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Relationship of Population Attributes of a Dominant Macrofaunal Species with Environmental Conditions in a Eutrophic Estuary (Guanabara Bay, Brazil)

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Abstract: Hydrobioid gastropods are abundant in coastal systems and ecologically important for ecosystem functioning. We aimed to unravel the relationship between usual and stress-related population attributes of the dominant macrofaunal species *Heleobia australis* with indicators of environmental quality and coastal pollution. Using Guanabara Bay (GB, Brazil) as a model of a multi-impacted coastal system, our hypothesis is that increased amounts of rainfall during the warm season reduce the bay's environmental quality and induce shifts in snail population attributes. A suite of environmental variables, population attributes, and sediment quality descriptors was assessed by combining field and laboratory evaluations with literature compilation. Results indicate high organic pollution levels with environmental degradation and reinforce GB status as a severely contaminated system. Some environmental conditions can be applied as seasonal predictors of changes in warm-rainy (rainfall and salinity), intermediate (silicate), and cold-dry seasons (nitrite and nitrate). Three selected usual population attributes (snail density, fecundity, and recruitment) were not affected by changes in environmental conditions, but significant effects were detected on two stress-related attributes (relative penis length index and shell deformity). For the first time, shell deformity was recorded in *H. australis* snails. Low variation in usual population attributes highlight the high tolerance of *H. australis* to shifts in environmental conditions.

Keywords: bioindicators; pollution; eutrophication; Guanabara Bay; macroinvertebrate; stress-related effects

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1. Introduction

Environmental changes (e.g., deforestation and habitat modifications) and wastewater released by human activities have historically impacted coastal ecosystems worldwide. Various anthropogenic pressures, including dense human populations and inputs of large amounts of residues from domestic, industrial, agricultural and aquaculture sources have been detected in coastal areas for a long time [1–3]. The impacts of anthropogenic activities on the biological components and environmental health depend on their intensity and frequency (i.e., spatial and temporal) and the interactions among distinct stressors [4]. An emergent concern among researchers consists in the assessment of environmental quality at coastal areas subjected to multiple anthropogenic impacts.

The application of macroinvertebrates as bioindicators might be based on community predictors (e.g., richness and diversity), relative species contribution, comparison of assemblage structure and composition (e.g., indices of species similarity or dissimilarity), and biotic indices based on sensitivity levels of a particular taxon to specific stressors [5–10]. Benthic macroinfaunal organisms are sensitive to changes in the upper sediment surface layer induced by stressors (e.g., sewage pollution, and metal contamination) [7]. For example, exposure to environmental contaminants can induce changes in shell morphology, structure and composition of molluscan assemblages in marine and estuarine systems [11–14]. Among biomarkers, the evaluation of imposex levels (i.e., superimposition of male sexual characters onto female gastropods) has been recognized as a tool for monitoring organotin compounds, mainly tributyltin—TBT [15–18].

Guanabara Bay is one of the most polluted aquatic systems along the Brazilian coastline [19], consisting of a model of a coastal ecosystem chronically impacted by multiple anthropogenic activities in South America [20]. The major sources of the bay's impacts include inputs of municipal wastewater, deforestation, changes in land use for agricultural and/or industrial purposes, urban runoff, and industrial effluents [21]. A severe eutrophication process was evidenced as a result of a historical release of domestic residues into the bay [22], with an input of approximately 18 m^3s^{-1} of untreated sewage produced by inhabitants around the bay's hydrographic region [23]. The mud snail *Heleobia australis* (Caenogastropoda: Cochliopidae) comprises small estuarine animals that dominate the benthic macroinfauna in the estuarine region of Guanabara Bay, accounting for 77% of total molluscan abundance in this ecosystem [24]. Deposit-feeder snails are often the main group of benthic macroinvertebrates in aquatic ecosystems [25–28], where they have an important role in aquatic food webs and in nutrient cycling [29–31]. Furthermore, an effect-response of sediment contamination by organotin compounds was evidenced by imposex development in *H. australis* snails at Guanabara Bay [18].

Environmental conditions are known to affect the community (e.g., diversity, composition) and population (e.g., abundance, structure, growth, and recruitment) attributes of molluscs [32–35]. Despite a few studies that have registered punctual impacts of anthropogenic disturbances on populations of the mud snail *H. australis* [18,36], the relationship between its population and the environmental quality of coastal systems is barely known. Thus, the present study aimed to unravel the relationship of usual and stress-related population attributes of the benthic macroinvertebrate *H. australis* with indicators of degraded environmental quality and coastal pollution using Guanabara Bay as a multi-impacted model system. This bay is influenced by the intrusion of the South Atlantic Central Water (SACW), a cold and nutrient-rich water body that is more intense in the southern summer, which often establishes a seasonal thermocline in response to the inflow of this oceanic water mass into the bay [37,38]. However, the water conditions of Guanabara Bay are also determined by tidal currents, precipitation regimes, and freshwater discharges [39]. Our main hypothesis is that increased amounts of rainfall during the warm season reduce the environmental quality in Guanabara Bay, leading to shifts in population attributes of the mud snail *H. australis*. Moreover, the estuarine region of the bay is within one of the most degraded and polluted areas by organic and chemical compounds [23]. Therefore, we have performed a compilation of environmental pollutants (e.g., metals, aliphatic and organotin compounds) detected in the sediment of Guanabara Bay estuarine region to assess the pollution levels in the snail sampling area. This study is the first attempt to understand how environmental quality and conditions at a multi-impacted estuarine system can affect the population attributes of a mud snail species. This study is within the context of the Sustainable Development Goal 14 (i.e., Life Below Water) of the United Nations, by increasing the research efforts in marine sciences.

2. Materials and Methods

2.1. Study Area

Guanabara Bay (Southeastern Brazil) is a 384 km^2 semi-enclosed estuarine system, surrounded by 91 rivers and channels and large urban zones corresponding to Rio de Janeiro metropolitan area [40] (Figure 1). The sampling location was carefully chosen for performing a species-driven sampling considering previous data on *H. australis* distribution at Guanabara Bay. Sampling was conducted in the intermediate sector of the bay, between Governador and Fundão Islands (22°50′8.6″ S; 43°2′7.3″ W) (Figure 1), in a shallow area (4–6 m) with low water transparency (0.3–1.5 m) [41]. This region has a tapering shape combined with a suffocated geometry that hinders water circulation and based on the stratification-circulation diagram bay's region it is characterized as Type 2b–a partially mixed system (i.e., turbulent tidal diffusion is the main process responsible for salt transport) with weak vertical stratification [42]. Snails of different size classes occur at high densities and all year round in this region [18], which is considered a "source area" of snails for other areas of the bay [43].

