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Larval fish assemblages in selected Brazilian estuaries: Species-environment relationships under different anthropogenic influences

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ABSTRACT

We investigate the effects of spatial changes in environmental conditions and anthropogenic influences on larval fish communities in two tropical estuaries with varying levels of human impact. Our findings revealed a distinct structure of larval fish assemblages between the two estuaries located in northeastern Brazil, and we observed that eutrophication, characterized by high concentrations of nutrients, had adverse effects on the abundance and richness of larval fish assemblages. Additionally, we observed that a decrease in rainfall had an impact on larval fish assemblages, particularly during the dry season, when intermittent upstream rivers lead to changes in salinity and species composition within the estuaries. This study contributed to evaluating the community descriptors of two tropical estuaries under different levels of human influence, providing insights into the vulnerability of larval fish assemblages to climate change, specifically in relation to human influences and hypersalinity and the effects of marinization in shallow tropical estuaries in this region.

1. Introduction

In tropical estuaries, salinity and precipitation are the main predictors of the abundance and occurrence of larval fish assemblages (Amorim et al., 2016; Spies and Steele, 2016; Lima et al., 2022). Salinity has an important influence on larval development and survival (Lima et al., 2016; Santos et al., 2017a, 2017b; Vanalderweireldt et al., 2020). For example, salinities that are outside the tolerance range of a species can cause adverse effects such as increased metabolic rates, reduced neural tissue mass, smaller eye size or poor visual acuity and smaller body size (Garrido et al., 2015). In addition, the energy burden of osmoregulation in extreme conditions can significantly reduce the overall energy budget available to complete development (Ackerly et al., 2023). Precipitation works as a driver of species reproduction and larval recruitment (Barletta-Bergan et al., 2002). Some authors suggest that river discharge plumes strongly influence fish larvae in coastal waters, playing a significant role in the ingress of species to estuaries (Vinagre et al., 2009; Amorim et al., 2016; Montenegro et al., 2020).

Human impacts mainly related to land pollution in coastal cities cause alterations in estuarine water quality and habitat conditions, which may in turn affect larval fish species distribution and abundance. Changes in land use (e.g., intensive agriculture, urbanization, and sewage disposal), together with reduced flow river can lead to an increase/longer residence time of the nutrient load, thereby favour eutrophication, which can cause several deleterious effects on the health of estuaries, including decreased dissolved oxygen, toxicity to biota through high concentrations of ammonia, and species loss (Mosley et al., 2023). In addition, structural alterations such as dredging activities, alteration of the banks and construction of ports can interrupt the connectivity between habitats (Halpern et al., 2015; Elliott et al., 2016; Tolf et al., 2018; Waltham et al., 2020), and linkage between local populations (Rodrigues et al., 2022), and limit larval transport and dispersal processes (Duan et al., 2016; Menegotto et al., 2019). Therefore, fish larval assemblages also respond to the impact of human activity on estuaries, resulting in losses to biodiversity and ecological functions (Lara-Lopez et al., 2020).

The eutrophication process is known as an emerging global issue associated with increasing anthropogenic nutrient loading (Courrat et al., 2009; Andersen et al., 2020). Ramos et al. (2015) concluded that younger fish stages were highly sensitive to sewage contamination and nitrogen load and can act as bioindicators reflecting the ecological status of estuaries. Habitat health is also a critical aspect in supporting

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appropriate growth and high survival rates of fish larvae, which is essential to maintain productive fish populations (Guerreiro et al., 2021). Thus, the high survival rates in early life stages directly affect the number of adults in the population (Arevalo et al., 2023). In this way, understanding the consequences of anthropogenic disturbances on the composition patterns of larval fish assemblages requires estimates of these early life stages, since anthropogenic disturbances may have a negative effect on the health of fish larvae, because larvae are more sensitive to changing environmental conditions and the effects of pollution (Ramos et al., 2012; Strydom, 2015; Camara et al., 2019). Continuous investigations on species-environment relationships are of great importance for understanding estuarine communities, in view of the increasing degradation of these ecosystems (Mouillot et al., 2013; Selig et al., 2014; Riera et al., 2018).

The estuaries of the northeastern Brazil ecoregion are characterized by high salinity values due to elevated temperatures and low rainfall (Maia et al., 2018). Several studies have emphasized that these estuaries are recurrently affected by episodes of drought that intensify the hypersaline conditions (Gillanders et al., 2022; Soares et al., 2021; Whitfield, 2021). Recently, Mosley et al. (2023) suggested that increased hypersalinization of the Coorong Lagoon exacerbated eutrophication and contributed to a significant decline in ecosystem quality. In addition, hypersaline conditions can result in the loss of freshwater species, the decline of estuarine-dependent species, and establishment of marine species in the lower reaches of estuaries (Arevalo et al., 2023).

The aim of the present study was to examine changes in the spatial distributions of larval fish assemblages in two of the most important estuaries in Paraíba state (Paraiba do Norte and Mamanguape estuaries), which are exposed to different degrees of anthropogenic pressure. Thus, we postulated the following questions: 1) What are the potential environmental drivers of larval fish distribution and abundance in both estuaries? 2) How might the location along the inorganic nutrient and salinity gradient, and temporal differences in rainfall associated with low freshwater supply explain larval abundance? We predicted that the highest concentration of inorganic pollutants limits the abundance and richness of larval fish in both estuaries. Our general hypothesis was that the richness and spatial distributions of fish larvae differ between estuaries are modulated by large variations in environmental parameters.

2. Methods

2.1. Study area

The study was carried out in Paraíba do Norte (PNE) (6°47′45.3″S and 34°59′05.9″O) and Mamanguape (ME) (6°43′02″S and 35°67′46″O) estuaries that are located on the north coast of Paraíba State, Brazil (Fig. 1). The regional climate is classified by Köppen as As-type, i.e., hot and humid (Alvares et al., 2013). The rainy season begins in March and lasts until August, with maximum rainfall occurring from April to June. The dry season occurs between the months of September and February, with the lowest rainfall between October and December (Alvares et al., 2013). The two rivers draining semi-arid areas, and occurs reduced flushing and seawater inflows to estuaries principally during dry season.

