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MACROBENTHIC RESPONSE TO SEWAGE DISCHARGES IN CONFINED AREAS FROM COASTAL LAGOONS: IMPLICATION ON THE ECOLOGICAL QUALITY STATUS

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MACROBENTHIC COMMUNITY
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WASTEWATER
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M-AMBI

ABSTRACT. – We studied the effect of wastewater on macrobenthic assemblages and local Ecological Quality Status (EcoQS) along a pollution gradient. The study consisted of six sampling sites surrounding the discharge channel of a Wastewater Treatment Plant located within Ria Formosa coastal lagoon. The total number of individuals was significantly higher at the discharge point comparatively to the control site, while total number of species, species richness and diversity values revealed the opposite pattern. Deposit-feeding were the most abundant trophic group, particularly at the inner sites, while the highest contributions of suspension-feeding and carnivory groups were observed at external ones. The organic matter content was positively correlated with the deposit-feeding group conferring a structural character of this variable near the plant discharge. M-AMBI index was consistent with the ANOSIM and SIMPER analysis, successfully separating the sampling stations according to the distance from the discharge and distinguishing the lower EcoQS of the inner sites not complying with the requirements of the Water Framework Directive of a "Good" quality status. In addition, changes in ecological and biotic indices were negatively correlated with variations of organic matter and positively correlated with salinity, corroborating the effect of these variables on the composition and structure of macrobenthic communities.

INTRODUCTION

Coastal lagoons are highly productive environments and, due to the high availability of natural resources, preferential areas for human exploitation (Castel *et al.* 1996). Anthropogenic pressure in the vicinity of coastal lagoons has been pointed out as a strong contributor to the decrease of water quality and the consequent degradation of these habitats (Newton *et al.* 2003). Worldwide, discharges of Wastewater Treatment Plants (WWTPs) into these systems constitute one of the main pollution sources (Gray 2004), whose effects can be particularly severe in Regions of Restricted Water Exchange (RRE), where discharges can drastically change the water quality (Tett 2003). As a consequence, receiving communities are threatened by the typical organic enrichment (Pearson & Rosenberg 1978, Austen *et al.* 1989) and variations of water salinity (Tett 2003, Gray 2004). In fact, several authors have found changes in diversity and trophic structure when studying the effect of organic matter content (Weston 1990, Austen & Widdicombe, 2006, Klaoudatos 2006) and salinity (Teske & Wooldridge 2001, Gamito 2006, Zettler *et al.* 2007) variations on soft-bottom communities' structure and functioning.

Macrobenthic communities have an important role on the structure and functioning of ecosystems (Gray 1974, Pearson & Rosenberg 1978). They are, however, in direct contact with the sediment, where multiple contaminants tend to accumulate (Gray 1974). In fact, due to their limited mobility, benthic organisms are sensitive to local disturbance and because of their permanence over seasons, they integrate the recent history of disturbances that might not be detected in the water column (Warwick 1993). As well as salinity and organic matter content, environmental variables affecting macro-benthic communities in transitional waters include inorganic contaminants concentration, water residence time, temperature and freshwater discharges (Gray 1974, Snelgrove & Butman 1994). The response of each community to these stressors depends on its resilience and specific environmental conditions of the ecosystem (Jorgensen & Richardson 1996). The presence/absence of these organisms can provide information about temporal and spatial disturbances, since they integrate the conditions of the water-sediment interface (Reiss & Kröncke 2005). Hence benthic assemblages are broadly recognised as proper tools to describe the ecological conditions of marine and brackish systems.

Macrobenthic invertebrate fauna integrates biological quality elements for the definitions of ecological status proposed by the Water Framework Directive (WFD 2000/60/CE) (Teixeira *et al.* 2007). This directive established that all surface waters should have a "Good" ecological quality status (EcoQS) until 2015 by implementing the necessary integrated programs of measures (Mostert 2003). Nevertheless, despite the evaluation of pollution sources and their effects on benthic communities being well known, it has been difficult to reach a generalized and uniform qualification of the EcoQS (Teixeira *et al*. 2007, Prato 2009). As a result, different biotic indices were designed to establish the ecological quality of coastal and transitional waters, analysing the response of soft-bottom communities to natural and man-induced changes in water and sediment quality (Borja *et al*. 2000).

The estimation of biotic indices allows the assessment of the Ecosystem Quality status (EcoQS) in coastal systems. In this respect, the Shannon-Wiener index (Shannon & Weaver 1949), and the AMBI (Azti Marine Biotic Index, Borja *et al*. 2000) are amongst the indices generally used for EcoQS classification. The AMBI index provides information about the relative abundances of the sensitive species faced with increasing organic matter in the sediment and those of the species that are resistant or indifferent or even favored by such conditions (Dauvin 2007). This index is particularly useful in detecting time and spatial impact gradients but can sometimes be misleading because of the abundance of stress tolerant species that can be tolerant against natural stressors and lead to a natural increase in opportunistic species and, subsequently, to an increase in the AMBI values (Dauvin 2007). In order to minimize these problems some authors have tried to use a multi-index approach in order to avoid misclassifications of the EcoQS.

M-AMBI (Muxika *et al*. 2007) is a derived statistical tool which combines AMBI, diversity and richness for the EcoQS assessment of previous classification of water bodies and typologies, together with the definition of reference conditions allowing the distinction between impacted and undisturbed/reference sites. M-AMBI provides a classification of the system that matches adequately the one established by the WFD (Salas *et al*. 2004) and it has been applied at different geographic areas such as Europe (Ruellet & Dauvin 2007, Prato *et al*. 2008), Africa (Bigot *et al*. 2008, Bakalem *et al*. 2009) and North America (Borja *et al*. 2007, Borja & Tunberg 2010).

The Ria Formosa lagoon is a highly productive ecosystem and represents a relevant site for several economic activities such as fisheries, aquaculture and sand extraction (Mudge & Bebianno 1997, Newton *et al*. 2003). These activities, together with the tourist impact, intensive agriculture and sewage discharges at lagoon vicinities have increased pressures on the ecosystem, contributing to the degradation of water quality (Bebianno 1995, Mudge & Bebianno 1997, Newton & Mudge 2005). The confined characteristic of many channels in this lagoon makes these areas particularly sensitive to organic enrichment since water circulation is limited and the residence time of the pollutants is high (Mudge & Duce 2005); it is then urgent to evaluate the local water quality and the effects of the discharges on benthic communities.

