Original Research Article

Littoral Benthic Macroinvertebrates of the Martín García Island Nature Reserve (Upper Río de la Plata, Argentina): Their use for the evaluation of water quality

.ABSTRACT

At Martín García Island—a Natural Reserve located at the confluence of the Paraná and Uruguay rivers—we used benthic-macroinvertebrate biotic indices to elucidate the structure and community parameters of the littoral benthos and their response to environmental variables and to evaluate the island's coastal-water quality. Seasonal campaigns were carried out (March/1995-March/1996) at eight sites of the island's littoral, selected according to the substrate characteristics (fine sands, sandy-silty, reedbed, and silty with great hydrophyte development). From the sites with a soft substrate, triplicate samples were extracted with a 225-cm^2 Ekman manual dredge. The relative abundances of 71 taxa were measured: Nematoda, Turbelaria, Oligochaeta (23 sps.), Mollusca (21 sps.), Crustacea (11 sps.), Insecta (12 families), and Tardigrada. Macroinvertebrate density: 15-58,800 ind.m^{-2}. Analysis of variance revealed significant differences between the average annual macroinvertebrate-population densities among the five sites (F: 3.059; p<0.05). Site 7 exhibited the highest density at 38,700±19,000 ind.m^{-2}. Community parameters: diversity (H'; 0.77-2.16 bits); equitability (J; 0.16-1.02), and species richness (S; 3-29). Canonical-correspondence analysis indicated the environmental variables experiencing the greatest fluctuation to be: dissolved-oxygen concentration, pH, temperature, and conductivity. The results of this analysis suggest that macroinvertebrate-species distribution involves the physicochemical conditions of the water. Of the correlation between species and environmental variables, 95.5% were distributed on the cannonical-correspondence ordering diagram's Axis 1. Functional feeding groups: In all sites and seasons, the gathering collectors predominated, followed by the scrapers, filtering collectors, shredders, and predators. Macroinvertebrate Index of Pampean-Rivers: values generally ranged from weak to weak-to-very-weak to zero pollution.

Keywords: benthos, macroinvertebrates, water quality, Martín García Island.

1. INTRODUCTION

The Río de la Plata estuary—220 kilometers long, 35 to 230 kilometers wide, and a temperate microtidal system—contains a freshwater environment in the upper region where the Paraná and Uruguay rivers converge, with an average discharge of 20,000–25,000 m^3.s$^-$

[1] . The river's coasts border on both Uruguay and Argentina. This system is one of the major estuaries in South America and the first in terms of economic relevance and the magnitude of the human populations linked to its coasts (1).

The Martín García Island Multiple-use Reserve is located at the confluence of the Paraná and Uruguay rivers (the uppermost portion of the Río de la Plata) at 34º 11' S and 58º 15' W. The 1990s marked the beginning of studies on the biota of the Island that were particularly characterized by the contributions of (2) on tree and shrub trees; followed by investigations on vertebrates (3, 4, 5), aquatic invertebrates (6) molluscs, temnocephalic flatworms (7), and aquatic and semiaquatic insects (8) among others. Since 2000, studies on the invertebrate fauna have been intensified, both along the island's coast and in the interior ponds (9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23).

The aim of this work was to gather further information about the structure of the littoral benthos with respect to the community parameters along with their response to environmental variables and also to evaluate the water quality along the island's coast by using biotic indices based on the benthic macroinvertebrates.

2. MATERIAL AND METHODS

2.1. Description of the environment

This island (see fig.,1) consists in an outcropping of the crystalline basement, unevenly overlaid by Pleistocene and Holocene sediments (24, 25, 26). Ever since the island's declaration as a natural reserve in 1969, Martín García has been under the administration of the provincial agency for sustainable development of the province of Buenos Aires.

The littoral of the island exhibits a marked asymmetry, with the western and northern coasts receiving clayey silt sediments, which particles over time become initially deposited on the otherwise rocky substrate and then consolidated by marsh-vegetation growth. In contrast,

Comment [U4]: 3.Most information should be moved to material and methods.
4.The theoretical framework should be based on macroinvertebrates' ecology/water quality rather than the study area.
5. The introduction deeply cares conceptual backgound. Note the discrepancy between this secton and the discussion section.

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along the eastern and southern coasts, the rocky substrate has little silty sediment and instead is covered mainly by fine sands, with that desposit resulting in part from the action of strong winds from the southeast. This coastal functional asymmetry favors the differential distribution of vegetation. Thus, on the northern coasts (see site 7, figure 1) extensive grasslands and reedbeds have developed, containing approximately 16 hydrophyte species. The benthos farther along the west coast (see site 8, figure 1) is characterized by mixed sediments of sand and silt and lies in the vicinity of a dump, constituting a source of anthropic impact. To the south of the island is site 1, a sandy beach with the development of reedbeds of *Schoenoplectus californicus*. Site 2 is situated in the southeastern end along with site 3 (the water-intake area of the island). The substrate there is mainly rocky, whereas a little farther north, a small beach of clean sand (site 4) has developed along with pools at site 5 farther up the coast. To the north, extensive reedbeds of *S. californicus* are present at site 6.

2.2 The Sampling

Five seasonal campaigns were carried out from summer, autumn, winter, spring 1995 to summer 1996, at the eight sites along the island's coast (see Fig. 1), selected according to the characteristics of the substrate (fine sands, sandy-silty, reedbed, and silty with great development of hydrophytes). In sites with soft substrate (sand or silt), triplicate samples per site were extracted with an Ekman manual dredge (225 cm^2) and then fixed with 10% (v/v) aqueous formaldehyde.

In Sites 3 and 5, the material was collected as flakes of 15 cm in diameter with a 0.14-mm mesh by the methodology of capture per unit of effort (at individuals/30 min/per person) to obtain the relative abundance of individuals per hour.
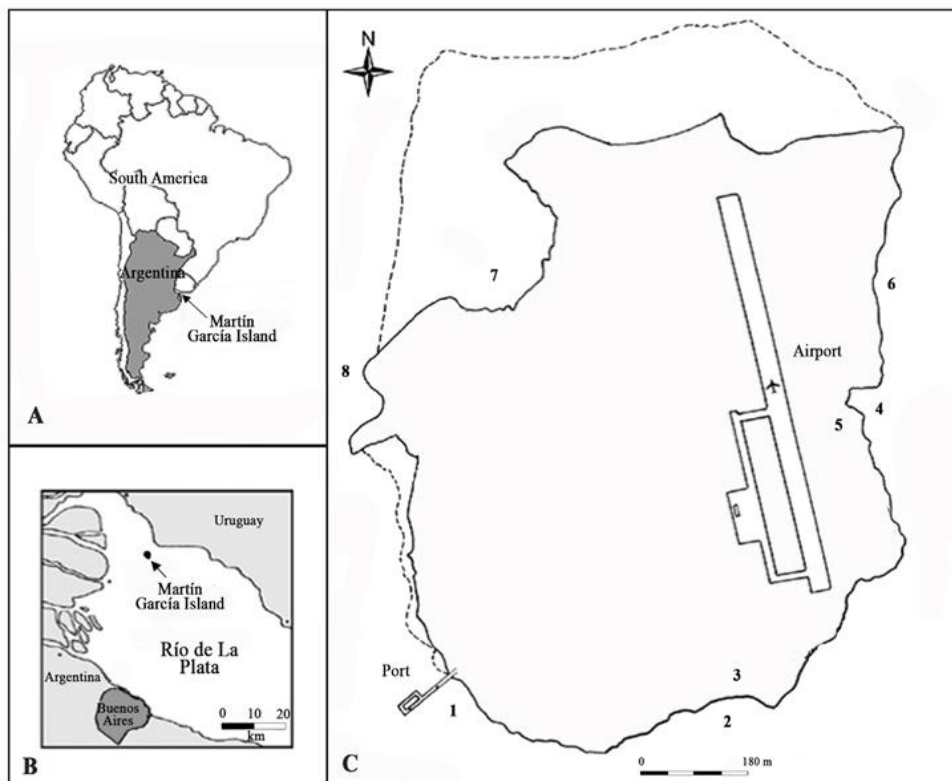
Fig. 1. Location of the Natural Reserve island of Martín García (Argentina) in a: South America b: the Estuary of ~~Rio de~~ la Plata River. C: Map of the island showing study sites 1-8.