The Paraíba do Norte estuary has an area of approximately 3102 ha of mangrove forest and extends for 22 km in a south-north direction (Fig. 1 a). The estuary channel is relatively shallow, with a maximum depth of 11 m and estuarine mouth size of 1.4 km (Nishida et al., 2008; Dolbeth et al., 2016; Moura et al., 2016). In geomorphological terms, the estuary is situated along a fluvial-marine plain formed by the North Paraíba River (principal river) and seven tributaries that contribute to the entry of fresh water into the estuary (Guedes, 2002). The Mamanguape estuary is part of the Environmental Protection Area of the Mamanguape River (CERHPB, 2004), with an area of 5721 ha of well-preserved mangrove forest and extends for 25 km in an east-west direction (Nobrega and Nishida, 2003; Silva et al., 2011). In the estuary entrance, a long reef barrier exists perpendicular to the shoreline (~13 km), creating a semiclosed bay with calm waters and a mix of fresh water and seawater (Campos et al., 2015). In these estuaries, the impacts of various human activities on estuarine water quality are attributable to large diffuse inputs from agricultural activities (e.g., areas of sugarcane fields), shrimp farms and industrial plants (for example, plants for cosmetics, concrete, paper, and weaving production) (Lacerda et al., 2014; Dolbeth et al., 2016). Specifically, the increased maritime shipping activity in the Paraiba do Norte estuary has necessitated the development of the Port of Expansion Program. This initiative entails the construction of a multipurpose terminal, a cargo terminal, a warehouse, and piers to meet the rising demand.

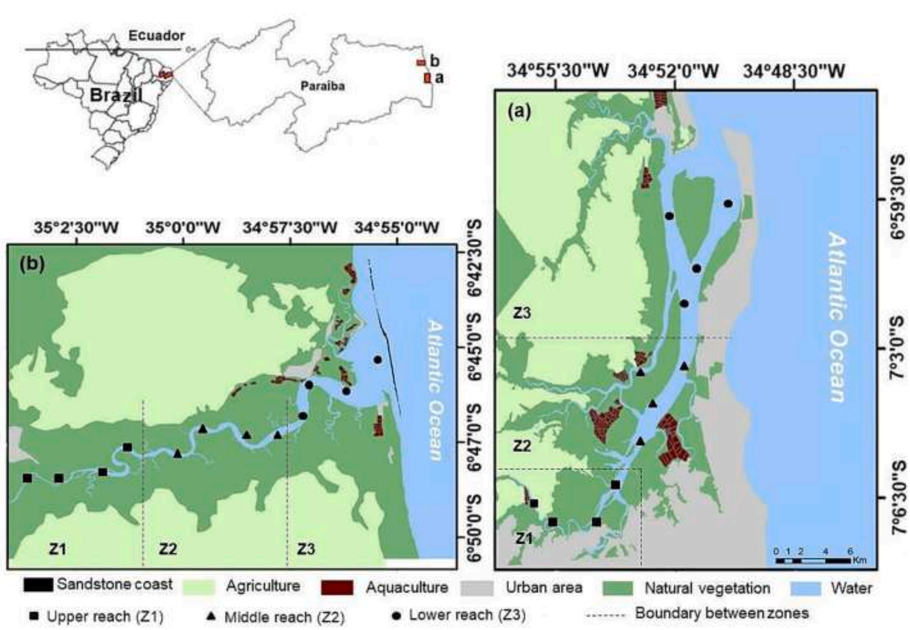

Fig. 1. Study area with indication of the three reaches throughout the Paraíba do Norte (a) and Mamanguape (b) estuaries, as well as characteristics of the landscape around these ecosystems located in Brazilian Northeastern.

2.2. Anthropogenic pressure index

To categorize estuaries based on the extent of human impact on ichthyoplanktonic assemblages, a pressure index (PI) was developed, adapting the methodology proposed by Aubry and Elliott (2006). This index takes into account landscape use metrics and incorporates seven additional descriptors, resulting in a total of 11 impact indicators: habitat loss, dredging, proportion of agricultural area, proportion of urban settlement, presence of marinas, presence of ports, proportion of aquaculture, fishing activities, tourism and recreation, sewage discharge, and population density. These descriptors may apparently include elements of double counting or redundancy, which can lead to overweight at certain pressures, but a single number of indicators cannot measure the complexity involved in the environmental state (Purvis and Hector, 2000; Derous et al., 2007; Borja et al., 2014; Santos et al., 2017a, 2017b). The 11 selected indicators were classified with the following scores: no change (0), very low (1), low (2), moderate change (3), high (4) and very high (5). The PI resulted from the weighted average value of the individual pressure scores (Ojaveer and Eero, 2011). The landscape use variables were obtained for each estuary from vector layers (scale 1: 120,000; database: Google Earth 2015). Landscape use metrics (natural vegetation, agriculture, aquaculture and urban settlement) were obtained from the total area (km^2) of each estuary: 588.86 km^2 for the Paraíba do Norte estuary and 238.76 km^2 for the Mamanguape estuary. For each land use metric, they were calculated as percentages of the total area, using the raster function of ArcMap through the geographic information system (ESRI, 2013).

According to the pressure index (PI), the estuaries differed in relation to anthropogenic pressures, with the Paraíba do Norte estuary classified as a medium impact estuary and the Mamanguape estuary classified as a very low impact estuary (Table 1). The Paraíba do Norte estuary experiences several anthropic pressures, mainly in relation to habitat loss, a high percentage of land use for agriculture and sewage pollution. The Mamanguape estuary showed little pressure associated with habitat loss and land use for agriculture.

2.3. Sampling

The estuaries were divided into three estuarine reaches according to the salinity gradient: upper (0.5–22), middle (24–33), and lower (>35) reaches (Fig. 1). Four sites were sampled in each reach of the estuary with three replicates per month at each site in daylight under high tide conditions (2 estuaries × 3 reaches × 4 sites × 3 replicates × 12 months = 864 samples).

