J. Mar. Biol. Ass. U.K. (2007), **87**, 377–381
Printed in the United Kingdom
doi: 10.1017/S0025315407050126

Ecological boundaries in estuaries: macrobenthic β-diversity in the Río de la Plata system (34–36°S)

D.A. Giberto*†§, C.S. Bremec*†, A. Cortelezzi‡, A. Rodrigues Capitulo*‡, and A. Brazeiro∫

*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). †Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP). ‡Instituto de Limnología 'Dr. Raul A. Ringuelet' (ILPLA). ∫Universidad de la República, Facultad de Ciencias, Uruguay. §Corresponding author, present address: Laboratorio de Bentos, Instituto Nacional de Investigación y Desarrollo Pesquero (INDEP), V. Ocampo no.1, B7602HSA Mar del Plata, Argentina. E-mail: diegogiberto@inidep.edu.ar

In this study, we analyse spatial patterns of macrobenthic communities of the Río de la Plata system, and assess the species turnover or beta diversity and its relationship with environmental gradients. Macrobenthic samples and physico-chemical parameters were collected from 20 sampling sites along a transect of 560 km, including the freshwater (FW), estuarine (ES) and marine (MA) sectors. Three main assemblages corresponding to the above mentioned sectors were defined with multivariate analysis (cluster, MDS). In total 134 taxa were recorded, 81 in MA, 33 in FW and 38 in ES, represented mainly by polychaete, mollusc and crustacean species. Depth, salinity and %clay showed the strongest correlation with the observed faunal patterns (ρ_w=0.62; BIO-ENV analysis). Beta diversity varied between dominant taxonomic groups and was positively correlated with changes in salinity. The high variability in the composition of assemblages was reflected in beta diversity, reaching its highest values at the boundaries between the defined sectors. This study suggests that beta diversity represents a useful tool to define ecological boundaries for benthic communities in the Río de la Plata.

INTRODUCTION

Ecological boundaries are defined as zones between contrasting habitat patches that delimit the heterogeneity of a landscape (Cadenasso et al., 2003). Understanding factors shaping species ranges is a central question in ecology and evolutionary biology (Holt & Keitt, 2005). This is particularly true for estuaries, which are recognized as transitional zones between freshwater and marine environments. Physico-chemical variables, such as the gradient of salinity from the sea to the river as well as the often-associated sedimentary changes or alterations in turbidity, have been typically used to establish apparently objective distinct zones in estuaries worldwide (McLusky, 1993; Elliot & McLusky, 2002). The separation of transitional areas of an estuarine system based on faunal communities remains more subjective (Elliot & McLusky, 2002). Several schemes were used, from those that consider the estuarine biota as a single entity separating marine and freshwater systems, to those that consider series of distinct communities along the estuary (Remane & Schlieper, 1971; Attrill & Rundle, 2002). If similarity in species composition between assemblages reflects similar ecological conditions, and environmental changes cause turnover in species composition, a beta diversity analysis could help to identify and to delimit ecological boundaries in estuarine systems. Within this context, two possible scenarios could be expected: (a) increasing values of species turnover at boundaries of freshwater and marine waters with the mixohaline system (a 'discrete' estuarine assemblage), or (b) increasing values of species turnover from sea or freshwater into the mixohaline zone (mixing of species, without an estuarine assemblage *per se*). Therefore, the purpose of this study is to provide a framework that combines a classical multivariate approach and beta diversity analyses, for the identification of ecological boundaries in estuaries, using data of macrobenthic assemblages of the Río de la Plata system.

MATERIALS AND METHODS

Sampling

Samples were collected from 20 sampling sites in the Río de la Plata system between October and November 2001. The transect covered approximately 560 km, throughout the freshwater, estuarine and marine environments (Figure 1). At each site, the following was collected:

Physical data: salinity (Practical Salinity Scale), temperature and depth with a Sea Bird-19 CTD (Conductivity-Temperature-Depth profiler), and three replicate sediment samples with a snapper Dietz-Lafond (0.02 m^2) for analysis of median grain size, sorting and composition (% fractions of sand, mud and clay). Additional details of sediment analysis are specified in Lopez Laborde (2002).