The aim of this study was to assess the effect of a WWTP discharge into a confined channel of the Ria Formosa coastal lagoon evaluating the macrobenthic community structure an local EcoQS.

METHODS

Study area: Ria Formosa is a mesotidal lagoon and one of the most important wetland at both European and International levels namely by its acceptance as a Natura 2000 and a Ramsar site (Newton *et al*. 2003). This lagoon is an extremely rich zone in ecological terms and a dynamic and complex system,

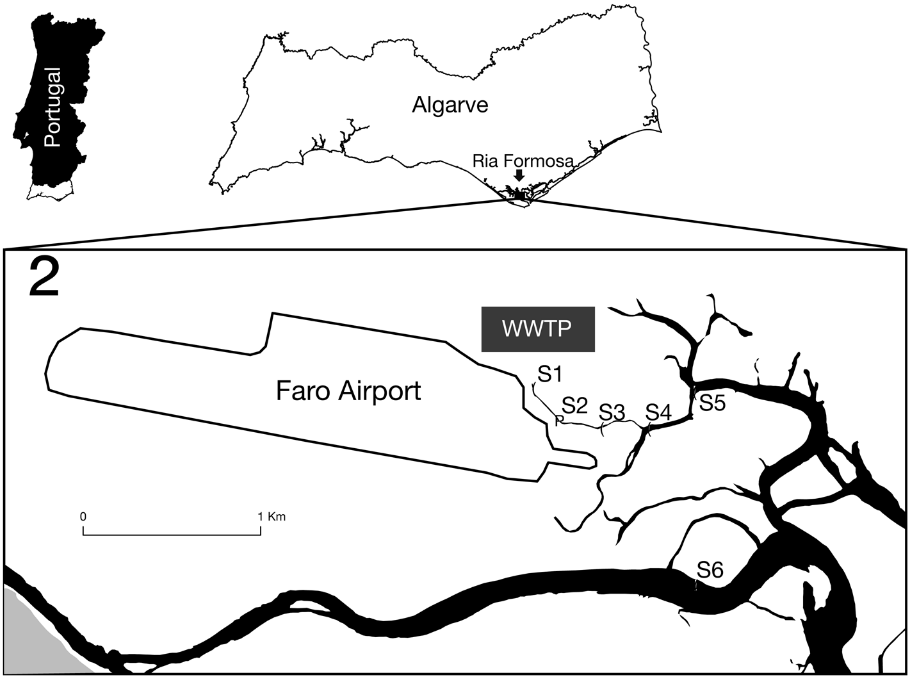

Fig. 1. – Geographic location of the Ria Formosa coastal lagoon showing the location of WWTP and sampling sites.

with high primary production and important live resources (Mudge & Bebianno 1997, Newton *et al*. 2003, Loureiro *et al*. 2005). The lagoon is located on the southern coast of Portugal (Algarve), extending for about 55 km and occupying a surface area of approximately 16 300 ha (Fig. 1).

There is no significant natural freshwater input in to the system and the salinity remains around 36 throughout the year, except during sporadic short run-off periods (Falcão & Vale 1990, Newton & Mudge 2003). The tidal amplitude varies from a maximum of 3.5 m at spring tide to 0.5 m at neap tide, which causes important semidiurnal and fortnightly fluctuations of the water volume inside the system (Falcão & Vale 1990). The lagoon presents a high water renewal rate with an average daily renewal of 2/3 of its volume (Águas 1986). However, in confined zones with reduced water circulation and renewal, sedimentation processes occur, due to a high residence time of the compounds in the system (Mudge & Duce 2005).

There are several WWTPs within the Ria Formosa lagoon national park, five of which serve populations above 10000 people. Faro North-western WWTP is placed in Faro near the international airport (37º01'04,08" N; 7º57'26,89" W), serving an area of 12000 inhabitants with a sewage flow of 0.12 m^3s^{-1} of domestic sewage, treated with primary and secondary treatment levels (Source: Águas do Algarve-ALGAR) (Fig. 1). The wastewaters are discharged directly into a confined secondary/inner open channel, draining directly to the lagoon. The area surrounding the discharge channel is characterised by muddy sediments covered by salt marsh vegetation, namely *Zostera noltii* Hornem.

Sampling strategy: Six sampling stations were selected in order to develop a pollution gradient, covering an area from the discharge point of the Faro North-western WWTP to the nearest main/primary channel of the lagoon. Sampling sites were named S1 to S6 where S1 was located upstream the discharge point (inner station) and S2 coincided with the discharge point. S3 was located 200 m downstream, but still at the inner channel and S4 was an intermediate point located at the intersection of the sewage channel with a secondary channel perpendicular to it. S5 was also located on this latter channel, at a more external zone and S6 was located on the main channel of the lagoon. Due to its location on the main open channel and its proximity to the Faro inlet, where the water renewal rate is higher, S6 is the point least affected by organic enrichment. Therefore, for the purposes of the present study, it can be regarded as a control site (Fig. 1)

Sampling was carried out every trimester during 2006 always at low tide, when the WWTP influence was more evident. In each site, three replicates of sediment samples were collected using a hand-corer (15 x15 cm; 0.0177 m^2). Each replicate was compound by 3 corers being subsequently placed into a meshed bag (500 μm) and sieved *in situ*. The retained material was preserved in ethanol previously coloured with Rose Bengal. Additionally, at each site, three smaller sediment corers (5 x 10 cm; 0.0019 m^2) were collected for quantification of organic matter in the sediment. Additionally, at each sampling site the temperature and water salinity values were measured using an YSI Multi-parameter probe.

In the laboratory, macrobenthic organisms were counted and identified under a binocular stereomicroscope (x6 magnification). Polychaetes and crustaceans were identified to the family level while gastropods and bivalves were identified to the species level. The sediment organic matter content was determined by the ash-free dry weight of the sediment samples after 6 h at 450º C.