Samplings were conducted between April 2018 and March 2019. Ichthyoplankton samples were collected using a conical-cylindrical plankton net (total length 1.50 m, 60 cm mouth opening and a mesh net size of 200 μm). A mechanical flow meter (General Oceanic) attached to the centre of the net was used to determine the volume of filtered water. This value was used to calculate the larval density (number $\times 100\ m^{-3}$) (Lima et al., 2015). At each sampling station, subsurface horizontal plankton hauls were performed during the day in the middle of the main channel at spring high tides. All hauls were standardized in a 5 min time, with a boat speed of 1.5 knots, to avoid individual escape as much as possible. All samples of plankton were stored and immediately preserved in 4 % formaldehyde/seawater (Barletta et al., 2003).

Table 1
Pressure descriptors used to calculate the human pressure index (PI) for the Paraíba do Norte and Mamanguape estuaries, Brazilian semi-arid region. The PI ranged from 0 (no change), 1 (very low), 2 (low), 3 (medium), 4 (high) and 5 (very high). Arcgis 10.0, MapBiomas (www.mapbiomas.org) GE: Google Earth, ANA: Agência Nacional das Águas (www.ana.gov.br), SUDEMA: Superintendência de Administração do Meio Ambiente (www.sudema.pb.gov.br), EJ: expert opinion, IBGE: Instituto Brasileiro de Geografia e Estatística (www.ibge.gov.br).

Indicators	Data source	Paraíba do Norte	Mamanguape
Habitat loss	MapBiomas; ArcGis; GE	3	3
Dredging	SUDEMA	4	0
% Agriculture area	ArcGis; GE	4	4
% Urban settlement	ArcGis; GE	3	1
Presence of marinas	ArcGis; GE	1	0
Presence of ports	ArcGis; GE	2	0
Aquaculture	ArcGis; GE	1	1
Fishing activities	EJ	1	1
Tourism and recreation	SUDEMA	4	2
Sewages	ANA, EJ	4	1
Population density	IBGE	4	2
Pressure index		**3**	**1**

2.4. Environmental parameters

The environmental parameters were selected based on their relevance to larval fish assemblages following the model proposed by Camara et al. (2019): local and landscape variables. Following each haul, local variables were measured and included salinity, water temperature (°C), transparency (cm), chlorophyll-*a* (μg/l), nitrate (mg/l), nitrite (mg/l), ammonia (mg/l), orthophosphate (mg/l) and total phosphorus (mg/l). Environmental parameters were measured in situ: temperature (through a mercury thermometer), salinity (using a refractometer), and water transparency (using a Secchi disk). Concentrations of nutrients were measured and determined in the laboratory, as described in Strickland and Parsons (1972). Primary production was also estimated by analysing chlorophyll-*a* content in the water following the methodology proposed by Wetzel and Likens (1990).

We used the parameters of total phosphorus and chlorophyll-*a* to calculate the trophic status index (TSI) of each estuary, following the standards established by Carlson (1977), modified by Toledo et al. (1983), which updated the original formula for tropical environments. The trophic status index categorization according to the TSI follows the following scale: (i) $TSI < 47$: ultraoligotrophic; (ii) $47 < TSI \leq 52$: oligotrophic; (iii) $52 < TSI \leq 59$: mesotrophic; (iv) $59 < TSI \leq 63$: eutrophic.

To characterize the landscape variables of the estuaries we measured canal width (m) and site distance from the estuary mouth (km) using Google Earth Pro software. Depth (cm) was measured using an Echotest depth sounder, and precipitation (mm) and wind intensity (km/h) data were compiled from the National Institute of Meteorology (INMET website 2019: www.inmet.gov.br).

2.5. Data analysis

2.5.1. Environmental variables and structure of larval assemblages

A permutational multivariate analysis of variance (PERMANOVA) (with 9999 permutations) was used to examine the spatial and temporal variations in the environmental parameters and larval assemblages (density, species richness and diversity). PERMANOVA was applied to three factors: estuary (two fixed levels: Paraíba and Mamanguape), reach (three fixed levels: upper, middle, and lower) and season (two fixed levels: rainy and dry) (Anderson et al., 2008). A univariate permutational analysis of variance (PERMANOVA) was used to investigate significant differences among the estuarine reaches and seasons, and a posteriori pairwise comparisons were performed to determine significant differences among factors. All univariate tests were based on Euclidean distance matrices (Anderson et al., 2008).

Logarithmic transformations Log (x + 1) were performed on the environmental variables, while the landscape variables were normalized (Su et al., 2011). Prior to analysis, the predictor variables were checked for multicollinearity (draftsman plot and Spearman correlation matrix), and redundant variables (Pearson's $r > 0.7$) were removed to maximize the parsimony of the models (Clarke and Gorley, 2006). The full set of 12 environmental variables was tested for collinearity, and five redundant variables were omitted (temperature, nitrate, orthophosphate rainfall and intensity of wind). In addition, for the larval fish assemblage data, richness was quantified as the number of species, and diversity was calculated through the Shannon-Wiener index (H′) (Shannon and Weaver, 1963).

2.5.2. Ordinary kriging interpolation

To verify the horizontal distribution of environmental variables along the estuaries, an ordinary kriging interpolation was performed using ArcGIS software in ArcMap. We use a theoretical spherical model. The reference values for analyses were based on Ordinance No. 357/05 in the National Water Quality Management Strategy - Australian and New Zealand Guideline for fresh and Marine Water Quality (2000). The ammonia, nitrite and phosphorus concentrations were classified into 3 classes according to Brazilian legislation (CONAMA No. 357/05). The first class represents water bodies in the best state for conservation, class two represents the range where some recreational and commercial activities are suitable, and class 3 represents lower-quality waters that can be used for landscaping. Chlorophyll-*a* concentrations, on the other hand, were based on the recommendations made for tropical Australian and New Zealand estuaries, where two classes were established, the reference class with chlorophyll-*a* values lower than 2 μg/l and the second class with values higher than 2 μg/l.

