrations during the dry season (0.262 mg L^{-1} in water; 0.43 ± 0.12 mg L^{-1} in intravalvular fluid), pointing to localized organic enrichment and anthropogenic pressures.

The adapted micro-volume (250 µL) Griess method proved to be a viable, rapid, and low-cost analytical alternative for quantifying nitrite in limited-volume oyster intravalvular fluid, significantly reducing chemical waste while maintaining analytical reliability (LOD 0.01 mg L^{-1}). Within the methodological scope of a rapid preliminary screening, targeting nitrite alone served as a practical proxy for identifying recent estuarine organic input and hypoxia-associated variations without requiring complex nitrate-reduction steps.

Notwithstanding these methodological advantages, the conclusions of this study are bounded by its spatial scope (three monitoring sites) and temporal design (two seasonal sampling events). Therefore, continuous monitoring at impacted sites like S2 is strongly recommended to support local management and sustainable extractivism. Future studies incorporating broader spatial scales, multi-season monitoring, and complementary physiological or microbiological analyses are encouraged to fully elucidate the long-term ecological implications of nitrogen dynamics in tropical estuarine bivalves.

Declarations

Language Editing

Aline S. Varela reviewed and edited the final English manuscript.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Meta AI (Muse Spark model) to assist with language editing, reference formatting and creation of conceptual diagram for Fig. 3.

The prompt used for Fig. 3 was: "conceptual diagram of nitrogen cycle in tropical estuary with mangrove oysters, showing $NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^- \rightarrow N_2O \rightarrow N_2$ pathways, anthropogenic inputs from urban area (S3), farm area (S1) and extractivism (S2)". Conceptual diagram was drafted with assistance of Meta AI and edited / re-drawn in Inkscape (v1.3). All scientific data, interpretations and conclusions were validated by the authors. The authors take full responsibility for the content of the publication.

Conflict of interest

The authors declare no competing interests.

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Author Contribution

G.S.C. and S.M.C wrote the main manuscript text. G.S.C. and E.N.S.T Colleted the data. E.N.S.T. wrote and designed the figures. L.C.M., R.N.F.C.N. and L.T. analysed the data I.V.F performed the final review. S.F.L.C.N gave final approval. All authors contributed critically to the paper.

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Data Availability

Publicly available in a repository:SciELO Data: https://doi.org/10.48331/SCIELODATA.DTHKYH