Data analysis: the total number of species (S), total abundance (N), density (ind/m^2) as well as the Margalef's species richness (d) (Margalef 1968); Shannon-Weaver diversity (H') (Shannon & Weaver 1949) and Pielou equitability (J) (Pielou 1968) indices were computed at each sampling site in every sampling periods. Differences in ecological explanatory variables among stations were analyzed by a parametric one-way ANOVA (Zar 1996). The Student-Newman-Keuls (S-N-K) pair wise tests were used to identify the differences among the sampling sites and periods. A significance level of $\alpha = 0.05$ was used in all tests.

Multivariate data analysis was performed using the statistical package PRIMER v5.2.4 (Clarke & Warwick 1994). A data matrix with the abundance values of the different taxa found in this study was performed. A two-way crossed ANOSIM was carried out to determine if there were significant differences between sample similarities from different sampling sites and periods. Community similarity among sampling sites and periods was analysed by multidimensional ordination techniques (non-metric MDS). Matrix data was based on the Bray-Curtis dissimilarity coefficient after square-root transformation. Species contributions to dissimilarities among sites and sampling periods were investigated using the similarity percentages routine (SIMPER).

For macrobenthic trophic structure analysis, the functional trophic group was determined according to other local studies (Fauvel 1977, Fachauld & Jummars 1984, Sprung 1994, Fish & Fish 1996, Chícharo *et al*. 2002) and performed by assigning each taxon to at least one of the following trophic groups: suspension-feeding, deposit-feeding, carnivory and herbivory. The assignment of taxa to a group was performed, according to Boaventura *et al*. (1999), by distributing the number of individuals of that taxa in the number of functional groups in which it could be included. Total number of individuals in each trophic category was converted in to percentage values. A Cluster analysis was used to demonstrate the spatial differences according to the trophic group indicator. Pearson correlation test ($\alpha = 0.05$) was used to achieve the relation among the different trophic groups and the sediment organic matter content.

An approach to the EcoQS assessment of the different sampling sites and periods in the Ria Formosa lagoon was performed by applying the AMBI (Borja *et al*. 2000) index and its multivariate extension M-AMBI (Borja *et al*. 2007, Muxika *et al*. 2007). AMBI is exclusively based on species sensitivities where *taxa* are assigned to five ecological groups based on their sensitivity to organic enrichment (EGI: species very sensitive;

EGII: species indifferent; EGIII: species tolerant; EGIV: second-order opportunistic species; EGV: first-order opportunistic species) as defined by Grall & Glémarec (1997). The M-AMBI method relies upon a statistical multivariate tool, Factor Analysis (FA), which includes richness (S), Shannon's diversity (H´) and AMBI. The M-AMBI application requires the definition of reference conditions related to the typology under study (Muxika *et al*. 2007, Borja *et al*. 2008). For the determination of AMBI and M-AMBI, the AZTI's software package version 4.1, with the species list version of February 2010 (available at AZTI's web page http://www.azti.es) was used, following the guidelines from the authors (Borja & Muxika 2005). These indices were calculated by the software according to a data matrix with the different taxa abundances at each study site and sampling period. In our analysis, and due to the lack of studies on macrobenthos from the Ria Formosa, no reference conditions were yet defined. Thus, for M-AMBI calculations, the reference conditions defined by the software were used.

The effects of both salinity and organic matter on habitat ecological status were assessed with correlations (Pearson test, $\alpha = 0.05$) with environmental variables measured and respective values for ecological indices.

Table I. – Values of water temperature (ºC) and salinity plus sediment organic matter content (g) measured at all sampling sites and periods.

	Site	TºC	S	OM
January	S1	20.1	4.7	4.7
	S2	17.8	3.6	5.8
	S3	14.3	4.0	6.0
	S4	15.7	26.1	1.8
	S5	21.5	36.5	1.8
	S6	19.9	36.7	3.5
April	S1	22.2	3.2	6.4
	S2	23.0	4.4	4.0
	S3	23.0	3.3	1.3
	S4	16.1	32.6	2.1
	S5	21.6	36.9	2.3
	S6	20.8	36.8	1.6
June	S1	23.7	8.7	2.2
	S2	23.0	4.4	2.4
	S3	18.7	9.1	2.0
	S4	24.1	32.8	2.1
	S5	21.9	36.9	2.4
	S6	20.9	36.9	1.8
October	S1	25.8	7.2	3.1
	S2	22.9	6.9	2.9
	S3	24.4	10.9	2.4
	S4	26.2	23.5	1.6
	S5	21.1	36.7	2.4
	S6	20.6	36.8	0.9

RESULTS

Environmental Variables

Water temperature displayed seasonal variation throughout the sampling periods with highest values in June and October (Table I). There was no visible spatial pattern of temperature values among sites. Salinity showed strong variations among sites with lower values at S1, S2 and S3 and higher values at S4, S5 and S6; this tendency was uniform during all sampling periods. The organic matter content in the sediment was higher at S1 and S2 and the lower values were found at S6, particularly *t* in January and April (Table I).

Macrobenthic community structure

A total of 47 taxa were identified during the study, distributed in four main taxonomic groups (polychaetes, bivalves, gastropods and crustaceans). Polychaetes comprised the highest number of taxa (15) as well as the highest overall abundance (57.9 %) followed by bivalves (11 taxa and 29.7 %), gastropods (13 taxa and 8.7 %) and crustaceans (8 taxa, 3.6 %) (Table II). The S1 and S2 sites were strongly dominated by polychaetes (78.0 % and 84.0 % respectively) while the highest abundance of bivalves was found at S3 (89 %). The highest proportions of gastropods were found at S4, S5 and S6, (20.8 %, 9.7 % and 35.4 % respectively). The crustaceans were limited to three sites, S1, S5 and S6, with a particularly large abundance at S6 (24 %). Only two taxa (Capitellidae and *Scrobicularia plana*) had a generalized distribution in all sampling sites, contributing, along with the Nereididae, for 57.1 % of the overall abundance (Table II).