The following physicochemical variables were measured with portable digital sensors: water and air temperature (°c), dissolved-oxygen concentration (mg.l^{-1}), percent oxygen saturation, electrical conductivity (μs.cm^{-1}), total concentration of dissolved solids (mg.l^{-1}), and pH.

2.3. THE SAMPLES PROCESSING

~~I~~n the laboratory, the samples were washed on a 125-μm mesh screen, or flotation techniques were applied (27) according to the type of substrate. ~~t~~The material was then stained with Erythrosin b. The specimens were characterized qualitatively and quantitatively

by microscopy and conventional scoring techniques. The taxonomical determinations were made through the use of the specialized literature pertaining to the particular groups (28, 29, 30, 31).

Comment [U9]: The authors identified the organisms to species, genus, family or order level. Please specify.

2.4. The Data Analyses

The species diversity (H') was calculated according to the Shannon-Wiener index, the species richness S (32), and the equitability (J) = H'/ ln S (33). Graphs were constructed with the excel software to determine the relative abundance per sampling site and season of the year. The taxa included correspond to those that exceeded a 1% representation for the purpose of greater graphical clarity.

To ascertain the variation in the average annual density of the macroinvertebrates among the sampling sites, an Analysis of Variance (ANOVA) supplemented with a Bonferroni post-hoc test was performed (p <0.05) by means of the statistical software SPSS v. 22. Data were previously log-transformed in order to meet the requirements of the analysis.

Comment [U10]: Not all sampling sites. Explain here which sites were included in this analysis and why only them.

For an analysis of the trophic structure of the benthos, the functional ~~food~~ feeding groups were characterized ~~in each sampling site and for the two years~~ according to preexisting classifications—namely, a grouping into: shredders, gathering collectors, filtering collectors, scrapers, and predators (34, 35, 36, 37, 38, 39).

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Because Martín García island had been classified as a Nature Reserve, we examined the water quality along the coast, applying the biotic index Macroinvertebrate Index of Pampean-Rivers (mipr) (40, 41, 42); based on the sum of the values of ecologic sensitivity (vx), varying from 0 to infinity with the lowest values corresponding to highly disturbed environments:

$$MIPR = \sum_{X=1}^{N} (VX)$$

Taxa   *Sites*

The relationships between species and environmental variables were examined by Canonical-Correspondence Analysis (CCA) (43, 44, 45, 46), with the statistical package MVSP 3.1 (47).

Of the 71 taxa found, only those being represented by more than 1% of the total invertebrates collected were considered (48) ~~along with the 15 most abundant and most frequent species~~ in conjunction with the corresponding 4 most relevant environmental variables. ~~For the cca, t~~The average value of each environmental variable was used together with the abundance data of the species from the 8 sampling sites. A Pearson correlation analysis was performed and the significance values tested by the student t Test.

The associations between species and sites were examined by the grouping-analysis technique Unweighted Pair Group Method with Arithmetic Mean (UPGMA), through the application of the Jaccard index (49), with the species considered being the same as those used for the CCA.

3. RESULTS

3.1. RELATIVE ABUNDANCE

For estimating the relative abundance of the benthic macroinvertebrates present, a total of 71 taxa were collected (as shown in table 1 and fig. 2). At site 1 (see fig. 2, a), in the autumn 1995, the only taxa recorded were the gastropods and bivalves; with the highest abundances represented by *Heleobia piscium* (~~d'orbigny~~d'Orbigny), *Biomphalaria straminea* (~~dunker~~Dunker) *Hebetancylus moricandi* (~~d'orbigny~~d'Orbigny), *Uncancylus concentricus* (~~d'orbigny~~d'Orbigny), and *Corbicula fluminea* (~~müller~~Müller).

Table 1. List of the benthic taxa (presence-absence) of the Reserva Natural Isla Martín García during the studied period.

Comment [U12]: I presume that all abundant and frequent taxa had more than 1% of relative abundance. In this case, you shoould only say «only those being represented by more than 1% of the total invertebrates collected were considered (XX)»

Comment [U13]: What did the authors consider «relevant environmental variables»?

Comment [U14]: Why did use this analysis? CCA was enough to demostrate the association of spp with abiotic variables.

Comment [U15]: Descriptor names always capitalized!

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	1	2	3	4	5	6	7	8
Nematoda	x	x	x	x	x	x	x	x
Turbelaria	x			x			x	
Annelida								
Hirudinea	x					x	x	
Oligochaeta								
Pristina rosea	x			x		x	x	x
P. breviseta							x	x
P. osborni				x		x	x	x
P. longiseta				x			x	x
P. aequiseta						x	x	
P. acuminata				x			x	
Amphichaeta leydigi	x			x		x	x	x
Eiseniella tetraedra						x	x	x
Nais variabilis	x					x	x	x
Slavina evlinae	x						x	
Stephensoniana trivandrana	x					x	x	x
Aulophorus furcatus							x	
Dero obtusa							x	
D. sawayai							x	
Chaetogaster diastrophus				x		x	x	x
C. diaphanus							x	x
Stylaria lacustris							x	x
Narapa bonettoi	x			x		x		
Limnodrilus hoffmeisteri	x			x		x	x	x
L. udekemianus	x						x	x

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Taxon	C1	C2	C3	C4	C5	C6	C7	C8
Aulodrilus pigueti	x					x	x	x
Aelosomatidae	x			x		x	x	x
Enchytraeidae	x			x		x	x	x
Mollusca								
Pisidium sterkianum		x			x		x	
P. taraguyense			x					
Limnoperna fortunei		x			x	x	x	
Corbicula fluminea	x	x	x	x	x	x	x	x
Heleobia parchapii	x	x	x		x	x	x	x
H. piscium	x				x	x	x	
Uncancylus concentricus	x		x	x		x	x	
Hebetancylus moricandi	x	x	x	x	x	x	x	
Potamolithus bushii	x	x	x	x	x	x		
P. lapidum		x	x		x			
P. agapetus		x	x		x			
Pomacea canaliculata		x	x		x		x	x
P. megastoma								x
Asolene platae	x	x	x		x	x	x	x
Biomphalaria straminea			x		x		x	
Drepanotrema kermatoides					x		x	
D. depressisimun					x			
Chilina fluminea		x			x	x		
Ch. megastoma			x					
Ch. rushi			x					
Stenophysa marmorata			x					
Tardigrada	x	x				x	x	x

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|	1	2	3	4	5	6	7	8
Ostracoda								
Cytheridella argentinensis		x		x		x	x	x
Limnocytere paranensis	x	x		x	x			x
Darwinula stevensoni	x			x		x	x	x
Iliocypris gibba	x			x		x	x	x
Cypridopsis vidua						x		
Isopoda								
Pseudophaeroma platense	x	x	x	x	x	x		x
Peracarida								
Claudicuma platensis	x	x		x				
Sinelobus stanfordi	x			x		x	x	x
Amphipoda								
Hyalella curvispina		x	x			x	x	x
Decapoda								
Aegla uruguayana				x		x		
A. platensis		x		x				
Diptera								
Chironomidae	x	x		x	x	x	x	x
Ceratopogonidae	x	x				x	x	x
Culicidae		x	x					x
Coleoptera								
Psephaenidae		x	x	x	x	x		
Elmidae		x	x					x
Ephemeroptera								
Baetidae		x	x		x	x		x
Leptophlebiidae		x				x		