2.5.3. Correlation of environmental variables in larval assemblages

For the multivariate analysis, the larval fish assemblage densities were transformed by square root, and the results were used to generate a Bray-Curtis similarity matrix. To identify correlations between the environmental gradients and the variations in the fish data, a distance-based linear model (DistLM) was used (Legendre and Anderson, 1999; McArdle and Anderson, 2001). To choose the final model, the "best" selection procedure used the Akaike information criteria (AIC) to identify the most parsimonious explanatory models. A distance-based redundancy analysis (dbRDA) was performed (McArdle and Anderson, 2001) to visualize the relative contributions of each of the predictor variables to the larval fish assemblage structure. The Pearson correlation was used for the selection of the most correlated variables ($r > 0.2$) from the axes of the dbRDA. All analyses were performed using the statistical package PRIMER v6 + PERMANOVA (Clarke and Gorley, 2006; Anderson et al., 2008).

Generalized mixed linear models (GLMMs) were used to investigate the effects of local variables on the density, species richness and diversity of larval assemblages in estuaries. The estuarine reaches and seasons were included as random factors to control for possible effects of spatial dependence and temporal variability on residual variance, respectively (Gelman and Hill, 2007; Bolker et al., 2009). Thus, the effects of sampling units were removed from the species-environment relationships, and the samples were considered independent. All models, density, species richness, and diversity values were included as Poisson variables, since the Poisson distribution is appropriate for count data, with a log link function that makes the expected response linear and the variance expected homogeneous (Gelman and Hill, 2007; Bolker et al., 2009).

At each structural level, GLMMs with all possible combinations of predictor variables (salinity, transparency, ammonia, nitrite, total phosphorus, chlorophyll-*a* and trophic status index) were performed. All models, including null models with no fixed effects, included the estuarine reaches and seasons as random effects. The pseudo-R^2 for the GLMMs was calculated to express the variance explained by the fixed effects (marginal R^2 - marg. R^2) and the whole model, including fixed and random effects (conditional R^2 - cond. R^2) (Nakagawa and Schielzeth, 2013; Johnson, 2014). Model selection based on information theory was applied to compare fitted models and identify the best supported models (Burnham and Anderson, 2002). The candidate models with negligible pseudo-R^2 values were not excluded because model selection was focused on evaluating the performance of each model compared to the dataset included in the full model.

Model selection was based on Akaike's corrected information criterion (AICc), which corrects for bias resulting from small sample sizes (Burnham and Anderson, 2002). The best models were those with the lowest AICc, that is, with less loss of information and a simpler structure (Burnham and Anderson, 2002). The models were classified according to the AICc weight (wi), which represents the probability that the model is the best among the set of candidate models, that is, the relative likelihood of the model (Wagenmakers and Farrell, 2004). The ΔAICc is the difference between the smallest AICc and the AICc of the model and represents the probability that the model minimizes the loss of information (Burnham and Anderson, 2002; Wagenmakers and Farrell, 2004). All models with $\Delta AICc < 2$ were considered to have substantial support for interpretation (Burnham and Anderson, 2002). Additionally, 85 % confidence intervals were also calculated because model selection using the AICc supports additional variables over a null model at this level (Arnold, 2010). Therefore, a parameter was considered informative if the 85 % confidence interval did not overlap with 0. Finally, the relative importance of the variable (RVI) for the parameter estimates in the model was also calculated by summing the wi of the selected models (that is, recalculated without the other candidate models), including the predictor variable (Burnham and Anderson, 2002).

The predictor variables were centered and standardized for all models (Legendre and Legendre, 2012). All analyses were performed in the R environment (version 3.5.2; R Core Team, 2018), with the packages vegan (version 2.5–3; Oksanen et al., 2012), car (version 3.0–2; Fox and Weisberg, 2011), lme4 (version 1.1–19; Bates et al., 2015), AICcmodavg (version 2.1–1; Mazerolle, 2017), and MuMIn (version 1.42.1, Barton, 2018).

3. Results

3.1. Environmental heterogeneity

PERMANOVA revealed that there were significant differences in environmental parameters between the estuaries (Pseudo-F = 32.152; *P* = 0.0001). In the Paraíba do Norte estuary significant differences were observed between estuarine reaches and seasons (PERMANOVA: Reaches: Pseudo-F = 33.279; P = 0.0001; Seasons: Pseudo-F = 40,393; P = 0.0001). Nitrite, ammonia, and total phosphorus showed the highest values in the middle reach during the dry season, while transparency showed high values in the lower reach in the rainy season (Fig. 2; Table S1 in the Supplementary Material). Chlorophyll-*a* and salinity showed high values in upper and lower estuaries, respectively, in both seasons. The TSI showed high values in the upper and middle reaches during the dry season and was classified as eutrophic (Fig. 2).

In the Mamanguape estuary, there were also significant differences between estuarine reaches and seasons (PERMANOVA: Reaches: Pseudo-F = 27.843; *P* = 0.0001; Seasons: Pseudo-F = 25.462; P = 0.0001). Ammonia and total phosphorus showed the highest values in the upper reach, while chlorophyll-*a* showed the highest values in the middle reach in both seasons (Fig. 3; Table S1 in the Supplementary Material). Transparency and salinity showed high values in the lower estuary during the rainy and dry season, respectively (Fig. 3; Table S1 in the Supplementary Material).

The depth was higher in the middle reach in the Mamanguape estuary (6.53 ± 0.27 m), whereas the canal width was higher in the Paraiba do Norte estuary (33.23 ± 1.381,73 m) (Fig. S1 in the Supplementary Material). Landscape variables revealed that there were significant differences between estuaries (Pseudo-F = 70.68; *P* = 0.0001) and estuarine reaches (Pseudo-F = 70.68; P = 0.0001).