The total number of taxa (S) per station varied between 1 and 22 with a mean value of 8.3. S1, S2, and S3 showed a lower mean number of taxa compared to S4, S5 and S6 (Fig. 2). Diversity (H´) varied between 0.0 and 3.9 with a mean value of 2.0 (Fig. 2). Diversity was significantly lower at S1 and S2 comparing to S4, S5 and S6 at April, June and October samplings (Table III).

The highest abundance was found in the January sampling with 35.6 % of the total abundance, and the lower abundance was found in October. The maximum density was found at S2 (5348 ind/m^2) in June and the minimum at S6 in October with 169 ind/m^2 (Fig. 2). The one-way ANOVA found significant differences between S2 and S6 in June and October with higher values for the first (Table III).

Evenness (J') ranged between 0.55 and 1.00 with a mean value of 0.75 being statistically lower at S2 in April, June and October comparing to S5 and S6 at the same months. The Margalef index (d) ranged between 0.24 and 3.38 (Fig. 2). This index was significant lower at S1 and S2 comparing with S4, S5 and S6 in April, June and October (Table III).

Table II. – Abundance (%) of the taxa found at the study sites comprising more than 90 % of the total abundance; taxonomic groups: TXG (P, Polychaetes; B, Bivalves; G, Gastropods; C, Crustaceans), trophic groups:TG (DF, Deposit feeding; FF, Filter-feeding; C, Carnivory; H, Herbivory), AMBI functional group: AG (I, II, III, IV, V, NA (not assigned)) and total contribution (%): TC, of each taxon in the study.

Taxa	TXG	TG	EG	TC	S1	S2	S3	S4	S5	S6
Capitellidae	P	DF	V	15.8	32.6	7.5	1.2	26.7	27.6	3.5
Cirratullidae	P	DF	IV	5.0				11.7	18.7	2.1
Eunicidae	P	DF	II	1.1				4.6	0.8	2.1
Glyceridae	P	C	II	2.4			3.3	4.5	2.0	7.6
Nereididae	P	C	III	23.0	44.3	69.1	2.7			
Spionidae	P	FF	III	1.4		4.7			1.0	
Terebellidae	P	DF	I	1.5					9.2	
***Cerastoderma edule* (Linnaeus, 1758)**	B	FF	III	1.3				5.0	2.3	
***Parvicardium scabrum* (Philippi, 1844)**	B	FF	I	2.6				12.3	1	4.1
***Scrobicularia plana* (da Costa, 1778)**	B	DF	III	18.3	16.1	12.6	83.1	6.9	3.6	2.1
***Venerupis senegalensis* (Gmelin, 1971)**	B	FF	I	1.7					9.6	16.0
***Bittium reticulatum* (da Costa, 1778)**	G	DF	I	12.3					8.6	18.8
***Nassarius nitidus* (Jeffreys, 1867)**	G	C	II	0.4						6.9
***Nassarius pfeifferi* (Philippi, 1844)**	G	C	II	3.8				19.5		4.8
Anthuridae	C	C	II	1.5					2.6	9.0
Calliopiidae	C	C	NA	0.5					1.0	4.2
Corophiidae	C	DF	III	0.6					1.0	2.1
Diogenidae	C	C	II	0.4					1.5	6.9
			Cumulative abundance		93.0	93.9	90.3	91.2	90.5	90.6

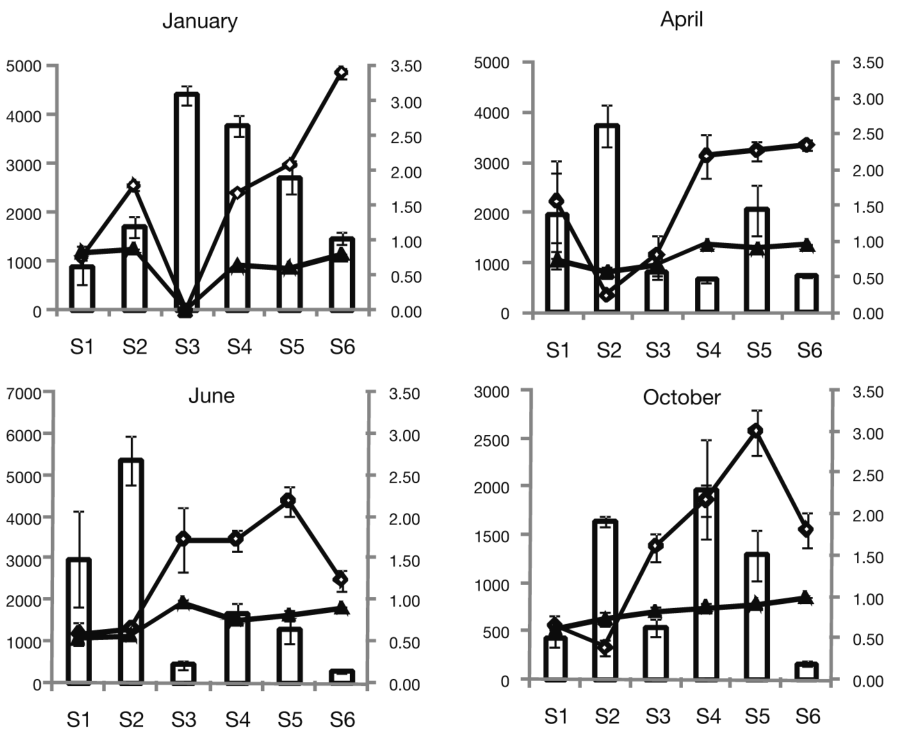

Fig. 2. – Mean and standard deviation of density (ind/m²), species richness (d) and evenness (J') in each site, across the four sampling periods.

The 2-way ANOSIM indicated significant differences among sites ("Site" factor; R = 0.862 p = 0.001) and among sampling periods ("Month" factor; R = 0.780; p = 0.001). The MDS analysis plot has distinguished the sites placed at the discharge channel (S1, S2 and S3) from the external stations (S4, S5 and S6) (Fig. 3). The same analysis did not show any pattern among months. The Cluster analysis corroborated the MDS results also show-

Table III. – Statistical analysis results of the one-way ANOVA among sampling sites (S1 to S6) and periods (January, April, June and October) for mean univariated indices (dependent variables): H, Shannon-Wiener diversity; Abundance, J', Pielou Evenness index; d, Margalef index. *P*-value: ** < 0.05, * < 0.01. The one way S-N-K ANOVA normality test was used in all analysis. The columns marked with "-"represent no significant difference results.