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Hemiptera						
Pleidae	x				x	x
Odonata						
Coenagrionidae		x	x			x
Protoneuridae		x	x			
Libelullidae					x	
Trichoptera						
Hydroptilidae		x			x	

During the winter 1995, the ~~nematoda~~ Nematoda predominated with lower relative abundances of Turbelaria and Oligochaeta, the last of these represented by the family Enchytraeidae plus a further 4 species among which *Limnodrilus hoffmeisteri* Claparède of the Tubificidae was the most abundant. The bivalves were represented by *C. fluminea*; and *Sinelobus stanfordi* (Richardson) was the most abundant crustacean, along with certain members of the phylum Tardigrada in addition to larvae of the Diptera Chironomidae. In spring of 1995, in addition to the benthos sampling, the reeds were surveyed by means of the capture-per-unit-of-effort technique. Of the benthic macroinvertebrates, the highest relative abundance corresponded to the crustaceans, the copepods, and the ostracod *Darwinula stevensoni* (Brady & Robertson) followed by the Chironomidae and *Narapa bonettoi* Righi and Varela (Oligochaeta). The Nematoda and the ostracods exhibited essentially the same abundance, while the harpacticoid and calanoid copepods likewise manifested similar abundances. The results of the capture per unit of effort demonstrated a clear predominance of gastropods such as *Potamolithus buschii* (Fraunfeld), *U. concentricus*, and *H. piscium*. For the summer 1996, the highest relative abundances corresponded to the Nematoda, the bivalve *C. fluminea*, and the Chironomidae.

At site 2 (as shown in fig. 2, b), in the summer 1995, the gastropods *Potamolithus agapetus* Pilsbry, *Potamolithus lapidum* (~~d'orbigny~~d'Orbigny), and Chironomidae —contributed the highest relative abundances. During the autumn, the bivalves *Limnoperna fortunei* (Dunke) and *C. fluminea* became prominent in addition to the gastropods *B. straminea* and *Chilina megastoma* Hylton Scott. In the spring of that year, the gastropods *P. agapetus* and *P. buschii* manifested the highest relative abundances followed by the isopod *Pseudosphaeroma platense* (Giambiagi). The summer 1996 was characterized by the presence of gastropods at the greatest relative abundance, particularly *P. buschii*, *P. agapetus*, and *H. moricandi*.
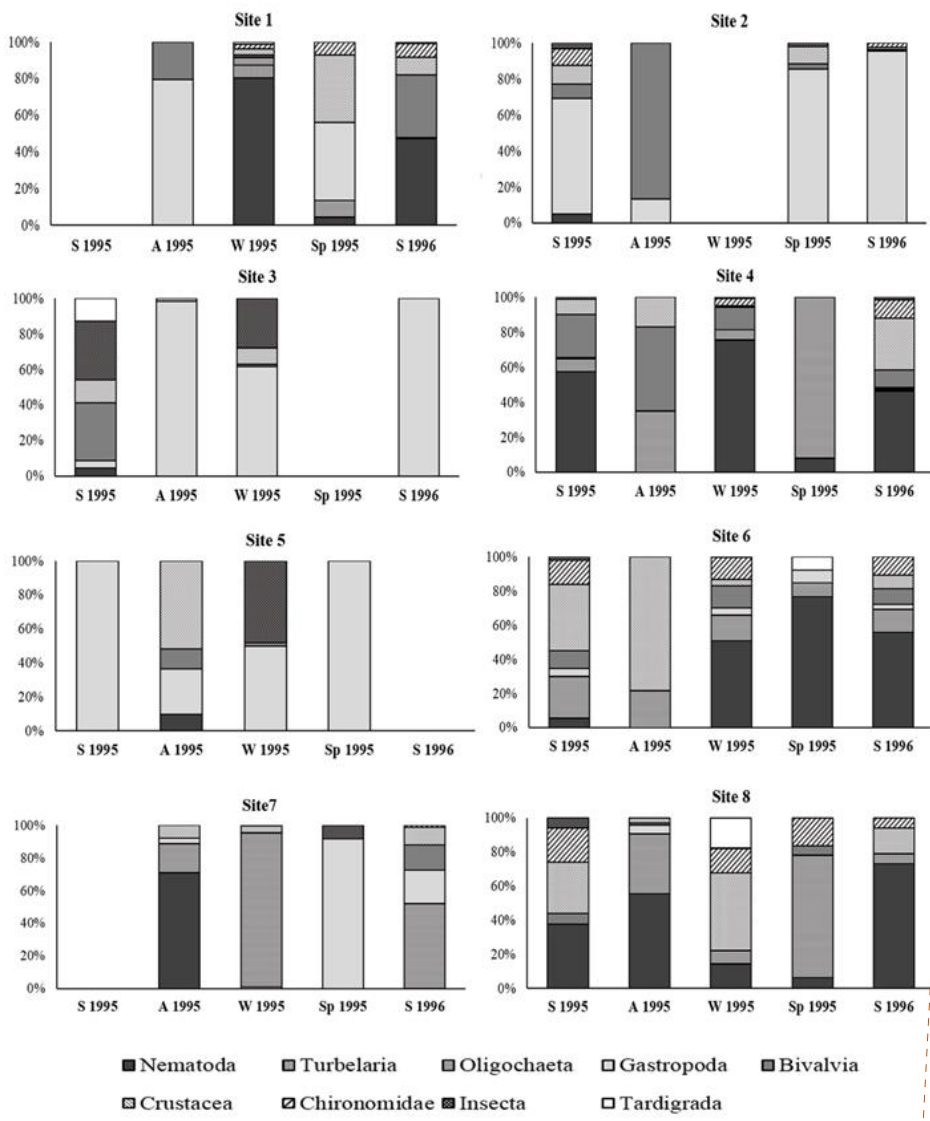
Fig. 2. Temporal changes in abundance of the most relevant taxa recorded at the sampling sites during the study.

At Site 3 (see Fig. 2, C) in the summer 1995, *C. fluminea* was the species of greatest

relative abundance, followed by *P. platense* and the insects represented by the Coleoptera Psephenidae and Elmidae. In the fall, 1995 a predominance of gastropods (96%) featured *Drepanotrema depressissimum* (Moricand) as the species of highest abundance followed by *Stenophysa marmorata* (Guilding) and *Pomacea canaliculata* (Lamark). In the winter 1995, gastropods, and particularly to *Heleobia parchapii*, manifested the highest relative abundance along with the insects, especially represented by the predominance of the Psephaenidae. The other species of gastropods were *B. straminea* and *Chilina rushi* Pilsbry along with the amphipods *Hyallela curvispina* Shoemaker and *S. marmorata* at similar densities. In the spring, no taxa were found. In the summer 1996, only the gastropods *P. agapetus*, *H. moricandi*, and *B. straminea* were recorded, and of these three the first exhibited the greatest relative abundance.

At Site 4 (as shown in Fig. 2, D), in the summer 1995, the highest abundances corresponded to the Nematoda, *C. fluminea* and immature oligochaetes of the Tubificidae. In autumn, the bivalve *C. fluminea* contributed the greatest relative abundance, followed by *N. bonettoi* and the ostracod *Limnocythere paranensis* Ferguson. During the winter, the Nematoda again predominated followed by *C. fluminea*, *N. bonettoi*, and the Chironomidae. In the spring, *N. bonettoi* predominated over a background of lower-abundance groups. Whereas, in the summer 1996, the macroinvertebrates with high relative abundances were the Nematoda, harpacticoid copepods, and Chironomidae plus the bivalve *C. fluminea* and the ostracod *L. paranensis*.