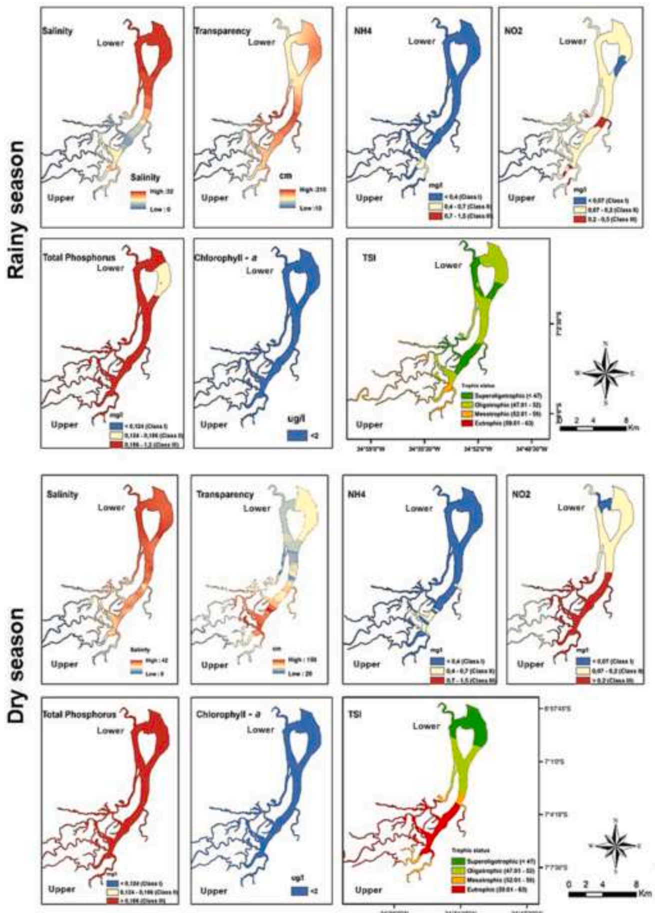

Fig. 2. Horizontal distribution of environmental variables along the Paraíba do Norte river estuary during the rainy and dry seasons. Class I: great state of conservation; class II: medium state of conservation and class III: low state of conservation. TSI < 47: Superligotrophic; (ii) 47 < TSI ≤ 52: Oligotrophic; (iii) 52 < TSI ≤ 59: Mesotrophic; (iv) 59 < TSI ≤ 63: Eutrophic; (v) 63 < TSI ≤ 67: Supereutrophic; (vi) TSI > 67: Hypereutrophic.

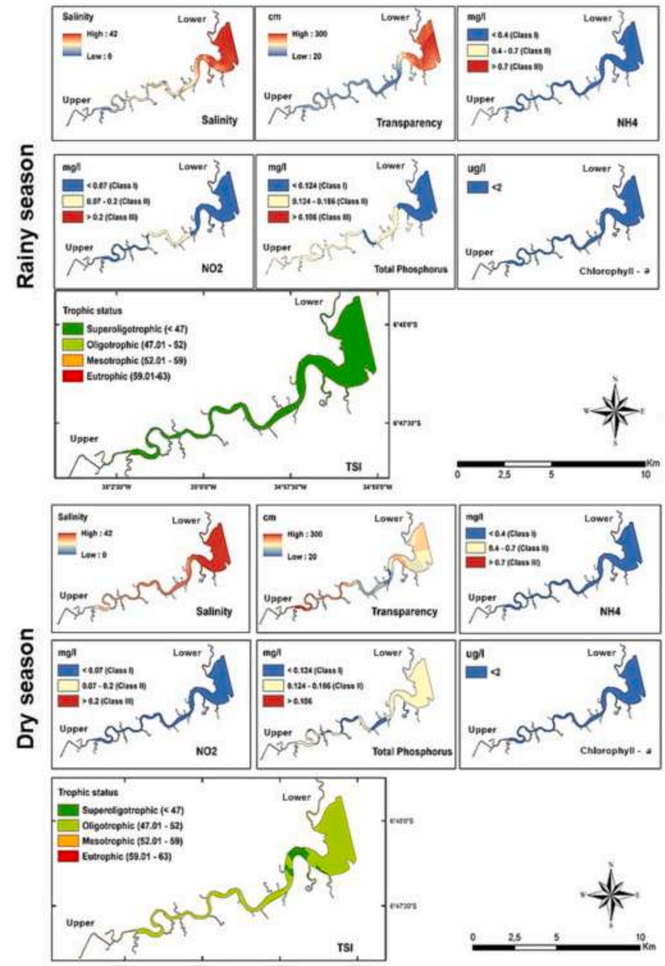

Fig. 3. Horizontal distribution of environmental variables along the Mamanguape river estuary during the rainy and dry seasons. Class I: great state of conservation; class II: medium state of conservation and class III: low state of conservation. TSI < 47: Superligotrophic; (ii) 47 < TSI ≤ 52: Oligotrophic; (iii) 52 < TSI ≤ 59: Mesotrophic; (iv) 59 < TSI ≤ 63: Eutrophic; (v) 63 < TSI ≤ 67: Supereutrophic; (vi) TSI > 67: Hypereutrophic.

3.2. Structure and composition of ichthyoplankton assemblages

PERMANOVA showed that there was a significant difference in larval fish assemblages among estuaries and indicated variability in density (Pseudo-F = 5.5768; $P = 0.0033$), species richness (Pseudo-F = 9.4364; $P = 0.0002$) and diversity (Pseudo-F = 13.484; $P = 0.0004$) (Fig. 4). The Paraíba do Norte estuary recorded a density of 0.24 individuals per 100 cubic metre, represented by 14 families distributed in 32 species. Among these, Engraulidae exhibited the highest number of species ($n = 7$), followed by Clupeidae (3) and Gerreidae (3). The sole *Trinectes paulistanus* was the most abundant species and accounted for 19.65 % of the total larval fish catch, followed by *Bathygobius soporator* (18.73 %), and *Rhinosardinia bahiensis* (12.46 %). In the lower reach, the highest densities of *Anchoa hepsetus*, *R. bahiensis* and *Bathygobius soporator* were recorded during dry season (see Table S3 in the Supplementary Material).

At the Mamanguape estuary, a distinct pattern of larval fish structure was observed, characterized by a high density (1.71 ind.100m^3), comprising 18 families and 49 species. Engraulidae had the highest number of species ($n = 9$), followed by Clupeidae (5), Gerreidae (5), Carangidae (4) and Sciaenidae (4). *Rhinosardinia bahiensis* was the most abundant species and accounted for 32.41 % of the total larval fish catch, followed by *Anchoa hepsetus* (22.01 %), *Anchoa januaria* (16.40 %) e *Bathygobius soporator* (12.21 %). In the middle reach the highest density was recorded for *Lycengraulis grossidens*, *Anchoviella lepidentostole* and *Anchoa januaria* during the rainy season, and the highest density was recorded for *R. bahiensis*, *A. hepseutus*, *Hyporhamphus unifasciatus* and *Anchovia clupeoides* during the dry season (see Table S3 in the Supplementary Material).