	H		Ab		J'		d	
	F	**p-w tests**	**F**	**p-w tests**	**F**	**p-w tests**	**F**	**p-w tests**
Jan	-	-	-	-	-	-	-	-
Apr	F(5,N = 3) = 23.2 (*)	$S_{1,2,3} < S_{4,5,6}$	-	-	F(5,N = 3) = 9.5(*)	$S_{2,3} < S_{4,5,6}$	F(5,N = 3) = 15.3(*)	$S_{1,2,3} < S_{4,5,6}$
Jun	F(5,N = 3) = 4.5 (**)	$S_{1,2} < S_{4,5}$	F(5,N = 3) = 2.7(**)	$S_2 > S_6$	F(5,N = 3) = 3.7(**)	$S_{1,2} < S_{5,6}$	F(5,N = 3) = 4.4(**)	$S_{1,2} < S_{4,5}$
Oct	F(5,N = 3) = 27.3(*)	$S_{1,2} < S_{3,4} < S_{5,6}$	F(5,N = 3) = 9.1(*)	$S_2 > S_6$	F(5,N = 3) = 7.1(**)	$S_{1,2} < S_{3,4,5,6}$	F(5,N = 3) = 27.7(*)	$S_{1,2} < S_{3,4} < S_{5,6}$

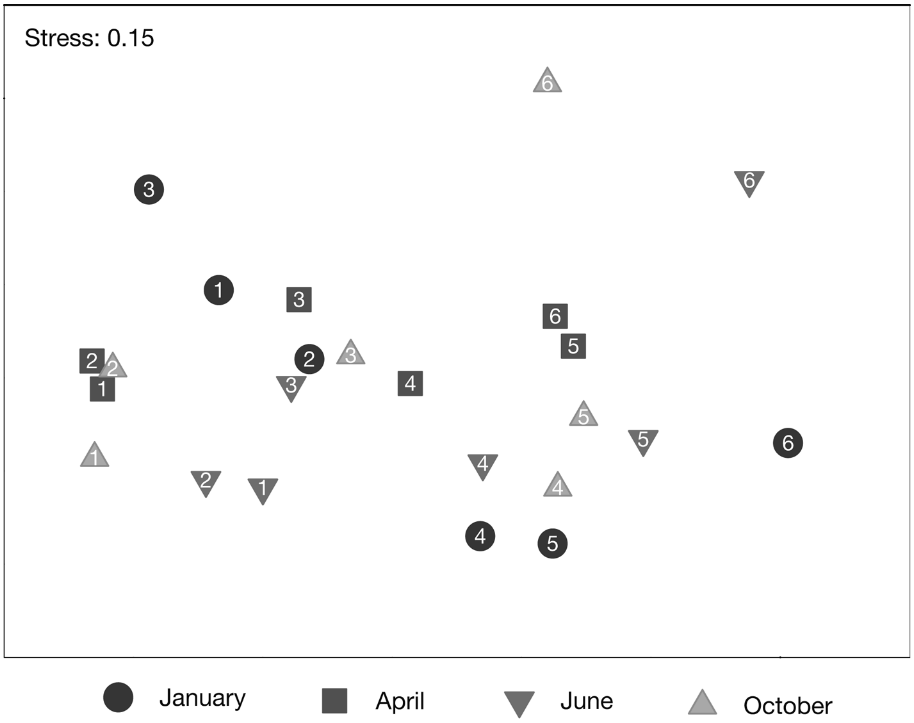

Fig. 3. – No metric multi-dimensional scaling (MDS) on square-root-transformed abundance data for the different sites (1, 2, 3, 4, 5 and 6) and sampling periods (January, April, June, October). The replicates were aggregated in the MDS analysis in order to allow a good visualization of the plot.

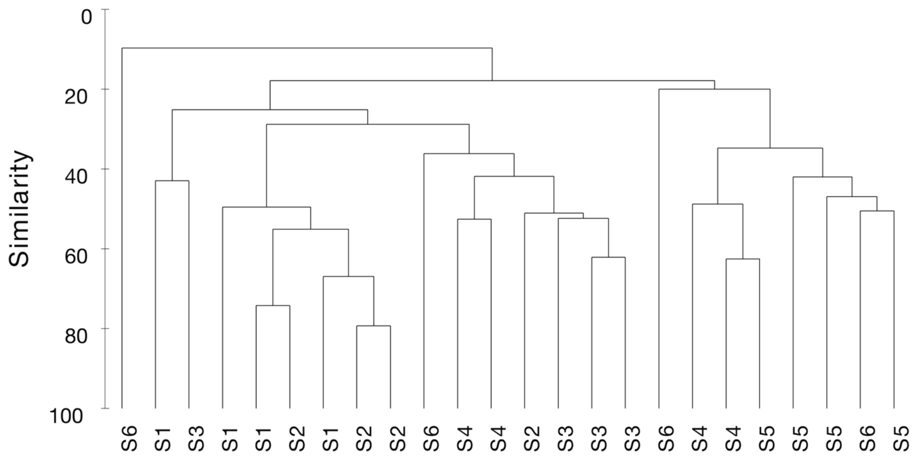

Fig. 4. – Cluster analysis for the "Site" factor according to the taxa abundances. The replicates were aggregated by averaging.

ing a separation between S1 and S2 from S4, S5 and S6 despite some fluctuation of the S6 samples (Fig. 4).

The SIMPER analysis for the "Site" factor showed higher dissimilarities between the stations nearby the discharge point (S1, S2 and S3) relative to the further ones (S4, S5 and S6) (Table IV). Those differences where mainly due to the high abundance of Nerediidae, *Scrobicularia plana* and Capitellidae that comprised more than

Table IV. – SIMPER analysis for the "Month" and "Site" factors.