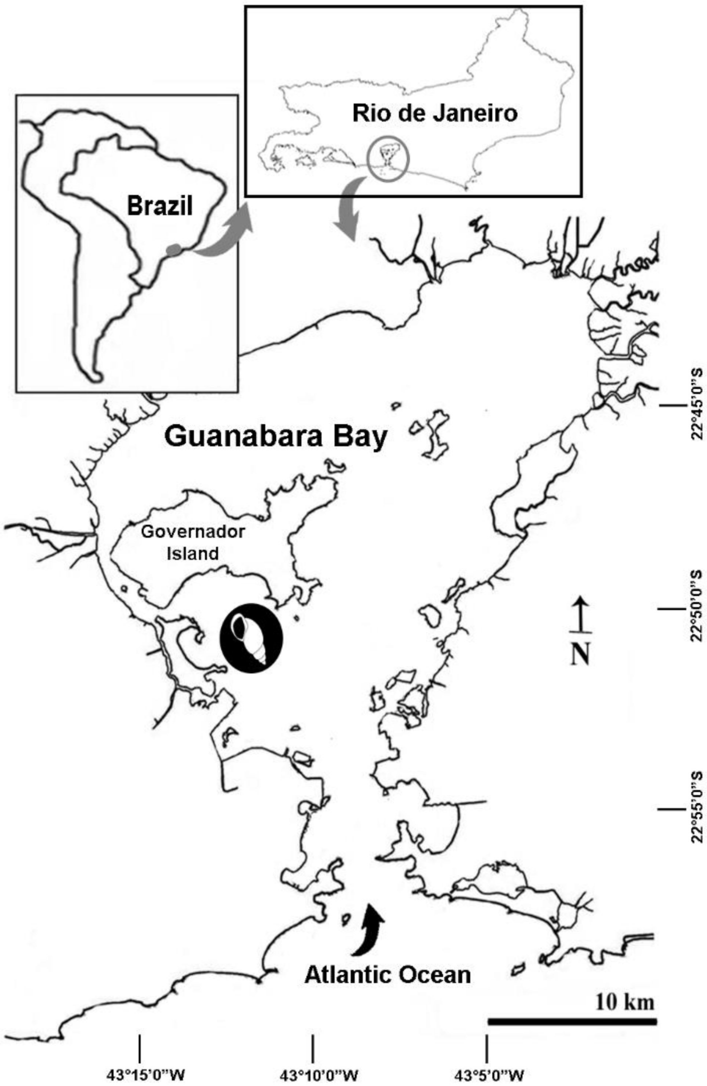

Figure 1. Geographic location of Guanabara Bay (Rio de Janeiro, Brazil), showing the sampling region (i.e., black ellipse with a snail inside).

In order to characterize the sediment contamination by chemical pollutants, the concentrations of pollutants detected in Guanabara Bays sediments were compiled from available literature for the same sampling area and presented in Table 1. Moreover, pollutant concentrations were compared to available sediment quality guidelines to indicate environmental concentrations above threshold effects level (TEL), probable effects level (PEL), and apparent effects threshold (AET) [44].

Table 1. The concentration of metals, hydrocarbons, and organotin compounds detected in the surface sediments of the estuarine region of Guanabara Bay, where snails were collected. Data compiled from the same study are presented as mean (±standard deviation) of replicates of sediment collected at the estuarine region of Guanabara Bay in the same period (dry or rainy season).

Environmental Pollutant ($mg\ kg^{-1}$ Sediment)		Dry Season	Rainy Season	References
Metal	Cr	158.3 (±100.36) [1,2]	227.5 (±76.43) [1,2,3]	[40,45]
	Cu	77.7 (±15.63)[1]	65.5 (±14.53) [1]	
	Ni	54.3 (±3.30)	91.25 (±17.01)	
	Pb	80.7 (±4.19) [1]	88.0 (±8.12) [1]	
	Zn	310.0 (±53.54) [1,2]	547.5 (±436.47) [1,2,3]	
Hydrocarbon	Total aliphatics	624.0 (±313.46)	442.75 (±156.69)	[46]
	UCM	585.5 (±295.75)	411.5 (±141.97)	
Organotin compound	Sn	298.0 (±106.63) [1]	456.9 (±171.6) [1]	[47]

Cr = chromium; Cu = cooper; Ni = nickel; Pb = lead; Zn = zinc; UCM = Unresolved Complex Mixture; Sn = tin. [1] value above the threshold effects level (TEL), [2] value above the probable effects level (PEL), and [3] value above the apparent effects threshold (AET) [44].

2.2. Biological and Environmental Sampling

Three independent replicates of sediment samples were monthly collected using a Van Veen grab (0.05 m^2) at the sampling station from March 2011 to March 2012 (n = 13 samplings). Sediment samples were sieved through a 100 μm net mesh to retain the recently settled and adult snails of *H. australis*. All the retained material was conditioned into plastic flasks with water enough to cover it for laboratory procedures. Simultaneously to specimens sampling, sediment samples were collected using the Van Veen grab (0.05 m^2) equipped with two lids that permit the removal of the upper sediment surface without emptying the whole sample, for the analyses of organic matter content (OM) and grain size (n = 3 replicates). Samples of the bottom water (4–6 m) were collected using a Niskin bottle (10 L) for dissolved oxygen (DO) and inorganic nutrient analyses (silicate, phosphate, ammonia, nitrite, and nitrate). Water samples were kept on ice until laboratory analyses. Temperature (°C) and salinity data were obtained in situ using a thermosalinometer. Our scientific research was registered in the Brazilian registration system to access genetic heritage and its traditional knowledge (SISGEN n° AGFBA19).

2.3. Analyses of Environmental Variables

Before analyses, all the macrofaunal organisms were removed from the sediments. The upper layer (~10 cm) of the sediment was separated in triplicates and dried for 48 h at 60 °C, and its organic matter content was determined from the weight loss after ignition at 500 °C for 4 h [48]. The grain size was analyzed by laser diffraction (Malvern Hidro 2000). Dissolved oxygen was analyzed by Winkler titration [49], using a Metrohm Titrino automatic burette system. The concentrations of the inorganic nutrients nitrate, nitrite, ammonium, silicate, and phosphate (μM) were determined using a flow injection FIAstar 5000 autoanalyzer (Foss Tecator, Denmark), following FOSS analytical methods, and unit conversions were performed using their molar weights (i.e., μM to $mg\ L^{-1}$). All the chemical analyses were performed using two analytical replicates. Data of monthly accumulated rainfall (mm) and atmospheric temperature (°C) were obtained from the meteorological station located at the bay's area proximity (A603: 22°35′23.0″ S, 43°16′56.0″ W; n = 13) compiled from the Brazilian Institute of Meteorology (INMET) database [50]. Assessment of environmental quality for brackish systems was based on the current Brazilian legislation, determined by the resolution n° 357 of the National Environmental Council (CONAMA), which disposes of the classification of water bodies and environmental guidelines for their framing, as well as establishes the conditions and standards for effluents disposal [51]. Considering the current multiple uses of Guanabara Bay, its water parameters must agree with those established for brackish water bodies class 1 (i.e., safe for the protection of aquatic communities; primary contact recreation; irrigation of vegetables, fruit, and garden plants; and aquaculture and commercial fishing activity).

2.4. Analyses of Biological Data

In the laboratory, a solution of magnesium chloride (3.5% $MgCl_2$) was added to the plastic flasks with samples for the specimen's narcotization. The solution was removed with a Pasteur pipette after 2 h of incubation, then, samples were preserved with 70% alcohol. Every month, snails were sorted, counted, measured (total shell length—SL) and distinguished by size (i.e., SL smaller and larger than 2 mm), based on size classification for adults and juveniles previously established for the hydrobioid *Hydrobia ulvae* [52]. Snail's measurements (±0.01 mm precision) were performed using the Axio Vision program (Zeiss) with a camera attached to the stereoscopic microscope (Zeiss). Sex determination (i.e., penis presence) was monthly performed in snails with SL larger than 2 mm (n = 100 per month) after shell cracking with a mini vice using a stereomicroscope (Zeiss). Density was calculated using the abundance of snails expressed per m^2, and recruitment consisted of the density of individuals smaller than 2 mm. Female individuals of *H. australis* deposit their egg masses over other snail shells [53]; therefore, all the eggs found attached to shells were counted by direct counting, and egg density was monthly calculated using the total number of eggs found attached to the snails per m^2. Realized fecundity was expressed by the total number of eggs over the total number of females per month [54].