Biological data: faunal samples were collected from three replicates taken with a Van Veen grab (0.1 m^2) for soft bottoms and an epibenthic dredge (200×50 cm) for hard bottoms at one site, sieved through a 0.5 mm mesh to retain the macrobenthic fauna and then fixed on board in 5% formalin. In the laboratory, all invertebrates were identified to the lowest level possible and counted.

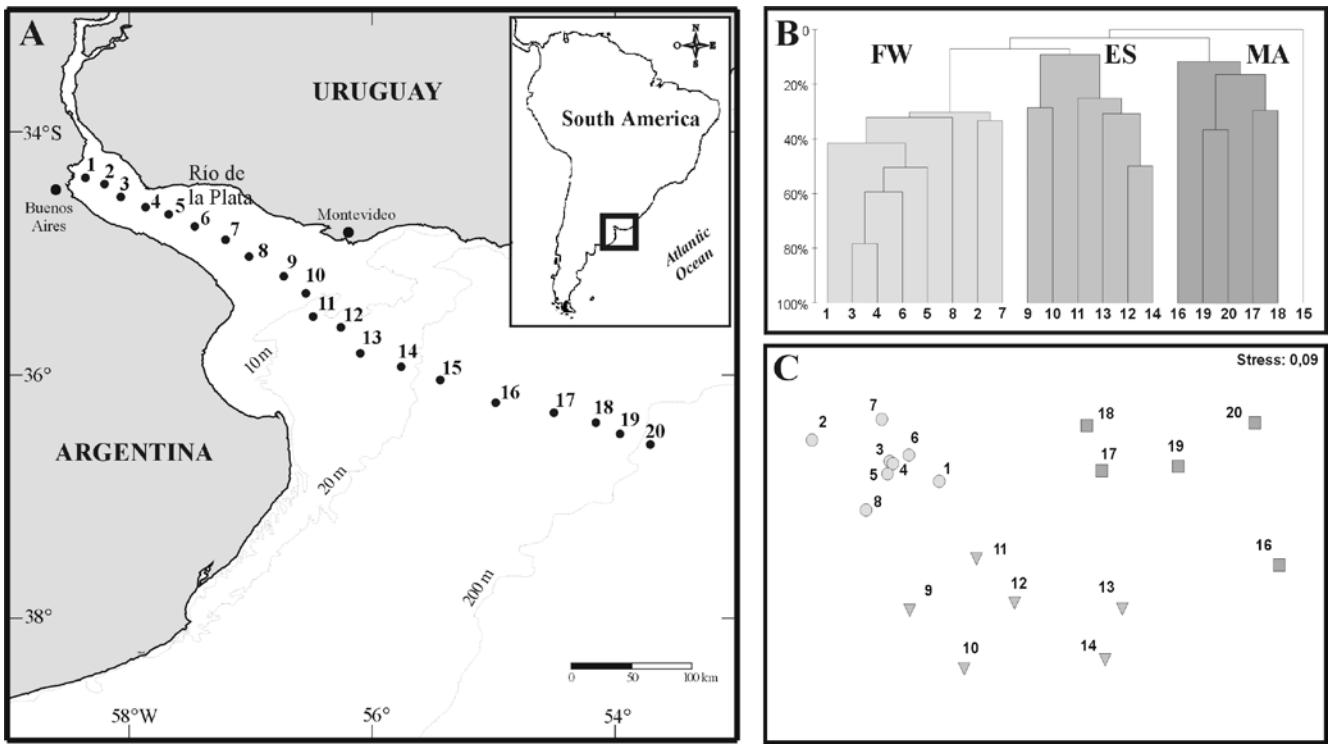

Figure 1. (A) The Río de la Plata system, with location of sampling sites (•) and isobaths (m); (B) classification (group average sorting of the Bray–Curtis similarity measure); and (C) MDS ordination, of stations using presence–absence data of species. Three major groups are indicated at a similarity level of 15%. Station 15 is not shown in the MDS plot because it was clearly separated from all the stations (see the cluster analysis). FW, freshwater (●); E, estuarine (▼); M, marine (■).