Regarding the Shannon diversity index, the lowest values were observed in the Paraíba do Norte estuary ($H' = 0.44 \pm 0.49$), while the highest average values were recorded in the Mamanguape estuary ($H' = 0.71 \pm 0.49$) (Fig. 4).

3.3. Environmental heterogeneity influences on larval fish assemblages

The linear model based on distance (DistLM) revealed a significant relationship between fish larvae in the Paraíba do Norte estuary and several environmental variables, including salinity, chlorophyll-*a*, ammonia, transparency, and total phosphorous (Table 2). The model with the best fit provided an explanation for 80.7 % of the variation observed in larval fish density ($R^2 = 0.807$). Ammonia showed the highest correlation and served as the best predictor for the upper reach, while total phosphorous emerged as the best predictor for the middle reach. On the other hand, salinity was the most influential factor in the lower reach. All these variables showed high values during the dry season (Fig. 5 a and b). In the case of the Mamanguape estuary, salinity, transparency, and nitrite emerged as the strongest predictors,

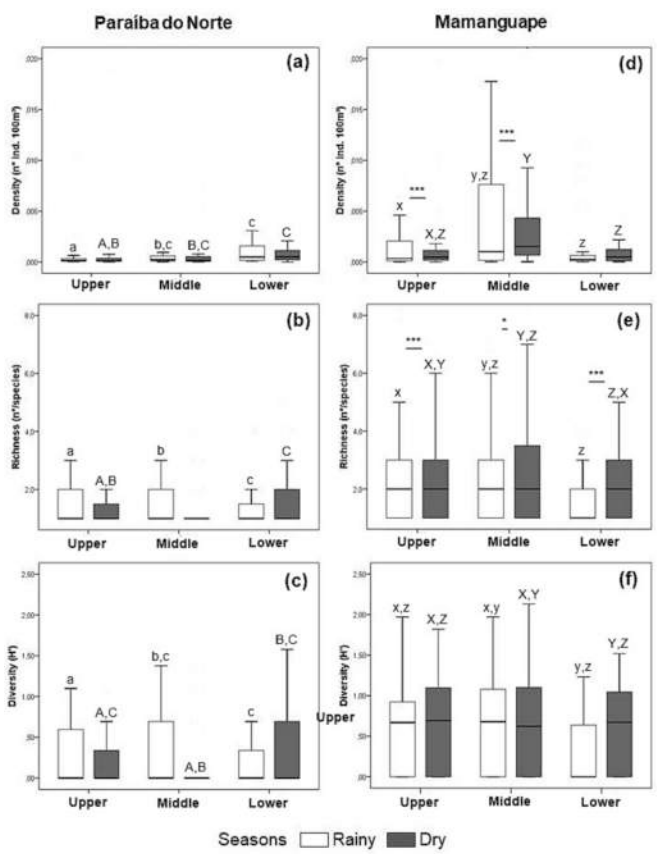

Fig. 4. Box-plots with median for variations in larvae density, number of species (richness), and Shannon diversity in different reaches of the Paraíba do Norte and Mamanguape estuaries, during rainy season (blue column) and dry season (purple column). $*p < 0.05$; $**<0.001$; $***<0.0001$. Graphs with the same lowercase letters and cases are not significantly different between estuarine reaches by the pair-wise test. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

collectively accounting for a large amount of the observed variation ($R^2 = 0.799$) (Table 2). Nitrite was the primary environmental variable that explained the variability in the middle reach during the rainy season, whereas transparency and salinity were the best predictors in the upper and lower reaches during the dry season, respectively (Fig. 5 c and d).

3.4. Structural levels of larval assemblages and variation in environmental effects

According to the model selections, it was found that multiple models were plausible for the structural levels of larval assemblages in both estuaries ($\Delta AICc < 2$). In the Paraíba do Norte estuary, multiple models were selected for the species richness density parameters, with the random effects being more expressive in the residual variation. The density of larval assemblages was found to be negatively influenced by chlorophyll-*a* and total phosphorus, while it exhibited positive effects from the TSI. Species richness, on the other hand, was positively influenced by nitrite and ammonia, but negatively affected by total phosphorus (see Table S5 in the Supplementary Material). For diversity, only one model was selected, indicating a negative effect of the TSI (see Tables S4 and S5 in the Supplementary Material).

4. Discussion

The results of the present study demonstrate significant differences in the structure of larval fish assemblages between the two estuaries. Our findings indicate that estuarine eutrophication, characterized by high concentrations of nutrients such as ammonia, nitrite and total phosphorous, had adverse effects on the abundance and richness of larval fish assemblages. In this regard, the community descriptors in the upper and middle reaches of Paraiba do North estuary were significantly impacted by deteriorating water quality, leading to alterations in the composition and distribution of larval assemblages.

Santos et al. (2017a, 2017b) conducted a study investigating the ecological status of four Brazilian estuaries, revealing that larval fish assemblages were adversely impacted by the compromised health of the ecosystems. Several studies have provided evidence for the direct negative effects that impact larval fish assemblages (Courrat et al., 2009; Borja et al., 2010; Ramos et al., 2012; Lima et al., 2015). These studies suggest that nutrient concentrations are among the primary environmental stressors in estuarine systems, and the abundance patterns of larval fish exhibit an inverse relationship with increasing eutrophication levels in estuaries. Additionally, pollutants may potentially damage the survival and distribution of larvae, as they are extremely sensitive to environmental changes (Andersen et al., 2020).

The spatial organization of the larval fish assemblages was notably influenced by different predictive factors in each estuary. Our DistLM analysis successfully discerned the correlations of each factor with species abundance, contributing to the establishment of distinct larval assemblage patterns in the estuarine reaches. For instance, in the Paraiba do Norte estuary, the upper and middle reaches exhibited higher

Table 2
DistLM marginal test showing the influence of environmental local variables on density of ichthyoplankton in the three reaches (upper, middle and lower) of the Paraíba do Norte and Mamanguape estuaries. Prop = Proportion (%).