Any level of shell deformity in adults was verified and counted on a subsample snail population (n = 100 per month) and its incidence was monthly calculated as the density of affected individuals by total evaluated. Shell deformity in individuals consisted of scalariform shell—detachment between whorls, malformation of shell spire, asymmetry in shell shape with spire curved to the left or right sides, based on previous studies with gastropods [55]. Imposex incidence (i.e., the density of affected females per total female density) and relative penis length index—RPLI (i.e., the ratio of imposexed-female penis length to male penis length multiplied by 100) were monthly calculated based on inspection of the distal reproductive tract by cracking the shells with a mini vice (n = 100 adult individuals by month). This criterium for imposex detection was adopted based on [18], since the observation of vas deferens and gonads in this species is not feasible using a stereomicroscope for monitoring purposes. The sexual characteristics of imposex-affected females of *H. australis* are morphologically different from males, as well as comparatively reduced in size, which makes it possible to distinguish this phenomenon by the inspection of the distal reproductive tract in *H. australis* snails [18]. In total, ten different population variables were obtained from biological samples: total density, female density, male density, recruitment, egg density, realized fecundity, incidence of shell deformity, imposex incidence, imposexed-female penis length (FPL), and relative penis length index (RPLI).

2.5. Statistical Analyses

Principal component analysis (PCA) was applied to the environmental data matrix (n = 10), after Hellinger transformation, to evaluate which atmospheric (accumulated rainfall), bottom water (salinity, temperature, dissolved oxygen, ammonium, nitrate, nitrite, phosphate, and silicate), and sediment variables (organic matter) mostly contributed to explaining the seasonal patterns of environmental quality. The Broken-Stick criterion was used to retain the PCA significant axes for sampling ordination. A multivariate analysis of permutational variance (PERMANOVA) was applied, using Euclidean distances (999 permutations), for comparisons of the PCA significant scores among rainy, dry, and eventual intermediate periods.

Spearman correlation tests were applied to assess the autocorrelated variables within usual (n = 6) and stress-related (n = 4) population attributes before performing the multivariate ordination analyses. Only non-autocorrelated variables (Spearman's $r < 0.60$; $p > 0.05$) and those significantly (Spearman's $r > 0.60$; $p < 0.05$) autocorrelated variables that explained most of the data variation (i.e., highest standard deviation) within each group (i.e., usual or stress-related population attributes) were retrieved for further analyses. Two constrained Redundancy Analyses (RDA) were applied to assess the relationship of the usual and stress-related population attributes to environmental variables and seasonal patterns. RDA is a linear multivariate method that combines regression and principal component analysis and models multivariate relationships of environmental and biotic data [56,57]. A forward model selection routine was applied for all RDAs to select the independent variables with significant contributions (9999 permutations; $p < 0.05$) for the best model explanation (i.e., the highest adjusted R^2). The data matrixes of population attributes and environmental variables were Hellinger-transformed to standardize the variables' units and improve the performance of the RDA linear method [58]. PERMANOVA was applied, using Euclidean distances (999 permutations), for univariate comparisons of population attributes among rainy, dry, and eventual intermediate periods. PCA and RDA analyses were performed in the software PC-ORD 6.0 and CANOCO 4.5, respectively, and PERMANOVA analyses were applied using the software PERMANOVA 1.6 [59].

3. Results

3.1. Environmental Data

The first two PCA axes were selected by the Broken-Stick (BS) method, with axis 1 and 2 explaining, respectively 37.1% (BS eigenvalue = 2.93) and 21.8% (BS eigenvalue = 1.93) of the total variance (Figure 2). PCA of environmental data evidenced the formation of three distinct groups, which were named (1) warm-rainy, (2) intermediate, and (3) cold-dry considering the main seasonal characteristics of groups. Significant differences among formed groups were overall supported by the results of the PERMANOVA test ($F = 7.19$, $p < 0.001$). PCA axis 1 accounted for most of the seasonal gradient in the southern hemisphere between samples collected during the warm-rainy season (e.g., late spring and summer months: from November to March) and samples from both intermediate (e.g., mid-seasons corresponding to spring and fall months: April-May and October) and cold-dry seasons (e.g., winter months: June-September). Samples from the warm-rainy season (▲) were located on the right side of the biplot and correlated with high values of salinity ($r = 0.85$), temperature ($r = 0.67$) and dissolved oxygen ($r = 0.64$) in the bottom water, and high amounts of accumulated rainfall ($r = 0.62$) and organic matter in the sediment ($r = 0.45$). PCA axis 2 mostly evidenced differences between intermediate (●) and cold-dry seasons (▼), in which samples were located at the upper (●) and lower (▼) on the left side of the biplot, respectively. Samples from the intermediate season were correlated with high values of silicate ($r = 0.30$) and phosphate ($r = 0.19$) in the bottom water. In contrast, samples collected during the cold-dry season correlated with high values of nitrogen-compounds in the bottom water: nitrite ($r = 0.72$), nitrate ($r = 0.62$) and ammonium ($r = 0.12$). PCA axis 2 also discriminated the rainiest month (i.e., January that is located closer to the rainfall vector) and the month that presented the highest content of organic matter in the sediment (i.e., December is located closer to the OM vector) within the group of the warm-rainy season (▲).