Data analysis

Classification (group average sorting of the Bray–Curtis similarity measures based on presence–absence data) and ordination (multi-dimensional scaling (MDS) on the above similarity matrices) methods were performed to define biological grouping of sampling sites (Field et al., 1982; Clarke & Warwick, 2001).

The BIO-ENV procedure was used to determine which set of environmental variables (similarity calculated with the Euclidean distance coefficient) best explains the biological matrices (presence–absence data, using Bray–Curtis similarity measure) (Clarke & Warwick, 2001). Prior to this analysis, a draftsman plot (scatter plots between pairs of environmental variables) was used to assess the linearity of the data and the inter-correlation between variables. Salinity, temperature, median size grain and sorting were transformed with the log(x+1). Log temperature and %mud were omitted from the BIO-ENV analysis because of very high degree of correlation with depth and % of sand fraction, respectively (Spearman's correlation coefficient $\rho_w > 0.9$). Multivariate analysis were carried out using PRIMER software (Clarke & Warwick, 2001).

Differences in environmental values among areas were analysed by a Kruskal–Wallis test followed by a Dunn test for multiple comparisons (Zar, 1984). A non-parametric approach was used because of significant data departures from homoscedasticity and normality.

Following Gray (2000), alpha diversity (sample species richness, SR_S) and gamma diversity (large area species richness, SR_L) were calculated for the 20 sampling sites and for the three major groups of sampling stations clustering together in the multivariate analysis. The three large areas constituted the largest scale studied (total area species richness, SR_T).

Several metrics for measuring beta diversity are distinguished: broad sense measures incorporating differences in composition attributable to species richness gradients, and narrow sense measures that focus on compositional differences independent of such gradients (Koleff et al., 2003). To investigate the patterns of turnover diversity in the transect, two approaches were used: (1) we analysed the differences in composition attributable to richness gradients with the single measure of Whittaker (1960), $\beta_W = (\gamma/\alpha) - 1$ (where γ=number of species resulting from merging a number of samples and α=average number of species in a sample) and the differences attributable to compositional differences (magnitude of species gains and species losses) with $\beta_{SIM} = \min(b, c)/\min(b, c) + a$ (where a=total number of species shared by two quadrants b and c) (Lennon et al., 2001, re-expressed in Koleff et al., 2003). Both measures were applied to the adjacent pairs of sampling stations of the transect following the sequence from freshwater to the sea. The β_W was also calculated to explore differences in species richness between each large area and the average samples within it and between each large area and the average of the total study area. (2) Spearman rank correlation coefficients were used to examine the relationships between turnover diversity (β_W and β_{SIM}) and differences in diversity, depth, salinity, temperature and sediment characteristics for all possible pairs of sampling stations.

https://doi.org/10.1017/S0025315407050126 Published online by Cambridge University Press

Table 1. *Range and mean (in parentheses, plus standard deviation) of environmental variables at the three areas defined in the cluster analysis.*

							Sediment composition		
Area	Sites	Depth (m)	Salinity	T(°C)	Md (mm)	So (mm)	%Sand	%Mud	%Clay
FW	8	3–9 (5.7 ±2.6)	0–4.5 (0.03 ±0.04)	19.1–23.9 (20.8 ±1.6)	0.032–0.185 (0.08 ±0.05)	1.114–2.817 (1.68 ±0.57)	44–95 (59.1 ±24.5)	1–54 (34.1 ±20.1)	0–12 (6.7 ±5.1)
ES	6	3–10 (7.3 ±2.8)	3–26 (14.9 ±9.0)	17.9–20.1 (19.1 ±0.9)	0.024–0.233 (0.1 ±0.1)	1.186–1.771 (1.52 ±0.25)	4–100 (48.3 ±47.2)	0–85 (45.2 ±42.9)	0–16 (6.4 ±5.3)
MA	5	24–270 (105 ±99.6)	32.3–34 (33.4 ±0.7)	4.7–16.7 (9.5 ±3.9)	0.025–0.219 (0.16 ±0.08)	1.238–1.336 (1.27 ±0.04)	5–100 (73.8 ±38.6)	0–77 (19.2 ±32.3)	0–18 (7 ±6.7)

FW, freshwater; ES, estuarine; MA, marine.