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Figures

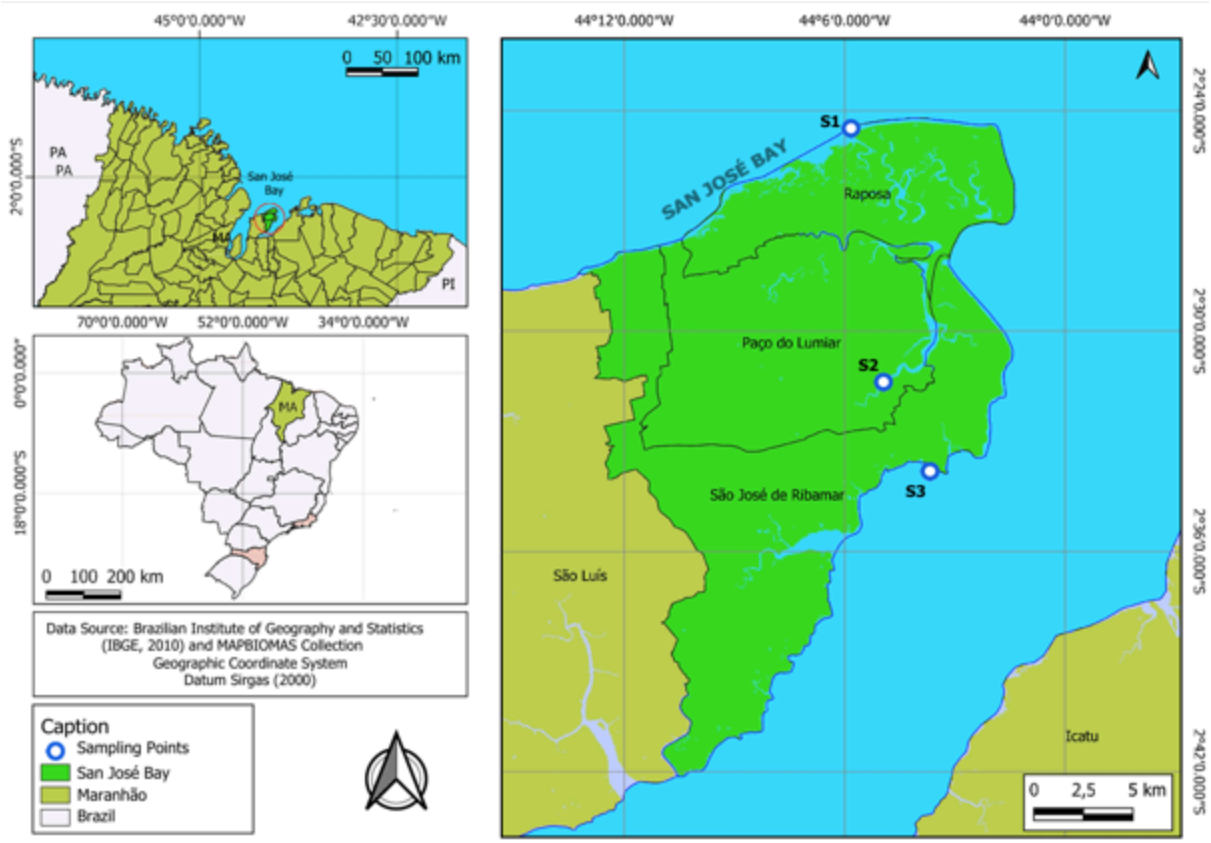


Figure 1

Map of the Study Area Located in São José Bay, Maranhão Island, Brazil, Showing the Sampling Stations. S1: Farm Area (Raposa-MA), S2: Extractive Area (Paço do Lumiar-MA) and S3: Urban Area (São José de Ribamar-MA).

Note. Geodesic Datum: SIRGAS 2000 / UTM Zone 23S. Cartographic Base: IBGE (2010) and MAPBIOMAS (Collection 8). Source: Elaborated by the author (2023).

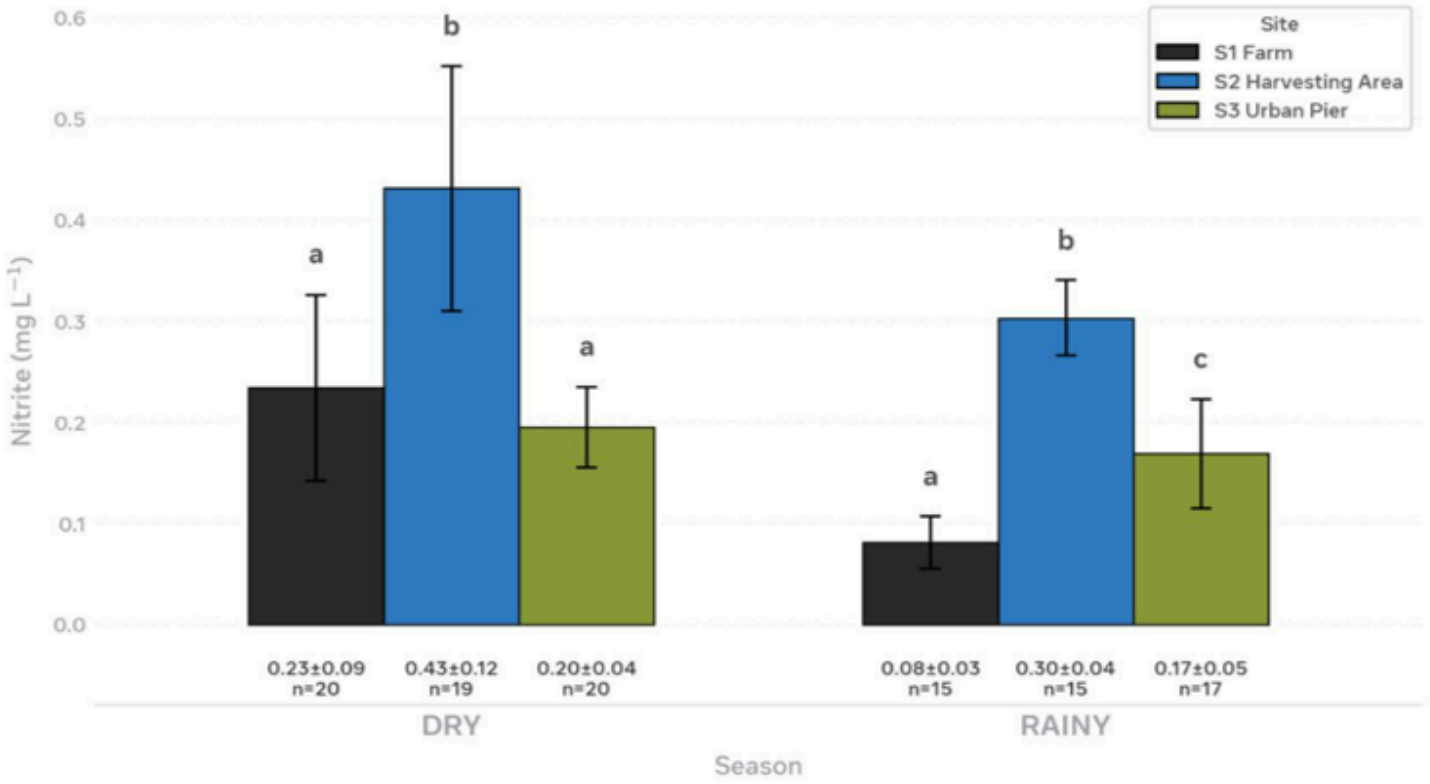


Figure 2

Nitrite Concentration (mg L⁻¹) in Oyster Intravalvular Fluid from Farm Area (S1), Harvesting Area (S2) and Urban Pier (S3) During Dry and Rainy Seasons, Maranhão Island, Brazil.