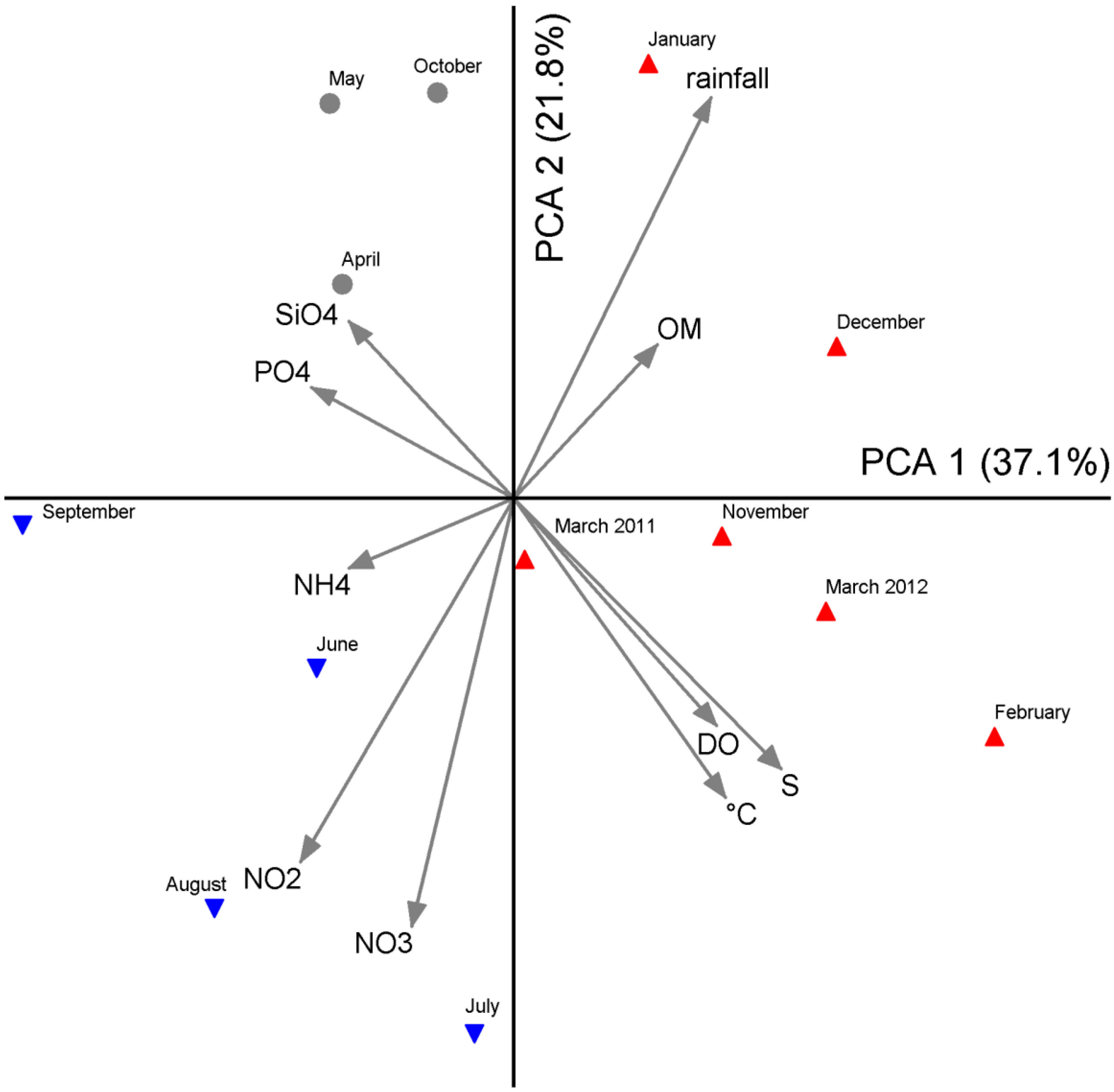

Figure 2. Biplot diagram of environmental variables collected during an annual cycle, from March 2011 to March 2012, in the estuarine region of Guanabara Bay. Environmental variables analyzed were accumulated rainfall (mm); salinity (S), temperature (°C), ammonium (NH_4), nitrate (NO_3), nitrite (NO_2), phosphate (PO_4), silicate (SiO_4), and dissolved oxygen (DO) in the bottom water; and organic matter in the sediment (OM). Distinct symbols and colors indicate that samples were collected in the warm-rainy (▲), intermediate (•), and cold-dry seasons (▼).

Minimum, maximum, and mean values (±standard deviation) of environmental variables from different ecosystem compartments during the warm-rainy, intermediate, and cold-dry seasons are shown in Table 2. The lower values of bottom water temperature were found during the warm-rainy season (i.e., summer in southeastern Brazil), when salinity and dissolved oxygen in the bottom water varied from 30–37.2 and 3.56–5.19 mg L^{-1}, respectively. The highest values of phosphate, nitrate and nitrite in bottom water were found in the late cold-dry season (September) and, just a month after, the highest value of ammonium was detected in the early intermediate season (October). The highest concentration of silicates was detected during the warm-rainy season (January), occurring simultaneously with low temperature (20–21 °C) and high salinity (33.8–34.1) in bottom water. Higher amounts of organic matter in the sediment were found during the rainy season, when accumulated rainfall was higher than 100 mm $month^{-1}$. The predominant sediment type was silt (muddy sand according to Folks' textural classification), with mean grain size ($^{\Phi}$) of 5.17 (±0.88), very poorly sorted ($^{\Phi}{}_{SD}$ = 2.48), mesokurtic (kurtosis$^{\Phi}$ = 0.998 ± 0.06), and positive skewed (skewness$^{\Phi}$ = 0.196 ± 0.027).

Table 2. Minimum, maximum (i.e., Min-Max) and mean (±standard deviation) values of environmental variables in the different compartments (atmosphere, superficial and bottom water, and sediment) in the estuarine region of Guanabara Bay (Rio de Janeiro, Brazil). Environmental data are shown by group evidenced in the Principal Component Analysis (PCA). Local depth varied between 3.5 and 6 m according to the tide. * Below the minimum and ** above the maximum limit value established by the Brazilian legislation [51] for brackish water bodies class 1 (i.e., with uses appropriated for the protection of aquatic communities; primary contact recreation such as swimming, water skiing, and diving; irrigation of vegetables, fruit, and garden plants; aquaculture and commercial fishing).

	Environmental Variable	Warm-Rainy		Intermediate		Cold-Dry	
		Min-Max	Mean (±SD)	Min-Max	Mean (±SD)	Min-Max	Mean (±SD)
Atmosphere	Temperature (°C)	13.9–38.4	24.70 (±4.21)	12.1–36.1	22.47 (±4.45)	8.5–36.6	19.77 (±5.03)
	Rainfall (mm)	62.8–220.9	112.97 (±55.77)	109.8–164.5	130.8 (±29.48)	9.3–40.8	24.73 (±14.54)
Superficial water	Dissolved oxygen (mg L^{-1})	3.34–7.15	4.41 (±1.62) *	2.70–4.96	3.83 (±1.57) *	2.37–8.0	4.39 (±2.58) *
	pH	7.90–8.15	8.03 (±0.18)	7.82–7.87	7.85 (±0.03)	7.71–8.25	7.98 (±0.38)
	Ammonium—NH_4 (mg L^{-1})	0.274–1.48	0.578 (±0.513) **	1.142–1.752	1.447 (±0.431) *	0.667–2.30	1.51 (±0.788) **
	Nitrate—NO_3 (mg L^{-1})	0.101–0.40	0.224 (±0.126)	0.187–0.339	0.263 (±0.107)	0.321–0.644	0.478 (±0.171) **
	Nitrite—NO_2 (mg L^{-1})	0.035–0.552	0.234 (±0.27) **	0.010–0.011	0.011 (±0.001)	0.004–0.111	0.048 (±0.052)
	Phosphate—PO_4 (mg L^{-1})	0.190–0.315	0.242 (±0.05) **	0.280–0.419	0.349 (±0.098) **	0.150–1.01	0.478 (±0.388) **
	Silicate—SiO_4 (mg L^{-1})	0.09–1.29	0.469 (±0.482)	0.861–0.995	0.928 (±0.095)	0.194–0.999	0.687 (±0.345)
Bottom water	Water temperature (°C)	20.0–21.1	20.7 (±0.54)	20.0–23.8	21.93 (±1.90)	22.3–26.0	23.45 (±1.73)
	Salinity (S)	30–37.2	32.68 (±2.85)	28–36.4	30.97 (±4.71)	29.7–38.0	35.05 (±3.87)
	Dissolved oxygen (mg L^{-1})	3.56–5.19	4.44 (±0.67)*	2.49–5.83	4.14 (±1.66) *	2.96–6.35	4.57 (±1.40) *
	pH	7.95–8.1	8.03 (±0.11)	7.84–7.93	7.88 (±0.06)	8.0–8.16	8.08 (±0.11)
	Ammonium—NH_4 (mg L^{-1})	0.125–1.325	0.427 (±0.481) **	0.667–1.613	1.009 (±0.524) **	0.599–0.776	0.664 (±0.079) **
	Nitrate—NO_3 (mg L^{-1})	0.130–0.248	0.188 (±0.05)	0.197–0.310	0.268 (±0.062)	0.372–0.608	0.475 (±0.117) **