At Site 5 (see Fig. 2, E), in the summer 1995, only gastropod molluscs were recorded with the highest relative abundance represented by *P. buschii* followed by *H. moricandi*, *B. straminea*, *P. canaliculata*, and *Drepanotrema kermatoides* (D'Orbigny). In the autumn, the crustacean copepods of the order Cyclopoida were dominant over the gastropods *H. piscium* and *C. fluminea*. In the winter, the Coleoptera –Psephenidae along with the gastropods *P. buschii* and *P. agapetus* exhibited a greater relative abundance, with those latter species being present at comparable relative densities. In the spring, only the gastropods were

registered, with H. parchapii and *H. piscium* having the highest abundances. No records were obtained during the summer of 1996.

At Site 6 (see Fig. 2, F), in the summer 1995, the tanaids *S. stanfordi*, the Chironomidae family, and the bivalve *C. fluminea* were the most abundant. In autumn, the greatest abundance was registered with the crustaceans *H. curvispina* and *S. stanfordi* followed by the Enchytraeidae oligochaetes. In winter, the Nematoda predominated followed by the family Chironomidae, *C. fluminea*, and *Amphichaeta leydigi* Tauber. The spring was characterized by a clear predominance of the Nematoda; whereas *N. bonettoi*, *H. piscium*, and the species of Tardigrada present exhibited similar, though lesser abundances. For the summer 1996, the Nematoda manifested a still greater relative abundance followed by the Chironomidae, the oligochaete *Stephensoniana trivandrana* (Aiyer), and the bivalve *C. fluminea.*

At Site 7 (as shown in Fig. 2, G), in the fall 1995, the greatest relative abundance was registered with the Nematoda. During the winter, the oligochaetes *A. leydigi* and *N. variabilis* predominated. *H. parchapii* was the species that predominated, followed by the insects Pleidae. Whereas, in the summer 1996, *S. trivandrana* reached the highest relative abundance followed by *P. agapetus*, *L. fortunei*, *Dero sawayai* Marcus, the cyclopoid copepods, and *L. hoffmeisteri*.

At Site 8 (see Fig. 2, H), in the summer 1995, the Nematoda were distinguished by their high relative abundance, followed by the Chironomidae, Cyclopoida copepods, the ostracod *D. stevensoni*, and the isopod *P. platense*. In the fall, along with a notable abundance of S. stanfordi followed by the harpacticoid copepods and the oligochaetes *L. hoffmeisteri*, species of the Enchytraeidae, and *Nais variabilis* Piguet; ostracod species such as *D. stevensoni*, *C. argentinensis*, and *Iliocypris gibba* Brady & Norman contributed to the assemblage at a relative abundance of 10%. In the winter, the harpacticoid copepods exhibited the highest relative abundance followed by the Tardigrada, Nematoda, Chironomidae, Cladocera, and the ostracod *D. stevensoni.* In the spring, the species with

the highest relative abundances were *L. hoffmeisteri*, *Aulodrilus pigueti* Kowalewski, and those of the Chironomidae. Whereas, in the summer 1996, the Nematoda manifested the greatest relative abundance followed by the Chironomidae, the calanoid copepods and the cyclopoids.

For Sites 1 and 7 we have no records for the summer-1995 campaign because the sudden rise in the tide prevented any sampling. Furthermore, at Site 2 no organisms were registered during the winter of 1995.

3.2. Macroinvertebrates density

The density of the benthic macroinvertebrates ranged from 15 to 58,800 ind.m^{-2}. The ANOVA revealed significant differences between the values for the average annual population density (log10 density) of macroinvertebrates among the five sites included in the analysis (F: 3.059; p <0.05). The highest average population density was recorded at Site 8 (38,700 ± 19,000 ind.m–2.) (Fig. 3).
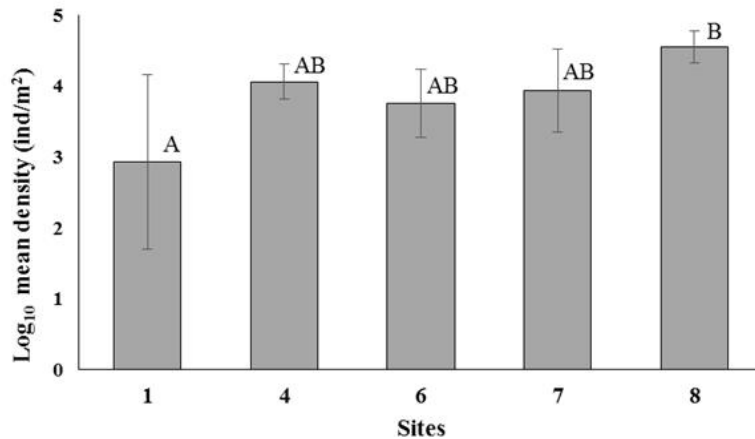
Fig. 3: Mean population density (log10 density) of benthic invertebrates in five study sites of the natural reserve of Martín García island. Bars represent the mean values and lines

indicate standard deviation. Bars with at least one letter in common don´t differ statistically (p>0.05).

3.3. Community parameters

The community parameters (see table 2) of diversity (H'), equitability (J), and species richness (S) were minimum and maximum, respectively, for H' at 0.77 and 2.2 bits at sites 2/3 and 3 (summer 1996) and at 2.16 bits at site 8 (winter 1995); for J at 0.16 at site 4, (spring 1995) and 1.02 at site 2 (autumn 1995); and for (S) at 3 at site 3, (summer 1996) and 29 at site 7 (autumn 1995).

Comment [U19]: You already mentioned the maximum value (2.2. at site3)

Community parameters	Seasons*	Sites							
		1	2	3	4	5	6	7	8
Species richness	S-95	–	13	11	16	7	25	–	17
	A-95	5	6	9	8	15	12	29	25
	W-95	20	–	13	13	6	26	24	26
	Sp-95	15	7	-	9	7	5	10	9
	S-96	13	7	3	17	16	13	23	11
Diversity index	S-95	–	1.29	2.08	1.33	1.59	2.10	–	1.96
	A-95	1.44	1.83	1.49	1.26	1.82	1.56	1.53	1.47
	W-95	1.00	–	2.2	1.09	1.27	1.62	1.35	2.16
	Sp-95	1.95	1.14	-	0.35	1.29	1.13	0.92	1.76
	S-96	1.38	0.77	0.77	1.6	1.76	1.57	1.95	1.12
Evenness index	S-95	–	0.50	0.87	0.48	0.82	0.65	–	0.69
	A-95	0.90	1.02	0.68	0.61	0.67	0.63	0.46	0.46
	W-95	0.33	–	0.86	0.42	0.71	0.50	0.43	0.66
	Sp-95	0.72	0.59	-	0.16	0.66	0.70	0.40	0.80
	S-96	0.54	0.40	0.70	0.56	0.63	0.61	0.62	0.47

Table 2. Community parameters (species richness, S; diversity, H′, and evenness, J′) for the eight aquatic sampling sites on Martín García island, analyzed seasonally (summer 1995 through summer 1996); *s, summer; a, autumn; w, winter; sp, spring.