		Dissimilarity between months			
15.15	**October**	87.66	83.48	88.08	**October**
	16.19	**June**	84.63	87.38	**June**
Similarity		21.85	**April**	85.28	**April**
within months			22.22	**January**	

	S1	41.94			**Similarity**		
S2	68.72	**S2**	50.33		**within sites**		
S3	81.54	80.76	**S3**	33.84			
S4	92.13	89.91	84.45	**S4**	31.28		
S5	92.59	92.53	91.97	79.03	**S5**	24.3	
S6	96.11	97.78	93.9	92.01	89.84	**S6**	17.44
				Dissimilarity between sites			

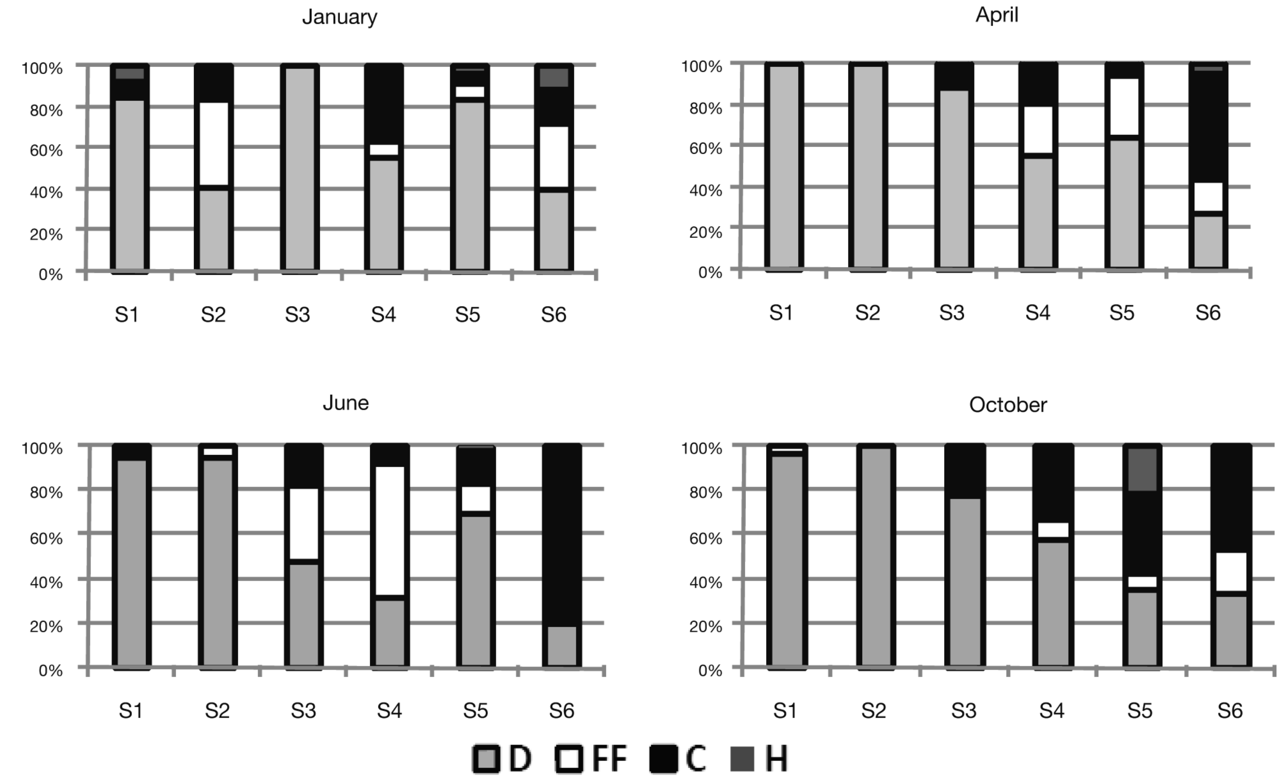

Fig. 5. – Contributions (%) of the different feeding modes (DF, Deposit feeding; FF, Filter feeding; C, Carnivory and H, Herbivory) for every sites and sampling periods.

90 % of the total abundance contribution at S1, S2 and S3 contrary to S4, S5 and S6 where the higher abundance of these organisms were replaced by a more heterogenous distribution of total abundance for a higher number of taxa as *Nassarius pfeifferi*, *Bittium reticulatum,* Cirratullidae and *Parvicardium scabrum*. For the "Month" factor the same analysis has revealed high dissimilarities between all months. The similarities within months have however presented uniform low values (Table IV).

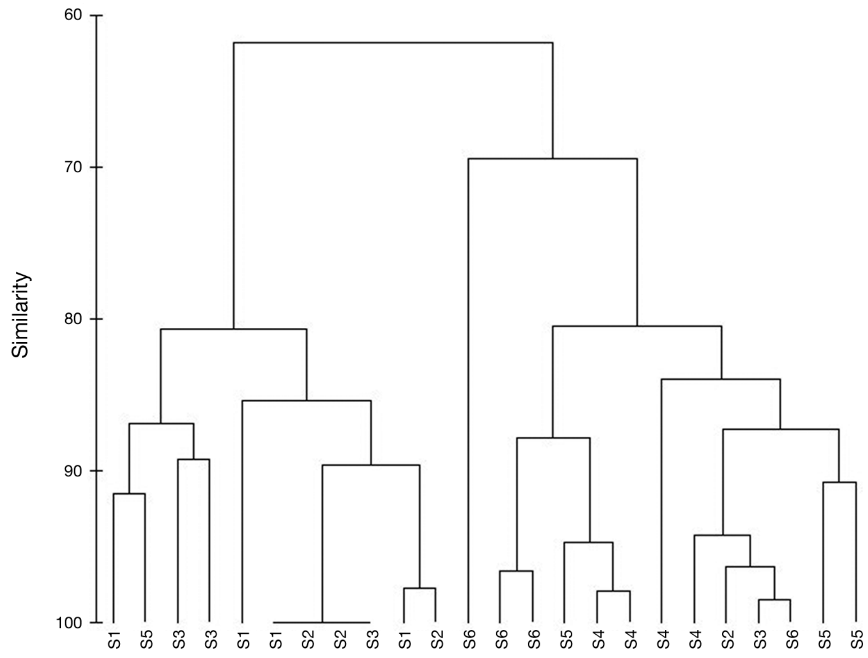

Fig. 6. – Cluster analysis for the "Site" factor per trophic function according to the taxa abundances. The replicates were aggregated by averaging.