3.2. Biological Data

Total density of *H. australis* snails varied from 872 to 39,400 ind. m^{-2}, with a higher contribution of females in the adult population, and recruit density showed a wide variation from 0 to 3101 ind. m^{-2}. Realized fecundity and egg density varied from 0 to 1847 and 0 to 6579 10^3 eggs m^{-2}, respectively. Imposex incidence varied from 1.6% to 11.1% in the *H. australis* population of Guanabara Bay, and the affected females showed a mean shell length of 3.08 ± 0.73 mm; imposex was not detected in January 2012. The sexual character of imposex-affected females differed from the males in morphology and size, the mean penis lengths of imposex-affected females and males were, respectively, 0.36 ± 0.2 mm and 1.13 ± 0.09 mm. Shell deformity affected snails with a mean shell length of 3.02 ± 0.83 mm and its incidence ranged from 0.43% to 15.93%; except for two months (April and June

2011) when no snails with shell deformity were recorded. Mean values of all the biological variables monthly obtained in the present study are available in Table 3.

Table 3. Mean values of all the biological variables obtained for the *Heleobia australis* population from Guanabara Bay (Rio de Janeiro, Brazil).

Sampling Month	Density (ind. m^{-2})				Egg Density (10^3 Egg m^{-2})	Fecundity	Incidence (%)		Index	
	Total	Recruits	Male	Female			Shell Deformity	Imposex	FPLI	RPLI
March 2011	15,924	243	6262	9394	1048	111.6	0.43	3.42	0.001	6.73
April 2011	6146	36	2261	3849	254.3	66.1	0	1.6	0.001	6.73
May 2011	2793	141	981	1671	52.7	31.5	0.62	11.1	0.007	14.35
June 2011	39,420	3101	13,438	22,881	0.016	0	0	6.6	0.024	40.78
July 2011	8593	351	3050	5192	429.0	82.6	0.68	3.8	0.011	25.38
August 2011	4936	0	1826	3110	247.2	79.5	1.86	4.5	0.007	16.04
September 2011	9756	120	3565	6071	2350	387.2	2.77	1.6	0.011	51.79
October 2011	7906	77	2897	4932	1992	403.9	4.37	3.5	0.021	46.67
November 2011	3110	0	1151	1959	3619	1847	4.57	1.9	0.011	32.61
December 2011	12,426	107	4558	7761	6579	847.8	4.76	2.0	0.008	43.37
January 2012	872	23	314	535	87.8	164.1	15.93	0	0	0

Three usual population attributes (total density, recruitment, and realized fecundity) and three stress-related attributes (incidence of shell deformity, the incidence of imposex, and relative penis length index—RPLI) were applied in multivariate analyses after the variable's selection to control data collinearity. A constrained Redundancy Analysis (RDA) was applied to summarize the variation in biological data (i.e., response variables) that can be explained by environmental conditions (i.e., explanatory variables), following a stepwise forward criterion for the selection of environmental variables. RDA axes 1 and 2 showed species-environment correlations of 0.449 and 0.387, respectively, accounting for a percentage variation of species-environment relation of 74% (Eigenvalue RDA 1 = 0.138) and 24.7% (Eigenvalue RDA 2 = 0.046) (Figure 3). However, all the canonical axes of this RDA were statistically non-significant (Monte Carlo permutation, F-ratio = 0.321; $p = 0.966$).

Overall, RDA evidenced a particular environmental condition for high snail densities, which were mostly related to the cold-dry season, in contrast to the better environmental conditions for high *H. australis* fecundity, which was positively correlated with organic matter content in the sediment during the warm-rainy season (Figure 3). Recruitment of young snails was most closely related to environmental conditions of higher dissolved oxygen and salinity in the bottom water during the intermediate and warm-rainy seasons (Figure 3). Despite the patterns evidenced by RDA for usual population attributes and environmental conditions with higher explicability for the model, no statistically significant difference was found for these population attributes among seasonal periods (PERMANOVAs, $F = 1.587$; $p \geq 0.22$).

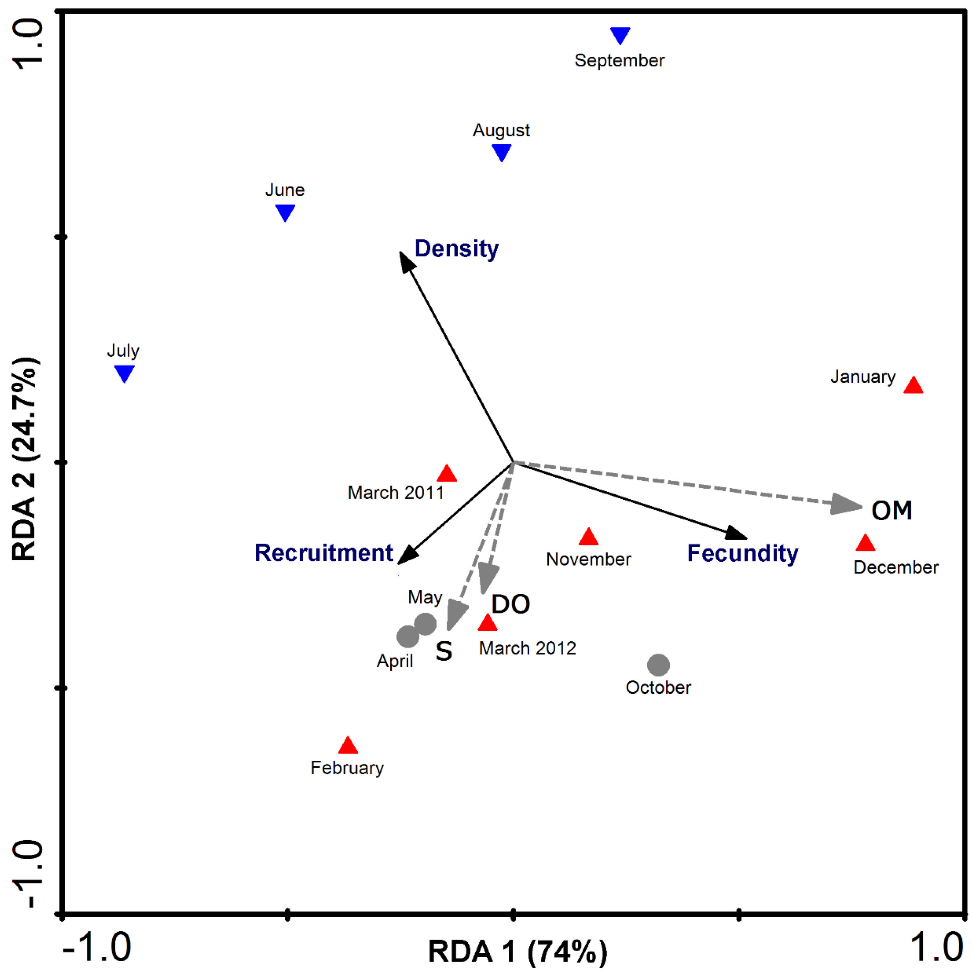

Figure 3. Biplot diagram of constrained RDA with data of usual population attributes (full black arrow) and environmental variables (dotted gray arrow). Biological variables (total density, recruitment, and fecundity) were previously selected to control for autocorrelation. A stepwise forward selection criterion has included only three environmental variables in the RDA that contributed significantly to the model explanation: salinity (S), dissolved oxygen (DO) in the bottom water, and organic matter in the sediment (OM). Distinct symbols and colors indicate that samples were collected in the warm-rainy (▲), intermediate (●), and cold-dry seasons (▼).