Note. Values are mean ± SD. Different letters above bars indicate significant differences by Tukey test ($p < 0.05$). Two-way ANOVA: site $F = 86.25$, season $F = 49.88$, interaction $F = 7.62$, $p < 0.001$; MS error = 0.00531; df error = 100; total N = 106.

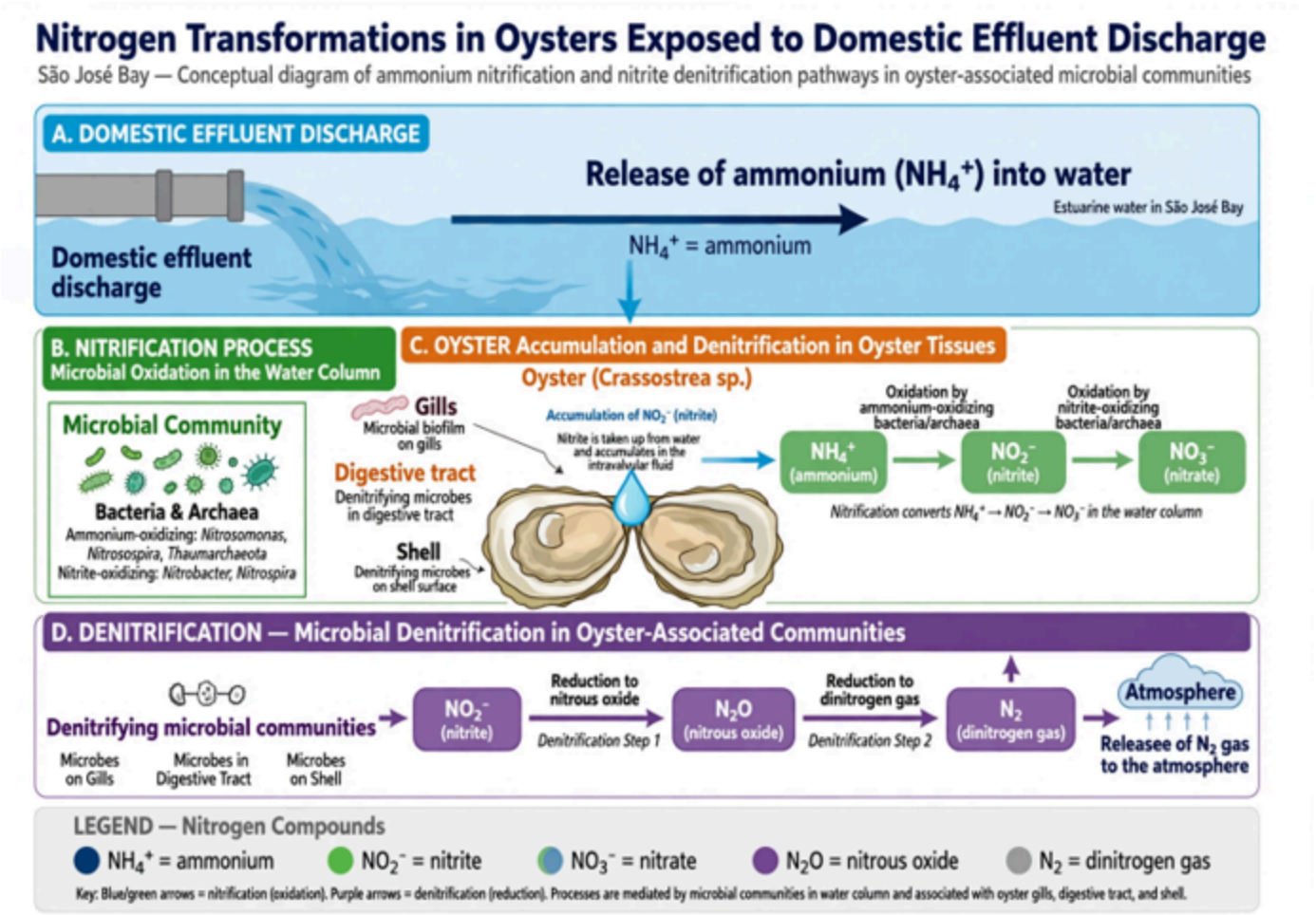


Figure 3

Conceptual Diagram of Nitrogen Transformations in Oysters (Crassostrea sp.*) Exposed to Domestic Effluent Discharge in São José Bay, Maranhão Island, Brazil.*

Note. A = effluent discharge and ammonium release; B = nitrification in water column; C = accumulation and nitrification in Crassostrea sp. tissues; D = denitrification in oyster-associated microbiome with N_2 release. NH_4^+ = ammonium; NO_2^- = nitrite; NO_3^- = nitrate; N_2O = nitrous oxide; N_2 = dinitrogen gas. Conceptual figure generated with assistance of Meta AI (2026).