RESULTS

Classification and ordination analysis separated sampling stations, at 15% of similarity, into three main groups, reflecting freshwater (FW), estuarine (ES) and marine (MA) environments (Figure 1). Freshwater comprised higher similarities (35.53% average similarity) than the ES (13.70%) and MA (17.89%) groups. The FW and ES comprised shallow sites with low salinity and muddy bottoms, while MA clustered deeper sites from sandy marine areas (Table 1). A significant difference was found between areas for depth (Kruskal–Wallis test, H=10.99, P=0.041), salinity (H=16.088, P=0.003) and temperature (H=13.48, P=0.012). The multiple comparisons between areas indicated a higher depth for MA (Dunn test, P>0.005), significant differences in salinity between FW–ES (P>0.01) and MA–FW (P>0.001), and significant differences in temperature between ES–MA (P>0.05) and MA–FW (P>0.001).

The SR_T (total area species richness) reached 134 species along the transect of 560 km. Gamma diversity (large area species richness, SR_L) at the three areas defined in the cluster analysis was variable, ranging from 33 species in FW to 38 species in ES and 81 species in MA. Alpha diversity (sample species richness, SR_S) ranged from 1 to 40 species, with a mean number of 12.0 ±10.01. The FW ranged between 3 and 12 (8.0 ±3.4), ES ranged from 1 to 20 (10.0 ±8.2) and MA between 1 and 40 (23.0 ±12.3).

The assemblages presented a different composition of taxa. Insects (6 spp.) and oligochaetes (6 spp.) were only present in FW. Crustaceans (13 spp.), molluscs (12 spp.) and polychaetes (7 spp.) characterized ES, while in MA polychaetes (22 spp.) were dominant, followed by molluscs (18 spp.) and crustaceans (15 spp.). These three groups were the dominant taxa in the whole study area (73.1% of the species).

Depth ranged from 3 to 270 m and salinities between 0 and 34 (Table 1; Figure 1). The subset of environmental factors that displayed the strongest correlation with faunal patterns (BIO-ENV) comprised salinity, depth, and clay content (ρ_w= 0.62), followed by a second subset with the same factors and the addition of median size grain (ρ_w=0.596). Among single environmental factors salinity showed the highest correlation (ρ_w=0.59), followed by depth (ρ_w=0.387), median size grain (ρ_w=0.303), clay content (ρ_w=0.250), % of sand fraction (ρ_w=0.174) and sorting (ρ_w=0.04).

Whittaker's (1960) beta diversity varied between the three major taxa and the large areas defined (FW, ES and MA).

Table 2. *Relationships between species turnover (β_w and β_{sim}) and changes in species number (N_0) and environmental variables, expressed as pairwise Spearman rank correlations for all possible pairs of sampling stations (N=190). Significant correlations are indicated with an asterisk.*

	β_W	β_{SIM}	N_0	DBS (km)	Depth (m)	T (°C)	S	%SA	%M	%CL	MD (mm)	SO (mm)
β_W	–											
β_{SIM}	0.97**	–										
N0	0.25**	0.18*	–									
DBS (km)	0.56**	0.54**	0.46**	–								
Depth (m)	0.38**	0.36**	0.59**	0.59**	–							
T (°C)	0.39**	0.37**	0.58**	0.78**	0.84**	–						
S	0.59**	0.56**	0.52**	0.87**	0.53**	0.60**	–					
%SA	0.14	0.13	−0.04	0.12	−0.06	−0.01	0.11	–				
%M	0.12	0.11	−0.05	0.12	−0.09	−0.02	0.12	0.98**	–			
%CL	0.24**	0.25**	−0.08	−0.02	−0.08	−0.06	−0.03	0.55**	0.46**	–		
MD (mm)	0.29**	0.27**	0.13	0.32**	0.15	0.22*	0.31*	0.76**	0.75**	0.35**	–	
SO (mm)	−0.01	−0.02	−0.14	−0.13	−0.23*	−0.20*	0.02	0.39**	0.42**	0.37**	0.28**	–