A second constrained RDA was applied to detect the relationship of stress-related variables (i.e., response variables) to environmental data (i.e., explanatory variables), following a stepwise forward selection model of environmental variables. RDA axes 1 and 2 showed species-environment correlations of 0.946 and 0.878, respectively, accounting for a percentage variance of species-environment relation of 99.5% (Eigenvalue RDA 1 = 0.890) and 0.4% (Eigenvalue RDA 2 = 0.003) (Figure 4). All the canonical axes of RDA showed statistical significance (Monte Carlo permutation, *F-ratio* = 6.060; $p = 0.048$). A PERMANOVA was applied to test for differences in stress-related population attributes among seasonal groups. Overall, a significant influence of seasonal periods on the explicability of stress-related attributes was only detected between warm-rainy and cold-dry seasons (PERMANOVA, $F = 1.349$; $p = 0.039$).

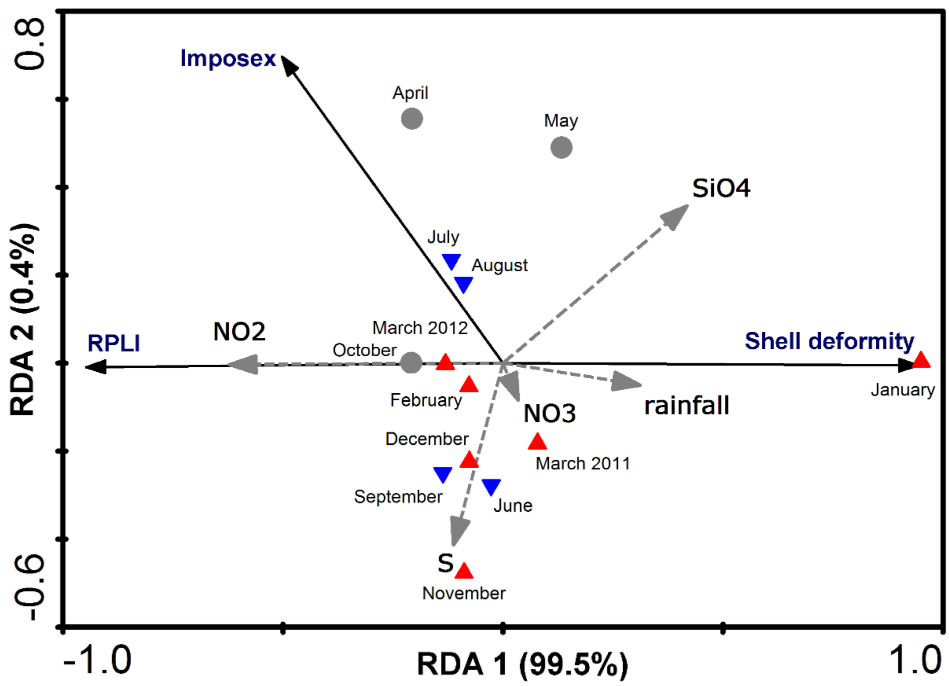

Figure 4. Biplot diagram of constrained RDA with data of stress-related population attributes (full black arrow) and environmental variables (dotted gray arrow). Biological variables (incidence of imposex and shell deformity, and the index of relative penis length—RPLI) were previously selected to control for autocorrelation. A stepwise forward selection criterion of environmental data has included six variables in the RDA that significantly contributed to the model explanation: accumulated rainfall (mm), and nitrogen compounds—nitrate (NO_3) and nitrite (NO_2), salinity (S), and silicate (SiO_4) in the bottom water. Distinct symbols and colors indicate that samples were collected during the warm-rainy (▲), intermediate (●), and cold-dry seasons (▼).

RDA Axis 1 accounted for almost the total of biplot explicability and evidenced three distinct patterns, posteriorly tested by PERMANOVA: (1) higher incidence of shell deformity showed closed relation to a particular month (January 2012) that exhibited a higher amount of accumulated rainfall during the warm-rainy season. Shell deformity showed significant variation among seasonal periods (PERMANOVA, $F = 15.33$; $p = 0.0003$), and a higher incidence was significantly detected during the warm-rainy season in comparison to intermediate (pairwise comparisons, $p = 0.0106$) and cold-dry seasons (pairwise comparisons, $p = 0.0046$) (Figure 5a); (2) higher values of relative penis length index (RPLI) was related to high amounts of nitrite in the bottom water. Values of RPLI showed significant differences among seasonal periods (PERMANOVA, $F = 4.334$; $p = 0.0436$), a significantly higher value was found during the intermediate season compared to values obtained in the warm-rainy (pairwise comparisons, $p = 0.0234$) and cold-dry seasons (pairwise comparisons, $p = 0.050$) (Figure 5b); (3) there was a tendency of increase in the imposex incidence in the intermediate and cold-dry seasons; however, no significant seasonal effect was detected in its incidence (PERMANOVA, $F = 1.859$; $p = 0.2187$).