DistLM marginal test									
Variables		Paraíba do Norte				Mamanguape			
		SS trace	Pseudo-F	P	Prop	SS trace	Pseudo-F	P	Prop
1	Salinity	17,363	5.6896	0.001	3.4439	9053.9	2.6585	0.011	1.0778
2	Transparency	10,882	3.5191	0.025	1.1521	9935.3	2.9204	0.008	1.1827
3	Chlorophyll-*a*	6525.5	2.0919	0.055	1.2906	2368.2	2.1061	0.03	8.5578
4	Nitrite	6140.2	1.9669	0.056	2.2144	14,963	4.4252	0.001	1.7813
5	Ammonia	11,178	3.6171	0.002	2.2107	7188.7	0.6898	0.739	2.8193
6	Total phosphorus	17,930	5.8824	0.001	3.5461	7051	2.0655	0.038	8.3938
7	Depth	1477.9	1.3319	0.148	1.2123	41,678	1.1279	0.209	1.8486
8	Channel width	19,411	0.9632	0.404	1.1137	84,086	2.5132	0.080	1.0755

Overall best resolution							
AIC	R^2	RSS	Selections	AIC	R^2	RSS	Selections
62.82	0.807	677.3	1,3,4,5,6	34.26	0.799	805.8	1,2,3,5

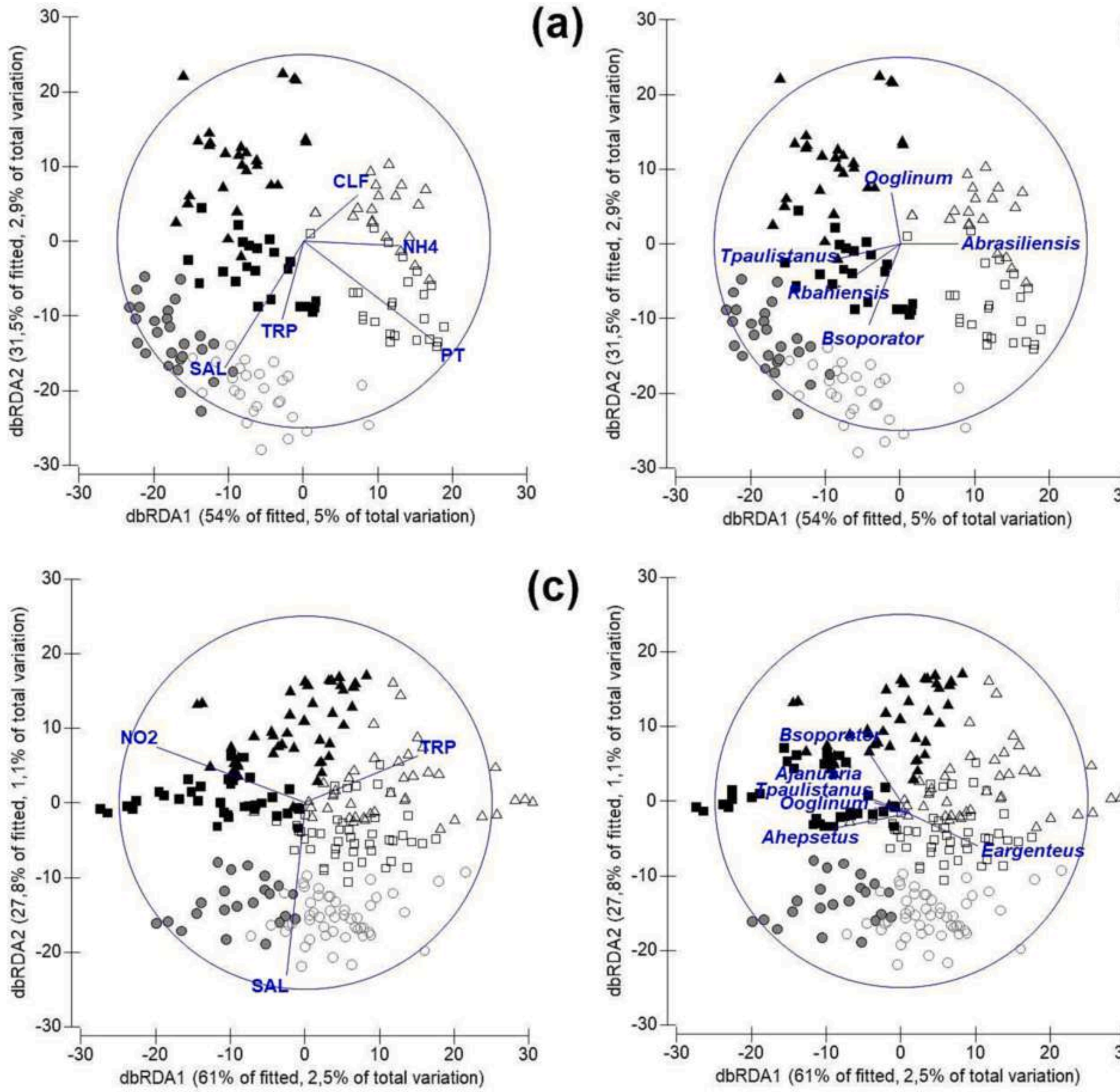

Fig. 5. Distance-based redundancy analysis (dbRDA) to larvae density in different estuarine recahes of the Paraíba do Norte and Mamanguape estuaries. Rainy season: upper (▲), middle (■) and lower (●). Dry season: upper (△), middle (□) and lower (◯). Environmental variables: salinity (SAL), transparency (TRP), chlorophyll-*a* (CLF), nitrite (NO_2), total phosphorus (PT). The most correlated fish species also indicated: Ahepsetus, *Anchoa hepsetus*; Ajanuaria, *Anchoa januaria*; Ooglinum, *Ophistonema oglinum*; Rbahiensis, *Rhinosardinia bahiensis*; Abrasiliensis, *Atherinella brasiliensis*; Eargenteus, *Eucinostomus argenteus*; Bsoporator, *Bathigoius soparator*; Tpaulistanus, *Trinectes paulistanus*.

levels of inorganic nutrients (ammonia and total phosphorous), which coincided with a significant decrease in the abundance of *R. bahiensis* and *O. oglinum*. Conversely, these two species showed peak abundance in the lower reach, contributing 53 % and 65 % of the numerical abundance, respectively. However, their contributions were considerably lower in the upper estuary, accounting for <15 % numerically. The mojarra *E. melanopterus* exhibited a similar abundance pattern to these species. Considering the substantial abundance of these species in lower reach, we hypothesize that the high nutrient concentrations may have adversely affected larval survival and growth and that they moved towards upstream reaches. Brownell (1980) noted that the mortality rate of marine fish larvae increases when exposed to high levels of ammonia, nitrite and nitrate.