3.4. Cluster Analysis

The results of the UPGMA cluster analysis (as shown in Fig. 4) indicated the existence of two main groups of species. The first was composed of *P. agapetus*, *P. buschii*, *N. bonetoi*, the Chironomidae, *H. parchapii*, and *C. fluminea* at a J = 0.60 that combined with the second group containing *Cytheridella argentinensis* and *Chaetogaster diastrophus* along with *D. stevensoni*, *A. pigueti*, *S. trivandrana*, *N. variabilis*, *S. stanfordi*, *L. hoffmeisteri*, and *A. leydigi* at a maximum similarity index of J = 0.80.
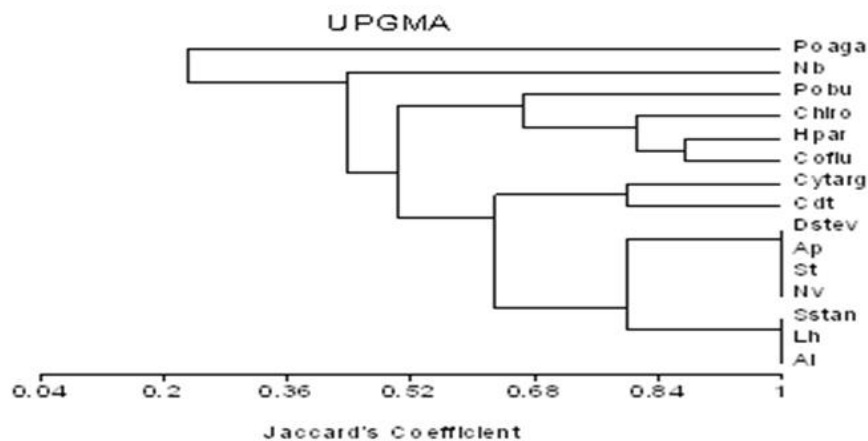
Fig. 4. Results of Cluster Analisys (UPGMA): Po aga, *P. agapetus*; N b, *N.* bonettoi; Po bu,

P. bushii; Chiro, Chironomidae; H par, *H. parchapii*; Co flu, *C. fluminea*; Cyt arg; *C.*

argentinensis; C cdt, *C. diastrophus*; D stev, *D. stevensoni*; A p, *A. piguetti*; S t, *S.*

trivandrana; N v, *N. variabilis*; S stan, *S. stanfordi*; L h, *L. hoffmeisteri*; A l, *A. leydigi*.

3.5. Average Aquatic Variables

During the study period, the average values for the environmental variables measured were: water temperature, 23.4 °C (SD = 9.08); dissolved-oxygen concentration 6.77 mg.L^{-1} (SD = 2.93); conductivity 135 µS.cm^{-1} (SD = 30.1), and pH 7.35 (SD = 1.33).

3.6. Canonical Correspondence Analysis Results

In the canonical-correspondence analysis (see Fig. 5, Table 3), the arrows in the figure represent the environmental variables and point to the maximum variation in the indicated parameter, with the length of each arrow being proportional to the magnitude of that variable in the ordering diagram. According to this analysis, the environmental variables that underwent the greatest fluctuation during the study period were the dissolved-oxygen concentration, the pH, the temperature, and the conductivity of the water. These results suggest that the distribution of the species is related to the physicochemical conditions of the water. Of the correlation between the species present and the environmental variables,

Comment [U20]: 1.These data are quite general. You should at least present the mean values for each sampling site, allowing comparison among them.
2.How about the variables: % of Oxygen Saturation and Concentration of Dissolved Solids? You mentioned them in M&M but did not present their values.

95.2% is distributed on Axis 1 of the ordering diagram (see Table 3, Fig. 5). The dissolved-oxygen concentration, the pH, and the temperature of the water correlated significantly with each other; but not with the electrical conductivity, which ~~parameter~~ became negatively related to these variables. The dissolved-oxygen concentration and the temperature were correlated with sites 4 and 2, respectively; whereas the pH became associated with sites 3 and 6 in the ordering diagram.

> **Comment [U21]:** Axis 1 or axis 2? Positively or negatively? Describe better the CCA

	Axis 1	Axis 2	Axis 3
Eigenvalues	0.112	0.038	0.14
Percentage	41.716	14.343	5.074
Cum. Percentage	41.716	56.059	61.133
Cum. Constr. Percentage	66.501	89.366	97.454
Spec .env. Correlations	0.952	0.824	0.595

Table 3. Main Results from the Canonical correspondence analysis (CCA)
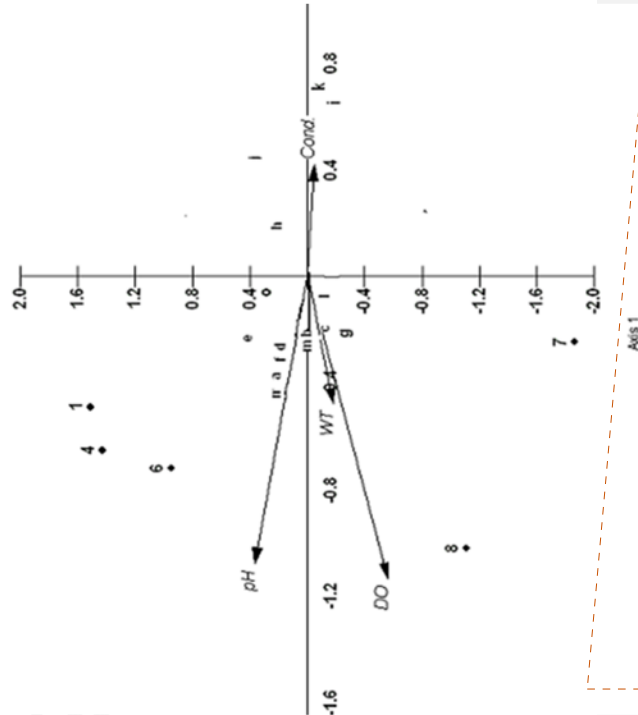
Fig. 5. Canonical-correspondence-analysis diagram: 15 species, and 4 environmental variables. Abbreviations: a, *A. leydigi*; b, *N. variabilis*; c, *S. trivandrana*; d, *C. diastrophus*; e, *N.bonettoi*; f, L. hoffmeisteri; g, *A. pigueti*; h, *C. fluminea*; I, *H. parchappii*; j, *P. buschii*; k, *P. agapetus*; l, *C. argentinensis*; m, *D. stevensoni*; n, *S. stanfordi*; o, Chironomidae. Sampling sites: 1–8

Table 4. Pearson correlations among four environmental variables, total number of species (ns), and the fifteen most abundant taxa analyzed.

Variable	T °C	DO	Conductivity	pH	NS
SN	0.06	**0.71	–0.23	**0.65	1
A l	–0.13	**0.53	0.06	0.07	**0.65
N v	–0.30	0.30	0.29	–0.17	**0.42
S t	–0.28	*0.35	0.26	–0.14	**0.45
Cd t	–0.17	0.32	0.07	**0.41	**0.49
N b	–0.05	0.11	**0.49	0.18	–0.03
L h	–0.15	**0.48	0.13	0.00	**0.56
A p	**0.69	**0.63	**–0.42	**0.54	*0.34
Co flu	–0.23	0.23	0.31	0.18	0.20
H par	**–0.45	–0.27	**0.61	**–0.55	0.01
Po bu	**–0.44	**–0.48	0.08	–0.30	**–0.47
Po aga	**–0.57	–0.04	0.21	**–0.44	–0.14
Cy targ	–0.11	*0.39	0.04	0.15	–0.02
D stev	**0.68	**0.64	**–0.41	**0.54	*0.36
S stan	**0.45	**0.68	**–0.56	**0.55	**0.56
Chiro	**0.45	**0.68	**–0.61	**0.59	**0.49

T, temperature; Do, dissolved-oxygen concentration

The numbers indicating strong correlations (*$p < 0.05$, **$p < 0.01$), where n = 40 for all variables. The abbreviations are the same as in the legend to Fig. 4.