DBS, distance between sites; T, temperature; S, salinity; %SA, sand; %M, mud; %CL, clay; MD, median grain size; SO, sorting. *, P<0.05; **, P<0.001

https://doi.org/10.1017/S0025315407050126 Published online by Cambridge University Press

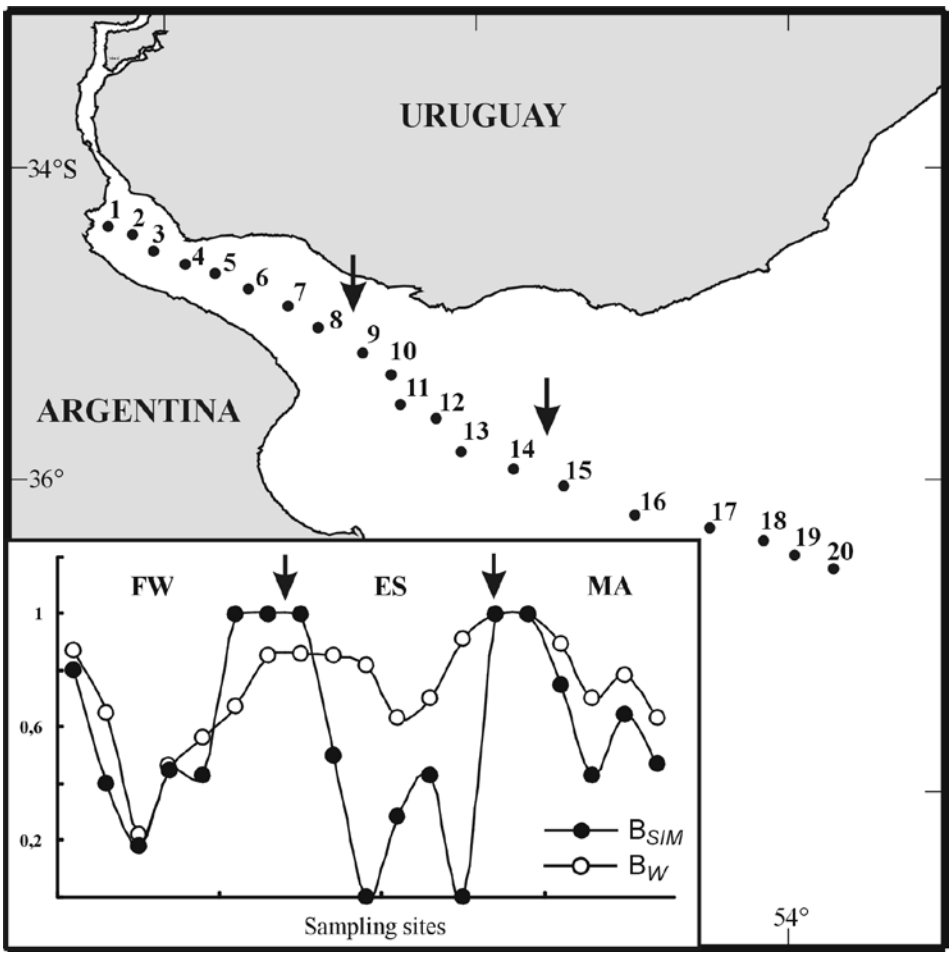

Figure 2. Beta diversity values (expressed as β_W and β_{SIM}, see text for details) along the study area, calculated for each adjacent pair of sampling sites, from Stations 1–2 to 19–20. FW, freshwater; ES,estuarine; and MA, marine. Black arrows indicate boundaries between each environment (Sites 8,9 and 14,15).

Species turnover was highest for crustaceans in FW (β_W=7.0, with values of 0.8 and 1.9 for molluscs and polychaetes, respectively) and ES (β_W=3.1, with 3.0 and 2.8 for molluscs and polychaetes, respectively), while in MA molluscs displayed the highest species turnover (β_W=3.8, with 2.5 and 2.0 for crustaceans and polychaetes, respectively). At the large scale (560 km), beta diversity was highest for all taxa pooled (β_W=10.2) than at the scale of each area (3.1, 2.7 and 2.6 for FW, ES and MA, respectively), reflecting the distinct composition of the three areas defined.