The physiological disturbances in fish in eutrophic estuaries are attributed to their active absorption of inorganic nutrients across their gills, which can impact survival and recruitment during the early life stages of marine fishes (Kroupova et al., 2005; Overstreet and Hawkins, 2017). A study carried out by Dolbeth et al. (2016), about functional diversity of the juvenile fish community in the Paraíba do Norte estuary, found that high concentrations of nutrients caused negative effects on fish diversity. Furthermore, Macedo et al. (2019) recorded high activity of multixenobiotic biomarkers in different species, mainly in the resident *A. brasiliensis*, being an indication of contamination in the environment by xenobiotics, which cause several harmful effects to organisms, contributing to the reduction of the diversity and abundance of species of fish, especially those in the larval stage due to their greater sensitivity to environmental changes.

A consistent inverse trend in community descriptors was observed in the Mamanguape estuary, with lower density and richness observed towards the downstream estuary. However, the results were primarily influenced by salinity rather than inorganic nutrient concentrations. In the upper reach, the abundance of herring and anchovy species could be attributed to lower salinity conditions, which appear to be attractive and favourable to larval concentration, providing a growth advantage to the larvae (Arevalo et al., 2023). This trend has been described in several studies focusing on herrings and anchovies (Joyeux et al., 2004; Pichler et al., 2015; Corrêa and Vianna, 2015; Sloterdijk et al., 2017). Other explanations for the utilization of the upstream estuaries include low predation pressure (Whitfield, 2020), increased food availability and shallow waters (Whitfield, 2021). On the other hand, the lower reach showed a high contribution of marine species to larval catches, which can be attributed to the strong oceanic influence at the mouth of the estuary. Species in the families Sciaenidae (*Bairdiella ronchus*, *Stellifer rastrifer*, *S. stellifer*, *Menticirrhus americanus*), Lutjanidae (*Lutjanus analis*, *Lutjanus cyanopterus*), Blenniidae (*Scartella cristata*, *Lupinoblennius paivai*), Carangidae (*Caranx latus*, *Oligoplites saurus*), and Belonidae (*Strongylura timucu*) represented the majority of marine species in this reach.

In general, larval fish assemblages exhibited variations in abundance and richness according to seasonal seasons. Our findings provide support for the notion that rainfall plays a significant role as an environmental driver affecting the abundance and survival of fish larvae. For instance, the highest density of *A. lepidentostole*, *A. hepsetus*, *A. januaria* and *L. grossidens* was observed during the rainy season in both estuaries. Santos et al. (2017a, 2017b) suggested that the abundance of Engraulidae larvae could be attributed to their spawning period in the coastal zone, with passive horizontal transport of eggs and larvae towards the estuaries, thereby enhancing larval distribution during this season. Similar patterns were also identified by Lima et al. (2020) in the Mamanguape estuary. Therefore, in tropical estuaries, it can be considered that the recruitment of ichthyoplankton is primarily influenced by rainfall (Barletta-Bergan et al., 2002; Lima et al., 2016).

We also suspect that a decrease in rainfall may be associated with the observed pattern in larval fish. During the dry season, both estuaries experienced reduced river flow input due to intermittent upstream rivers. This leads to changes in salinity and species composition within the estuaries. Pasquaud et al. (2012) describe how the "marinization" hypothesis, which relates to increasing salinity during drought periods, can alter the fish communities in estuaries. This hypothesis may also be applicable to the studied tropical estuaries. Our data suggest that stenohaline species such as *R. bahiensis*, *L. piquitinga*, *H. unifasciatus*, *C. latus*, *Sardinella brasiliensis*, and *Stellifer rastrifer* become more abundant during the dry season in both estuaries. This may be due to the associated saline intrusion, primarily influenced by tides, which could promote the transport of larvae from seawater into the estuary (Lima et al., 2022). Lima et al. (2020) observed a similar pattern during an atypical climatic event in 2015 in the Mamanguape estuary. Similar results have also been reported, showing an increase in the diversity of marine fish species due to saline intrusion in temperate estuaries (Garcia et al., 2001; Martinho et al., 2007; Plavan et al., 2010).

In conclusion, this study revealed significant differences in larval fish assemblages between different estuaries primarily due to variations in habitat quality. It was evident that eutrophication conditions have a noticeable impact on larval fish assemblages, particularly in the Paraíba do Norte estuary. Specifically, we have demonstrated that nutrient pollution can have an inverse effect on the richness and abundance of species, significantly influencing the success of fish recruitment. Recent studies have highlighted that anthropogenic impacts on estuaries compromise their connectivity with adjacent coastal areas, thus impacting their function as nurseries for the early life stages of fishes (Rodrigues et al., 2022; Arevalo et al., 2023). Furthermore, we observed a pronounced increase in salinity during the dry season, influenced by reduced freshwater supply from rivers characterized by intermittent flow regimes, and increased evaporation. This explains the simultaneous increase in the number and density of marine species. Consequently, monitoring community descriptors can serve as an effective approach to understanding the vulnerability of larval assemblages to climate change, particularly regarding hypersalinity and the effects of marinization in shallow tropical estuaries in this region.

CRediT authorship contribution statement

Lidiane Gomes de Lima: Conceptualization, Methodology, Writing – original draft. **Francisco Gerson Araújo:** Formal analysis, Supervision, Writing – review & editing. **Breno Silva Macário:** Investigation, Methodology, Writing – original draft. **André Luiz Machado Pessanha:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that there is no conflict of research interests in this work.

Data availability

Tradução de textos Detectar idioma Inglês Português Espanhol Português Inglês Espanhol Texto de origem O amterial suplementar ja tem dados sobre o trabalho.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2023.115858.

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