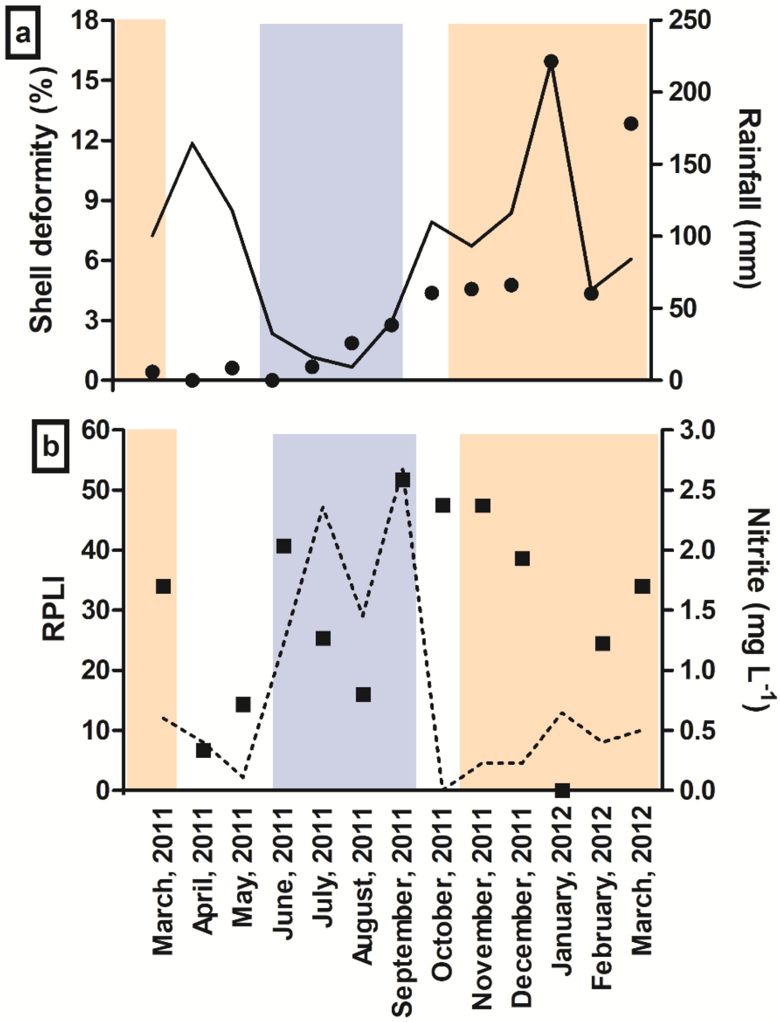

Figure 5. Stress-related population attributes of *Heleobia autralis* (left Y-axis) differed significantly among seasonal groups (PERMANOVA, $p \leq 0.05$) and the environmental variable of higher influence (right Y-axis) by month throughout the study period. (**a**) Incidence of shell deformity (%, •) and accumulated rainfall (mm; full line); (**b**) Relative Penis Length Index (RPLI, ■) and nitrite concentration (mg L^{-1}; dotted line). The months corresponding to distinct seasonal groups, as evidenced by PCA of environmental data, are indicated by different colors: warm-rainy (orange), intermediate (white), and cold-dry (blue).

4. Discussion

The mud snail *Heleobia autralis* presents particular characteristics usually found in species described as "efficient bioindicators" [5]): high abundance, wide distribution in South American brackish water systems, functional importance in aquatic systems, and low mobility. In this sense, particular environmental conditions—mainly represented by changes in accumulated rainfall, nitrogen compounds (mainly nitrate and nitrite), salinity, and silicate in the bottom water—were significantly associated with stress-related population attributes. Similarly, those environmental variables showed high correlations with seasons, which suggests their application as good predictors of changes in environmental conditions associated with the warm-rainy season (i.e., rainfall and salinity), intermediate season (i.e., silicate), and cold-dry season (i.e., nitrite and nitrate). Population attributes reflect species' adaptation to the environment and thus, individuals exposed to adverse and/or unfavorable conditions are expected to shift their birth, mortality, and recruitment rates [60]. Moreover, some tolerant estuarine gastropods can survive extremes of environmental conditions, such as *Amphibola crenata* [33], *Hydrobia ulvae* [61] and *H. australis* (this study), and might be expected that their biological attributes would reflect changes in environmental conditions. Environmental-mediated responses to usual population attributes of the gastropod *A. crenata* (e.g., abundance, structure, and growth rate) have been detected in New Zealand estuaries [33]. In the present study, no significant effect of environmental quality and conditions in Guanabara Bay was detected on the usual population attributes (density, fecundity, and recruitment) of *H. australis*, which may indicate low variation in *H. australis* population attributes. Our findings also suggest that all the life history stages of this mud snail (i.e., intracapsular embryonic development, planktonic larva, young recruits, and adults) are tolerant to the degraded habitat quality provided by Guanabara Bay.

In the present study, this typical bay's water was marked by increased temperature (22–26 °C) and higher levels of inorganic nutrients (phosphate, silicate, and nitrogen compounds) both in the superficial and bottom water. During the warm season (late spring and summer), this bay is regulated by a complex equilibrium between the intrusions of South American Central Water (SACW) and the higher frequency and intensity of heavy rainfall and rainstorm events which, consequently, increase the riverine inflows from its drainage basin [37,38]. Despite the SACW intrusion of cold and nutrient-rich seawater into the bay, increased inputs of freshwater from severely polluted riverine systems [62–64] seem to reduce or even neutralize the positive effects of SACW, mainly in the intermediate and inner estuarine regions of the bay. In contrast, the combination of decreased precipitation levels and restricted influence of oceanic water during the cold-dry season results in more homogeneity of the bay water conditions [41]. Therefore, the complex combination of the tidal regime, precipitation, riverine inflows, and intrusion of oceanic water alters the physical and chemical conditions of the water column and sediment compartments, as well as nutrients and organic matter levels, which consequently affects biological community composition and population structure of living biota at the Guanabara Bay, as detected in the present study and previously shown by other studies [41,65–67]. Irrespectively of a seasonal effect on the environmental conditions, the water quality of our study area in Guanabara Bay showed undoubtedly characteristics of highly eutrophic brackish water bodies [51]: both the superficial and bottom water showed low concentrations of dissolved oxygen (i.e., <5 mg L^{-1}), high concentrations of phosphate (i.e., >0.124 mg L^{-1}) and nitrogen compounds—nitrite (i.e., >0.07 mg L^{-1}), ammonia and nitrate (i.e., >0.4 mg L^{-1} for both compounds). Our environmental data corroborate previous findings [23,41,68,69] and highlights the high levels of organic pollution and water quality degradation in the estuarine region of Guanabara Bay.

Considering the environmental quality of estuarine systems, multiple compartments (e.g., water, sediment, atmosphere) and components (e.g., habitat, hydrodynamics, biota, physical and chemical variables, contamination) must be assessed in a holistic manner using integrated approaches [70]. Environmental quality assessment must address not only water parameters, but also integrate the sediment as an important component of aquatic ecosystems and a source of contaminants in the benthic food chain [71]. Organic matter (OM) levels in the surface sediments have been applied as proxies of ecosystem vulnerability, since OM enrichment affects nutrient fluxes, oxygen demand for bacterial respiration, and re-oxidation of anaerobic respiration products [72]. Excessive OM may induce hypoxia or anoxia in the water–sediment interface and/or the accumulation of toxic reduced compounds in the superficial sediments, which negatively affect macrofaunal organisms [73,74]. In the present study, all the sediment samples showed extremely high levels of organic matter (12–32%) in comparison to other eutrophic bays worldwide, such as Bahía Blanca -Argentina (2.63–5.86%) [75], Montevideo Bay—Uruguay (5.76–10.53%) [76], Odiel-Tinto Estuary—Spain (0.56–13.77%) [77]. In this way, our results indicate a highly eutrophic condition in the benthic compartment of the bay estuarine region.

The biotic integrity of water bodies is not exclusively affected by physical and chemical parameters that indicate environmental quality [78], but a broader perspective must be considered. Therefore, it is expected that species still inhabiting the estuarine region of Guanabara Bay are very persistent and resistant to organic and chemical pollution [21,42,79].

Heleobia australis is a dominant macroinvertebrate species that occur in high abundance in coastal lagoons and estuaries of South America [25,28,36,76,80,81]). In addition, this species tolerates adverse conditions of salinity stress [82], eutrophication [24], exposure to hydrocarbons (e.g., petroleum and diesel) [82–84] and to the emergent pollutant bisphenol A [85]. Usual population attributes (e.g., density, recruitment, fecundity) of this species were not significantly affected by shifts in environmental conditions of Guanabara Bay, possibly because of the snails' high tolerance to environmental changes and chronic exposure to detrimental conditions in this bay. Furthermore, high concentrations of inorganic nutrients in the bottom water and organic matter deposited in muddy sand sediments of Guanabara Bay seemed to favor this mud snail species. *Heleobia australis* showed all year-round input of eggs and juveniles (i.e., assessed by realized fecundity and recruitment rates), despite the detection of reproductive peaks (as shown in Table 3), and reached huge densities in patches (e.g., 39,420 ind. m^{-2}), the highest ever reported for the species in aquatic systems [86] and at Guanabara Bay [24,43]. Our results agree with predictions for hydrobioid populations as opportunistic mud snail species that attain high densities in eutrophicated aquatic systems [36,87,88].