Trophic groups

Deposit feeding was the dominant feeding group accounting for more than 75 % of the total abundance (Fig. 5). This feeding mode was particularly relevant at the stations located near the discharge point. This prevalence was mainly noted at S1 and S2 in the April, June and October samplings where this group was almost exclusively representing more than 90 % of the total abundance. The filter-feeding group represented 11.4 % of total organisms mainly represented at S3, S4, S5 and S6, being particularly abundant in June where it reached 50 % (S4). The carnivory group had a low contribution at S1, S2 and S3 and a higher presence at the external sites, mainly at S6 where it reached more than 80 % of the abundance in April. The cluster analysis based on trophic composition confirmed the general separation of stations located near (S1, S2 and S3) from those located far (S4, S5 and S6) from the source of disturbance (Fig. 6). The Pearson correlation between organic matter content and the abundance of each trophic group significant positive between the deposit-feeding group and the organic matter ($R = 0.45$; $p < 0.01$). A negative significant correlation was

Table V. – M-AMBI values and consequent EcoQS for all sites and sampling periods according to diversity (H'), richness (S) and AMBI values.

	Stations	Diversity	Richness	AMBI	M-AMBI	Status
January	S1	1.30	3	3.00	0.42	**Moderate**
	S2	2.44	7	2.91	0.67	**Good**
	S3	0.00	1	3.00	0.22	**Poor**
	S4	1.90	8	3.81	0.58	**Good**
	S5	1.90	9	4.72	0.55	**Good**
	S6	2.81	12	0.60	0.99	**High**
April	S1	0.72	2	3.00	0.37	**Poor**
	S2	0.47	2	3.00	0.35	**Poor**
	S3	1.19	5	2.82	0.49	**Moderate**
	S4	3.10	11	2.34	0.81	**High**
	S5	3.36	16	2.07	0.95	**High**
	S6	3.46	14	1.66	0.96	**High**
June	S1	1.42	6	4.13	0.42	**Moderate**
	S2	1.19	4	3.56	0.39	**Moderate**
	S3	2.28	6	2.63	0.63	**Good**
	S4	2.81	15	1.86	0.92	**High**
	S5	3.03	14	2.36	0.89	**High**
	S6	1.76	4	1.13	0.63	**Good**
October	S1	1.01	5	2.90	0.36	**Poor**
	S2	0.90	3	2.98	0.32	**Poor**
	S3	2.51	9	3.08	0.53	**Good**
	S4	3.17	15	2.97	0.68	**Good**
	S5	3.95	22	1.94	0.91	**High**
	S6	2.95	8	0.67	0.72	**Good**

Table VI. – Pearson correlation coefficients for salinity and organic matter crossed with the values of the several biotic indices used for the EcoQS. *: $p < 0.001$; **: $p < 0.05$.

	S	Ab	d	J	H	AMBI	M-AMBI
MO	-0.30**	-0.30**	-0.42**	-0.27*	-0.39**	0.41*	-0.41*
Salinity	0.68*	0.68*	0.78*	0.45*	0.69*	-0.52*	0.82*

found between this variable and filter-feeding (R = -0.22 $p < 0.05$), in carnivory (R = -0.8; $p < 0.01$) and herbivory (R = -0.26; $p < 0.05$) groups.

Biotic indices

The multi-index analysis M-AMBI distinguished different EcoQS according to the several sites and periods of the study (Table V). The higher ecological status was found systematically at S4, S5 and S6, presenting a satisfactory status ("Good" or "High") at every sampling periods due to a high diversity and richness and to the fact the majority of that taxa present within this sites belong to the AMBI groups I and II. To the contrary, the S1 and S2 presented a low EcoQS with "Poor" (April and October) and "Moderate" (June) classifications, due to the low diversity and richness as well as the tolerant and opportunistic character of the few species presented at these sites. In January S2 presented a "Good" status mainly due to an exceptional high diversity and richness. S3 shown the most variable tendency of EcoQS since it presented "Poor" (January), "Moderate" (April) and "Good" (June and October) classifications.

The Pearson correlation between the different biotic indices used in this study revealed that biotic indices were negatively correlated with the organic matter content and positively correlated with the salinity (Table VI).

DISCUSSION

The WWTPs discharges a large amount of nutrients (Newton *et al.* 2003, Tett *et al.* 2003, Loureiro *et al.* 2005) and organic matter (Bebiano 1995, Hewitt & Mudge 2004, Loureiro *et al.* 2005) directly into the Ria Formosa lagoon also inputting freshwater in the area (Tett *et al.* 2003). Worldwide, several authors have focused on the evaluation of soft-bottom communities under the effect of organic enrichment as Weston (1990) (North America), Klaoudatos *et al.* (2006) (Mediteranean), and on variations of water salinity as Zettler *et al.* (2007) (Baltic Sea), Dauer *et al.* (1987) and Kennish *et al.* (2004) (North America) or Teske & Wooldrigde (2001) (South Africa). Interactions of the structural factors of these communities may however show a pattern difficult to interpret or may be masked by other factors such as biological interactions or pollutants (Kennish *et al.* 2004). This issue is particularly relevant for WWTP located in sites characterized by variable physical-chemical conditions, which render difficult the evaluation of the source of changes on benthic communities (Gray 2004). Additionally, some authors (Weisberg *et al.*1997) have related the difficulty to differentiate habitat-induced variation from variation caused by natural and anthropogenic stresses which can also constitute a limitation in evaluating the abundance, diversity, distribution and trophic structure of benthic assemblages.

The macrobenthic characterization of the present study showed a pattern similar to several studies that focused on pollution gradients in other areas (Pearson & Rosenberg 1978, Weston 1990), pointing out a significant higher abundance of individuals at the pollution source (S2) when compared to the control site (S6). The diversity and species richness also followed the normal tendency of higher values at the sites far from the pollution source (S4, S5 and S6) and lower values near the WWTP (S1, S2 and S3), as well as the occurrence of shifts in the relative dominance of trophic guilds along the pollution gradient, as predicted by Pearson & Rosenberg (1978). Moreover, trophic structure at the external stations presented a more heterogenous composition of different trophic groups when compared to the inner sites, namely the increase of suspension-feeding and herbivory groups. Carvalho *et al.* (2010) has also noted an increase of contribution of other trophic functional groups in areas of higher water circulation in the Ria Formosa. The water renewal in these areas seems to reduce the amount of organic compounds (Tett *et al.* 2003), decreasing the relative contribution of opportunistic deposit-feeding found around the WWTP discharge.