Both measures of beta diversity (β_W and β_{SIM}) were strongly correlated, and consequently they displayed a similar trend (Table 2). In general, beta diversity increased continuously when either distance between sites or differences in salinity values were higher, but these values were also strongly correlated (Table 2). Weaker relationships with changes in depth, temperature, %clay, and median grain size were also found (Table 2). The differences between sites attributable to species richness gradients (β_W) or to species composition (β_{SIM}) displayed similar trends when they were applied to the adjacent pairs of sampling stations (Figure 2). To a certain extent, both measures increased at the boundaries between assemblages (FW, ES and MA), but this general trend was more obvious for the β_{SIM} metric, which had high rates of species turnover among Sites 7 to 9 and 14 to 16 (Figure 2).

DISCUSSION

Three major assemblages (representing freshwater, estuarine and marine environments) and the boundaries between them were identified using classical multivariate analysis and beta diversity indices (β_W and β_{SIM}). In particular, β_{SIM} identified areas of high spatial turnover correlated with changes in environmental heterogeneity, thus representing a potential tool to define ecological boundaries in transitional zones along the Río de la Plata system.

Salinity is usually the major factor responsible for diversity patterns in estuaries (Remane & Schlieper, 1971). Beta diversity (β_{SIM}) was affected strongly by changes in salinity in our study. This is supported by the spatial pattern of areas of high species turnover, both coincident with the presence of two quasi-permanent hydrological features of the estuary, the bottom salinity front and the surface salinity front (Guerrero et al., 1997; Acha et al., 2004). Our results must be interpreted with caution because of the limitation of the data set, yet they are in accordance with previous works in mixohaline and marine waters of the study area, in which salinity fronts proved to be key factors affecting the distribution and abundance of fish, ichthyoplankton and benthic assemblages (Berasategui et al., 2004; Giberto et al., 2004; Jaureguizar et al., 2004).

https://doi.org/10.1017/S0025315407050126 Published online by Cambridge University Press

Beta diversity varied between the dominant taxonomic groups and the scales considered, reflecting the fact that they may respond in different ways to similar environmental gradients; this supports the notion that a single taxonomic group cannot be taken to represent overall beta diversity of assemblages (Ellingsen & Gray, 2002). Boundaries along fronts change rapidly, they are usually sites of intensified biological production, with several organisms adapted to live over such dynamic boundaries, presenting high turnover of populations (Margalef, 1997). The analysis of β_W proved to be of limited use for detecting boundaries of ecological change because it only assumes extreme values when differences in richness between sites are large (Koleff et al., 2003). On the contrary, β_{SIM} displayed clear differences at the boundaries of defined areas, which suggests a high rate of change in species composition.

To conclude, our results indicate the presence of a 'discrete' estuarine assemblage. An important point to consider here is the large extent of mixohaline waters in the Río de la Plata system (32,000 km^2), which are defined by two salinity fronts. The surface salinity front changes annually from near 2.2 psu/10 km to 1.3 psu/10 km, while the bottom salinity front usually has less variation (Piola et al., 2003). These conditions may indicate lower salinity stress compared to smaller estuaries, where several studies of estuarine diversity have been carried out and extreme salinity fluctuations over shorter distances could be found (see Remane & Schlieper, 1971; Atrill & Rundle, 2002). Further evidence, with adequate replication at several estuarine scales, is needed in order to properly understand processes shaping ecological boundaries in estuaries worldwide.

Special thanks to Dr A. Brand (PEML) for critical reading of the MS. This work was supported by PNUD-GEF-99G31/22, PICT-8424 and 01-15080, PEI-6026, PIP 5009 and Antorchas 13900-13. This is an INIDEP contribution no. 1431.

REFERENCES

Acha, E.M., Mianzan, H.W., Guerrero, R.A., Favero, M. & Bava, J., 2004. Marine fronts at the continental shelves of austral South America. Physical and ecological processes. *Journal of Marine Systems*, **44**, 83–105.

Attrill, M.J. & Rundle, S.D., 2002. Ecotone or ecocline: ecological boundaries in estuaries. *Estuarine, Coastal and Shelf Science*, **55**, 929–936.