Stress-related population attributes of *H. australis* seem to be induced by the chronic environmental exposure of snails to high levels of contaminants (e.g., metals, hydrocarbons, emerging pollutants from effluents) in the sediment. Considering the threshold effect sediment quality guidelines [44,70], all the compiled concentrations for metals (Cr, Ni, Zn, Pb, and Cu) in sediments of the bay estuarine region were above the maximum limit values. For the first time, this study has shown the incidence of shell deformities (e.g., scalariform shell, malformation of shell spire, and asymmetry in shell shape with spire curved to the left or right sides) in the *H. australis* population from a highly contaminated system (Guanabara Bay). A higher incidence of shell deformities in *H. australis* snails was more evidenced during the mid- and late warm-rainy season, in conditions of increased rainfall amount that induce shifts in the local environmental conditions (as previously shown). In addition, the rainy season is responsible for changes in total metal concentrations (Cr, Ni, and Pb) and their fractionation within different phases in Guanabara Bay sediments [40], as well as increases in the inputs of freshwater from polluted riverine systems [62]. Despite the irreversible characteristic of this stress-related attribute, snails of *H. australis* have the ability to "escape" from stressful environmental conditions by creating a gas bubble inside its shell and floating on the water column being carried along by tide and wind currents [43,89]. Since outmigration is possible for the *H. australis* population exposed to stressful conditions, a possible explanation for the positive relationship between the incidence of shell deformity and rainfall amount may be related to a higher dispersion of snails without deformity during high rainfalls, which is reinforced by a great reduction in the total density of *H. australis* in January 2012 (Table 3). None is known about biological and ecological constraints related to shell deformity in aquatic gastropods. Moreover, the shell morphology of hydrobioids (ectomorphs) has been applied as a palaeoecological indicator to detect climatic and environmental changes that occurred during the late Quaternary [90]. In the present study, it is thus supposed that shell asymmetry might limit the dispersal of snails exposed to stressful conditions by potentially affecting bubble formation inside their shell or reducing floating capacity and equilibrium in the water column, which may explain the higher incidence of shell deformity simultaneously with the lowest population density and drastic shifts in environmental conditions at Guanabara Bay. The action mechanisms and exposure time that induce changes in the shape and composition of molluscan shells in the presence of environmental contaminants are still unknown. Shells of molluscs from multi-impacted systems can differ in shape and composition in relation to individuals from reference sites [11,13]. Until now, 18 different molluscan species (i.e., 6 bivalves and 11 gastropods) were found exhibiting shell alteration in response to contamination [12]; according to our results, *H. australis* integrates the list, totaling 19 species. Further studies are needed to assess the biological and ecological impacts of shell deformity on gastropod populations.

International resolutions against the use of organotin compounds have globally prohibited their use as biocides in anti-fouling systems on ships by January 2003, and totally banned by January 2008, which was later adopted as the International Convention on the Control of Harmful Anti-Fouling Systems on Ships [91,92]. These international resolutions have led to a reduction in recent tributyltin (TBT) concentrations and its biological effects worldwide [93–95]; however, this is not the common scenario for coastal systems in Brazil and other Latin American countries. Despite the attempts of local authorities to control the use of TBT-based antifouling paints, organotin compounds and their biological effects are still detected in Latin American coastal areas under influence of commercial ports, marinas, and shipyards [96–102]. Chronic exposure to high levels of organotin compounds, mainly TBT, has been related to alterations in endogenous steroid levels combined with imposex development in gastropods [103–106]. The occurrence of this phenomenon, previously described for the soft-bottom snail *H. australis* from Guanabara Bay [18], is not surprising considering their chronic exposure to high concentrations of organotin compounds found in the sediments of the bay estuarine region (as shown in Table 1). High levels of imposex have been already described for the carnivorous gastropod *Stramonita haemastoma* that inhabits hard bottoms (e.g., rock shores, naval base, and marina) at Guanabara Bay [17,107]. In the present study, despite the low incidence of imposex found in the soft-bottom snails (1.6–11.1%), previous studies have reported incidences within this range for other gastropod populations worldwide, for example: 2.56% and 5% for populations of *Pareuthria plumbea* [108]; 4.54% and 7.69% for populations of *Prunum martini* [108]; 1% for a population of *Thais brevidentata* [109]; 11% for a population of *Nassarius coppingeri* [110]; 10.3% for a population of *N. mutabilis* [111]; 6.3% for a population of *N. reticulatus* [112]; and 6.7% for a population of *N. vibex* [113]. Irrespective of imposex intensity in a gastropod population, it is important to consider its occurrence to monitor TBT pollution in different system compartments and to detect the ecological issues related to imposex development in affected populations. Moreover, the *H. australis* population of Guanabara Bay is known to be chronically exposed to multiple pollutants (including organotin compounds), which is expected to negatively affect the sensitive individuals and favor the resistant ones, modulating population response to pollutants.

A significant seasonal effect was detected in the imposex index (RPLI) for the *H. australis* population of Guanabara Bay. Higher values of RPLI have been related to increased concentrations of organotin compounds accumulated in individuals [114–117]. In the present study, high values of penis length of imposex-affected females (3.71 ± 0.83 mm) and RPLI (47.5–51.8) were found in larger snails (i.e., older ones) from September to November (i.e., during the late cold-dry, fall and early warm-rainy seasons). These larger individuals might have accumulated more organotin compounds throughout their life cycle compared to smaller snails. Organotin compounds have a close relationship with inorganic nitrogen compounds, since debutylation is promoted by nitrate reduction to nitrite [75,118], thus high concentrations of nitrogen compounds in the bottom water of Guanabara Bay may promote organotin compounds transformation in the sediments altering their bioavailability. As demonstrated for other gastropod species, seasonal variations can significantly affect imposex intensity and index related to differences in species sensitivity and in penis length (mainly in males) that are affected by intrinsic biological factors related to organisms [106,119–121]. Therefore, the sampling season must be standardized when assessing imposex in *H. australis* from Guanabara Bay, and preferentially in months when this phenomenon was more detected in the present study (from September to November).

5. Conclusions

In conclusion, most changes in environmental conditions in Guanabara Bay occur during the rainy season, when high levels of rainfall and/or stormwaters lead to high continental flows of domestic and industrial residues into the bay in combination with SACW intrusion with a cold and nutrient-rich seawater (i.e., high salinity, and silicate and phosphate concentrations). Usual population attributes of *H. australis* are not significantly affected by environmental changes in the estuarine region of Guanabara Bay, which seems to be related to high species tolerance to environmental shifts and deteriorated conditions. Irreversible stress-related attributes of *H. australis* show high correspondence with environmental conditions, which reinforces the importance to assess seasonal effects on these attributes to avoid misinterpretation of results. The incidence of shell deformity in *H. australis* (not previously described for the species) was more detected in the mid- and late warm-rainy season (i.e., higher environmental variability and continental influence). In contrast, the imposex index was more evident in the months of September to November in Guanabara Bay. Our results highlight the importance to assess stress-related attributes of populations chronically exposed to multiple pollutants. In this way, further studies must be performed to detect the triggers and identify which pollutants induce shell alterations in molluscs, and to determine the biological and ecological implications of morphological changes and other stress-related responses to contamination in molluscan populations. Moreover, further comparative studies must be performed to assess the feasibility of *H. australis* application as a pollution indicator in impacted estuaries and coastal systems.

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