3.6. Functional Feeding Groups

As to the functional feeding groups (see Fig. 6), at all the sites and in all the seasons of the two years; the gathering collectors predominated, followed by the scrapers, filtering collectors, shredders, and predators.
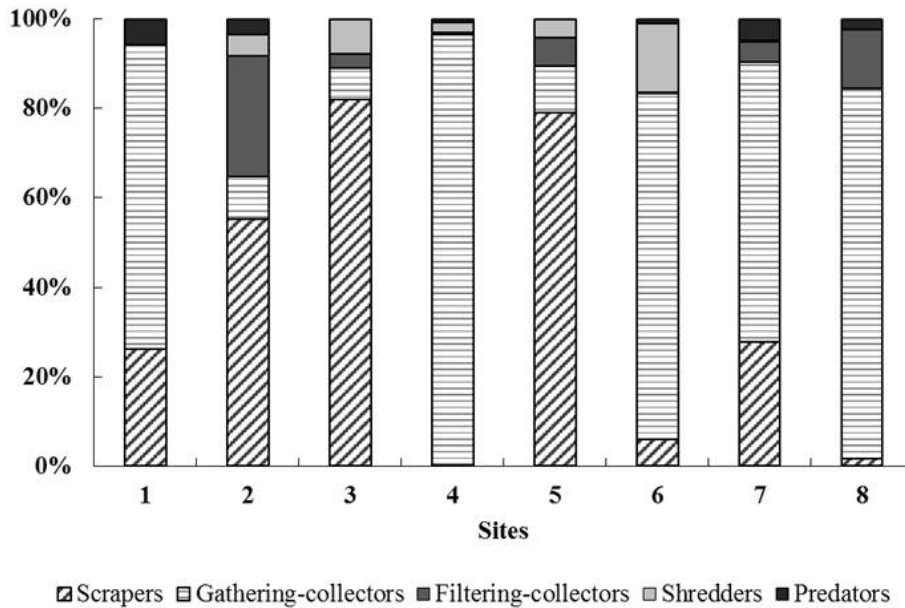
FIG. 6. Functional feeding groups of the benthic community.

3.7. Macroinvertebrate Index of Pampean-Rivers (MIPR)

Calculations of the MIPRS (see Fig. 7) at 6 of the 8 sampling sites—with sites 2 and 3 and

sites 3 and 4 being combined because of their proximity and intermixing during high tides—

gave maximum and minimum values of 19.2 (i. e., a very low degree of pollution to none at

all) and 1.90 (i. e., a strong contamination). Site 1 exhibited a moderate level of

contamination (at 3.00) in the winter 1996 and a considerably lower degree in the

following autumn (at 8.25) along with somewhat higher levels (5.85 and 7.00) in the spring

and early summer of that year. Site 3 manifested an extremely low pollution to none at all at

16.1 (summer 1995), a modest degree in autumn and winter at 8.80 and 9.30, respectively,

and a considerably higher level at 4.40 and 5.20 in autumn and winter, respectively;

whereas, in the spring and summer 1996, similarly high respective degrees of pollution were

recorded at 4.40 and 5.20. At site 4, the contamination was extremely low to none at all at from 13.8 to 19.2) in summer and autumn 1995, modest in the winter and summer 1996 (at 8.20 and 9.30, respectively), and higher in the spring of that year (6.60). The contamination at site 6 was only very slight at 12.4 in the summer 1995, somewhat greater at 6.10 in autumn of that year, once again quite low at 10.9 in the winter, high (2.10) in the spring, and finally much lower at 5.75 in the summer 1996. Site 7 was usually substantially free of contamination at values between 8.25 and 11, while site 8 exhibited comparable levels in the summer 1995 at 7.80 along with progressively lower degrees at 8.15 in the following autumn and 11.1 in the winter, though manifesting a high value of contamination (2,4 and 1,5) in the spring of that year.
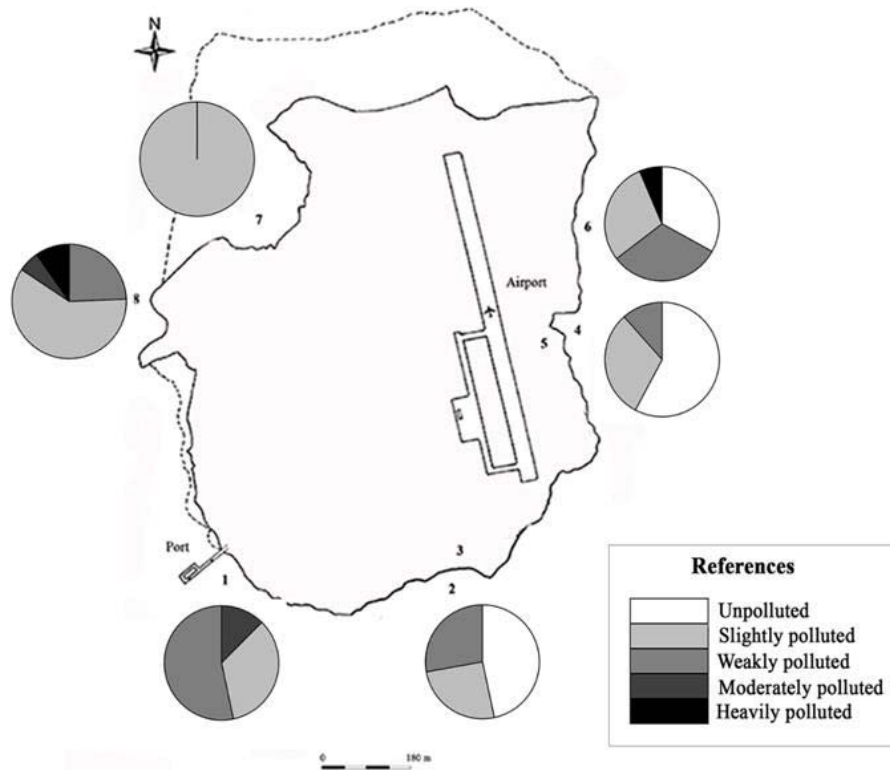
Fig. 7. Percent representation of each category established by the ~~MAcroinvertebrate~~ Macroinvertebrate INDEX of Pampean Rivers (MIPR) for the sampling sites during the study period. Sites 2 and 3 and sites 3 and 4 are represented in single pie charts because of their proximity and intermixing during high tides.

4. DISCUSSION.

In the Martín García Island Nature Reserve, the most abundant and representative macroinvertebrates in the littoral sites and seasons were the Nematoda, the oligochaete annelids, and the gastropods and bivalves among the Mollusca. The Nematoda evidenced a conspicuous representation at sites 1, 4, and 6 through 8 (see Table 1) and were found

Comment [U25]: The discussion section should be re-elaborated. Most of the text describe the results found (what should be presented only at previous section).

mainly in summer, autumn, and winter.

The Oligochaeta constitute an abundant group within the benthic organisms because the hydrosedimentologic dynamics of this and other great rivers create a wide heterogeneity of habitats as reflected in the spatial and temporal structure of the benthic communities (49, 47). This subclass has been recorded in almost all freshwater environments and is particularly abundant in the Paraná-Plata basin (51, 52, 53, 54, 55, 56, 57).

In the littoral of this Nature Reserve, these annelids were represented by 21 species in addition to the families Aelosomatidae and Enchytareidae at sites 4 and 6 through 8 (Table 1 and Fig. 2); of the total taxa 19 species, Aelosomatidae and Enchytraeidae were present at Site 7 during the autumn and winter 1995 and the summer 1996. This Site is characterized by a great development of grasslands, reeds, and a total of 16 species of hydrophytes (2). At Site 8—it containing a major development of reedbeds associated with mixed sediments of sand and silt—14 species of oligochaetes and the families Aelosomatidae and Enchytraeidae were recorded.

The oligochaete abundances were higher in autumn and winter 1995. Moreover, as has also

been observed in areas of the Paraná River, *N. bonettoi* was linked to sandy sediments (58, 49, 56, 59); and particularly at Site 4—that area characterized by medium and fine sands free of vegetation (9)—the species reached a relative abundance of 35% in the autumn as well as one of 98% in the spring of that year. In studies conducted by Ambrosio (39) covering a sector of the southern coastal strip along the Argentine side of the Río de la Plata estuary, all the species mentioned for the Martín García Island were registered; and their findings along the coast confirmed that the majority of oligochaete species is linked to areas with vegetation, such as was found at the present sites 7 and 8.