Berasategui, A.D., Acha, E.M. & Fernandez Araoz, N.C., 2004. Spatial patterns of ichthyoplankton assemblages in the Rio de la Plata Estuary (Argentina-Uruguay). *Estuarine, Coastal and Shelf Science*, **60**, 599–610.

Cadenasso, M.L., Pickett, S.T.A., Weathers, K.C. & Jones, C.G., 2003. A framework for a theory of ecological boundaries. *BioScience*, **53**, 750–758.

Clarke, K.R. & Warwick, R.M., 2001. *Change in marine communities: an approach to statistical analysis and interpretation*. Plymouth: PRIMER-E.

Ellingsen, K.E. & Gray, J.S., 2002. Spatial patterns of benthic diversity: is there a latitudinal gradient along the Norwegian continental shelf? *Journal of Animal Ecology*, **71**, 373–389.

Elliot, M. & McLusky, D.S., 2002. The need for definitions in understanding estuaries. *Estuarine, Coastal and Shelf Science*, **55**, 815–827.

Field, J.G., Clarke, K.R. & Warwick, R.M., 1982. A practical strategy for analyzing multispecies distribution patterns. *Marine Ecology Progress Series*, **8**, 37–52.

Giberto, D.A., Bremec, C.S., Acha, E.M. & Mianzan, H.W., 2004. Large-scale spatial patterns of benthic assemblages in the SW Atlantic: the Río de la Plata estuary and adjacent shelf waters. *Estuarine, Coastal and Shelf Science*, **61**, 1–13.

Gray, J.S., 2000. The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. *Journal of Experimental Marine Biology and Ecology*, **250**, 23–49.

Guerrero, R.A., Acha, E.M., Framiñan, M.B. & Lasta, C.A., 1997. Physical oceanography of the Río de la Plata estuary, Argentina. *Continental Shelf Research*, **17**, 727–742.

Holt, R.D. & Keitt, T.H., 2005. Species' borders: a unifying theme in ecology. *Oikos*, **108**, 3–6.

Jaureguizar, A.J., Menni, R., Guerrero, R.A. & Lasta, C.A., 2004. Environmental factors structuring fish communities of the Río de la Plata estuary. *Fisheries Research*, **66**, 195–211.

Koleff, P., Gaston, K.J. & Lennon, J.J., 2003. Measuring beta diversity for presence-absence data. *Journal of Animal Ecology*, **72**, 367–382.

Lennon, J.J., Koleff, P., Greenwood, J.J.D. & Gaston, K.J., 2001. The geographical structure of British bird distributions: diversity, spatial turnover and scale. *Journal of Animal Ecology*, **70**, 966–979.

López Laborde, J., 2002. Campaña de prospección ambiental del Río de la Plata y su Frente Marítimo. Sedimentos superficiales de fondo. Proyecto de las Naciones Unidas para el Desarrollo. PNUD Project/Gef-RLA/99/G31, 32 pp.

Margalef, R., 1997. Boundaries. In *Excellence in Ecology Series: our biosphere* (ed. O. Kinne), pp. 59–70. Oldendorf (Luhe), Germany: Ecology Institute.

McLusky, D.S., 1993. Marine and estuarine gradients-an overview. *Netherlands Journal of Aquatic Ecology*, **27**, 489–493.

Piola, A.R., Guerrero, R.A., Osiroff, A.P. & Molinari, G., 2003. Climatología de frentes y estratificación del Río de la Plata. *V° Jornadas Nacionales de Ciencias del Mar, Mar del Plata, Argentina, 8–12 December* [Extended abstract.]

Remane, A. & Schlieper, C., 1971. *Biology of brackish water*. New York, Toronto, Sydney: Wiley Interscience Division: John Wiley & Sons, Inc.

Whittaker, R.H., 1960. Vegetation of the Siskiyou Mountains, Oregon and California. *Ecological Monographs*, **30**, 279–338.

Zar, J.H., 1984. *Biostatistical Analysis*. Englewood Cliffs: Prentice-Hall Inc.

Submitted 15 February 2005. Accepted 13 November 2006.