The opportunistic character of dominant taxa in this study was corroborated by the trophic group analyses, which clearly showed a positive correlation between organic matter content and the prevalence of deposit-feeding characteristics. Previous works carried out at the Ria Formosa (Hewitt & Mudge 2004, Gamito 2006) reported that sediments in external areas present larger grain-size, which contributes to a larger interstitial water renewal and reduces the deposition of detritus on sediments. Such conditions favor the establishment of filter feeders, competition with other taxa, enhance the presence of predators and decrease the densities of opportunistic organisms, increasing local diversity and species richness (Gray 1974, Pearson & Rosenberg 1978). However, the high amount of suspended solids in the water column in discharge areas can be detrimental to the feeding structure of some organisms, namely the suspension feeders (Terlizzi 2005). This factor is particularly important due to the restricted nature of the discharge zone plus the small grain-size of the sediments that can lead to a high sedimentation rate (Terlizzi 2005) and the occurrence of anoxic processes.

The natural variations of water salinity values found from the inner channels of the Ria Formosa to the open channels (Newton & Mudge 2003) seem to be increased by the WWTP discharges inducing very low values at the inner stations. This fact has also contributed to the low diversity found at these sites since only a few macrobenthic taxa are able to support such salinity conditions.

The two-way ANOSIM found significant differences for the "Site" factor, reflected in an evident separation of two distinct groups of sites: S1, S2, S3 and S4, S5, S6 in the MDS analysis. As for the "Month" factor, despite the significant differences revealed by the ANOSIM analysis, there was no heterogeneity in the representation of the "Month" samples. This fact, together with the low similarities found within months in the SIMPER analysis for this factor, suggests that in this study the spatial variability overlaps the temporal factor. This tendency should be however considered with caution since the sampling has not complied replication within months focusing mainly on spatial differences along the pollution gradient. The SIMPER analysis for the "Site" factor suggested that the dominance of the worms Nereididae and of the bivalve *S. plana* at the inner stations has contributed to the dissimilarities among sites.

The *Scrobicularia plana* and Nereididae populations dynamics found in this study highly contributed to the macrobenthic community structure, dominating the sites near the discharge and having a lower contribution at external sites. These two taxa are recognized as tolerant to relatively high organic matter concentrations and while they may occur under normal conditions, these taxa populations are stimulated by organic enrichment due to their ability to feed on organic compounds (AMBI list - www.azti.es). The high amount of organic matter near the WWTP constitutes an exceptional food source these organisms, explaining the high abundance of these taxa near the WWTP. The strong presence of *S. plana* in these areas is possible due to its multitrophic behavior, deposit and filter feeder (Cheggour *et al.* 2005), allowing it to prevail in the absence of filtering conditions. The low values of salinity measured near the discharge point could also have contributed to the dominance of *S. plana* over the scarce abundance of other taxa, due to its strong haline tolerance (Bryan & Uysal 1978). Several authors pointed out the deposit-feeder characteristics of the species within the Nereididae family, its typically high abundance in organic enriched systems (Carvalho *et al.* 2007), and its haline tolerance (Scaps 2002). In the present study Nereididae were not identified at the species level, not allowing the complete interpretation of the local dynamics of these organisms.

The M-AMBI index globally made a distinction among the sites near the discharge channel in relation to the distant ones, following a gradient of anthropogenic pressure. Biotic indices analysis has attributed lower values of EcoQS to S1, S2 and S3 that did not satisfy the requirements of the WFD. The inclusion of *S. plana* and Nereididae in the EGIII associated with lower diversity values was decisive to such status. In the other hand, at S4, S5 and S6 the water quality seemed to be unaffected by the WWTP and showed a satisfactory EcoQS. In fact, the increase of EcoQS from the inner sites to the external ones was in accordance to the differences in community structure found with ANOSIM, SIMPER, and the trophic group analysis. At the S4 station, the intersection of the discharge with an external water channel seems to dilute the effects of pollution by the WWTP, normalizing the organic content of sediments and water salinity values, and consequently increasing the EcoQS. This fact was corroborated by the presence of a higher number of bivalves (e.g. *P. scabrum*, *C. edule*, *Venerupsis pullastra*), gastropod species (*N. pfeifferi* and *B. reticulatum*) and the appearance of crustaceans that were included in EGI and II. Borja & Muxica (2005) pointed out some difficulties in interpreting AMBI results in areas with strong salinity variations and/or low diversity. In the present study that problem seems to have been avoided by the use of a multi-index approach adding the diversity and species richness to the final analysis in M-AMBI, as recommended by the same authors (Borja & Muxica 2005).

Overall the results suggest that the WWTP discharge induces a structural change on the macrobenthic communities and local EcoQS, especially before the dilution of the discharge channel into the secondary channel at S4. In the scope of WFD, new water management measures should be applied in order to mitigate the "Moderate" status waters verified until S3. According to the ecohydrology concept, the properties of ecosystems should serve as management tool to improve water quality (Wolanski *et al.* 2004). One possible solution could be to extend the discharge channel through a pipeline system, to a more hydrodynamic zone, such as S4, where the greater water renewal will increase the dispersion of pollutants, reducing the impact of the discharge. These aspects should be taken into consideration when evaluating the WFD criteria in areas affected by WWTPs to avoid misleading results and unnecessary restoration efforts.

This study has tried to evaluate the response of macrobenthic communities to an organic and haline stress using the qualification of the EcoQS approach, still scarcely applied in local studies. A widespread study focused on the EcoQS along all the Ria Formosa ecosystem is however needed in order to detect the sensitive areas affected not only by the local WWTPS but also by other impact sources allocated there (fish farming, dredging processes, sand extraction), assessing the global range of this stress factors on the structure and functioning of the local communities.

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