The portion of the Río de la Plata estuary included in the Brazilian subregion (60) has been found to exhibit one of the highest species-richness values of Gastropoda (31) (61). The mollusks on the Martín García Island (Table 1, Fig. 2)—represented by 21 species, 4 of the Bivalvia and 17 of the Gastropoda, that were found at all the sampling sites and in all the

seasons—along with the oligochaetes, constituted the most abundant and diverse of the macroinvertebrate groups registered in the present survey. Ambrosio (39) had cited 10 species of gastropods linked to soft sediments and vegetation plus 4 species of bivalves along the coast. On the island, in the present work, species of the families Chilinidae and Lithoglyphidae were also registered adhering to the rocky substrate.

The Tardigrada were found at sites 1, 2, and 6 through 8; though always in low percentages except for Site 8 in the winter of 1995 (then at 17%). In addition to the Copepoda Cyclopoida, Calanoida, Harpacticoida, and Cladocera, eleven crustacean species were recorded at all sites and in all the seasons (see Table 1, Fig. 2); manifesting the highest abundances at sites 4, 5, and 6 through 8. Especially abundant at Site 6 was *S. stanfordi* (at 42% in the summer of 1995 and 30% in the autumn, though only 3% in the winter, which species was linked to the sediment and the presence of reeds, as mentioned by (39) for the Argentine coast.

The crustacean *Claudicuma platensis* (Cumacea) was recorded at only Sites 1, 2, and 4 and always at abundances less than 1%. Five species of Ostracoda were registered at Sites 1, 2, and 4 through 8 in 1995: *C. argentinensis* (4%) at Site 4 and *D. stevensoni* (13%) and *I. gibba* (1%) at Site 8 in summer along with *L. paranensis* (14%) at Site 4 and *D. stevensoni* (2%) at sites 7 and 8 in autumn. Members of the Ciprididae and Darwinulidae were recorded both in vegetated sites and at Site 4 having sandy sediments. *Pseudosphaeroma platense* exhibited a maximum relative abundance of 10% in spring 1995 at Site 1, there also in relation to the reedbed of that part of the island. The amphipod *H. curvispina* was recorded in both vegetated and nonvegetated sites. Among the decapod crustaceans, *A. uruguayana* was collected with a crab net at sites 4 and 6, while *A. platensis* was recorded at sites 2 and 4 along with members of the genus *Temnocephala*, commensals of that species (7).

Twelve families of insects belonging to 6 orders were identified (Table 1). The larvae of Chironomidae dipterans were recorded at all the sites except Site 3, with the highest relative abundance corresponding to the winter season at Sites 6 and 8, there in association

Comment [U28]: Results

Comment [U29]: results

Comment [U30]: results

with mixed sediments and a vegetation of *S. californicus*. The coleopteroid famiily Psephenidae and the ephemeropteran family Baetidae manifested relatively higher distributions at those sampling sites. The families Coenagrionidae, Psephenidae, Baetidae, and Elmidae in combination were present at a relative abundance of 33% at Site 3 in summer 1995; the Pleidae and Ceratopogonidae together at one of 6% at Site 8 in that same summer; the Psephenidae at one of 26% at Site 3 and 48% at Site 5, both in the winter; and the Pleidae at one of 8% at Site 7 in the spring. The remaining families were at relative abundances lower than those cited. All of these families of insects have been observed on the island, had been cited on the sector of the coast studied by (39), except for the Psephenidae, whose larvae inhabit littoral rocks.

With regard to species richness, 20 were registered at Site 1 in the winter and 22 at Site 2 in the summer 1995. Athough sites 3 through 5 contained lower numbers, sites 6 though 8 exhibited a greater richness—especially in autumn, winter, and spring. These sites offered a greater refuge for the development of benthic macroinvertebrates because of the presence of mixed sediments (silt plus sand) and an abundant development of hydrophytes. The diversity—as in other southern-coastal-strip sectors of the Río de la Plata—was higher wherever a greater differentiation of habitats occurred in association with marsh vegetation (22). In contrast. the equitability varied in accordance with the climatic conditions and seasons.

Within the functional feeding groups (Fig. 5), the macroinvertebrate gathering collectors were represented in the benthos of the island's littoral by the oligochaete, nematode, crustacean and insects taxa. With the last two of these groups exemplified by the harpacticoid copepods, ostracods, *C. platensis*, the amphipod *H. curvispina*, and the tanaidacean *S. stanfordi*; among the insects, the Elmidae family of the order Coleoptera plus certain Chironomidae of the Diptera, the Hydroptilidae of the Trichoptera, and Baetidae of the Ephemeroptera were present. This feeding category characterized more than 80% of the macroinvertebrates at sites 1, 4, and 6 through 8 in almost all the seasons,

an observation that had been made previously for different lotic environments of the Paraná-River basin (49, 62, 63,). Ambrosio (39) reported for the coast of Punta Lara and La Balandra within the southern coastal strip, dominances of such gathering collectors at higher than 80% in sediment-free vegetation. On the Martín García Island, this situation occurs in both the free sediments (*e. g.*, at Site 4) and the vegetated ones (*e. g.*, at sites 1 and 6 through 8).

The scrapers, represented principally by gastropod mollusks, were found in a large percentage at sites with rocky substrates such as Site 2 (68–97%), Site 3 (77–100%), and Site 5 (52–99%}. Sites 1 and 7 (those vegetated) had respective abundances of 55 and 82% during most of the seasons. Pavé & Marchese (62) had reported very few macroinvertebrates belonging to this functional-feeding category in the environments of their study on the Paraná River. The investigations of (39), whose results coincided with those the previous authors cited for that sector of the Argentine coast of the Rio de la Plata, reported a scraper representation of 14%.

Comment [U33]: results

The filtering collectors were represented by the bivalve molluscs such as *L. fortunei*, *P. taraguyense*, and *P. sterkianum* along with certain cyclopoid and cladoceran copepods. The highest representations of filtering collectors were recorded at Site 2 at 67% (*e. g.*, *L. fortunei*, autumn 1995), Site 7 at 15% (*e. g.*, *L. fortunei*, *P. sterkianum*, and cyclopoids; summer 1996), Site 8 at 15% (*e. g.*, cladocerans, winter 1995), and Site 5 at 13% (*e. g.*, cladocerans and *P. sterkianum*, spring 1995). The filtering collectors were not registered at Site 1 and in the remainder were present at only low percentages. These overall percentages were similar to those reported by (63) and (39) for sites with the best water quality.

Comment [U34]: results

In the shredder category, the decapods *Aegla platensis* and *Aegla uruguayana* were recorded at sites 2 and 4 and at sites 4 and 6, respectively, in the summer, autumn, and winter. The predators, in contrast were characterized by low percentages (at a maximum of 15%) at sites 1, 2, 3, and 6 through 8—represented by turbelaries, hirudineans, *Hellobdella*

sp., oligochaetes such as *Chaetogaster diastrophus* and *C. diaphanus*, isopods such as *P. platense*, Ceratopogonidae dipterons, hemipterans, Pleidae, odonates, Coenagrionidae, and Libellulidae. Ambrosio (39) had reported similar values for those same coastal sampling sites.

The results of the UPGMA cluster analysis would appear to indicate that the coastal macroinvertebrate species of Martín García Island formed two main groups. Group 1— containing *P. agapetus*, *P. buschii*, *H. parchapii*, *N. bonetoi*, *C. fluminea*, and the Chironomidae—links those two species of *Potamolithus* to sites with rocky substrates (sites 2, 3, and 5) along with individual vegetated habitats—*i. e.*, Site 7 for *P. agapetus* and Site 1 for *P. buschii*. *Heleobia parchapii* was registered in all but 4 sites and the Chironomidae in all sites except Site 3; while *C. fluminea* was common to all the sites as an infaunal bivalve, the distribution of which is closely related to abiotic parameters such as the type of substrate (64). At a J = 0.60, all of these taxa are linked to the second group containing the ostracod *C. argentinensis* at sites 2, 4, and 6 through 8 plus the annelid *C. diastrophus* at sites 4 and 6 through 8 along with the rest of the species cited in the Results section that combine as a cluster with an index of maximum similarity (*i. e.*, J = 0.80).

With respect to the behavior of the species in the face of environmental variables, of the 15 species considered in this analysis, the majority were ordered close to the average dissolved-oxygen concentration, pH, and temperature; with *C. argentinensis*, the species located closest to the centroid of the diagram (as shown in Fig. 5), evidencing preferences for the mean values of the variables under consideration. *Darwinula stevensoni* (see Table 4) had a highly significant positive correlation (p <0.01) with the temperature, dissolved-oxygen concentration, and pH along with a negative association with the electrical conductivity. *Sinelobus stanfordi* (Tanaidacea) was sequestered like *D. stevensoni*. Of the Oligochaeta, *A. leydigi* exhibited a highly significant association with the dissolved-oxygen concentration; whereas, *S. trivandrana* also maintained a significant correlation with that parameter (see Table 4) (p <0.05); *C. diastrophus* was associated with the pH (p <0.01), and

N. bonettoi with the conductivity (p <0.01); *L. hoffmeisteri* was correlated with only the dissolved-oxygen concentration (r = 0.48); and *A. pigueti* was highly positively associated with the temperature, dissolved-oxygen concentration, and pH—but negatively so with the conductivity.

Among the mollusks, *H. parchapii* was highly positively correlated with the conductivity, but negatively associated with the temperature and the pH (see Table 4). *Potamolithus buschii* evidenced highly significant negative correlations with the temperature and the dissolved-oxygen concentration as did *P. agapetus* with the temperature and the pH. The Chironomidae family maintained high positive associations with the temperature, dissolved-oxygen concentration, and pH, but was negatively correlated with the \conductivity. The species richness, in general, varied positively and to a high degree with the dissolved-oxygen concentration and the pH.

The temperatures of the system were quite variable over time, being directly linked to the seasonal variation and the hydrodynamics of the ecosystem; whereas the average conductivity value in the littoral water of the island of *ca.* 135 μS.cm^{-1} was considerably lower than the average figure of 259 μS.cm^{-1} reported for the water of the southern coastal

strip. The guideline levels for quality as a function of the different uses of the island's resources, as indicated by the Use-IV range of parameters appropriate for the protection of the aquatic life indicated on the Water Quality meter of the (64), involve pHs of 6.5 to 8.5 and a dissolved-oxygen concentration of at least 5 mg.L^{-1}. The work reported here has confirmed that the average values of the environmental variables recorded on the island are still within that recommended range.

Living organisms are widely used as biotic indicators for the monitoring and evaluation of water quality, with the benthic macroinvertebrates being the most frequently recommended for aquatic ecosystems (66, 67, 68, 63, 69, 70, 71). In Argentina, the benthic entomofauna have been used to evaluate the water quality of rivers and mountain streams

by the application of the biotic indices Biological-Monitoring–Working-Party and Average-

Store-per-Taxon (72, 73, 74, 75, 76). In studies monitoring the ecologic quality of the Matanza-Riachuelo basin (Buenos-Aires province) indices involving the meso- and macroinvertebrates (40), the diversity (H'), and the deficit-of-species have been applied (77). Based on such observations, the MIPR was formulated specifically for the rivers of the Argentine pampas (40, 41). In the present study, we observed a variation in the quality and quantity of the meso- and macroinvertebrates, as evidenced upon application of that index, that reflected the effect of contamination in the system.

Along the littoral of Martín García Island, the MIPR values (see Fig. 7) fluctuated both among the sampling sites and during the seasons of the year: The highest values were observed in the summer and autumn 1995 at sites 2, 4, and 6 (very weak contamination down to none at all), with the value of Site 2 being particularly relevant since that area corresponds to the intake of water that flows directly to the water-treatment plant of the island supplying the resident population of the island (in years of the study, about 150 inhabitants) along with tourist contingencies that visit the island.

Comment [U42]: result

In general, we can state that the MIPR values ranged from only weak to weak-to-very-weak to zero pollution. Two exceptions, however, were registered at values reflecting strong contamination, both in the spring and at sites 6 (2.4) and 8 (1.9). At Site 6, this discrepancy could have resulted from the use of only 4 taxa with values of low ecologic sensitivity and a predominance of nematodes (at 77%). The explanantion for Site 8 is similar to that of Site 6, but with the further aggravating circumstance of the site's proximity to the island's garbage dump, whose refuse could have entered the water during high tides. The results of (39) are quite similar to those calculated for the island although with values indicating higher pollution levels.

Comment [U43]: results

The work reported here constitutes the first investigation considering the use of macrobenthic invertebrates as indicators of water quality in the Martín García Island Nature Reserve. These findings can serve as the basis for additional investigations that, after a lapse of 23 years, are being carried out on the current state of the island's community and

the quality of the internal and coastal water. We trust that these observations and conclusions will be of assistance in the development of the appropriate environmental-management policies by the relevant enforcement authorities.

5. CONCLUSION

A total of 71 taxa were collected in the Martín García island Nature Reserve during the study period. The most abundant and representative macroinvertebrates in the littoral sites and seasons were the nNematoda, the oligochaete annelids, and the gastropods and bivalves among the mollusca.

The density of the benthic macroinvertebrates ranged from 15 to 58,800 ind.m^{-2}. The ANOVA revealed significant differences between the values for the average annual population density of macroinvertebrates among the five sites included in the analysis (F: 3.059; p <0.05). The highest average population density was recorded at Site 8 (38,700 ± 19,000 ind.m^{-2}).

The community parameters of diversity (H'), equitability (J), and species richness (S) were minimum and maximum, respectively, for H' at 0.77 and 2.2 bits at sites 2 and 3 (summer 1996) and at 2.16 bits at site 8 (winter 1995); for J at 0.16 at site 4, (spring 1995) and 1.02 at site 2 (autumn 1995); and for (S) at 3 at site 3, (summer 1996) and 29 at site 7 (autumn 1995).

The results of the UPGMA cluster analysis indicated the existence of two main groups of species.

According to CCA, the environmental variables that underwent the greatest fluctuation were the dissolved-oxygen concentration, the pH, the temperature, and the conductivity of the

water. These results suggest that the distribution of the species is related to the physicochemical conditions of the water. Of the correlation between the species present and the environmental variables, 95.2% is distributed on Axis 1 of the ordering diagram.

As to the functional feeding groups, at all the sites and in all the seasons of the two years; the gathering collectors predominated, followed by the scrapers, filtering collectors, shredders, and predators.

Along the littoral of the island, the MIPR values fluctuated both among the sampling sites and during the seasons of the year: the highest values were observed in the summer and autumn 1995 at sites 2, 4, and 6 (very weak contamination down to none at all), with the value of site 2 being particularly relevant since that area corresponds to the intake of water that flows directly to the water-treatment plant of the island supplying the resident population of the island. In general, we can state that the MIPR values ranged from only weak to weak-to-very-weak to zero pollution.

The work reported here constitutes the first investigation considering the use of macrobenthic invertebrates as indicators of water quality in the Martín García Island Nature Reserve.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

As per international standard written ethical permission has been collected and preserved by the authors.

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